Universidade Federal do Rio Grande do Sul
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Dinâmica da comunidade microbiana entre múltiplas escalas espaciais e temporais em lagos rasos costeiros do extremo sul do Brasil

Marla Sonaira Lima

Defesa de Tese de doutorado submetida ao Programa de Pós-Graduação em Recursos Hídricos e Saneamento Ambiental da Universidade Federal do Rio Grande do Sul, como requisito à obtenção do título de DOUTORA.

Orientador: David da Motta Marques

Porto Alegre, 19 de dezembro de 2016

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Descobrir consiste em ver o que todos viram e pensar o que ninguém pensou.”

Albert Szent Gyorgy

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Abreviaturas e Símbolos

AF – Atributos funcionais

AH – Ácidos húmicos

ARISA – Automated ribosomal intergenic spacer analysis

BCC – Bacterial community composition

BDEHR – Beta diversity-environmental heterogeneity relationship

BNES – Componente da diversidade beta - aninhamento

BSIM- Componente da diversidade beta – *turnover*

BSOR – Índice de diversidade beta

CCB – Composição da comunidade bacteriana

CDOM – Chromophoric dissolved organic matter

Chla – Clorofila a

ChlF – Chlorophyll fluorescence

CI – Curve integration index

COD – Carbono orgânico dissolvido

COT – Carbono orgânico total

DNA – Deoxyribonucleic acid/ácido desoxirribonucleico

DO – Dissolved oxygen

DOC – Dissolved organic carbon

FT – Functional traits

HS – Humic substances

MODC - Matéria orgânica dissolvida cromofórica

NH_4^- - Amônia/ammonium

NO_3^- - Nitrato/nitrate

NT – Nitrogênio total

OD – Optical density

ORP – Oxidation-redution potential

OTUs - Operational taxonomic units

PCNM – Principal Coordinates of Neighbour Matrices

PERANOVA – Permutation ANOVA

PERMANOVA – Permutation MANOVA

PT – Fósforo total

RDA – Redundance analysis

RNA – Ribonucleic acid/ácido ribonucleio

SHT – Sistema Hidrológico do Taim

SIMPER – Similarity percentage analysis

SRP – Soluble reactive phosphorus

TN – Total nitrogen

TOC – Total organic carbon

TP – Total phosphorous

TSS – Total solid suspensed

TURB – Turbidity

VIF – Variation inflation factor

WT – Water transparency

WV – Wind velocity

Resumo

A comunidade bacteriana aquática foi estudada quanto à composição, biomassa e à atividade de consumo de carbono (perfil de consumo potencial de substratos orgânicos através de *Ecoplates* Biolog™) ao longo de diferentes escalas espaciais e temporais em 26 lagoas costeiras do sul do Brasil. Com o objetivo de investigar a influência de distintos padrões temporais e espaciais na dinâmica da comunidade, investigou-se *i*) a variação da composição da comunidade bacteriana (CCB) e de seus atributos funcionais (AF) em função de efeitos exclusivos do ambiente, do espaço, do tempo, ou de todos os fatores na Lagoa Mangueira; *ii*) os padrões espaciais de distribuição da diversidade beta bacteriana aquática e os fatores influenciando tais variações ao longo de 25 lagoas com diferentes graus de conectividade, no Sistema do rio Tramandaí; e *iii*) a dinâmica temporal da composição e biomassa bacteriana ao longo de um distúrbio provocado por frentes frias na Lagoa Mangueira. Dessa forma, três perguntas principais foram compostas: *i)* há variação da composição da comunidade bacteriana (CCB) e de seus atributos funcionais (AF) em função de efeitos exclusivos do ambiente, do espaço, do tempo, ou de todos os fatores? *ii)* a diversidade beta bacteriana aquática apresenta padrão de distribuição aninhado ou turnover e quais processos influenciam tais padrões? *iii)* a composição e biomassa da comunidade bacteriana muda em resposta a distúrbios de curta duração ocorridos pela entrada de frente fria polar? Como resultados consistentes observaram-se que: *i)* a CCB e seus AF variam temporal e espacialmente, sendo sua distribuição explicada principalmente pelos filtros ambientais (*species-sorting*) e pela distância espacial, respectivamente; *ii)* a diversidade beta bacteriana entre e dentro das lagoas é principalmente o resultado da substituição de espécies (*turnover*), sendo que a relação entre a diversidade beta bacteriana e a heterogeneidade

ambiental entre as lagoas parece ser resultado de *species-sorting*, enquanto que dentro das lagoas pode ser o resultado do efeito de massa, devido à alta conectividade e dispersão dentro das lagoas; *iii*) a comunidade bacteriana em termos de biomassa apresenta-se resiliente ao distúrbio, enquanto que a composição da comunidade apresenta-se resistente ao distúrbio provocado por frente fria. Assim, esta tese contribui no incremento de discussões acerca dos padrões ecológicos da comunidade bacteriana entre múltiplas escalas espaciais e temporais, mostrando que a menor escala de heterogeneidade bacteriana detectada pode estar positivamente relacionada ao tamanho do lago. Além disso a comunidade apresenta distintas respostas de estabilidade em termos composicionais e biomassa em eventos que causam distúrbios ao sistema. Finalmente, esse estudo conseguiu verificar variação espacial e temporal da comunidade bacteriana entre multiplas escalas no sistema aquático, em resposta às variações nas características ambientais nas diferentes escalas estudadas. Portanto, esse estudo identifica a resposta bacteriana aos distintos padrões espaciais e temporais, e quais mecanismos influenciam a comunidade bacteriana, evidenciando a importância em se considerar múltiplas escalas para a compreensão da biogeografia microbiana e a sua habilidade de responder a perturbações.

Palavras-chave: ARISA, biogeografia microbiana, composição da comunidade bacteriana, diversidade beta, ecoplate, lagoas costeiras, metacomunidade, resistência, resiliência.

Abstract

The aquatic bacterial community was studied in terms of composition, biomass and carbon consumption activity (physiological profile - Ecoplate) over different spatial and temporal scales in 26 coastal lagoons in southern Brazil. In order to investigate the influence of different spatial and temporal patterns in community dynamics, we investigated *i*) the variation of the bacterial community composition (CBB) and its functional traits (FT) as a function of exclusive effects of the environment, of the space, of the time, or of all the factors in Lake Mangueira; *ii*) the patterns of distribution of the aquatic bacterial beta diversity and the factors influencing such variations along 25 lakes with different degrees of connectivity in the Tramandaí River System; and *iii*) temporal dynamics of bacterial composition and biomass along a disturbance provoked by cold fronts in Lake Mangueira. In this way, three mainly questions were made: *i)* is there variation in the BCC and its FT as a function of the sole purpose of environment, of space, time, or all of the factors? *ii)* Does aquatic bacterial beta diversity presents a nested pattern of distribution or turnover and which processes influence these patterns? *iii*) Do the composition and biomass of the bacterial community change in response to short-term disturbs due to the polar cold front event? As a consistent result, it was observed that: *i*) the BCC and its FT varied temporally and spatially, and its distribution is explained mainly by environmental filters (species-sorting) and spatial distance, respectively; *ii*) the bacterial beta diversity between and within lake is primarily the result of species turnover, and the bacterial beta diversity-environmental heterogeneity relationship (BDEHR) among lakes appears to result from species-sorting, while within-lake the beta diversity should be a result of the mass effect, due to the high connectivity and dispersion within-lake; *iii*) the bacterial community, in terms of biomass is resilient

to the disturbance, whereas the bacterial composition is resistant to the disturbance. Thus, this thesis contributes in increasing discussions about the ecological patterns of the bacterial community between multiple scales showing that the smaller scale of bacterial heterogeneity detected can be positively related to the lake size. In addition, the community presents distinct stability responses in terms of composition and biomass over events that cause disturbances to the system. This study was able to verify spatial and temporal variation of the bacterial community among multiple scales in the aquatic system, in response to different environmental factors among the multiple scales studied. Therefore, this study identified the bacterial response to the different spatial and temporal patterns, and the mechanisms that influence the bacterial community, evidencing the importance in considering multiple scales for the understanding of microbial biogeography and its ability to respond to disturbs.

Key-words: ARISA, bacterial community composition, beta diversity, coastal lakes, ecoplate, metacommunity, microbial biogeography, resistance, resilience.

Capítulo 1. Introdução

Justificativa

A compreensão de padrões e processos da biodiversidade é uma questão fundamental em estudos ecológicos. A biodiversidade governa e regula processos ecossistêmicos, influenciando a forma como os ecossistemas funcionam (Hillebrand e Matthiessen, 2009). Por isso, entender os mecanismos que a determinam é essencial para prever alterações nos ecossistemas.

Nesse contexto, as bactérias representam grande fração da diversidade do planeta (Pace, 1997). Essa diversidade inclui tanto a composição genética dos microrganismos, como seu papel ecológico ou funcional dentro do ecossistema (Hunter-Cevera, 1998), assumindo papel essencial no seu funcionamento. Esses microorganismos atuam nos processos de remineralização de nutrientes orgânicos, participando de toda a respiração anaeróbica e de grande parte da aeróbica, da decomposição e regeneração de nutrientes e também como importantes componentes das teias tróficas (Cole, 1999; Pomeroy, 1974; Azam *et al.*, 1983). Tais serviços bacterianos prestados aos ecossistemas dependem fortemente da riqueza de espécies e da composição taxonômica (Bell *et al.*, 2005), sendo assim decisivo identificar quais fatores afetam a estrutura das comunidades bacterianas.

Dada a importância dessas comunidades para os processos metabólicos nos ecossistemas, e sabendo-se que as bactérias podem representar mais de 90% dos microorganismos no ambiente aquático (Hahn, 2006) torna-se relevante identificar a influência dos padrões espaciais e temporais na sua distribuição e organização e entender como variam entre diferentes escalas de tempo e de espaço para reconhecer os processos que governam a assembléia bacteriana e a sua habilidade de responder a

perturbações. Estudos com este enfoque nos permitem, além de descrever padrões de distribuição e de diversidade de espécies em função de gradientes ambientais e geográficos, reconhecer os mecanismos que promovem variações na estruturação das comunidades (Logue *et al.*, 2011).

Em 1934, Bass-Becking, referindo-se à distribuição dos microorganismos, postulou: "*Everything is everywhere, but the environment selects*", indicando que os microorganismos distribuem-se ubiquamente no planeta em função da seleção de fatores locais, ou seja, que as condições ambientais e interação entre espécies são mais relevantes que fatores regionais, como a distância entre ecossistemas. O reconhecimento da importância da seleção ambiental para os taxa bacterianos, por Bass-Becking, destaca a relevância de *trade-offs* em traços ecológicos entre as espécies bacterianas, incorporando o conceito de nicho em um ambiente heterogêneo, o qual constitui um dos quatro paradigmas de metacomunidade (species-sorting) delineados por Leibold *et al.* (2004). Desde então se evoca o pensamento de padrões biogeográficos para populações microbianas, o qual vem tornando-se cada vez mais significativo devido ao advento de técnicas moleculares para o estudo da diversidade microbiana. Com o aperfeiçoamento de técnicas para acessar a diversidade microbiana, constatou-se que essa diversidade é muito superior à obtida através de técnicas cultiváveis, uma vez que cerca de 99% da diversidade microbiana não pode ser cultivada (Muyzer, 1999). Assim, vem crescendo o debate acerca dos fatores responsáveis pelos padrões biogeográficos microbianos, que têm sido considerados importantes estruturadores dessas comunidades (Azevedo e Farjalla, 2010).

Apesar da visão clássica de ampla distribuição desses microorganismos no planeta, alguns estudos apontam para a estruturação espacial das comunidades bacterianas, indicando padrões biogeográficos na distribuição desses organismos (e.g.

Fenchel e Finlay, 2004; Martiny *et al.*, 2006; Verleyen *et al.*, 2009; Heino *et al.*, 2010). Porém, outros autores contrariam a equivalência entre padrões biogeográficos em microbiologia e macroecologia, devido às taxas de dispersão e evolução extremamente distintas em bactérias (Milici *et al.*, 2016).

Atualmente os estudos de diversidade perpassam diferentes escalas, desde a escala local, os quais incluem a descrição da riqueza de espécies (diversidade alfa), as diferenças na diversidade entre escalas temporais e espaciais (diversidade beta) e a diversidade regional (diversidade gama) (Jones *et al.*, 2012). Nesse sentido, a variação na composição de espécies e na estrutura de comunidades biológicas entre sítios (i.e. diversidade beta) é consequência de fatores locais e regionais, que afetam sua dispersão (Leibold *et al.*, 2004; Logue *et al.*, 2011). Ou seja, comunidades microbianas aquáticas variam composicional e funcionalmente entre escalas espaciais e temporais, como resposta à interação de múltiplos fatores ambientais embutidos nessas diferentes escalas, tais como condições físico-químicas (van der Gucht *et al.*, 2007; Souffreau *et al.*, 2015), interações bióticas, principalmente entre fitoplâncton e zooplâncton (Kent *et al.*, 2004; Kent *et al.*, 2006; Kent *et al.*, 2007), estado trófico (Yannarell *et al.*, 2003), paisagem e morfometria da lagoa (Lear *et al.*, 2014). Mas ainda é pouco compreendido o quanto cada fator contribui para a resposta bacteriana. Tradicionalmente esses fatores vêm sendo estudados em conjunto, sem a preocupação de se avaliar a contribuição isolada de cada um, através da partição da variância desses fatores, por exemplo. No entanto a análise da influência pura de cada fator na variação composicional e funcional do bacterioplâncton vem revelando respostas contrastantes na estruturação das comunidades. Alguns estudos apontam para efeitos significativos exclusivos do ambiente (van der Gucht *et al.*, 2007; Logue e Lindström, 2010), exclusivos do espaço

(Lear *et al.*, 2014) ou de ambos do ambiente e do espaço (Langenheder e Ragnarsson, 2007; Schiaffino *et al.*, 2011; Liu *et al.*, 2015).

Nesse contexto, é importante reconhecer como diferentes gradientes ambientais afetam a diversidade beta bacteriana, por exemplo, uma menor heterogeneidade ambiental - seja por causas naturais (e.g. menor variação das características limnológicas, menor área, menor conectividade, etc) ou antrópicas, tendem a resultar em menos espécies do *pool* regional, reduzindo a diversidade beta em comparação a ambientes com maior heterogeneidade ambiental, que tendem a promover maior diversidade de espécies (Ricklefs e Schluter 1993). Nessas condições ambientalmente homogêneas, as diferenças na composição de espécies entre duas comunidades podem refletir a perda de espécies (aninhamento). Isso ocorre quando a composição de espécies em comunidades pobres é um subconjunto da composição de espécies de comunidades ricas (Wright *et al.*, 1998), provavelmente devido à exclusão de espécies suscetíveis à perda de determinadas características ambientais e de habitats. Entretanto, em lagoas que diferem nas suas características ambientais, mas apresentam riqueza de espécies similar, as diferenças na composição de espécies entre duas comunidades podem ser atribuídas à substituição de espécies (*turnover*) imposta por diferentes filtros ecológicos (Wright *et al.*, 1998; Baselga, 2010).

Mundialmente, é crescente o número de estudos investigando os fatores que afetam a dinâmica e estruturação das comunidades bacterianas aquáticas. No Brasil, esse número é igualmente crescente, entretanto concentra-se principalmente em estudos de metabolismo microbiano, produção e crescimento microbiano (e.g Farjalla *et al.*, 2006; Amado *et al.*, 2006; Farjalla *et al.*, 2009; Vidal *et al.*, 2011; They *et al.*, 2012; Amado *et al.*, 2013), sendo ainda muito incipientes estudos focando os padrões biogeográficos, principalmente avaliando-se diferenças da diversidade e composição

bacteriana entre múltiplas escalas. De maneira geral, alguns autores sugerem ampla distribuição taxonômica ao redor do globo, demonstrando similaridade dos níveis taxonômicos de bacterioplâncton entre sistemas aquáticos do Brasil e do mundo (Tessler *et al.*, 2016), outros indicam a noção de espécies e de processos bacterianos ecologicamente definidos, suportando as relações entre diversidade bacteriana e parâmetros ambientais na região tropical (Silveira *et al.*, 2011) e verificam relação positiva entre a heterogeneidade do bacterioplanctôn (composição, morfotipos, biomassa, biovolume, produção) e a área do lago (Haig-They *et al.*, 2010).

Como se percebe, há ainda para a microbiologia aquática uma lacuna para o entendimento da ideal escala de variação característica das comunidades microbianas aquáticas (Lindstrom e Langenheder, 2012). Reconhecer como as comunidades microbianas variam em função do tempo e do espaço é uma peça chave para o entendimento básico da diversidade bacteriana aquática, o que dificulta o desenvolvimento de teorias acerca da sua estabilidade (Jones *et al.*, 2012), principalmente frente à distúrbios, por exemplo.

A discussão acerca de distúrbios ambientais vem recebendo bastante destaque em estudos ecológicos, porém menor ênfase tem sido dada aos padrões de resposta da comunidade microbiana a esses distúrbios (Shade *et al*. 2012). Com as mudanças climáticas globais, estima-se o aumento em frequência de eventos climáticos intensos (IPCC, 2007), promovendo maior pressão sobre rios, lagos e, pequenos corpos d'águas, pela variação abrupta de temperatura, carga de nutrientes, alteração da hidrologia, etc (Graham e Vinebrooke, 2009; Carey *et al*. 2012). Tais variações no sistema acabam por afetar significativamente a comunidade planctônica (White e Picket, 1985). Nesse contexto, a estabilidade da comunidade planctônica frente a distúrbios, como de frentes frias, por exemplo, reflete sua sensibilidade ou insensibilidade ao agente estressor

(Ryckiel, 1985). Mudanças na composição microbiana frequentemente associam-se a mudanças nas taxas de processos ecossistêmicos e vice-versa (e.g Schimel e Gulledge, 1998; Gulledge *et al.* 1997).

Nesta tese procurou-se investigar a variação espacial e temporal da comunidade microbiana entre múltiplas escalas e quais fatores influenciam tais variações. Os estudos foram conduzidos em dois sistemas aquáticos distintos: *i*) sistema hidrológico do Taim, estudando-se a comunidade microbiana da lagoa Mangueira e *ii*)sistema hidrológico do Rio Tramandaí, analisando-se as comunidades microbianas ocorrentes em 25 lagoas pertencentes a esse sistema. Ambos os sistemas são compostos de lagoas rasas e costeiras, de clima subtropical.

A Lagoa Mangueira caracteriza-se por seu extenso comprimento (90km) e grande área total (820 km^2). Esse extenso espelho d'água é cercado por dunas fixas no lado costeiro, ao norte é dominada por macrófitas emergentes e ao sul por um denso leito de macrófitas submersas, ao sul. Na porção ocidental da lagoa, há principalmente o cultivo de arroz em antigas zonas húmidas. Esse sistema sofre grande influência de alta freqüência de frentes frias originárias da Antártida, causando mudanças significativas nas condições hidrometeorológicas e sendo a força dominante nesse ecossistema aquático (Fragoso et al., 2008). Essas frentes apresentam maior freqüência e intensidade durante o inverno e a primavera (Brito et al., 1996) e têm como principal efeito a mistura e homogeneização da coluna de água dessas lagoas (Tundisi 1983, Tundisi et al.2010). Por essas características, a Lagoa Mangueira foi palco para o desenvolvimento dos estudos apresentados nos capítulos 2 e 4 desta tese.

Com relação às lagoas pertencentes ao sistema do Rio Tramandaí, elas foram escolhidas para o desenvolvimento do capítulo 3 da tese por abrangerem uma ampla gama de condições ambientais ao longo da zona costeira do litoral norte do Rio Grande

do Sul, com variados graus de conectividade, idade, influência marinha e paisagem (áreas de pastagem / agricultura, florestas, arrozais e influência urbana), contribuindo para a abordagem de avaliação de variação da diversidade beta bacteriana entre e dentro das 25 lagoas.

Por essas considerações, surgem importantes questões a respeito da estrutura e função das comunidades bacterianas entre diferentes escalas espaciais e temporais que nortearam esse estudo, tais como:

i) a comunidade bacteriana varia em sua composição e função dentro do lago, como resposta aos efeitos exclusivos do ambiente, do espaço, do tempo, ou de todos os fatores? Qual a escala apropriada para amostragem bacteriana?

ii) quais os padrões de distribuição da diversidade beta bacteriana aquática e quais os mecanismos influenciam tais variações?

iii) qual a dinâmica da comunidade bacteriana em resposta a distúrbios de curta duração ocorridos pela entrada de frentes frias polares ? Essa comunidade pode se apresentar sensível e consequentemente resiliente ao distúrbio ou insensível e, portanto, resistente?

Dessa forma, este estudo visa contribuir com informações relevantes acerca da dinâmica da comunidade microbiana aquática em lagoas costeiras rasas que sofrem freqüente influência dos fatores locais, do regime de ventos, da paisagem, da morfologia entre outros, para compreender, suas variações espaciais e temporais e suas respostas frente a distúrbios que influenciam a ecologia desses sistemas.

Hipóteses

Pelas questões referenciadas acima foram formuladas hipóteses para a realização deste trabalho. A seguir são apresentadas as hipóteses desenvolvidas para cada capítulo da tese.

Capítulo 2

Uma lagoa de grande extensão, com grande influência do vento, frequentemente misturada e com grande conectividade, como a Lagoa Mangueira, não apresentará barreiras de dispersão para taxa bacterianos e seus atributos funcionais, até mesmo em grandes meso escalas. Assim, as variações espaciais e temporais na composição e função bacteriana devem ser reflexo dos filtros ambientais impostos.

Capítulo 3

Dentro e entre as lagoas o padrão de distribuição das comunidades terá prevalencia do *tunover* (substituição de espécies), devido à heterogeneidade ambiental apesar da alta conectividade das lagoas. Também previmos que a beta diversidade dentro e entre as lagoas será positivamente relacionada à heterogeneidade de habitats (ambiental, fitoplâncton, a paisagem e a distância espacial), nas escalas intra-lagoa e entre lagoas.

Capítulo 4

A comunidade bacteriana da Lagoa Mangueira, sob efeito de distúrbios durante o inverno, apresentará uma mudança composicional e de biomassa, seguida de uma recuperação (resiliencia) à medida que os distúrbios diminuem.

Objetivo Geral

O objetivo geral desta tese de doutorado foi investigar os padrões espaciais e temporais da composição filogenética, biomassa e dos atributos funcionais da comunidade bacteriana em lagoas costeiras rasas do Sul do Brasil. Dessa forma, contribuir para o entendimento dos padrões biogeográficos e da resposta frente a distúrbio das comunidades bacterianas entre múltiplas escalas e avaliar os fatores responsáveis por esses padrões.

Nesse contexto, esta tese desenvolveu objetivos específicos que foram desenvolvidos em diferentes capítulos.

Objetivos Específicos

Capítulo 2

Investigar a heterogeneidade espacial e temporal da composição da comunidade bacteriana (CCB) e de seus atributos funcionais (AF) e avaliar o efeito isolado das condições ambientais, da biomassa fitoplanctônica, do espaço e do tempo, através da partição da variância da CCB e AF na lagoa Mangueira.

Capítulo 3

Identificar os padrões de distribuição da diversidade beta bacteriana aquática e seus componentes. Também avaliar quais fatores, entre ambientais, biológicos, espaciais ou de paisagem, promovem tais padrões na comunidade bacteriana.

Capítulo 4

Analisar a dinâmica bacteriana em termos da composição da comunidade e biomassa ao longo de distúrbios decorrentes de entradas de frentes frias polares. E

avaliar a sensibilidade bacteriana aos distúrbios, considerando se sua biomassa e composição são resiliente ou resistente a ele.

Material e Métodos

Área de Estudo

O presente estudo foi conduzido em dois sistemas lacunares costeiros rasos, do Sul do Brasil. Um é a Lagoa Mangueira, pertencente ao sistema hidrológico do Taim. Essa lagoa é rasa, costeira, possui uma grande área de cerca de 820 km^2, grande extensão, 90 km e sofre intensa ação de ventos, provocando frenquentes distúrbios na coluna d'água. Por tais características, ela foi escolhida para investigar as questões referentes ao capítulo 2 e capítulo 4, uma vez que sua morfometria e hidrologia, com intensa influência de ação de ventos pode influenciar o padrão de distribuição espacial e temporal e a dinâmica das comunidades. O segundo sistema estudado, no capítulo 3, é o Sistema Hidrológico do Rio Tramandaí composto por 41 lagoas costeiras rasas no litoral norte do Rio Grande do Sul, que historicamente se formaram durante o período Quaternário como resultado de transgressões e regressões marinhas (Holz, 1999). Das 41 lagoas pertencentes a este sistema foram analisadas 25. As características consideradas na escolha das lagoas foram a proximidade ao mar, diferentes graus de conectividade, área, perímetro e facilidade de acesso, através de visualização em cartas cartográficas de escala 1:50.000. Tais características foram definidas para compreender o padrão espacial da distribuição da diversidade beta e seus componentes ao longo do sistema de lagoas.

Delineamento Amostral

O delineamento amostral variou em função das questões formuladas especificamente para cada capítulo, focando principalmente nas diferentes escalas

espaciais e temporais para a coleta de dados. Nesse contexto, foram coletados dados ambientais e biológicos, cujo detalhamento de análise é apresentado nos capítulos que seguem.

Estrutura da Tese

A presente tese de doutorado representa um conjunto de estudos, que foram realizados em dois ambientes aquáticos distintos, realizados no âmbito de diferentes projetos com objetivos afins. Assim, nesta tese, são apresentados três capítulos que se referem aos artigos desenvolvidos como resultado da pesquisa de doutorado:

Capítulo 2. Contrasting factors drive within-lake bacterial community composition and functional traits in a large shallow subtropical lake

Neste capítulo é apresentado o artigo publicado na revista Hydrobiologia. Ele investiga os padrões temporal e espacial da comunidade bacteriana aquática e apresenta a contribuição relativa dos fatores ambientais, biológico, espacial e temporal sobre a composição da comunidade.

Capítulo 3. Bacterial beta diversity among lakes, but not within lakes, is positively related to different aspects of habitat heterogeneity

Este capítulo é um manuscrito de artigo submetido para a revista The ISME Journal. Neste estudo, a comunidade bacteriana aquática foi analisada com o objetivo de compreender a distribuição da diversidade beta e seus componentes ao longo e dentro

de 25 lagoas rasas, e verificar quais fatores da heterogeneidade de habitat influenciam essa variação.

Capítulo 4. Resistance and resilience of aquatic bacterial community over a cold front event

Este capítulo é um manuscrito de artigo ainda não submetido para publicação. Neste estudo, a comunidade bacteriana aquática foi analisada, em termos de composição e biomassa, com o objetivo de compreender a sua sensibilidade frente a distúrbio provocado por frente fria polar, e verificar os fatores influenciando seus padrões.

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Capítulo 2. Contrasting factors drive within-lake bacterial community composition and functional traits in a large shallow subtropical lake

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Contrasting factors drive within-lake bacterial community composition and functional traits in a large shallow subtropical lake

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Abstract

Lakes are considered as “islands” for assessing microbial biogeography, but bacterial community composition (BCC) and function may vary significantly within lakes, with

the roles of scale and connectivity still unclear. This study investigated the spatial and temporal heterogeneity of the BCC (Automated Ribosomal Intergenic Spacer Analysis) and Functional Traits (FT, carbon-source utilization), and the contribution of: (i) environmental variables, (ii) phytoplankton, (iii) season and (iv) space, through variance partitioning in the large and well-mixed Lake Mangueira. The BCC and FT differed in time and space, with BCC being explained by environmental variables and phytoplankton, whereas FT explained only by space. The smallest scale of variability detected for the BCC and FT (~49 km) was the largest in comparison with findings in other studies, suggesting an effect of lake size (fetch and connectivity). Our results indicate that barriers to bacterial dispersal due to long distances are overcome by high connectivity, reinforcing the role of species sorting for BCC. FT were probably driven by gene dispersal and/or the effects of local conditions on migrant bacterial taxa and resuspended bacteria. Our results highlight the role of within-lake heterogeneity for ecosystem functioning and the implications for the appropriate scale for sampling bacterial communities.

Keywords: Automated Ribosomal Intergenic Spacer Analysis, Ecoplate, within-lake biogeography, variance partitioning, species sorting, dispersal, Lake Mangueira

Introduction

Microbes represent a large fraction of the biosphere's diversity (Pace, 1997) and are key players in aquatic ecosystem functioning (Cole, 1999). Consequently, understanding the mechanisms controlling their diversity and function in aquatic ecosystems is of paramount importance.

Lakes are often assumed to have a homogeneous microbial community and are therefore considered as "islands" in most biogeographical analyses that test factors shaping microbial diversity and distribution. Consequently, the factors driving variation among lakes is relatively well known and lake microbial communities change rapidly in space and time in response to habitat size (Horner-Devine et al., 2004; Yannarell & Triplett, 2004; Bell et al., 2005; Reche et al., 2005; Green & Bohannan, 2006; Crump et al., 2007). Yet, increasing evidence for within-lake variability in factors such as physical and chemical conditions (van de Gucht et al., 2007; Souffreau et al., 2015) biotic interactions (Kent et al., 2006), and trophic status (Yannarell et al., 2003; Wu et al., 2007), suggests microbial communities within lakes are also heterogeneous. As environmental variables tend to be structured across the landscape within space and time, the disentanglement of the drivers of microbial diversity and function is therefore a key topic for research (Lear et al., 2014). Studies using approaches based on variance partitioning have obtained contrasting results, indicating significant pure effects of the environment (van de Gucht et al., 2007; Logue & Lindström, 2010), space (Lear et al., 2014), or a combination of both environment and space (Langenheder & Ragnarsson, 2007; Schiaffino et al., 2011; Liu et al., 2015; see the thorough discussion by Lindström & Langenheder, 2012).

Several studies have described the temporal and spatial dynamics of bacterial functional traits using patterns of substrate utilization as a proxy for bacterial activity in freshwater (e.g. Grover & Chrzanowski, 2000; Christian & Lind, 2006; Tiquia, 2010), sediment-water interfaces (Christian & Lind, 2007) and the marine environment (Sala et al., 2006). All these studies found that substrate utilization was strongly associated with environmental conditions. In contrast, studies on smaller scales in freshwater ponds (Lear et al., 2014) and small to meso-scales in soil (Bissett et al., 2010), have found either a higher contribution of geographical distance (Lear et al., 2014) or an absence of relationships between function and environmental variables (Bissett et al., 2010). These differences suggest that other factors such as the degree of connectivity, rates of dispersal and life history of microorganisms can drive bacterial functional traits in space and time (Bissett et al., 2010; Severin et al., 2013; Lear et al., 2014).

Few studies address biogeographical questions within individual lakes since rates of dispersal and connectivity are typically assumed to be high within lakes. Surprisingly, these studies have found not only an important additional role of within-lake heterogeneity of environmental conditions (Wu et al., 2007; Shade et al., 2008; Tian et al., 2009), but also evidence for barriers to dispersal on scales as small as 20 m in a lake (Lear et al., 2014). Given such small-scale heterogeneity within lakes, a key question in the study of microbial diversity and function is the most appropriate scale for sampling.

Similarly to large-bodied organisms, microbes exhibit a distance-decay relationship between species composition and geographic distance (see review in Green and Bohannan, 2006), which suggests an increasing role of environmental heterogeneity and barriers for dispersal in larger lakes. However, the relative role of local factors (i.e., environmental variables) versus space (i.e., barriers to dispersal) in shaping the bacterial

community composition and functional traits within a lake remains unknown. We hypothesized that a wind-driven and well-mixed large lake with high connectivity will not present barriers to the dispersal of bacterial taxa and their functional traits, even across larger meso-scales.

In this study we investigated the spatial and temporal heterogeneity of the bacterial community composition (BCC) and functional traits (FT), as well as the pure effects of lake environmental conditions, phytoplankton biomass, space and time, through variance partitioning of BCC and FT in Mangueira, a large, shallow, sub-tropical lake. The sampling design covered the 90-km length of the lake during one year, i.e., a biogeographical mesoscale (10-3000 km; Martiny et al., 2006). We tested whether there were consistent differences among lake regions (center, south and north) and seasons (winter, spring, summer and autumn) in BCC and FT, and also which explanatory variables significantly explained individual fractions of the variances of BCC and FT.

Materials and Methods

Study Area and Sampling

Lake Mangueira (Fig. 1) is located in the state of Rio Grande do Sul, southern Brazil (~30°31’22”S 53°07'48”W). It is a large shallow subtropical coastal lake, covering a total area of 820 km^2, 90 km long and 3-10 km wide, with a mean depth of 2.6 m and a maximum depth of 7.0 m. The trophic state ranges from oligotrophic to mesotrophic (Crossetti et al., 2013). Lake Mangueira is surrounded by fixed dunes on

the Atlantic Ocean side, by emergent macrophytes in the northern wetland, and a dense bed of submersed macrophytes on the southern side. The western side is used for agriculture, primarily rice production in former wetlands.

Water samples for environmental and biological variables including chlorophyll *a*, phytoplankton, and bacterial compositional and functional characterization were collected in summer (February 2010), autumn (May 2010), winter (August 2010) and spring (November 2010). In each season, the lake was sampled in an identical fashion for all variables, at 18 sampling points: 6 in the southern, 6 in the center and 6 in the northern part of the lake (Fig. 1 and Supplementary Table S1).

Location of Figure 1

Environmental variables and Chlorophyll *a*

Turbidity (Turb), pH, dissolved oxygen (DO), depth, water temperature and oxidation-reduction potential (ORP) were measured with a multiparameter probe (YSI 6600). Water transparency (WT) was estimated with a Secchi disk, and the amount of total suspended solids (TSS) was assessed gravimetrically by water evaporation in porcelain dishes (APHA, 1999). Nutrients, including total nitrogen (TN), nitrate (NO_3^-), and soluble reactive phosphorus (SRP) were measured through colorimetric methods following APHA (1999). Analyses of ammonium (NH_4^-) and total phosphorus (TP) followed Mackereth et al. (1989). A Carbon Analyzer (Shimadzu VCPH) was used to determine the dissolved organic carbon (DOC) of the fraction that passed through a 450ºC pre-combusted glass fiber filter (0.45 μm mean mesh size). Chlorophyll *a* (Chl*a*) was extracted from GF/F filters with 90% ethanol and measured by spectrophotometry

147 (Jespersen & Christoffersen, 1987). Humic substances (HS) were estimated as the ratio
148 of the absorption coefficients at 250 and 365 nm (Strome & Miller, 1978), using a
149 Varian Cary 1-E spectrophotometer with a quartz cuvette.

150 The meteorological data (wind velocity and direction; precipitation; nebulosity,
151 i.e. the percentage in tenths of the sky covered by clouds; insolation and evaporation)
152 were obtained from the Santa Vitória do Palmar Meteorological Station (INMET, Rio
153 Grande do Sul), located approximately 23 km from the lake, with data collected three
154 times per day (00:00, 12:00 and 18:00 h). Data were interpolated according to the time
155 when each sampling point was visited.

156

157 Phytoplankton biomass

158

159 Phytoplankton was counted according to Utermöhl (1958) after sedimentation
160 (Lund et al., 1958). At least 100 specimens of the most frequent species were
161 enumerated (counting error < 5%, Lund et al., 1958). Biomass ($mm^3\ L^{-1}$) was estimated
162 through biovolume, according to Hillebrand et al. (1999). For data analysis, the biomass
163 of all phytoplankton taxonomical classes was used.

164

165 Bacterial composition and functional traits

166

167 The bacterial community composition (BCC) was assessed through Amplified
168 Ribosomal Intergenic Spacer Analysis (ARISA). Bacterial cells were concentrated
169 through filtration of 250 ml of water from each sample onto cellulose acetate membrane
170 filters (0.22 µm pore size; 47 mm diameter; Sartorius). Total DNA was extracted from

the membrane filter using the PowerSoil DNA isolation kit (MO BIO). We used the filter in place of the 0.25 g soil recommended for this method. The region between the 23S and 16S ribosomal RNA genes from the total DNA extracted was amplified using the 6-FAM-labelled universal primer 1406F (5′- TGYACACACCGCCCGT-3′) and the bacteria-specific primer 23Sr (5′-GGGTTBCCCCATTCRG-3′) (Fisher and Triplett, 1999; Yannarell et al., 2003). ARISA profiles were analyzed according to Jones & McMahon (2009). Since this method under-represents rare member of the community, BCC hereafter refers to the most abundant taxa.

The functional traits of the heterotrophic microbial community were measured as carbon utilization patterns through Biolog Ecoplates® (Hayward, CA, USA). In the field, water samples were pre-filtered in a 20 µm-mesh sieve and placed in 50-ml amber glass bottles to prevent interference from phytoplankton. In the laboratory, samples were inoculated in the plates and incubated at approximately 22°C (Christian & Lind, 2007). The utilization of the substrates was assessed through optical density (OD) measured at 590 nm, with an automated plate reader (SpectraMax 5.0, Molecular Devices), immediately after the wells were filled (t=0 h) and every 24 h for 12 days, to ensure that saturation of carbon utilization was reached in all samples (Salomo et al., 2009).

Statistical Analyses

For BCC analyses we used relative abundance data, including OTUs with >5% frequency of occurrence (Shade et al., 2008). For functional traits, we corrected the raw absorbance data by subtracting each response well against its own first reading (t=0), to

compensate for the intrinsic absorbance of the carbon sources (Insam & Goberna, 2004). Then, we subtracted the control from the response well, and any negative values were considered zero (no oxidation). The areas under the curve of the three Ecoplate replicates were integrated for each of the 31 carbon sources in order to estimate the curve integration index (CI) (Guckert et al., 1996). The CI is a trapezoidal approximation, and this approach incorporates additional information from the absorbance versus incubation time (lag phases, rates of color development, and maximum absorbance) into a single number. The single value obtained for each substrate was expressed as the percentage area of the total area of the plate (sum of all areas of substrates).

Since the data were non-parametric, we performed PERMANOVAs (PERmutation MANOVAs) for BCC and for FT, and PERANOVAs (PERmutation ANOVAs) for environmental variables (Euclidean distance) and phytoplankton (Bray-Curtis distance) to test for differences over time (winter, spring, summer, autumn) and space (south, center and north). P-values were adjusted by Bonferroni correction for multiple testing. The OTUs (Bray-Curtis of transformed abundances) and substrates (Euclidean distance) that contributed most to the dissimilarity among groups of factors (time and space) were examined through Similarity Percentage Analysis (SIMPER), with a threshold of > 1.5% for OTUs and >5.5% for substrates.

The degree of association between the BCC and FT data was assessed through a Mantel test (Mantel, 1967). This is used to compare two independent dissimilarity (or similarity) matrices describing the same set of entities, and to test whether the correlation is higher than expected by chance (Sokal & Rolf, 1995).

For the variance partitioning of BCC and FT, we first performed Redundant Analyses (RDA) on each of the four explanatory matrices: environmental variables

(physical, chemical, and meteorological variables + chlorophyll *a*); time (using dummy variables to encode for summer, spring, winter and autumn); phytoplankton (biomass of phytoplankton taxonomical classes); and space (PCNM vectors obtained from latitude and longitude coordinates; Borcard & Legendre, 2002; Dray et al., 2006). The explanatory variables for the models were selected through variation inflation factors (VIFs), which identify collinear constraints. Variables with VIF > 10 were removed from the RDAs (Gross, 2003). The significance of the global RDAs was tested by permutation (P-value ≤ 0.05, 1,000 iterations) and the significant RDAs were then submitted to variation partitioning through Partial Redundancy Analysis (pRDA) (Borcard et al., 1992; Legendre & Legendre, 1998) to evaluate the significance of the contribution of each matrix after the effect of the others was removed. The BCC data were treated using Hellinger transformation prior to the analyses (Ramette, 2007). The PER(M)ANOVA analyses were performed in PAST software, the SIMPER analysis was carried out in PRIMER v.6.1.9 (Primer-E Ltd, Plymouth, UK) and the Mantel test, RDAs, pRDAs and graphical presentations were performed in the software Rstudio (R Core Team, 2013) using the vegan package (Oksanen et al., 2013).

Results

Temporal and spatial differences

Environmental variables, chlorophyll a *and phytoplankton*

The PERANOVA revealed differences among seasons for several variables. Most of them were higher in summer (chl*a*, TP and pH) or in summer and in another season: temperature, ORP and insolation (summer and spring); TSS, TN and HS (summer and autumn). Other variables were higher in other seasons: SRP (autumn and spring); DO and water transparency (winter); nitrate (winter and spring); precipitation, wind velocity (WV) and nebulosity (autumn). DOC was lowest in spring. Biomass of most phytoplankton groups were higher in spring (Cyanobacteria and Zygnemaphyceae) or in spring and autumn (Bacillariophyceae, Chlorophyceae). There was no seasonal variation for Dinophyceae, Chrysophyceae and Euglenophyceae. Among all groups, Cyanobacteria was dominant overall and was mainly represented by *Aphanocapsa* spp., *Chroococcus* spp. and *Planktolyngbya* spp. (Tables 1 and 2).

Spatial differences were also detected by PERANOVA. The north stations were generally shallower, with lower water transparency and higher turbidity, TP, NH_4^+ and nebulosity; while the south stations were generally deeper, with higher water transparency and lower turbidity and chlorophyll *a* concentrations. The center stations were more similar to the north stations in some variables, and to the south stations in others. For phytoplankton, no spatial differences were observed (Tables 1 and 2).

Location of Tables 1 and 2

Bacterial Community Composition (BCC) and Functional Traits (FT)

A total of 121 Operational Taxonomic Units (OTUs) were detected by ARISA; 116 of them had >5% of frequency of occurrence. Most of them were shared over time (78%) or locations (95%), but when taking into account the abundance of the OTUs, the PERMANOVA indicated that the temporal variation was more important than the spatial variation ($R^2 = 0.22$ and $R^2 = 0.06$, respectively). In this context, all seasons were different from each other, whereas only south × north displayed consistent differences in BCC (Table 3).

A similar response was found for FT, with all the 31 carbon sources being consumed in all samples; however, the degree of utilization varied over time and along the spatial scale. There were consistent differences among all seasons ($R^2 = 0.13$), while for the spatial variation ($R^2 = 0.06$) only south x center did not differ significantly (Table 3).

Location of Table 3

Based on the SIMPER analysis and the arbitrary cutoffs chosen, the OTUs 399, 403, 404, 438, 511, 520, 546, 547, 634, 650, 654 and 669, and the substrates phenylethylamine (G4), 2-hydroxy benzoic acid (C3), γ-hydroxybutyric acid (E3), glucose-1-phosphate (G2), α-D-lactose (H1), α-ketobutyric acid (E3), i-erythritol (C2), glycogen (F1) and L-phenylalanine (C4) made stronger contributions to the dissimilarity among seasons (see Supplementary Table S2 and Supplementary Figure S1 for details). Regarding spatial differences, the OTUs 399, 403, 404, 511, 546, 547, 634 and 654 and the carbon sources phenylethylamine (G4), i-erythritol (C2), glucose-1-phosphate (G2), γ-hydroxybutyric acid (E3) and 2-hydroxybenzoic acid (C3), most

notably G4 and C3, contributed most to the dissimilarity among sites (see Supplementary Table S3 and Supplementary Figure S2 for details).

Although BCC and FT showed similar temporal and spatial patterns, they were not significantly correlated (Mantel test, r = -0.05258, P=0.71), suggesting that even though they shared the same pattern, they responded to different drivers.

Variance Partitioning of BCC and FT

All explanatory matrices were significant for BCC when separate RDAs were run, and hence were included in the variance partitioning. However, when tested for their isolated effects through pRDA, only the matrices for the environmental variables and the phytoplankton biomass were significant. These matrices explained 7.3 and 4.7%, respectively, of the total inertia, and the residual variance was very high (63.6%) (Table 4). The contributions of the environmental variables to the BCC pattern after the removal of the other explanatory matrices were, in decreasing order of importance: I) First RDA axis: TP, SRP, turbidity, TN, chl*a* and depth (negatively); II) Second RDA axis: WT, NH_4^+, WD, pH, nitrate, TSS, and evaporation (negatively) and HS, nebulosity, ORP, WV and DOC (positively) (Fig. 2). The contribution of phytoplankton classes to the BCC patterns after the removal of the other explanatory matrices were, in decreasing order of importance: I) First RDA axis: Zygnemaphyceae and Bacillariophyceae (negatively) and Euglenophyceae (positively); II) Second RDA axis: Dinophyceae, Cyanobacteria, Chlorophyceae and Chrysophyceae (negatively) (Fig. 3).

For bacterial FT, all explanatory matrices run separately were significant except the phytoplankton matrix, which therefore was not included in the variance partitioning.

The pRDA revealed that only the spatial matrix (PCNMs) was significant after the effect of the other explanatory matrices was removed. Around 81.5% of the total inertia remained unexplained by the explanatory matrices tested (Table 5).

Location of Tables 4 and 5

Location of Figures 2 and 3

Discussion

Our results revealed a strong spatial and temporal heterogeneity in Lake Mangueira for environmental variables, chlorophyll *a* and phytoplankton taxonomical classes. Similarly, the BCC and FT showed strong temporal heterogeneity, while the spatial analysis showed differences between the north-south axis. When considering differences in BCC and FT as assessed by PERMANOVA, the effects of environmental variables and distance are confounded, and it is therefore necessary to compare their individual effects on the dynamics of BCC and FT. The pRDAs revealed that local factors (environment and phytoplankton) were the significant factors explaining the BCC dynamics; whereas, unexpectedly, the FT seemed to be driven by dispersal constraints.

Although 95% of OTUs were found in all three parts of the lake and 78% in all seasons of the year, the BCC differed significantly from the northern to the southern areas of the lake and across all seasons, suggesting that the majority of freshwater bacterial taxa are not confined to a subset of regions on the geographical or temporal scales. Our results suggests that ubiquitous taxa are prevalent in bacterial communities, and support previous observations of high percentage OTUs occurring across sampling

sites (85%, 76% and 77.6% of all OTUs) found by van der Gucht et al. (2007), Souffreau et al. (2015) and Lear et al. (2014), respectively. Even though the ARISA method is highly reproducible (Jones et al., 2012), it is biased toward the detection of the most abundant OTUs, a limitation of all fingerprinting methods (Jones et al., 2012). It is possible that the inability to detect a pure spatial effect for the BCC stemmed from the failure to detect rare members of the bacterial community, since rare OTUs may display strong biogeographical signals and significantly impact the results of the analysis if they are included (Yannarel & Triplett, 2004; Galand et al., 2009).

Assessing within-lake heterogeneity is a critical issue when considering the appropriate scale for sampling microbial communities. Such small scale variability was recognized early, and Palmer et al. (1967) reported patchiness in the distribution of bacterial plate counts at scales of approximately 20 m. More recently, a significant variability of within-lake BCC has been reported via molecular and culture-independent methods (e.g. Wu et al., 2007) and scales as short as 10 m (Jones et al., 2012) to < 20 m (Lear et al., 2014) have been pointed to be significant for dispersal of bacterial OTUs. Although the scale of heterogeneity seems to be positively related to lake size, within-lake variation in BCC occurs across all types of morphometry (Table 6). Another important factor controlling variability in BCC is the degree of connectivity, which depends on the mixing regime, wind (velocity, direction and fetch), the presence of physical barriers such as islands, embayments or constrictions (Yannarell & Triplett, 2004; Lear et al., 2014) and lake heterogeneity (e.g., presence of macrophytes) (Wu et al., 2007; Lear et al., 2014).

Given the high connectivity of Lake Mangueira due to its wind-driven and well-mixed nature, barriers to dispersal are expected to be weak or nonexistent, and this is reflected in the smallest scale of heterogeneity in BCC detected (~49 km), which is the

largest yet found in comparison to other studies (Table 6). Assuming that the fetch increases with lake size, we hypothesize that the relatively large scale of variability in BCC in Lake Mangueira is derived from its long fetch. The significant differences between the northern and southern parts of the lake can be explained by i) the long fetch of the lake due to prevailing northeast or southeast winds parallel to the main axis of the lake, which controls sediment resuspension between these two areas (Fragoso Jr. et al., 2008) and, ii) differences in the composition of dominant macrophytes (mostly emergent in the northern wetlands and mostly submersed in the southern part), which are known to affect BCC in many lakes (Wu et al., 2007; Lear et al., 2014) including Lake Mangueira (They et al., 2010).

The temporal patterns of BCC and FT were stronger than the spatial patterns. Even though patterns in time are not as often explored as patterns in space for BCC and FT, the few studies available are in agreement with our results. Stronger temporal variation of BCC was reported for a large estuarine area, where temperature changes had a stronger seasonal influence than the spatial dynamics (Kan et al., 2007). Alternative explanations may include a dependence on the phenological cycle of other components of the microbial loop such as phytoplankton and zooplankton (Kent et al., 2004). Considering that Lake Mangueira is a large lake, this pattern is in agreement with the positive relationship between the time similarity-decay and ecosystem size found for several aquatic species, including planktonic ones (Korhonen et al., 2010). Similarly, FT (assessed as substrate utilization) have been reported to vary on a seasonal basis in lakes, associated with patterns of stratification (Christian & Lind, 2007) temperature, and the availability of nutrients and organic substrates (Dickerson & Williams, 2014).

The higher explanatory power of local factors over dispersal has often been emphasized in studies that addressed BCC variation over geographic scales (Beisner et al., 2006; van der Gucht et al., 2007, Sommaruga & Casamayor, 2008; Souffreau et al., 2015). Other studies have found that both environmental and spatial factors explain bacterioplankton community dynamics (Schiaffino et al., 2011), or even that the interaction of environmental conditions, region, time and landscape influence BCC (Yanarell & Tripplet, 2005). Our study supports the view that the bacterioplankton community structure is primarily the result of species-sorting selection (Chase and Leibold, 2003; Leibold et al., 2004), which is in accordance with the high rates of dispersal expected for Lake Mangueira.

In Lake Mangueira, the significant environmental variables that contributed most to the RDAs explaining the BCC (total phosphorus, PO_4^{-3}, NH_4^{+}, water transparency, turbidity and water temperature) varied seasonally and spatially as a result of water withdrawal for agricultural irrigation and nutrient inputs (i.e., low watershed nutrient loads in winter and high loads in summer) (Fragoso Jr. et al., 2008; Rodrigues et al., 2015). These inorganic nutrients are essential and often limiting for bacterial growth (Carlsson and Caron, 2001; Caron, 1994), whereas water transparency and turbidity are inversely linked, and along with temperature (Lindström et al., 2005) can be an important source of variation of the BCC in lakes (e.g. Yannarell & Triplett, 2005).

In addition, the significance of the share of BCC variance explained by the biomass of phytoplankton taxonomical classes is in accordance with the influence of phytoplankton on bacterioplankton diversity and metabolism (Kamjunke et al., 1997; del Giorgio & Cole, 1998; Höfle et al., 1999; Cotner & Biddanda, 2002; Eiler and Bertilsson, 2004; Honer-Devine et al., 2003; Kent et al., 2007). Phytoplankton also competes with bacteria for nutrients (Cotner & Biddanda, 2002) and positively

influences bacteria through exudation of dissolved organic matter, which can fuel as much as half of the carbon required for bacterial production (Baines & Pace, 1991), determining patterns of BCC (Jones et al., 2009). Since phytoplankton production tends to be more important in larger lakes (Vadeboncoeur et al., 2008), especially in the pelagic zone, algae are expected to significantly influence BCC in Lake Mangueira. The biomass of large groups such as Cryptophytes and Chrysophytes has been found to be correlated with BCC in lakes (Lindström, 2000); however, since a wide metabolic repertoire in terms of exudates of organic carbon is expected for these broad classes of phytoplankton, it is impossible to link each specific algal class to the results found. Bacterivory by phytoplankton is another possibility, since mixotrophy is higher in more oligotrophic lakes (Saad et al., 2013) and prevalent in members of Dinophyceae and Chrysophyceae, among others (see review by Stoecker, 1998). Notably, in some systems mixotrophy may represent as much as 50% of the total bacterivory by flagellates (Unrein et al., 2007).

A similar pattern of spatial and temporal variation was found for FT, even though all substrates were oxidized in all seasons and locations, and the BCC and FT were not significantly correlated. This lack of correlation between BCC and FT has also been found by Lear et al. (2014) for individual ponds, and by Comte & del Giorgio (2010), who reported that even though BCC and FT were not directly correlated, their rates of change along environmental gradients did correlate. Several explanations such as those summarized by Lear et al. (2014) can be considered: i) inability of migrant bacteria to grow, thereby contributing to BCC but not to FT; ii) contribution of Archaea or picoeukaryotes to the substrate utilization pattern; iii) the potentially higher functional redundancy due to the generic metabolic pathways assessed by this study.

436 To our knowledge, this is the first evidence for dispersal of functional traits in
437 bacterial communities, as shown by the smallest scale of variation found for FT (~49
438 km). This unexpected dispersal driven FT may be explained by: i) indirect effect of
439 local factors, ii) exclusion of significant explanatory variables and iii) horizontal gene
440 transfer. Local factors act by modulating the FT due to the interaction of local
441 conditions and migrating bacteria. For example, Severin et al. (2013), found that the
442 ecosystem functioning (using bacterial secondary production as a proxy) was strongly
443 explained by the functional traits of bacterial communities along a dispersal-rate
444 gradient, with the receiving local environment being critical for the final effects on the
445 functional traits of the migrant cells. Hence, the adaptive success and subsequent
446 likelihood of immigrant bacteria contributing their original functional traits to the
447 receiving community is inversely proportional to the difference in the local conditions
448 (i.e., distance). In terms of potentially significant explanatory variables excluded, the
449 contribution of attached (sediment) bacteria stands out. Since we had significant
450 differences in wind velocity across seasons and the wind in shallow lakes often
451 resuspends the sediment, we believe that sediment bacteria may have had a role in the
452 FT patterns found. This was confirmed by the fact that wind velocity correlated
453 significantly with 4 of 9 substrates indicated by SIMPER as the most important for
454 differences among seasons (not shown). Additionally, attached bacteria may be richer in
455 oxidized Ecoplate® substrates compared to free-living bacteria (Lyons and Dobbs,
456 2012). A further indication that other important variables may not have been included in
457 the models tested is the large proportion of unexplained variation, since it is inherently
458 impossible to control field conditions and/or to measure all relevant environmental
459 variables. At last, horizontal gene transfer may be another factor that is unaccounted for
460 in this study, as gene flow can occur even under high phylogenetic dispersal limitation

(Parnell et al., 2010). In this case we may have detected a signal of gene dispersal, which unfortunately cannot be confirmed through patterns of substrate utilization.

In summary, this study showed significant differences in bacterioplankton community composition and function over spatial and temporal gradients in a shallow lake, with temporal differences being stronger than spatial ones. Compared to previous studies, the smallest scale of variability detected in BCC and FT was large (~49 km). Thus, our results suggest that the smallest scale of heterogeneity detected may be positively related to lake size. However, at least part of the results was likely due to the long fetch of Lake Mangueira, which may be responsible for higher connectivity through mixing. Variance partitioning revealed, however, that the bacterial community composition and functional traits were driven by environmental conditions and spatial factors respectively, and our results reinforce the role of local factors in structuring BCC in highly connected systems. Regardless of whether the spatial differences found were driven by environmental factors or dispersal, the results have important implications for sampling design and for understanding the dynamics of freshwater microbial communities. Specifically, spatial and temporal differences in microbial communities within a given lake may be comparable to differences among lakes. The current results are in line with a growing number of other studies (summarized in Table 6), which together highlight within-lake variability as an important component of the total variability of the landscape. Thus, biogeographical studies should be extended to include scales of variability within lakes for explaining biodiversity and ecosystem function.

Location of Table 6

Acknowledgments

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757

Figure captions

Fig. 1 Sampling points in Lake Mangueira, southern Brazil. South (points 1 to 6), center (points 7-12) and north (points 13-18).

Fig. 2 Biplot of the pRDA of the BCC constrained by the environmental variables matrix, after removing the effect of all other explanatory matrices.

Fig. 3 Biplot of the pRDA of the BCC constrained by the phytoplankton major classes' biomass matrix, after removing the effect of all other explanatory matrices.

Supplementary Fig. S1 Contour plots showing temporal variation of OTUs and carbon substrates that contributed most to BCC and FT dissimilarities, according to SIMPER. Black dots represent the sampling points along Lake Mangueira.

Supplementary Fig. S2 Contour plot showing spatial variation of OTUs and carbon substrates that contributed most to BCC and FT dissimilarities, according to SIMPER. Black dots represent the sampling points along the southern, center and northern parts of Lake Mangueira.

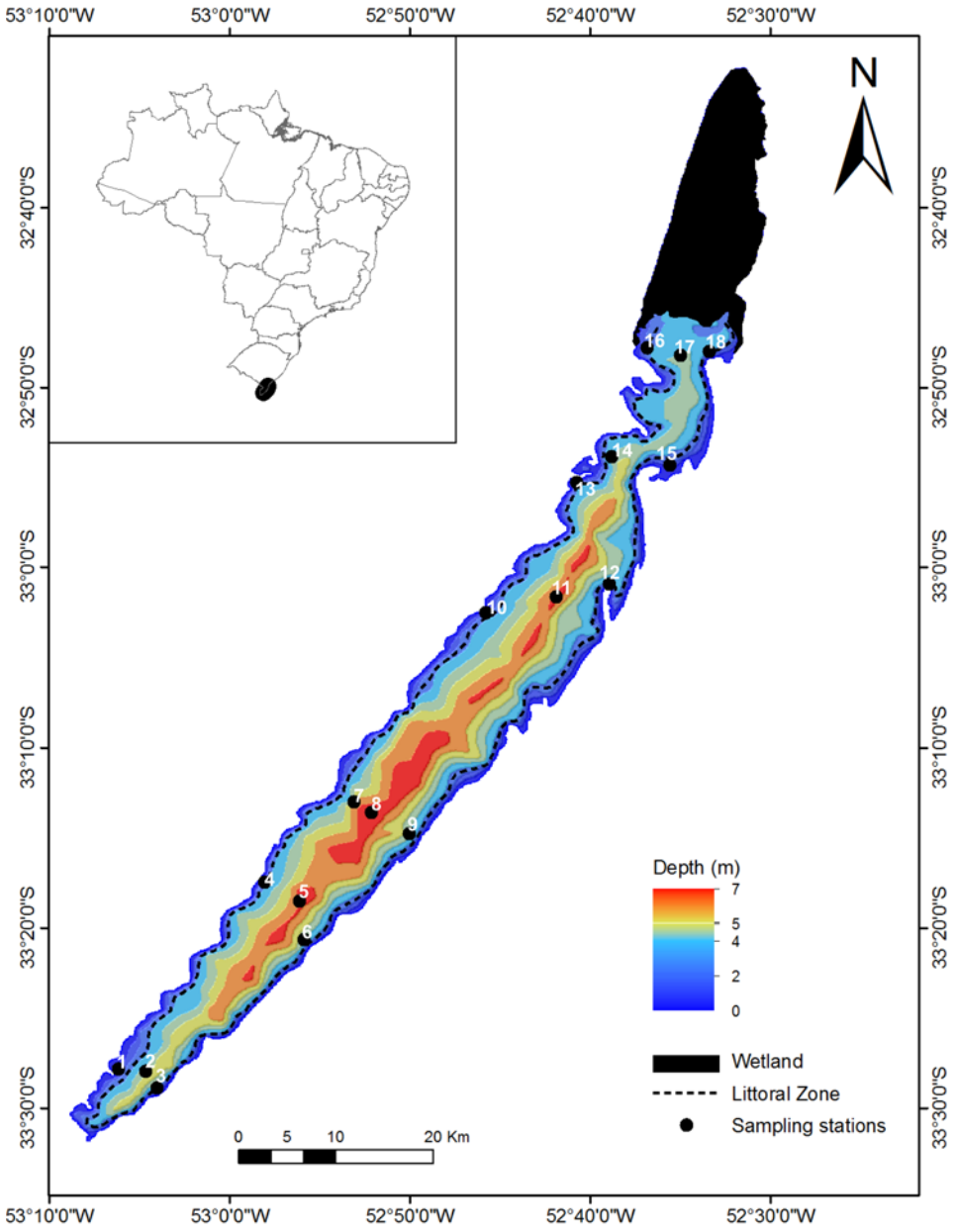

53°10'0"W
53°0'0"W
52°50'0"W
52°40'0"W
52°30'0"W
32°40'0"S
32°50'0"S
33°0'0"S
33°10'0"S
33°20'0"S
33°30'0"S
N
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Depth (m)
7
5
4
2
0
Wetland
Littoral Zone
Sampling stations
0 5 10 20 Km

Fig. 1

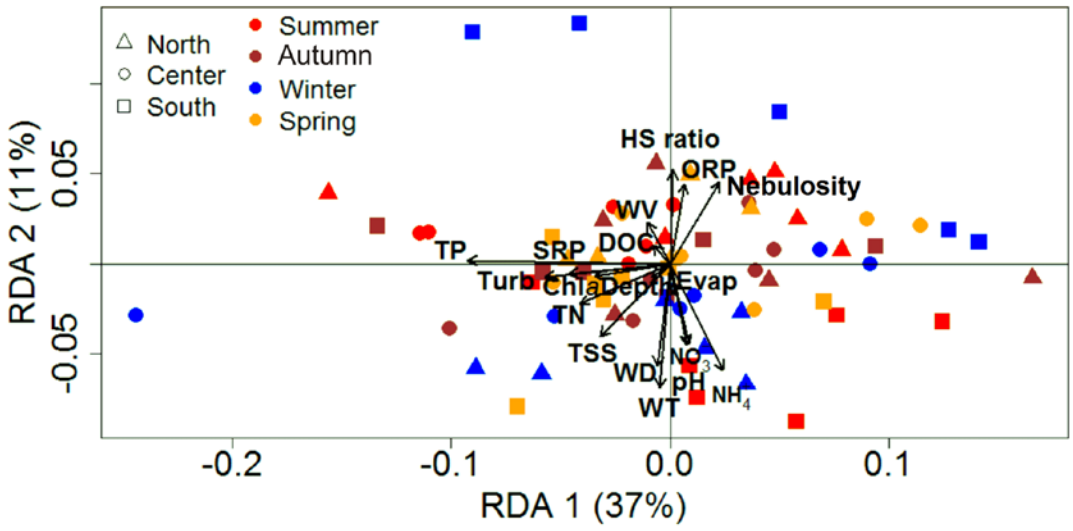

North
Center
South
Summer
Autumn
Winter
Spring
HS ratio
ORP
Nebulosity
WV
DOC
TP
SRP
Turb
Chla
Depth
Evap
TN
TSS
WD
NO3
pH
NH4
WT
RDA 2 (11%)
0.05
-0.05
-0.2
-0.1
0.0
0.1
RDA 1 (37%)

Fig.2

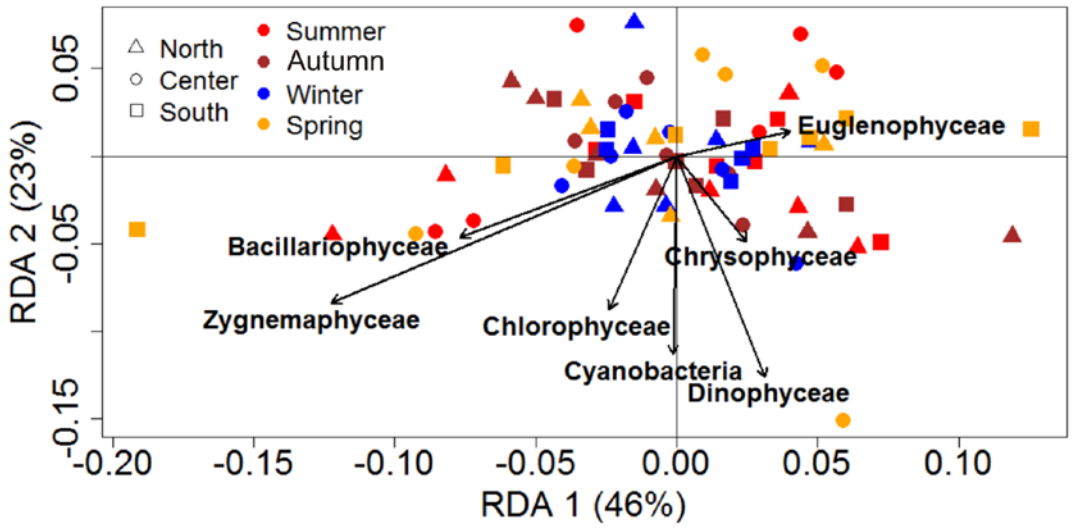

North
Center
South
Summer
Autumn
Winter
Spring
Euglenophyceae
Bacillariophyceae
Chrysophyceae
Zygnemaphyceae
Chlorophyceae
Cyanobacteria
Dinophyceae
RDA 2 (23%)
0.05
-0.05
-0.15
-0.20
-0.15
-0.10
-0.05
0.00
0.05
0.10
RDA 1 (46%)

Fig.3

Table 1 Mean (standard deviation) of environmental variables and chlorophyll *a* across seasons and locations for Lake Mangueira

	Season				Location		
	Summer	**Autumn**	**Winter**	**Spring**	**South**	**Center**	**North**
Temp (ºC)	22.75 (1.05)^{a}	17.79 (0.34)^{b}	11.66 (0.44)^{c}	22.16 (0.66)^{a}	18.18 (4.39)^{a}	18.63 (4.61)^{a}	19.27(4.54)^{a}
DO (mg L^{-1})	9.09 (0.43)^{a}	9.33 (0.19)^{a}	11.45 (0.31)^{b}	8.61 (0.16)^{c}	9.53(1.21)^{a}	9.65 (1.18)^{a}	9.61 (0.99)^{a}
ORP (mV)	170.37 (13.83)^{a}	137.74 (17.61)^{b}	96.01 (15.38)^{c}	166.19 (9.29)^{a}	145.62 (38.29)^{a}	142.64 (33.66)^{a}	141.37 (26.08)^{a}
Depth (m)	2.92 (1.53)^{a}	2.74 (1.52)^{a}	3.12 (1.68)^{a}	3.30 (1.64)^{a}	3.8 (1.47)^{a}	3.30 (2.04)^{ab}	2.36 (0.76)^{b}
WT (m)	1.16 (0.26)^{a}	0.94 (0.31)^{a}	1.63 (0.62)^{b}	1.08 (0.25)^{a}	1.41 (0.47)^{a}	1.12 (0.47)^{ab}	1.05 (0.34)^{b}
TSS (mg L^{-1})	14.50 (4.17)^{a}	14.31 (6.57)^{a}	9.71 (3.22)^{b}	10.06 (5.48)^{b}	10.71 (4.42)^{a}	13.06 (5.24)^{a}	12.78 (6.43)^{a}
Turb (NTU)	4.90 (2.11)^{a}	6.33 (2.81)^{a}	5.18 (2.14)^{a}	4.08 (3.07)^{a}	3.70 (1.78)^{a}	5.58 (2.15)^{b}	6.12 (3.26)^{b}
TP (mg L^{-1})	0.04 (0.01)^{a}	0.02 (0.01)^{b}	0.03 (0.02)^{b}	0.03 (0.01)^{b}	0.02 (0.01)^{a}	0.03 (0.01)^{ab}	0.03 (0.01)^{b}
SRP (µg L^{-1})	10.10 (2.02)^{a}	12.76 (5.06)^{ab}	11.54 (2.25)^{a}	15.46 (3.67)^{b}	11.30 (3.58)^{a}	12.96 (2.85)^{a}	13.21 (5.04)^{a}
TN (mg L^{-1})	0.48 (0.12)^{a}	0.43 (0.07)^{a}	0.25 (0.03)^{b}	0.22 (0.13)^{b}	0.35 (0.12)^{a}	0.32 (0.14)^{a}	0.37 (0.18)^{a}
NH_4^+ (mg L^{-1})	0.05 (0.11)^{a}	0.03 (0.02)^{a}	0.03 (0.02)^{a}	0.13 (0.09)^{a}	0.03 (0.03)^{ab}	0.03 (0.02)^{a}	0.06 (0.09)^{b}
Nitrate (mg L^{-1})	0.07 (0.05)^{a}	0.06 (0.01)^{a}	0.11 (0.02)^{b}	0.13 (0.09)^{b}	0.10 (0.06)^{a}	0.08 (0.05)^{a}	0.10 (0.07)^{a}
DOC (mg L^{-1})	2.07 (1.19)^{ab}	2.72 (0.86)^{a}	2.68 (0.81)^{a}	1.96 (0.55)^{b}	2.46 (1.01)^{a}	2.02 (0.92)^{a}	2.59 (0.78)^{a}
HS (nm)	8.06 (1.88)^{a}	8.18 (1.31)^{a}	4.33 (0.68)^{b}	5.12 (0.91)^{c}	7.14 (2.76)^{a}	6.34 (1.82)^{a}	5.84 (1.47)^{a}
chl*a* (µg L^{-1})	6.20 (2.01)^{a}	3.79 (1.58)^{bc}	4.36 (1.35)^{b}	2.95 (1.09)^{c}	3.52 (1.45)^{a}	4.81 (1.97)^{b}	4.66 (2.15)^{ab}
pH	8.54 (0.12)^{a}	8.13 (0.05)^{b}	8.04 (0.09)^{c}	8.01 (0.10)^{c}	8.19 (0.17)^{a}	8.17 (0.21)^{a}	8.28 (0.31)^{a}
WD (□)	S (S)^{a}	SSE (NE-WSW)^{ab}	SE (S-E)^{b}	SE (SE)^{ab}	SE (S-ENE)^{a}	S (SO-ESE)^{a}	SSE (SSW-ESE)^{a}
WV (m s^{-1})	1.75 (0.39)^{a}	7.78 (1.17)^{b}	1.94 (1.03)^{a}	3.17 (0.77)^{c}	3.44 (3.31)^{a}	3.46 (2.15)^{a}	4.17 (2.24)^{a}
Nebulosity (tenths)	1.58 (1.20)^{a}	9.50 (0.79)^{b}	3.00 (3.68)^{ac}	6.33 (3.28)^{c}	3.13 (4.31)^{a}	5.48 (4.10)^{ab}	6.87 (2.38)^{b}
Precip (mm)	0.00 (0.00)^{a}	9.19 (5.82)^{b}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.53 (0.93)^{a}	2.94 (5.65)^{a}	3.57 (6.15)^{a}
Insol (h)	10.80 (0.00)^{a}	0.55 (0.45)^{b}	5.44 (1.30)^{c}	5.60 (0.00)^{ac}	5.85 (3.96)^{a}	5.51 (3.67)^{a}	5.43 (3.72)^{a}

Different letters indicate significance at $P < 0.05$. Temp, water temperature; DO, dissolved oxygen; ORP, oxidation-reduction potential; depth, local depth; WT, water transparency; TSS, total suspended solids, Turb, turbidity; TP, total phosphorus; SRP, soluble reactive phosphorus; TN, total nitrogen; NH4+, ammonium; DOC, dissolved organic carbon; HS, humic substances ratio (Abs 250:365 nm); chl*a*, chlorophyll *a*; WD, wind direction; WV, wind velocity; Precip, precipitation; Insol, insolation

Table 2 Mean (standard deviation) of biomass (mg L^{-1}) of major classes of phytoplankton across seasons and locations for Lake Mangueira

	Season				Location		
	Summer	**Autumn**	**Winter**	**Spring**	**South**	**Center**	**North**
Bacillariophyceae	0.10 (0.33)^{a}	0.11 (0.09)^{b}	0.02 (0.02)^{c}	0.15 (0.15)^{b}	0.09 (0.10)^{a}	0.10 (0.28)^{a}	0.09 (0.14)^{a}
Chlorophyceae	0.21 (0.24)^{a}	0.43 (0.31)^{bc}	0.29 (0.08)^{b}	0.96 (0.83)^{c}	0.33 (0.31)^{a}	0.53 (0.61)^{a}	0.57 (0.64)^{a}
Chrysophyceae	0.00 (0.00)^{a}	0.01 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.00)^{a}
Cyanobacteria	4.31 (2.56)^{a}	4.86 (1.34)^{a}	6.00 (2.13)^{a}	9.67 (4.66)^{b}	4.95 (2.23)^{a}	6.26 (3.93)^{a}	7.48 (4.00)^{a}
Dinophyceae	0.02 (0.04)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.01 (0.04)^{a}	0.00 (0.00)^{a}	0.01 (0.04)^{a}	0.01 (0.03)^{a}
Euglenophyceae	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.01)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
Zygnemaphyceae	0.01 (0.01)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{b}	0.02 (0.03)^{c}	0.00 (0.01)^{a}	0.01 (0.02)^{a}	0.01 (0.02)^{a}

Different letters indicate significance at $P < 0.05$

Table 3 Differences among seasons and locations, assessed by PERMANOVA for BCC and FT in Lake Mangueira

	BCC			Functional Traits		
	F	**R^2**	**P**	**F**	**R^2**	**P**
Temporal	6.271	0.22	0.0001*	3.432	0.13	0.0001*
Summer *vs* Autumn	4.216	-	0.0414*	2.052	-	0.048*
Summer *vs* Winter	8.708	-	0.0006*	3.315	-	0.0006*
Summer *vs* Spring	5.205	-	0.003*	3.254	-	0.0006*
Autumn *vs* Winter	6.719	-	0.0006*	5.087	-	0.0006*
Autumn *vs* Spring	6.892	-	0.0006*	4.473	-	0.0012*
Winter *vs* Spring	6.135	-	0.0006*	3.091	-	0.0066*
Spatial	2.314	0.06	0.01*	2.126	0.06	0.001*
South *vs* Center	1.178	-	0.8394	1.193	-	0.7305
South *vs* North	3.267	-	0.0129*	3.183	-	0.0012*
Center *vs* North	2.272	-	0.1155	2.045	-	0.0279*

(*) significant at $P < 0.05$; (-), not available

Table 4 RDA and variation partitioning (pRDA) of the bacterial community composition (BCC) among four explanatory matrices in Lake Mangueira

		RDA		Variation Partitioning (pRDA)		
Explanatory Matrix	**Description**	**R^2adj**	**P**	**R^2adj**	**P**	**%**
Selected environmental variables	TSS, Turb, TP, SRP, TN, NH_4^+, Nitrate, Chl*a*, DOC, Depth, WT, pH, ORP, HS, WD, WV, Nebulosity, Evap	0.271	0.005*	0.073	0.017*	7.3
Spatial	PCNM Vectors	0.040	0.036*	0.005	0.410	0.5
Temporal	Dummy variables for summer, autumn, winter and spring	0.169	0.005*	0.017	0.140	1.8
Phytoplankton	Biomass of Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cyanobacteria , Dinophyceae, Euglenophyceae and Zygnemaphyceae	0.115	0.005*	0.047	0.031*	4.7
				Shared	0.221	22.1
				Residual	0.636	63.6
				Total	1.000	100

(*) significant at $P < 0.05$; Abbreviations as in Table 1

Table 5 RDA and variation partitioning (pRDA) of the bacterial functional traits (FT) among four explanatory matrices in Lake Mangueira

		RDA		Variation Partitioning (pRDA)			
Explanatory Matrix	**Description**	**R^2adj**	**P**		**R^2adj**	**P**	**%**
Selected environmental variables	TSS,Turb, TP, SRP, TN, NH_4^+, Nitrate, Chl*a*, DOC, Depth, WT, pH, ORP, HS, WD, WV, Nebulosity, Evap	0.118	0.005*		0.032	0.160	3.2
Spatial	PCNM Vectors	0.042	0.015*		0.062	0.005*	6.2
Temporal	Dummy variables for summer, autumn, winter and spring	0.093	0.005*		0.0004	0.500	0.0
Phytoplankton	Biomass of Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cyanobacteria, Dinophyceae, Euglenophyceae and Zygnemaphyceae	0.021	0.130		-	-	-
				Shared	0.090		9.1
				Residual	0.815		81.5
				Total	1.000		100.00

(*) significant at $P < 0.05$; Abbreviations as in Table 1

Table 6 Comparison of studies that have addressed within-lake heterogeneity in BCC through molecular approaches

Number of systems studied	Size (km^2)	Ecosystem	Mean Depth (m)	Max Depth (m)	Sampling layout	Nº sampling points	Smallest scale identified	Reference
1	820	Large, shallow subtropical lake	2.6	7.0	18 sampling sites, from ~1.9 to ~90 km apart	18	49 km*	This study
2	0.005 39.38	Small and shallow lake x Large and deep lake	1.7 12.8	2.5 25.3	32 sites	32 32	10 m	Jones et al. (2012)
3	0.00245 0.0028 0.0039	Shallow, small ponds	0.5 (all)	0.5 (all)	7×7 m grids	33 35 53	20 m	Lear et al. (2007)
1	2338	Large, shallow subtropical lake	1.9**	< 3.0	3 sampling sites 100 and 1000 m apart, located inside 6 sampling areas	3	>1 km	Wu et al. (2007)
13	0.005-39.38	Small to large, shallow to deep temperate lakes	NA	2.5-35.7	3 stations in each basin - equilateral triangle centered on the deepest point. The variability within lakes was determined at two scales: 10 m (station level) and 100 m (basin level)	90	100 m	Yannarell and Triplett (2004)

* - Considering the smallest difference between pairs of northern and southern sampling stations.

** - Qin, B., 2008. Lake Taihu, China: Dynamics and Environmental Change. Springer. 356 pp.

NA – not available

Supplementary Material

Supplementary Table S1: Geographical distance matrix (meters) between sampling points in Lake Mangueira. From south to center the longest distance was 65,378.1 m, between S1 and C12, and the shortest distance was 11,008.1 m, between S5 and C8. From south to north the longest distance was 89,726.4 m, between S1 and N18; and the shortest was 49,119.0 m, between S4 and N13. From center to north the longest distance was 55,978.6 m, between C9 and N18; and the shortest was 11,230.7 m, between C12 and N13.

	S1	S2	S3	S4	S5	S6	C7	C8	C9	C10	C11	C12	N13	N14	N15	N16	N17
S2	2354.2																
S3	3822.4	**1954.7**															
S4	23023.1	22031.2	23155.5														
S5	**23268.7**	21954.1	22813.1	3551.5													
S6	20789.0	19213.3	19834.0	6795.9	3972.1												
C7	34168.2	33023.0	33981.4	11257.6	11234.7	14798.4											
C8	34214.3	32955.4	33816.5	11621.8	**11008.1**	14279.9	**1885.**										
C9	34832.0	33352.2	34002.1	13405.1	11739.9	14168.5	5823.3	3939.5									
C10	56628.7	55542.2	56510.4	33610.8	33727.0	37079.4	22529.9	22803.3	23668.2								
C11	61479.5	60252.2	61096.6	38583.0	38302.9	41383.8	27338.2	27296.9	27438.4	6243.0							
C12	**65378.1**	64061.7	64822.5	42638.4	42122.2	45015.4	**31384.6**	31171.7	30901.1	11019.9	4777.0						
N13	72141.8	71090.1	72074.0	**49119.0**	49295.2	52628.3	38092.8	38360.8	39021.9	15568.2	12137.0	**11230.7**					
N14	75954.5	74856.4	75800.4	52936.3	52994.8	56247.8	41834.4	42017.3	42477.6	19325.9	15158.0	13085.2	4124.4				
N15	78224.4	77001.9	77838.7	55298.3	55051.4	58097.3	44069.0	44046.5	44067.6	21970.5	16749.9	13323.9	8613.1	5147.9			
N16	87234.3	86223.5	87232.0	64221.6	64470.6	67818.3	53256.2	53548.6	54178.5	30748.9	26957.9	24694.1	15190.4	11809.2	12384.3		
N17	88011.6	86930.0	87881.6	64989.4	65077.6	68326.7	53910.8	54100.5	54515.4	31389.3	27108.3	24369.1	15896.2	12083.4	11367.6	3011.0	
N18	**89726.4**	88601.3	89516.1	66715.5	66703.1	69885.2	55580.7	55705.3	**55978.6**	33108.1	28540.5	25490.0	**17773.4**	13801.1	12215.0	5373.2	**2509.2**

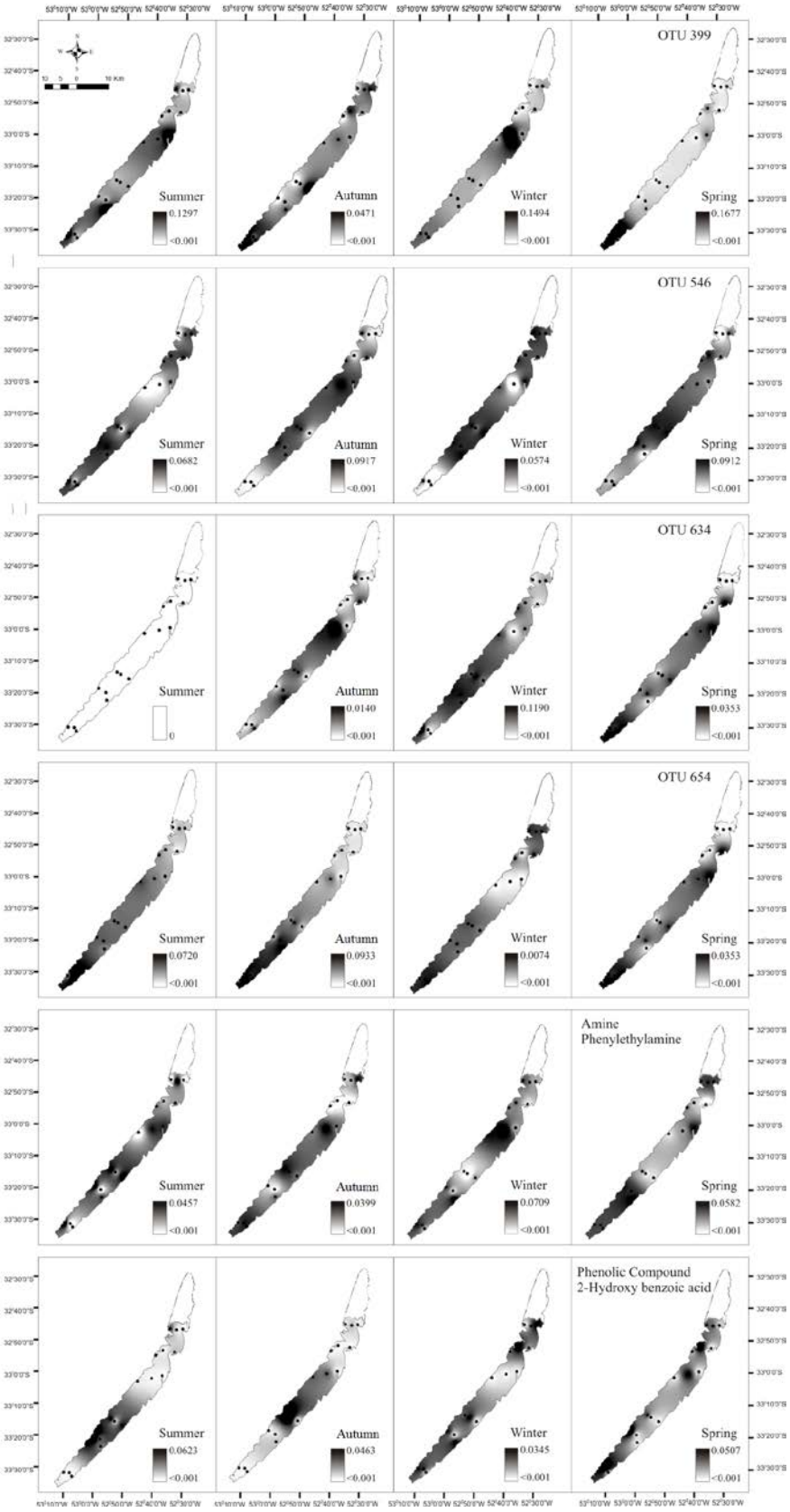
S, C, N represents South, Center and North stations, respectively. Red color: largest differences; Blue color: smallest differences; Gray color: distance within each station, within south, within center and within north

Supplementary Table S2 OTUs and substrates with the highest contribution to dissimilarity in BCC and FT among seasons, according to SIMPER. For the BCC, OTUs 399 and 546 contributed most to the dissimilarity between summer × spring and summer × autumn, respectively. OTU 634 contributed to dissimilarity between winter × summer and winter × autumn, whereas OTU 403 contributed most to the winter × spring dissimilarity. Differences between autumn × spring were mostly due to OTU 654. Overall, OTUs 399 and 547 increased in summer, 438, 650 and 654 increased in autumn, 511, 634 and 699 in winter and 404, 520 and 546 in spring. For the FT, the carbon source phenylethylamine (G4) and 2-hydroxy benzoic (C3) were the main substrates responsible for dissimilarities, as they showed high mean potential oxidation in winter and spring. Phenylethylamine contributed most to dissimilarities between summer × autumn, summer × winter, autumn × winter and summer × spring, whereas 2-hydroxybenzoic acid contributed most to dissimilarities between winter × spring and autumn × spring.

OTU	Average	Average	Cont.%	Cum.%	Carbon-Source	Average	Average	Cont.%	Cum.%
	Summer	**Autumn**				**Summer**	**Autumn**		
546	0.170	0.160	2.460	2.460	G4	0.116	0.120	9.270	9.270
399	0.200	0.120	2.070	4.530	C3	0.068	0.053	8.420	17.690
438	0.010	0.100	1.930	6.460	E3	0.131	0.120	6.330	24.020
654	0.140	0.150	1.820	8.280	G2	0.113	0.096	6.270	30.280
					H1	0.187	0.143	6.180	36.460
	Summer	**Winter**				**Summer**	**Winter**		
634	0.000	0.180	2.990	2.990	G4	0.116	0.181	11.990	11.990
669	0.010	0.140	2.150	5.140	C3	0.068	0.091	10.040	22.030
399	0.200	0.150	1.810	6.950	E3	0.131	0.052	9.140	31.170
					C2	0.084	0.151	8.850	40.020
					G2	0.113	0.143	5.770	45.790
	Autumn	**Winter**				**Autumn**	**Winter**		

OTU	Average	Average	Cont.%	Cum.%	Carbon-Source	Average	Average	Cont.%	Cum.%
634	0.040	0.180	2.700	2.700	G4	0.120	0.181	11.700	11.700
669	0.020	0.140	2.170	4.870	C3	0.053	0.091	10.520	22.220
546	0.160	0.180	2.170	7.030	E3	0.120	0.052	8.710	30.930
654	0.150	0.060	2.010	9.040	C2	0.092	0.151	6.850	37.780
438	0.100	0.000	1.900	10.940	G2	0.096	0.143	6.780	44.560
650	0.150	0.050	1.810	12.750	F1	0.221	0.174	5.670	50.230
	Summer	**Spring**				**Summer**	**Spring**		
399	0.200	0.100	2.620	2.620	G4	0.116	0.177	12.130	12.130
403	0.190	0.210	2.550	5.170	C3	0.068	0.089	12.080	24.200
654	0.140	0.010	2.220	7.390	E3	0.131	0.046	10.500	34.700
546	0.170	0.210	2.000	9.380	C2	0.084	0.122	6.460	41.170
547	0.300	0.280	1.880	11.270	G2	0.113	0.143	5.780	46.940
404	0.110	0.170	1.850	13.110					
	Autumn	**Spring**				**Autumn**	**Spring**		
654	0.150	0.010	2.360	2.360	C3	0.053	0.089	12.920	12.920
403	0.170	0.210	2.280	4.640	G4	0.120	0.177	12.090	25.010
404	0.070	0.170	2.160	6.810	E3	0.120	0.046	10.230	35.240
546	0.160	0.210	2.140	8.950	G2	0.096	0.143	6.920	42.160
	Winter	**Spring**				**Winter**	**Spring**		
403	0.210	0.210	2.610	2.610	C3	0.091	0.089	19.760	19.760
634	0.180	0.090	2.120	4.730	G4	0.181	0.177	10.580	30.350
669	0.140	0.010	2.120	6.850	G3	0.103	0.145	8.660	39.010
399	0.150	0.100	2.060	8.910	F1	0.174	0.201	8.190	47.190
547	0.250	0.280	1.960	10.880	C2	0.151	0.122	6.770	53.960
511	0.170	0.070	1.820	12.700	C4	0.103	0.140	5.520	59.480
520	0.030	0.140	1.800	14.490					

Carbon sources: phenylethylamine (G4), L-phenylalanine (C4), i-erythritol (C2), glucose-1-phosphate (G2), α-D-lactose (H1), γ-hydroxybutyric acid (E3), α-ketobutyric acid (G3), 2-hydroxy benzoic acid (C3), glycogen (F1). Average, abundance average in season or location; Cont. %, percentual contribution of the OTU or substrate for the difference; Cum. %, cumulative percentual contribution.



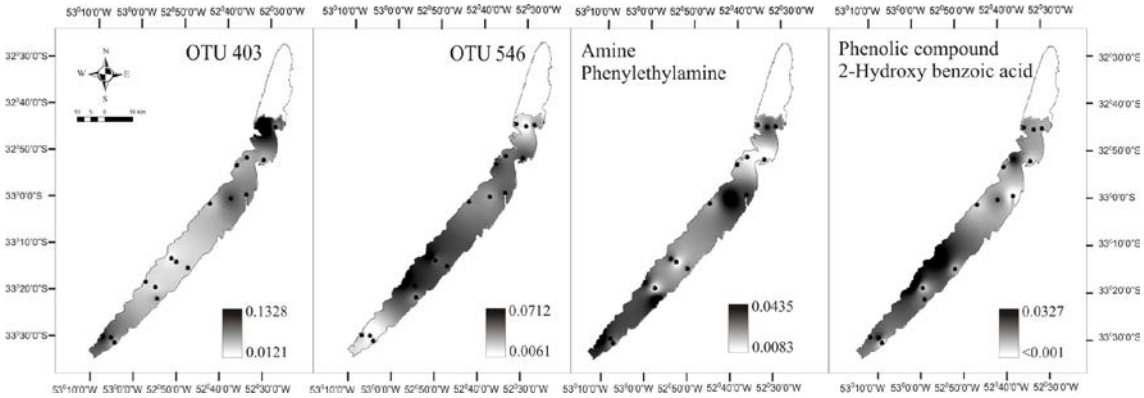
Supplementary Fig. S1 Contour plots showing temporal variation of OTUs and carbon substrates that contributed most to BCC and FT dissimilarities, according to SIMPER. Black dots represent the sampling points along Lake Mangueira.

Supplementary Table S3 OTUs and substrates with the highest contributions to dissimilarity in BCC and FT among locations, estimated by SIMPER. OTU 403 was the major one responsible for the dissimilarity between north × south. OTUs 399, 511, 547, 634, 654 showed higher relative abundance in the south, while 403 and 404 were higher in the north, and 546 in the center. For FT, the phenylethylamine (G4) and 2-hydroxybenzoic acid (C3) were the substrates that showed higher potential oxidation in the south compared to north and center, whereas i-erythritol (C2) and γ-hydroxybutyric acid (E3) predominated in the center. In the north, glucose-1-phosphate had the highest potential oxidation.

OTU	Average	Average	Cont.%	Cum.%	Carbon-Source	Average	Average	Cont.%	Cum.%
	South	**Center**				**South**	**Center**		
546	0.170	0.190	2.240	2.240	C3	0.080	0.073	13.060	13.060
654	0.130	0.080	2.070	4.310	G4	0.162	0.159	8.610	21.670
399	0.180	0.150	2.070	6.370	E3	0.086	0.102	8.310	29.980
634	0.090	0.090	2.040	8.410	G2	0.115	0.127	7.060	37.040
403	0.190	0.170	1.980	10.390	C2	0.105	0.116	6.300	43.340
	South	**North**				**South**	**North**		
403	0.190	0.230	2.120	2.120	G4	0.162	0.122	13.860	13.860
399	0.180	0.090	2.100	4.220	C3	0.080	0.072	11.230	25.090
546	0.170	0.170	1.970	6.190	E3	0.086	0.074	6.810	31.900
404	0.090	0.170	1.940	8.120	C2	0.105	0.113	6.290	38.180
547	0.300	0.260	1.790	9.910	G2	0.115	0.129	6.120	44.300
654	0.130	0.060	1.780	11.690					
511	0.170	0.090	1.710	13.410					
634	0.090	0.050	1.620	15.030					
	Center	**North**				**Center**	**North**		
403	0.170	0.230	2.150	2.150	G4	0.159	0.122	11.450	11.450

OTU	Average	Average	Cont.%	Cum.%	Carbon-Source	Average	Average	Cont.%	Cum.%
546	0.190	0.170	1.990	4.140	C3	0.073	0.072	10.630	22.080
404	0.100	0.170	1.950	6.080	E3	0.102	0.074	7.190	29.270
	Center	**North**				**Center**	**North**		
399	0.150	0.090	1.930	8.010	C2	0.116	0.113	5.570	34.830
547	0.300	0.260	1.720	9.730					
634	0.090	0.050	1.610	11.340					

Carbon sources: phenylethylamine (G4), L-phenylalanine (C4), i-erythritol (C2), glucose-1-phosphate (G2), α-D-lactose (H1), γ-hydroxybutyric acid (E3), α-ketobutyric acid (G3), 2-hydroxy benzoic acid (C3), glycogen (F1). Average, abundance average in season or location; Cont. %, percentual contribution of the OTU or substrate for the difference; Cum. %, cumulative percentual contribution



Supplementary Fig. S2 Contour plot showing spatial variation of OTUs and carbon substrates that contributed most to BCC and FT dissimilarities, according to SIMPER. Black dots represent the sampling points along the South, Center and North areas of Lake Mangueira.

Capítulo 3. Bacterial beta diversity among lakes, but not within lakes, is regulated by local factors

Apresentação

O capítulo apresentado a seguir é o manuscrito do artigo no formato requerido pela revista The ISME Journal ao qual será submetido.

Bacterial beta diversity among lakes, but not within lakes, is regulated by local factors

Running title: Bacterial environmental heterogeneity relationship

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Subject Category: Microbial population and community ecology

Abstract

Beta diversity and its components are important in explaining ecological patterns of species composition among and within sites. Considering the importance of bacterial community composition (BCC) to aquatic ecosystem functions, it is essential to understand the ecological patterns and spatial variation of BCC. Here, we explored the aquatic bacterial beta diversity and their components (turnover and nestedness) among and within 25 coastal shallow lakes and evaluated which factors of habitat heterogeneity

(environmental, phytoplankton composition, landscape and spatial distance) explained the variation in community composition. We found that spatial turnover component contributed more strongly to beta diversity than nestedness over all lakes (among and within). Among lakes, the Sørensen dissimilarity was positively related to phytoplankton dissimilarity, whereas Bray-Curtis dissimilarity and turnover component was positively related to environmental and phytoplankton dissimilarity. The turnover was also negatively related to geographic distance among lakes, whereas the nestedness component was only explained by geographic distance. Contrary to our expectation within-lake bacterial beta diversity was not related to any explanatory variable measured. We conclude that the bacterial beta diversity-environmental heterogeneity relationship among lakes is a result of species sorting, whereas within-lake should be the result of mass effects, due to high connectivity within-lakes.

Key words: aquatic bacteria, distance-decay, phytoplankton influence, mass effect, metacommunity; microbial biogeography, species-sorting

Introduction

Biological diversity can be partitioned into additive and hierarchical components α, β and γ, which measure within communities, among communities and the total or regional diversity, respectively (Whittaker, 1960; Whittaker, 1972; Lande, 1996). While most past efforts have focused on understanding patterns and drivers of α-diversity, in recent years efforts have concentrated on identifying the amount of variation in species composition among sites, the beta diversity (Legendre *et al*., 2005; Anderson *et al*., 2011), and determining which factors promote and maintain such variation.

Beta diversity may be decomposed into turnover and nestedness. The former refers to the true change in species composition from one community to another, while the later refers to the loss of species in some communities in such a way that the less diverse communities represent a species subset of the more diverse ones (Baselga, 2010). Different drivers may be responsible for the prevalence of turnover and/or nestedness patterns. For instance, nestedness may be related to dispersal limitation and recolonization-after-extinction such as glaciations events (Dobrovolski *et al*., 2012), while turnover may be driven by selection through environmental filters (Baselga, 2010).

Bacteria are among the most abundant and diverse organisms in the planet and their diversity drives many ecological functions and services (Pace, 1997; Cole, 1999; Bell *et al*., 2005). Hence, understanding the ecological patterns and spatial variation in bacterial community composition (BCC) in aquatic systems is crucial for understanding ecosystem functioning and for envisioning conservation and environmental monitoring (Jones *et al*., 2012). Most of the studies on bacterial beta diversity point to stronger contribution of environmental filters (Lindström *et al*., 2005; Yannarel and Tripplet, 2005, Logue and Lindström, 2010) and phytoplankton community composition (del Giorgio and Cole, 1998; Cotner and Biddanda, 2002; Eiler and Bertilsson, 2004; Kent *et al*., 2007; Jones *et al*., 2009; Lima *et al*., 2016; Tada and Suzuki, 2016), while dispersion limitation usually seems to be a less important factor (Beisner *et al*., 2006; van der Gucht *et al*., 2007; Jones and McMahon, 2009; Nelson *et al*., 2009; Souffreau *et al*., 2015).

In this context, we expect that in landscapes with high environmental heterogeneity the major contribution of the turnover component of bacterial beta diversity will be due to species sorting (Chase and Leibold 2003; Leibold *et al*., 2004).

On the other hand, in lakes with high connectivity, even strong environmental filters can be overcome by mass effects (e.g. Beisner *et al*., 2006; Jones and McMahon, 2009; Souffreau *et al*., 2015; Lima *et al*., 2016).

However, to disentangle the beta diversity-environmental heterogeneity relationship it is essential to consider different spatial scales, since most patterns in ecology are scale dependent (Levin, 1992), and thus important for the understanding of microbial biogeography (Martiny *et al*., 2011). Further, evaluating the importance of landscape and environmental filters on the beta diversity patterns across a region is essential to better understand the organization of aquatic bacterial communities. This approach demands analysis in several scales of variation of beta diversity (Martiny *et al*., 2006), including the within-lake variability, in which dispersal limitation is low, but also the landscape total variability (Yannarel and Triplett, 2004; Wu *et al*., 2007; Jones *et al*., 2012; Lear *et al*., 2014; Lima *et al*., 2016).

In this study we investigated beta diversity patterns of free-living bacteria within and among 25 coastal shallow lakes, encompassing a wide range of environmental conditions. Our hypotheses are that both within and among lakes the turnover component contributes most to total beta diversity, in spite of the high connectivity of the lakes. We also predicted that beta diversity would be positively related to habitat heterogeneity (environmental, phytoplankton, landscape and spatial distance) at the among-lake and within-lake scales.

Materials and Methods

Study Area

The 25 lakes comprising this study belong to the Tramandaí River System, located in the Coastal Plain of Rio Grande do Sul, southern Brazil (29º37’ to 30º30’ S, 49º’74’ to

50º24' W). (Figure 1). The process of evolution of this coastal plain occurred by marine transgressions and regressions, which formed a complex system of coastal lagoons during the Holocene (Schwarzbold and Schäfer, 1984; Holz, 1999). The Tramandaí system presents 41 lakes with variable degrees of connectivity, age, marine influence and landscape (grassland areas/agriculture, forests, rice paddies, and urban influence) (Guimarães *et al*., 2014).

We collected sub-surface water samples for environmental and biological variables, including bacterial community composition (BCC), phytoplankton biomass and chlorophyll *a*. The sampling was carried out in three sites within each lake in a longitudinal transect including littoral and pelagic areas (Figure 1).

Location of Figure 1

Bacterial community composition (BCC)

A volume of 400 ml of sub-superficial lake water was pre-filtered onto cellulose acetate membrane filters (0.65 µm pore size; 47 mm diameter; Sartorius) to remove phytoplankton and bacterivorous protozoa (Tada and Suzuki, 2006). The free-living bacterial cells were then concentrated by filtration onto polyethersulfone membrane filter (0.22 µm pore size; 47 mm diameter; Millipore) and the samples were frozen at -80 ^{o}C for further analyses. Total DNA was extracted following Lima *et al.* (2016) and the BCC was assessed through DNA fingerprinting by Amplified Ribosomal Intergenic Spacer Analysis (ARISA) according to Fisher and Triplett (1999). In summary, the intergenic spacer region between the 23S and 16S ribosomal rRNA genes was amplified using the 6-FAM-labeled universal primer 1406F (5'-TGYACACACCGCCCGT-3') and the bacterial-specific primer 23Sr (5'-GGGTTBCCCCATTCRG-3') (Fisher and

Triplett, 1999; Yannarell *et al*., 2003). The ARISA profiles were examined following Jones and McMahon (2009). The BCC data were obtained as operational taxonomic units (OTUs). Since this method is biased towards the detection of the most abundant members of the community, the rare members are underrepresented in the BCC (Jones *et al*., 2012).

Phytoplankton biomass and chlorophyll a

For phytoplankton biomass, 250 ml of sub-superficial lake water were fixed with 1% acetic lugol solution for the quantitative analysis following Utermöhl (1958) and the settling time and quantification accuracy of Lund *et al*. (1958). Biomass ($mm^3 L^{-1}$) was estimated by multiplying density by the biovolume of each species (Hillebrand *et al*., 1999). For data analysis, we used the biomass of each phytoplankton taxonomic class separately (Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Cyanophyceae, Dinophyceae, Euglenophyceae, Klebsormidiophyceae, Raphidophyceae, Xanthophyceae, Zygnematophyceae) (Guiry and Guiry, 2016) and also the total phytoplanktonic biomass. We determined chlorophyll *a* (Chla) by extracting pigments with 90% ethanol after filtration through a Whatman GF/F filter and measuring it spectrophotometrically (Jespersen and Christoffersen, 1987).

Environmental variables

Conductivity (Cond), pH and dissolved oxygen (DO) were measured *is situ* using a multiparameter probe. Water transparency (WT) was estimated with a Secchi disk. Suspended solids were estimated through filtration on glass fiber filters (GF/C 1.2 µm, Whatman), which were dried (total suspended solids, TSS) and ignited (fixed suspended solids, FSS) at 350 ºC > 3 h. Volatile suspended solids (VSS) were calculated as the

151 difference between TSS and FSS (APHA, 2012). Turbidity was measured by the
152 nephelometric method in nephelometric turbidity units (NTU), color (absorbance at 430
153 nm) and nutrients, including nitrate (NO_3^-), nitrite (NO_2^-), soluble reactive phosphorus
154 (SRP) and silicate (SIL), were measured through colorimetric methods following APHA
155 (2012). Analyses of total ammoniacal nitrogen or ammonium (NH_3+NH_4^+) and total
156 phosphorus (TP) followed Mackereth *et al*. (1989). Organic carbon, total nitrogen (NT)
157 and total dissolved nitrogen (TDN) were analyzed in a TOC analyzer (Shimadzu
158 VCPH). Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were
159 the fractions that passed through a 450 ^oC pre-combusted glass-fiber filter (0.45-µm
160 mean mesh size). DOC and total organic carbon (TOC) were measured through the
161 NPOC method (non-purgeable organic carbon) (burning after acid addition), while DIC
162 was estimated as the difference between acidified and non-acidified NPOC
163 measurements. Particulate organic carbon (POC) was obtained as the difference
164 between TOC and DOC.

165

166 *Landscape variables*

167 Landscape variables included lake area (ha), distance from the sea (m), and
168 connectivity. Connectivity was calculated taking into account the type, length and
169 number of connections between each lake and its neighboring lakes and the area of all
170 neighboring lakes (Guimarães *et al*., 2014). We also obtained the geographical
171 coordinates for each site.

172

173 *Data analysis*

174 Overall bacterial beta diversity

We estimated the overall bacterial beta diversity among-lakes using multiple-site Sørensen dissimilarity ($\beta_{SØR}$, or total beta diversity), which was partitioned into its two components: beta diversity due to species turnover (β_{SIM}, Simpson dissimilarity index) and beta diversity due to nestedness (β_{SNE}), where $\beta_{SNE} = \beta_{SØR} - \beta_{SIM}$ (Baselga, 2010). We then evaluated the relative contribution of turnover and nestedness to overall beta diversity by calculating the proportion of nestedness-resultant component to overall multiple-site dissimilarity: $\beta_{ratio} = \beta_{SNE} / \beta_{SØR}$. Values of β_{ratio} lower than 0.5 indicate that species turnover is responsible for most of beta diversity, whereas values greater than 0.5 indicate that the nestedness-resultant component is dominant (Dobrovolski *et al.*, 2012).

Evaluation of beta diversity among lakes

Beta diversity of the aquatic bacterial community was assessed using four dissimilarity pairwise coefficients: *i*) Bray-Curtis dissimilarity index applied to BCC relative abundance data after logarithmic (x+1) transformation (Faith *et al.*, 1987; Legendre and Legendre 1998); *ii*) Sørensen dissimilarity index applied to presence-absence data ($\beta_{sør}$) *iii*) Simpson dissimilarity index (β_{sim}) and *iv*) β-diversity due to nestedness (β_{sne}) (Baselga, 2010).

To evaluate beta diversity among the 25 lakes, we first generated matrices containing average values of the three sites sampled per lake for bacteria OTUs abundance, environmental variables and phytoplankton biomass. To calculate the spatial distance between lakes we used the central geographical coordinate of each lake. We then generated BCC dissimilarity matrices using the four coefficients described above and eight explanatory dissimilarity matrices using all environmental variables, geographic distance, phytoplankton composition (taxonomic classes), total

phytoplanktonic biomass (the sum of biomass among all taxonomic classes), chlorophyll *a*, lake area, connectivity, and distance from the sea. For phytoplankton composition we used the Bray-Curtis index on log (x+1) data, while for all other explanatory matrices we used Euclidian distance (Legendre and Legendre, 1998). Dissimilarity in chlorophyll *a* among lakes was excluded from analyses because it was highly correlated with environmental dissimilarity (r=0.89). Correlations between all other explanatory matrices were low ($r < 0.35$). Finally, we used multiple regressions on distance matrices (MRM, Legendre *et al*., 1994; Lichstein, 2007) to evaluate the relationship between dissimilarity in BCC and seven explanatory dissimilarity matrices. The significances of the standardized partial regression coefficients were evaluated with 9999 permutations. Following Martiny *et al*. (2011), we first performed the MRM test with all seven explanatory variables and then reran the test after excluding the nonsignificant variables to reduce the effect of spurious relationships. We show the results from this second test. Before MRM, all dissimilarity matrices were standardized (mean = 0; variance = 1).

Evaluation of beta diversity within lakes

Bacterial beta diversity within lakes was calculated as the average biological distance of the three sites within a lake to the group centroid (PERMDISP, Anderson *et al*., 2006) for each of the four dissimilarity indices already mentioned. PERMDISP evaluates the multivariate dispersion of the sites to their group centroid (here, each lake) in the full dimensional space calculated in a Principal Coordinates Analysis (PCoA), so the greater the average distance to the centroid, the greater the beta diversity of the lake. We also calculated environmental heterogeneity (Euclidean distance) and variation in phytoplankton composition (Bray-Curtis index) within lakes using the PERMDISP

approach. Differences in the within-lake bacterial beta diversity, environmental heterogeneity and phytoplankton heterogeneity among lakes were evaluated using permutation tests (9999 permutations). In all cases we tested the null hypothesis that within-lake bacterial, environmental and phytoplankton dispersion does not differ among lakes. Differences in within-lake turnover and nestedness components of the bacterial community were tested using a paired t-test.

Finally, we used multiple regressions followed by model selection to test the relationship between beta diversity (Bray-Curtis, Sørensen, Simpson and Nestedness-resultant distances to the group centroid) and environmental heterogeneity (Euclidian distance to a group centroid), heterogeneity in phytoplankton composition (Bray-Curtis distance to a group centroid), coefficient of variation of chlorophyll *a* (coefficient of variation within each lake), lake area, distance from the sea, and lake connectivity. Spatial extent (the average distance of sites to the lake centroid) and total phytoplanktonic biomass (coefficient of variation within each lake) were also calculated, but were excluded from the regressions because of their strong correlation with lake area (r=0.96) and heterogeneity in phytoplankton composition (r=0.72), respectively. Regression residuals were normally distributed, according to Shapiro-Wilk's tests. Further, spatial autocorrelation in the response variables and in the regression residuals were evaluated using Moran's *I* coefficient based on 10 distance classes and no significant spatial autocorrelation was detected using Bonferroni's correction (P = 0.05/10 distance classes).

The best models were selected using second-order Akaike's information criterion (AICc). We used AICc differences between each model and the best model to select all models with AICc differences <2, which were considered as having equivalent levels of support (Burnham and Anderson, 2002).

All analyses were performed in the R environment (R Core Team, 2016). The partition of beta diversity was calculated using the *betapart* package (Baselga and Orme, 2012). MRM was run using the *ecodist* package (Goslee and Urban, 2007), PERMDISP using the *vegan* package (Oksanen *et al*., 2016), spatial autocorrelation was tested with *pgirmess* package (Giraudoux, 2016), and AICc using *MuMIn* (Barton, 2016).

Results

Overall aquatic bacterial beta diversity

A total of 215 OTUs were identified by ARISA, 36 of which occurred in more than 50% of the samples. Only one OTU occurred in more than 90% of the samples and no OTU was common to all of them. Multiple-site Sørensen dissimilarity among all lakes was high ($\beta_{SØR}$=0.84) and was mainly determined by turnover (β_{SIM}=0.79), as the proportion of the nestedness component in overall beta diversity was lower than 0.5 (β_{ratio}=0.05).

Beta diversity among lakes

According to the MRM results, dissimilarity in BCC based on the Sørensen coefficient was positively related only to dissimilarity in phytoplankton composition (p=0.0002). On the other hand, dissimilarity in the BCC turnover component showed a positive relationship with both environmental (p=0.014) and phytoplankton composition (p=0.013) dissimilarities and a negative relationship with geographic distance (p=0.0001). The nestedness-resultant component was positively related only to geographic distance (p=0.005). Finally, using the Bray-Curtis coefficient, the dissimilarity in BCC showed a positive relationship with environmental (p=0.002) and phytoplankton composition (p=0.0003) dissimilarities. Environmental, chlorophyll *a* and phytoplankton data for all lakes are shown in Supplementary Table S1 and Table

S2. Landscape variables as lake area, distance from the sea and connectivity did not show any significant relationship with bacterial beta diversity

Beta diversity within lakes

Within-lake bacterial total beta diversity (Sørensen coefficient) measured as the mean distance to group centroid ranged from 0.128 to 0.338 (mean = 0.239 ± 0.058) (Table 1). Distance from centroid based on turnover varied from 0.061 to 0.292 (mean = 0.194 ± 0.067), while nestedness varied from 0.009 to 0.166 (mean = 0.059 ± 0.037), and the turnover components was significantly higher than the nestedness component (paired t-test, t = 7.495, P<0.0001, Figure 2). The structural variation of BCC (Bray-Curtis distance to group centroid) varied from 0.103 to 0.410 (mean = 0.271 ± 0.093). Environmental heterogeneity varied from 0.846 to 4.674 (mean = 1.912 ± 0.960) and phytoplankton composition heterogeneity ranged from 0.064 to 0.332 (mean = 0.153 ± 0.073). In all cases the mean distance to group centroid did not differ among lakes (Table 1).

Location of Figure 2

In this context, according to AICc, among the best models selected for each of the beta diversity measures (models with delta<2) none of the models outperformed the null model (without any explanatory variable and only the intercept) (Table 2).

Location of Table 1 and Table 2

Discussion

As expected, beta diversity at both scales (among and within lakes) resulted from spatial turnover, indicating that changes in species composition among lakes is mainly due to species replacement and less due to differences in species richness. Among lakes, the total beta diversity due to Sorensen dissimilarity was positively related to phytoplankton, whereas the Bray-Curtis dissimilarity and the turnover component were positively explained by environmental variables and phytoplankton. The turnover was also negatively explained by the geographic distance; the nestedness component was positively explained only by geographic distance. Within lakes, no model comprised of measured variables explained the variation of beta diversity better than a null model without variables. Thus, this result did not support the hypothesized relationship of within-lake bacterial beta diversity and any explanatory variable measured here, including environmental heterogeneity and heterogeneity in phytoplankton composition.

A higher contribution of turnover to total beta diversity is expected if environmental filtering is the major driver of BCC distribution, emphasizing spatial environmental niche separation (Chase and Leibold, 2003; Leibold *et al*., 2004). The positive relationship between environmental heterogeneity and beta diversity is expected because a greater variety of environmental conditions among sites allows the occurrence of species adapted to those different conditions. These local factors that include physical and chemical characteristics of the environment have been extensively reported to affect BCC (e.g Sommaruga and Casamayor, 2008; Jones and McMahon, 2009; Souffreau *et al*., 2015; Lima *et al*., 2016). Also, dissimilarities in community composition might be mediated by competitive interactions, reducing nestedness (Matthews *et al*., 2015; Xingfeng *et al*., 2015). Local factors could include phytoplankton community composition, since others have suggested it affects BCC

patterns (Horner-Devine *et al*., 2003; Kent *et al*., 2007; Lima *et al*., 2016), possibly through the exudation of different classes of dissolved organic matter (DOM) (Baines and Pace, 1991; Cole, 1999). Actively growing phytoplankton release a wide range of labile low molecular weight (LMW) DOM compounds like amino acids, carbohydrates and carboxylic acids (Fogg, 1983; Bjørrisen, 1988; Tada and Suzuki, 2016) that are able to stimulate bacterial growth (Bertilsson and Tranvik, 1998) and mediate shifts in free-living heterotrophic BCC (Tada and Suzuki, 2016).

Given that connectivity occurs to different degrees among lakes, although the lakes have areas ranging from 23.03 ha to 5065.22 ha, barriers to dispersion controlled by the landscape were not strong, which was evidenced by the lack of observed relationship between landscape variables and BCC heterogeneity. Nevertheless, we found unexpected negative relationships between Simpson dissimilarity (spatial turnover component) and geographic distance, suggesting a distance-increase similarity rather than distance-decay, where neighboring lakes have distinct BCC, and more distant lakes have similar community composition. Similar patterns have been found for polychaete communities, which have been attributed to directional dispersion by winds, for example (Moritz *et al.* 2013). Thus, the expected increase in dispersal limitation with increasing distances among sites leading to species replacement, once different regional species pools are assessed (Heino *et al*., 2013; Heino *et al*., 2015a), was not observed here. Instead, we observed that the distance-decay relationship (Nekola and White 1999) resulted from nestedness. Some alternative explanations can be raised for explaining this pattern: i) the failure to detect rare members of the BCC, which were underrepresented in fingerprinting methods such as ARISA (Jones *et al*., 2012), which might hide strong biogeographical signals from rare members (Galand *et al*., 2009); ii) a consequence of the interaction of species-specific environmental responses that are

spatially structured and were not accounted for in this study. For example, neighboring lakes, in which one lake receives sewage discharge and the other not, will harbor a distinct species composition that, may have similar species composition as a distant lake that also receive sewage discharge; iii) randomness in species distribution due to wind-driven disturbances (Shade *et al*., 2012), which is a strong influence in these coastal and shallow lakes (Fragoso *et al*., 2008; Cardoso and Motta-Marques, 2009), promoting intense effects or even temporary extinctions of some species from a lake. In this sense, the link between species loss and local extinctions with the nested pattern has been suggested in previous studies (Dobrovolski *et al*., 2012; Xingfeng *et al*., 2015).

Regarding the within-lake scale, we did not observe within-lake bacterial beta diversity variation among lakes, which was quite unexpected given the range in lake size and environmental characteristics. Also, no one of the wide array of explanatory variables measured here explained the variation in beta diversity, independent of the component evaluated ($\beta_{sør}$, β_{sim}, β_{sne} or Bray-Curtis). This suggests that within-lake mass effects may be masking any potential structuring role of species sorting, since an organism with high dispersion rates can occur even in places that do not correspond to their tolerances and environmental requirements (Leibold *et al*., 2004; Heino and Grönroos, 2013; Heino *et al*., 2013). However, it should be noted that in the context of our study, the role of species sorting may be underestimated considering that the ARISA technique does not discriminate dead and living cells. Similar lack of beta diversity-environmental heterogeneity relationship (BDEHR) was also observed for unpredictable species distributions of macroinvertebrates in streams, with no species-specific responses to environmental variables (Heino, 2013). Even though patterns of beta diversity may relate or not with habitat heterogeneity (Heino *et al*., 2015b), beta

diversity can be comparable and as large at smaller scales (local) than at larger (regional) scales (Comte *et al*., 2015).

In summary, our hypothesis that dissimilarity in community composition among lakes would be positively related to dissimilarities in local factors (e.g physical and chemical variables and phytoplankton composition) and that the larger share of beta diversity is due to species turnover was confirmed. Also we found an interesting inverse spatial relationship with turnover and positive relationship with nestedness. For within-lakes, the expected variation in within-lake bacterial beta diversity among lakes and the contribution of environmental factors and phytoplankton was not confirmed. We found that bacterial BDEHR among lakes appears to result from species sorting, whereas within-lake bacterial beta diversity was not related to within-lake habitat heterogeneity, probably being a result of mass effects masking species sorting. Nevertheless our analyses of variation in bacterial community composition and structure among-lakes (based on dissimilarity matrix) and within-lake beta diversity (based on mean distance to group centroid) represent different types of beta diversity, resulting in different conclusions about the bacterial BDEHR. In this sense, it is important to consider multiple spatial scales for understanding microbial biogeography, since the majority of ecological patterns and process are scale dependent (Levin, 1992; Logue *et al*., 2011; Moritz *et al*.,2013; Stein, 2014).

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Conflict of Interest

The authors declare no conflict of interest.

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Figure legends

Figure 1 Twenty-five shallow coastal lakes sampled in the Tramandaí River System, southern Brazil. Red dots: sampling sites.

Figure 2 Bacterial beta diversity components within-lakes based on mean distance to group centroid of three sites per lake in 25 subtropical shallow lakes.

Table 1 Mean distance to group centroid for bacterioplankton, environmental heterogeneity and phytoplankton composition heterogeneity for each lake. F- and *p*-values were obtained from tests for homogeneity of multivariate dispersions of the sites to their group centroid.

Lakes	Bray-Curtis	Sørensen ($\beta_{sør}$)	Simpson (β_{sim})	Nestedness- (β_{sne})	Environment	Phytoplankton
Ramalhete	0.325	0.236	0.138	0.089	1.628	0.154
Negra	0.124	0.148	0.114	0.033	1.410	0.102
Malvas	0.200	0.269	0.261	0.072	4.674	0.127
Passo	0.204	0.225	0.204	0.026	1.543	0.133
Caieira	0.103	0.128	0.126	0.029	1.662	0.310
Lessa	0.364	0.300	0.241	0.065	2.013	0.149
Traira	0.325	0.310	0.272	0.032	2.433	0.221
Caconde	0.379	0.338	0.292	0.042	2.208	0.106
Peixoto	0.143	0.176	0.069	0.166	1.891	0.096
Marcelino	0.347	0.226	0.201	0.052	3.519	0.175
Rincão da Cadeia	0.233	0.244	0.228	0.099	3.486	0.332
Pombas	0.135	0.157	0.104	0.075	1.452	0.111
Horacio	0.189	0.195	0.184	0.009	1.169	0.212
Tramandai	0.280	0.247	0.151	0.100	2.882	0.274
Dom Daniel	0.358	0.290	0.232	0.080	3.103	0.153

Lakes	Bray-Curtis	Sørensen ($\beta_{sør}$)	Simpson (β_{sim})	Nestedness- (β_{sne})	Environment	Phytoplankton
Emboaba	0.352	0.310	0.259	0.063	0.907	0.092
Emboabinha	0.167	0.176	0.061	0.109	0.846	0.081
Custodias	0.410	0.327	0.269	0.054	1.881	0.064
Gentil	0.271	0.204	0.167	0.028	1.251	0.134
Tapera	0.371	0.311	0.259	0.066	1.010	0.130
Manuel Nunes	0.192	0.190	0.121	0.084	1.784	0.241
Fortaleza	0.327	0.231	0.207	0.028	1.108	0.066
Suzana	0.334	0.230	0.199	0.051	1.011	0.136
Cidreira	0.302	0.242	0.235	0.010	1.718	0.124
Cerquinha	0.342	0.260	0.248	0.010	1.207	0.098
$\boldsymbol{F}_{24,50}$	0.588	0.536	1.101	1.454	1.394	0.775
P	0.923	0.950	0.376	0.121	0.152	0.759

Table 2 Summary of best models selected through second-order Akaike's information criterion (AICc) to explain within-lake variation in beta diversity. Env, environmental heterogeneity; Phyto, heterogeneity in phytoplankton composition; Chl, chlorophyll *a*; df, degrees of freedom; Delta, AIC difference to the best model; Weight, Akaike weight; Adj R^2, ordinary adjusted coefficient of determination.

	AICc	df	Delta	Weight	Adj. R^2
Bray-Curtis					
No variables	-44.2	2	0.00	0.374	0.000
Phyto	-43.4	3	0.78	0.254	0.030
Chl	-42.9	3	1.31	0.195	0.009
Phyto+Chl	-42.7	4	1.49	0.178	0.069
Sørensen ($\beta_{sør}$)					
No variables	-67.5	2	0.00	0.317	0.000
Chl	-66.4	3	1.09	0.184	0.018
Env	-66.3	3	1.16	0.178	0.015
Env+ Phyto	-66.3	4	1.19	0.175	0.080
Phyto	-65.9	3	1.55	0.146	-0.001
Simpson (β_{sim})					
Chl	-62.0	3	0.00	0.427	0.109
No variables	-60.7	2	1.36	0.216	0.000
Chl + Env	-60.5	4	1.57	0.195	0.116
Env	-60.1	3	1.95	0.161	0.037
Nestedness-resultant from richness difference (β_{sne})					
No variables	-90.3	2	0.00	0.489	0.000
Env	-89.4	3	0.92	0.308	0.241
Chl	-88.5	3	1.77	0.202	-0.009

Supplementary Table S1 Mean, maximum, minimum and standard deviation of environmental variables among all 25 lakes.

	Cond	pH	DO (mg l^{-1})	WT (m)	Color(mg l^{-1} Pt-Co)	Turb (NTU)	TSS (mg l^{-1})	FSS (mg l^{-1})	TP (mg l^{-1})	SRP (µg l^{-1})	SIL(mg l^{-1})	TN (mg l^{-1})	TDN (mg l^{-1})	NH_3+NH_4^+ (mg l^{-1})	NO_3^- (mg l^{-1})	NO_2^- (mg l^{-1})	TOC (mg l^{-1})	DOC (mg l^{-1})	DIC (mg l^{-1})	POC (mg l^{-1})
Ramalhete	63.90	6.59	9.71	0.55	40.47	11.33	21.67	14.33	43.72	4.35	3.58	0.34	0.20	0.03	0.01	0.00	5.09	4.23	2.43	0.86
Negra	69.83	6.70	7.89	0.83	51.53	5.67	18.67	11.67	18.62	1.60	3.73	0.34	0.29	0.12	0.01	0.00	7.65	6.20	2.31	1.45
Malvas	122.10	7.24	8.25	0.87	16.07	2.81	154.00	13.33	20.69	2.05	1.95	0.22	0.17	0.00	0.01	0.00	3.56	0.91	5.96	2.65
Passo	78.13	6.94	9.54	0.53	29.27	5.16	34.00	26.67	20.92	2.28	4.06	0.17	0.16	0.00	0.01	0.00	4.81	2.99	4.01	1.82
Caieira	69.43	6.73	9.85	0.40	42.80	14.33	20.00	12.33	39.80	1.82	1.71	0.44	0.36	0.02	0.01	0.00	5.50	4.65	3.69	0.85
Lessa	67.23	7.14	8.69	0.70	42.90	21.00	23.33	16.00	34.28	4.35	0.40	0.52	0.46	0.11	0.00	0.01	5.01	3.47	2.84	1.54
Traira	60.50	7.13	8.95	0.97	30.57	7.67	9.00	4.33	66.98	77.13	0.49	0.46	0.38	0.09	0.00	0.00	7.11	4.68	4.29	2.43
Caconde	60.83	7.09	9.70	0.93	25.60	7.33	12.33	6.67	11.25	1.60	0.58	0.45	0.37	0.02	0.01	0.01	7.48	5.09	5.14	2.39
Peixoto	153.27	8.68	7.93	0.73	33.70	3.01	61.67	7.33	91.39	25.07	0.37	0.48	0.43	0.00	0.01	0.00	6.17	4.97	2.76	1.19
Marcelino	359.67	10.45	14.07	0.12	80.37	10.73	63.00	17.67	599.66	186.74	5.75	4.18	1.74	1.05	0.01	0.01	10.42	5.13	2.34	5.29
Rincão da Cadeia	210.80	6.57	9.33	0.53	53.47	1.85	16.67	10.00	32.90	4.12	4.89	0.35	0.30	0.04	0.00	0.00	6.33	4.06	7.47	2.27
Pombas	59.80	6.85	9.16	0.70	31.37	7.33	11.67	7.67	8.95	1.60	3.02	0.28	0.25	0.05	0.01	0.00	4.73	2.66	3.06	2.07
Horacio	67.80	6.59	7.83	0.77	52.57	5.00	11.67	5.67	21.15	1.60	3.61	0.26	0.25	0.10	0.01	0.00	8.30	4.85	1.90	3.45
Tramandai	10382.67	8.16	7.34	0.73	20.47	4.33	14.33	8.67	33.36	1.60	3.57	0.20	0.15	0.21	0.01	0.01	6.93	5.24	2.12	1.68
Dom Daniel	5332.67	7.37	8.53	0.77	25.07	5.00	13.67	8.67	13.55	3.66	2.38	0.63	0.40	0.04	0.01	0.00	5.22	4.35	2.47	0.87
Emboaba	71.17	6.58	8.55	0.80	42.07	3.67	8.67	4.67	21.61	1.60	1.35	0.28	0.24	0.02	0.00	0.00	6.27	4.42	3.57	1.85
Emboabinha	62.07	6.59	8.42	0.80	46.83	4.00	7.33	4.00	11.71	1.60	1.59	0.30	0.27	0.02	0.00	0.00	6.21	5.29	2.67	0.92
Custodias	3920.33	8.96	6.66	0.87	21.70	2.28	37.00	21.00	8.48	1.60	1.47	0.81	0.64	0.11	0.01	0.00	6.06	3.23	2.21	2.83
Gentil	317.67	7.08	7.29	1.10	28.53	0.57	13.67	4.00	10.79	1.60	1.55	0.40	0.31	0.00	0.00	0.00	6.35	5.51	1.94	0.84
Tapera	78.93	6.28	8.45	1.10	26.17	4.33	9.67	8.00	10.33	1.60	1.29	2.48	0.20	0.00	0.00	0.00	4.23	3.76	2.20	0.47
Manuel Nunes	253.43	7.08	7.07	0.67	18.00	1.21	71.33	6.67	30.13	1.60	2.80	0.38	0.42	0.02	0.01	0.01	7.56	5.78	2.11	1.78

	Cond	pH	DO (mg l^{-1})	WT (m)	Color(mg l^{-1} Pt-Co)	Turb (NTU)	TSS (mg l^{-1})	FSS (mg l^{-1})	TP (mg l^{-1})	SRP (µg l^{-1})	SIL(mg l^{-1})	TN (mg l^{-1})	TDN (mg l^{-1})	NH_3+NH_4^+ (mg l^{-1})	NO_3^- (mg l^{-1})	NO_2^- (mg l^{-1})	TOC (mg l^{-1})	DOC (mg l^{-1})	DIC (mg l^{-1})	POC (mg l^{-1})
Fortaleza	196.67	7.21	8.86	0.78	22.10	3.33	26.67	16.67	18.62	1.60	0.89	2.52	0.17	0.07	0.01	0.00	2.45	0.57	1.21	1.89
Suzana	155.13	7.06	8.35	0.73	17.00	2.67	9.33	4.67	23.22	1.60	1.37	0.28	0.22	0.06	0.00	0.00	3.98	3.15	1.93	0.83
Cidreira	204.67	7.19	8.35	0.77	18.53	3.67	11.33	6.67	16.31	1.60	0.93	1.17	0.21	0.10	0.00	0.00	5.04	4.39	4.20	0.65
Cerquinha	213.67	6.99	7.79	0.67	16.87	3.33	19.33	11.33	27.37	1.60	1.07	0.30	0.23	0.09	0.00	0.00	5.23	4.95	3.79	0.29
Maximum	10382.67	8.96	9.33	1.10	53.47	7.33	71.33	21.00	33.36	4.12	4.89	2.52	0.64	0.21	0.01	0.01	8.30	5.78	7.47	3.45
Minimum	59.80	6.28	6.66	0.53	16.87	0.57	7.33	4.00	8.48	1.60	0.89	0.20	0.15	0.00	0.00	0.00	2.45	0.57	1.21	0.29
Standard Deviation	2941.01	0.68	0.78	0.15	13.00	1.68	16.46	4.77	8.73	0.81	1.20	0.77	0.12	0.06	0.00	0.00	1.48	1.35	1.51	0.92

Cond, conductivity; *DO*, dissolved oxygen; *WT*, water transparency; *TSS*, total suspended solid; *FSS*, fixed suspended solids; *TP*, total phosphorus; *SRP*, soluble reactive phosphorus; *SIL*, silicate; TN, total nitrogen; TDN, total dissolved nitrogen; NH_3+NH_4^+, ammonium; NO_3^-, nitrate; NO_2^-, nitrite; TOC, total organic carbon, DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; POC, particulate organic carbon.

Supplementary Table S2 Mean, maximum, minimum and standard deviation of chlorophyll *a*, and phytoplankton biomass of taxonomic classes among all 25 lakes.

	Chl ($\mu g\ l^{-1}$)	BAC	CHL	CHR	CRY	CYA	DIN	EUG	KLEB	RAP	XAN	ZYG
Ramalhete	5.56	0.21	162.80	0.01	0.13	0.64	0.00	0.00	0.00	0.00	0.00	1.79
Negra	5.56	1.66	126.97	0.00	0.08	0.74	5.11	0.05	0.00	0.00	0.00	0.01
Malvas	2.69	0.66	12.56	0.02	0.43	2.81	0.03	0.00	0.00	0.00	0.00	1.63
Passo	6.23	4.38	22.88	0.01	0.00	7.70	0.09	0.02	0.00	0.00	0.00	0.59
Caieira	15.16	4.45	245.52	0.00	0.22	0.58	0.00	0.01	0.00	0.00	0.00	0.33
Lessa	27.45	6.20	163.21	0.00	0.16	5.05	0.00	0.08	0.00	0.00	0.00	2.10
Traira	2.36	1.63	12.37	0.01	0.22	2.36	0.35	0.20	0.00	0.00	0.00	15.52
Caconde	5.73	2.00	3.89	0.00	0.07	5.24	0.57	0.23	0.00	0.00	0.00	51.33
Peixoto	24.08	9.98	118.91	0.03	1.12	8.14	0.25	0.12	0.00	0.00	0.00	2.20
Marcelino	418.81	4.03	100.46	0.00	3.92	53.90	2.46	0.00	0.00	0.95	0.00	0.13
Rincão da Cadeia	9.67	1.32	26.62	0.55	0.01	2.87	0.11	0.22	0.00	0.00	0.00	0.48
Pombas	5.73	6.00	335.93	0.00	0.05	1.06	0.02	3.20	0.01	0.00	0.00	0.05
Horacio	5.39	1.87	29.81	0.00	0.23	3.21	2.79	0.00	0.00	0.00	0.00	0.12
Tramandai	40.08	17.57	0.17	0.00	0.05	3.55	0.01	0.28	0.00	0.00	0.92	0.55
Dom Daniel	10.61	9.21	42.64	0.02	0.03	8.42	0.12	0.00	0.00	0.42	0.02	0.77
Emboaba	2.36	0.75	149.91	0.00	0.13	0.64	0.03	0.00	0.00	0.29	0.00	0.02
Emboabinha	8.42	4.09	165.47	0.00	0.08	0.75	0.03	0.10	0.00	0.00	0.00	0.03
Custodias	7.58	28.81	0.89	0.00	0.01	9.52	0.04	0.00	0.00	0.00	0.00	0.72
Gentil	4.72	4.86	11.72	0.00	0.13	28.65	0.06	0.02	0.00	0.00	0.00	0.57
Tapera	4.04	0.71	84.17	0.00	0.06	0.84	0.02	0.29	0.00	0.19	0.02	1.07

	Chl (μg l^{-1})	BAC	CHL	CHR	CRY	CYA	DIN	EUG	KLEB	RAP	XAN	ZYG
Manuel Nunes	5.39	1.07	1.98	0.02	0.00	32.53	0.07	0.41	0.00	0.00	0.02	0.41
Fortaleza	3.03	1.47	138.93	0.01	0.03	1.17	0.01	0.00	0.00	0.00	0.00	1.02
Suzana	2.53	0.21	61.16	0.00	0.01	0.86	0.01	0.01	0.00	0.00	0.00	0.15
Cidreira	3.03	1.21	97.61	0.01	0.04	1.02	0.00	0.00	0.00	0.00	0.00	0.59
Cerquinha	2.36	1.13	144.20	0.01	0.10	2.23	0.01	0.65	0.00	0.00	0.00	0.50
Maximum	40.08	28.81	335.93	0.55	0.23	32.53	2.79	3.20	0.01	0.42	0.92	1.07
Minimum	2.36	0.21	0.17	0.00	0.00	0.64	0.00	0.00	0.00	0.00	0.00	0.02
Standard Deviation	9.36	7.94	90.85	0.14	0.06	10.18	0.71	0.81	0.00	0.13	0.24	0.34

Chl, Chlorophyll *a*; *BAC*, Bacillariophyceae; *CHL*, Chlorophyceae; *CHR*, Chrysophyceae; *CRY*, Cryptophyceae; *CYA*, Cyanophyceae; *DIN*, Dinophyceae; *EUG*, Euglenophyceae; *KLEB*, Klebsormidiophyceae; *RAP*, Raphidophyceae; *XAN*, Xanthophyceae; *ZYG*, Zygnematophyceae.

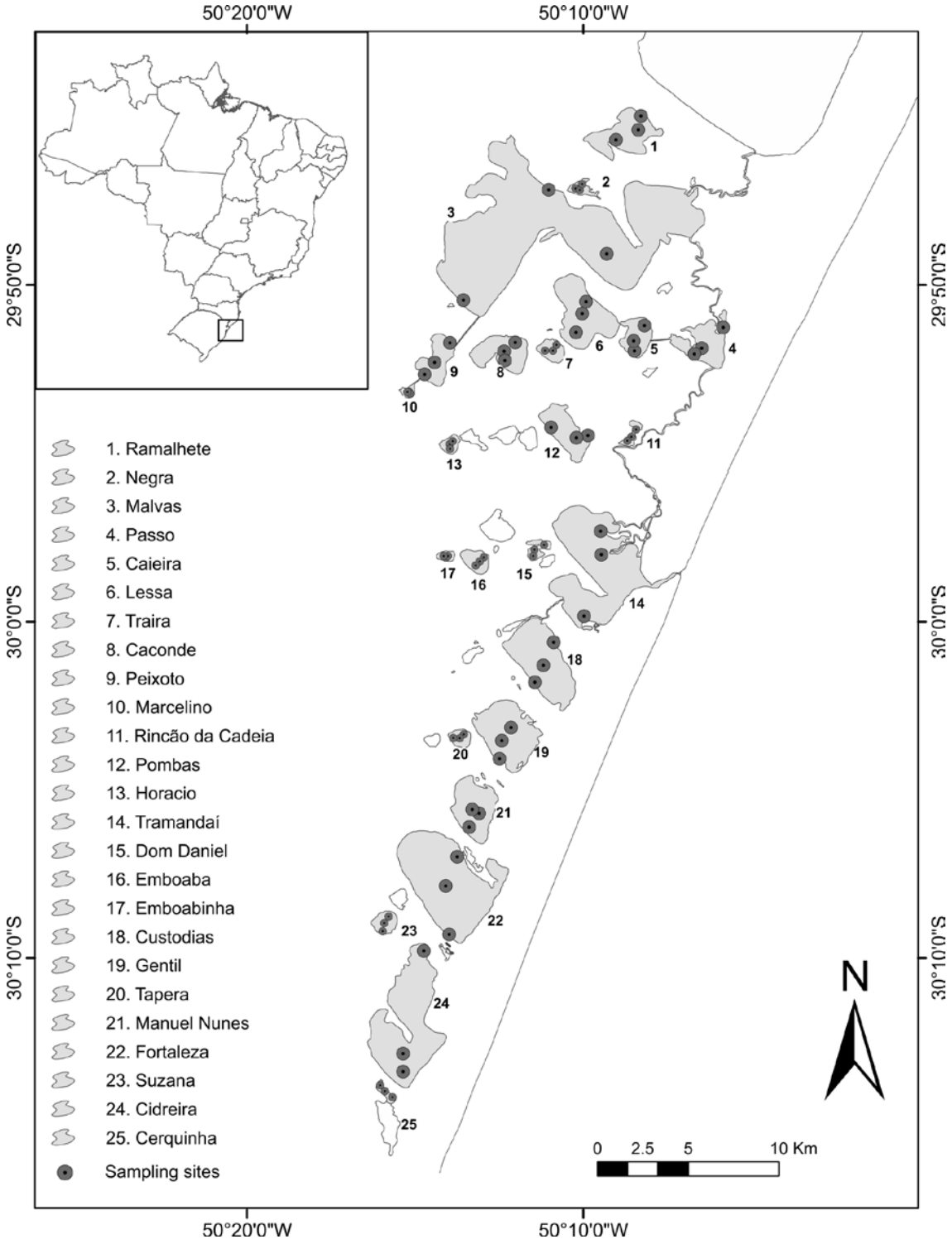
50°20'0"W
50°10'0"W
29°50'0"S
30°0'0"S
30°10'0"S
1. Ramalhete
2. Negra
3. Malvas
4. Passo
5. Caieira
6. Lessa
7. Traira
8. Caconde
9. Peixoto
10. Marcelino
11. Rincão da Cadeia
12. Pombas
13. Horacio
14. Tramandaí
15. Dom Daniel
16. Emboaba
17. Emboabinha
18. Custodias
19. Gentil
20. Tapera
21. Manuel Nunes
22. Fortaleza
23. Suzana
24. Cidreira
25. Cerquinha
Sampling sites
N
0 2.5 5 10 Km

Figure 1

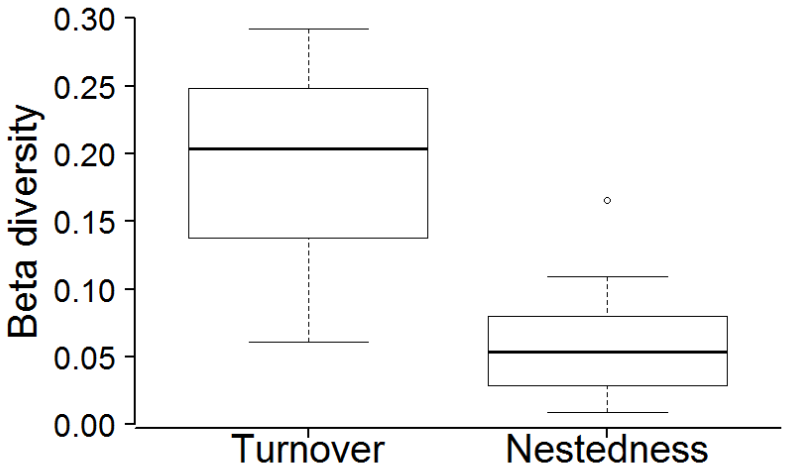
Beta diversity
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Turnover
Nestedness

Figure 2

Capítulo 4. Resistance and resilience of aquatic bacterial community over a cold front event

Apresentação

O capítulo apresentado a seguir é o manuscrito do artigo em preparação ainda não submetido para publicação.

Resistance and resilience of aquatic bacterial community over a short-term cold front event

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Abstract

The abrupt variation on climate affect microbial communities, but little is known about the influence of intense events on its structure and function. Given the microbial importance in the metabolic processes of aquatic ecosystems, a current issue in environmental studies is to understand the responses of these communities to intense weather events, such as the passage of cold fronts. However, it is still difficult to understand and predict how microorganisms behave in the face of such disorders. In this study, we investigate the dynamics of aquatic bacterial (composition and biomass), during a short-term cold front event. This work was conducted in Mangueira, a shallow, subtropical and coastal lake in the southern of Rio Grande do Sul, Brazil. This ecosystem is strongly influenced by cold fronts, mainly during winter. During the course of seven days, six daily water samples for analysis of bacterial community and environmental variables, totaling 42 samples were collected directly in the lake. To assess differences or similarities before, during and after the disturbance, one-way PERANOVA were performed for bacterial biomass, and one-way PERMANOVA for bacterial composition and environmental data. Spearman correlation and RDA were then performed to verify the relationship between environmental conditions and bacterial community. The results showed a significant variation of bacterial biomass and environment conditions, but not for bacterial community composition data over the

cold front event. At the beginning of the disturbance, there was a significant increase of biomass, comparing to the period before the event. After the front end, the biomass returned to the initial condition. In general, the biomass bacterial community of Lake Mangueira showed to be resilient after the occurrence of the disturbance and the BCC showed resistant.

Key-words: bacterial biomass, disturbance, stability, sensitivity, insensitivity shallow lake

Introduction

Disturbances are causal events that can immediately alter the environment, and reverberate indirect responses in the community, or may directly alter the community, causing relative changes in their abundance (Rykiel 1985; Lake 2000). The definition of disturbance depends on its context and scale (Shade *et al*. 2012a), its intensity and frequency, being characterized by temporal patterns, mainly as pulse or pressure disorders (Lake 2000; Shade *et al.* 2012a). Pulse disorders are short-term and often intense, sharply delineated, which rapidly decrease their severity for a short time, reflecting a condition in which the stressor returns to the pre-disturbance condition. Pressure disorders, on the other hand, are continuous and can start very well, but they reach a constant level that is maintained for a long period of time, that is, the stressor agent does not return to the pre-disturber condition (Lake 2000). Aquatic ecosystems oscillate in response to changing environmental conditions. This natural response of the system can occur smoothly and continuously or intensively and abruptly, promoting changes from one persistent regime to another (Scheffer *et al*. 2001). With global climate change, pulse disturbances such as extreme weather events (storms and cold fronts) will tend to increase in frequency, while pressure disturbances (eg increase in atmospheric carbon dioxide, acidification of the oceans, etc.) will be continuous (IPCC

2007). As a consequence of the increase in these extreme climatic events, it is expected that the frequency of climate effects on rivers, lakes and reservoirs will increase due to abrupt temperature variation, allochthonous nutrient loading, hydrology and longer periods of thermal stratification (Graham and Vinebrooke 2009; Carey *et al*. 2012). Such variations promote disorder in the system and significant effects on the planktonic community (White and Picket 1985).

Despite the great prominence of environmental disturbances studies in the ecological literature, few studies have evaluated the microbial community's response patterns to these disturbances (Shade *et al*. 2012b), and most in temperate regions (Yannarell et al. 2003; Allison 2004; Nelson 2009; Shade et al. 2010). Hence, the evaluation of resistance and resilience of microbial communities against perturbation remain rare (e.g. Wertz et al. 2007; McKew et al. 2011). Disturbance events may induce bacterial community changes, followed by post-disturbance return to the original composition and function.

The response of the aquatic microbial community in the face of disturbances associated with global climatic changes is extremely important, since in general, microorganisms are fundamental elements for the ecological processes that occur in aquatic ecosystems (Azam 1983, Cole 1999), and changes in microbial composition are often associated with changes in the rates of ecosystem processes (eg Schimel and Gulledge 1998; Gulledge *et al*. 1997). Therefore, it is important to understand and recognize the stability (resilience and resistance) of aquatic microbial communities and the factors underlying it, in order to predict how the structure and function of these communities respond to the disturbances (Shade et al., 2012a).

In this context, the present work was conducted in Lake Mangueira, a large, coastal and shallow lake system, which is highly influenced by intense cold front

events, and aimed to answer: *i*) does short-term disturb due to polar cold front event promotes changes in the aquatic bacterial community? *ii*) are the bacterial community resilient or resistant to these disorders? We expect that the bacterial community under the influence of disturbances during the winter will change in structure and biomass after a disturb followed by post-disturbance return to its original pre-disturb patterns as a response to hydrological changes over the disturbance event.

Material and Methods

Study area

Lake Mangueira is located in the Taim Hydrological System (SHT), a complex of lakes and wetlands, which are part of the perimeter of the Taim Ecological Station, an integral protection conservation unit. The lake is located at the southern of Rio Grande do Sul (~ 30^{o}31'22 "S 53^{o} 07'48" W), comprising stretches of the Municipalities of Santa Vitória do Palmar and Rio Grande between latitudes 32° 20'S and 33° 00' S, and Mirim Lake and the South Atlantic Ocean (52° 20'W and 52° 45'W) (Figure 1). It is located near Arroio Chuí, on the border with Uruguay. Lake Mangueira is a large aquatic system of the region, covering a total area of 820 km^2, 90 km long and 3 to 10 km wide, with an average depth of 2.6 m and a maximum depth of 6.0 m. Its trophic state varies from oligotrophic to mesotrophic (Crossetti et al., 2013). The climate of the region, according to the Köppen-Geiger System, is of type Cfa, presenting precipitation distributed in every month throughout the year. The temperatures of the warmer months are on average higher than 22 ºC, while those of the colder months range between 18 and -3 ºC (Ferreira, 2005). This region presents annual precipitation of 1,300 mm and undergoes strong action of the winds, whose predominant direction is Northeast - Southwest (Cardoso et al., 2012).

This system has a large influence of cold fronts originating in Antarctica, and affecting all coastal lakes in southern Brazil. These fronts occur with high frequency in the southern region of Brazil, causing significant changes in the hydrometeorological conditions and being the dominant force in subtropical coastal aquatic ecosystems of southern Brazil (Fragoso et al. 2008). These fronts present higher frequency and intensity during winter and spring (Brito et al., 1996) and have as main effect the mixing and homogenization of the water column of these ponds (Tundisi 1983, Tundisi et al.2010).

Sampling

Water samples were collected for environmental data and bacterioplankton composition and biomass. The collection occurred during the winter, during 7 days, from July 31 to August 6, 2013 at the central point of Lake Mangueira (Figure 1). Sampling occurred every four hours (2h, 6h, 10h, 14h, 18h, 22h), totaling 6 samples per day, using two ISCO 6712 automatic samplers with the insulating canister, containing twelve 1 liter samples containers, which were installed in the central tower of Lake Mangueira.

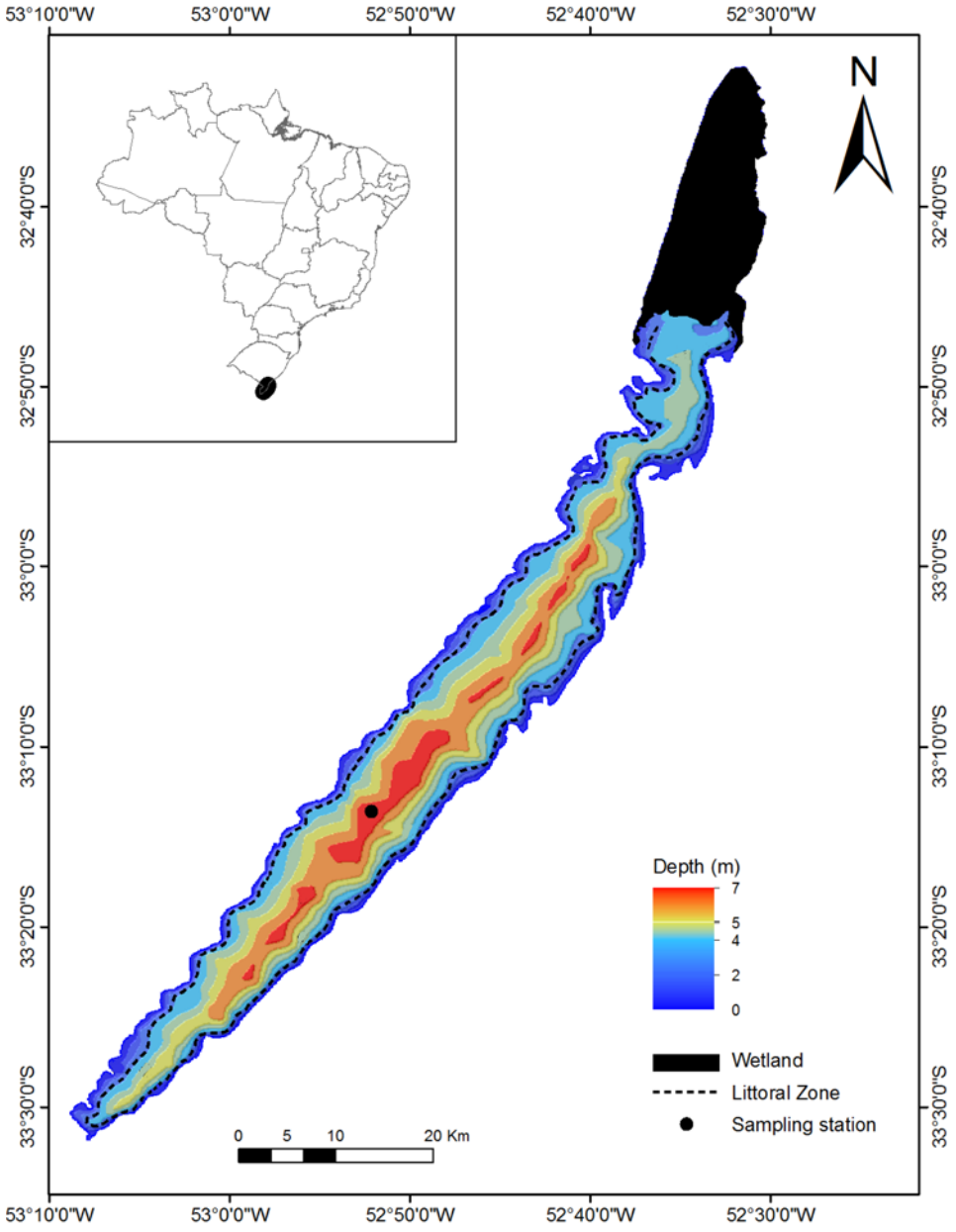

Figure 1 Study area, in particular the sampling station in the pelagic zone of Lake Mangueira.

Bacterial community

We first performed a pre-filtration of the crude sample directly from the automatic sampler, using a 10 μm mesh sieve. Samples destined for biomass analysis were then fixed in 40% formalin (9: 1 vol: vol) in plastic polyethylene bottles. Biomass,

biovolume and bacterial morphotypes were accessed by filtration of 5 ml of sample in a Vacuum Mainfold Filtration Tower (Millipore). The cells were concentrated onto black polycarbonate membrane sample of 0.22 μm pore size and 25 mm diameter (GE) and 1ml of 10% of acridine orange stain was added to the filters for 5 min, and then washed with pre-filtered Mili-Q (0.2μm) water and air dried. Filters were arranged in slides with mineral oil and images (MOTIC image 3.2) were captured through an inverted epifluorescence microscope, according to Massana et al (1997). These images were processed through the CMEIAS software of Image Tool 1.27 (Liu et al, 2001) for classification of morphotypes, density, biomass and biovolume values. By statistical classification the program allows to classify 11 bacterial morphotypes (Coccus, Spiral, Curved Rod, U-Shaped Rod, Regular Rod, Unbranched Rod, Ellipsoid, Club, Prosthecate, Rudimentary Branched Rod and Branched Filament). From the specific equations (Massana et al, 1997), the biovolume (μm^3) is calculated and converted to carbon, generating the biomass in pgC (Norland, 1993).

For the bacterial community composition (BCC), 500 ml of prefiltered water sample were field-filtered on polyethersulfone membrane with 0.22 μm pore size and 47 μm in diameter (Milipore). After filtration, the filter was cooled to -20 oC until it returned to the laboratory where it was conditioned at -80 oC until analysis. The composition of the bacterial community was accessed through DNA extraction as Lima et al. (2016). Ribosomal intergenic space amplification analysis (ARISA) was performed according to Fisher and Triplett (1999), Yannarell *et al.* (2003) and Jones and McMahon, (2009). The BCC data were obtained as operational taxonomic units (OTUs).

Environmental variables

Air temperature, wind velocity (WV) and direction (WD), chlorophyll fluorescence (ChlF), and chromophoric dissolved organic matter (cDOM) were collected directly from instruments installed in the center of the lake tower. Total suspended solids (TSS) was assessed gravimetrically by water evaporation in porcelain dishes (APHA, 1999). Nutrients, including total nitrogen (TN) was measured through colorimetric methods following APHA (1999), and total phosphorus (TP) followed Mackereth et al. (1989). A Carbon Analyzer (Shimadzu VCPH) was used to determine the dissolved organic carbon (DOC) of the fraction that passed through a 450ºC pre-combusted glass fiber filter (0.45 μm mean mesh size).

Data analysis

The microbial community composition and morphotypes were tested for its resilience and resistance after disturbances comparing the composition of the pre-disturbance bacterial assembly with each subsequent day (disturb and post-disturbance) by one-way non-parametric MANOVA (PERMANOVA), using the similarity of Bray-Curtis. Bacterial biomass, however, being a single variable, was tested by grouping pre, disturb and post-disturb values through one-way non-parametric ANOVA (NP MANOVA in univariate mode) (Anderson, 2001). The changes in environmental conditions were also evaluated in pre, disturb and post-disturb through PERMANOVA, comparing to the Euclidean distance data previously transformed into log (x + 1). The null hypothesis ($P > 0.05$) is that the community and the environment are resistant if there are no differences, and the alternative hypothesis ($P<0.05$) the community and environment are not resistant. A similarity percentage analysis (SIMPER) was perfomed

for morphotype data to evaluate the contribution of each morphotypes along the cold front event.

To evaluate the relationship between environmental conditions with the bacterial community, we performed a Spearman correlation between bacterial biomass and environmental conditions, and two redundancy analyzes (RDA): one between environmental variables and biomass and morphotypes and another between environmental conditions and BCC.

Results

The cold front period showed in figure 2. Through the local environmental conditions graphics, we verified that during the 7 days of short-term cold front event, the beginning of the cold front is marked mainly by temperature drop. Also the period is marked by an increase of wind speed, chlorophyll fluorescence, chromophoric dissolved organic matter, total solid suspended, total phosphorus, and dissolved organic matter from the second day of sampling, until, approximately the penultimate day (Figure 2). Accordingly, the environmental variation over seven days of event (including pre, during and post-disturbance) showed significant differences (PERMANOVA; $F = 6.46$; $P = 0.0019$).

Related to the aquatic bacterial community, a total of 158 OTUs were detected by ARISA, of which 96 OTUs occurred before the disturbance, 151 OTUs during the disturbance, and 102 OTUs post-disturb. The bacterial biomass and morphotypes varied significantly along the seven days of short-term cold front event (PERANOVA; $F = 68.82$, $P < 0.0001$; $F=11.56$, $P<0.0001$, respectively). However the BCC did not varied significantly (PERMANOVA, $F= 1.164$; $P=0.2622$).

The environmental conditions changed during the disturbance (P=0.0216) followed by return to its original conditions (P=0.19) (Figure 2). The bacterial biomass average increased significantly (P<0.0001) during the disturb (96.35±23.36) comparing to the pre-disturbance period (6.87±2.69) and post-disturbance (14.97±9.91). After the cold front ended, the biomass returned to the initial condition, that is, did not differ significantly from the pre-disturb period (P = 0.07) (Figure 3). In contrast, the bacterial morphotypes changed during the disturbance (P<0.0001) but did not return to its original (pre-disturb) condition (P=0.0117).

Overall, the coccus form was predominant in the community over all period, including pre, disturb and post-disturb. Nevertheless there was an increase in other morphotypes, along with a reduction of coccus, during disturb. Although at post-disturb, the coccus form increased their contribution, in relation to the reduction of the other morphotypes (Figure 4; Table 1), this variation did not represent a significant recovery to the preconditions.

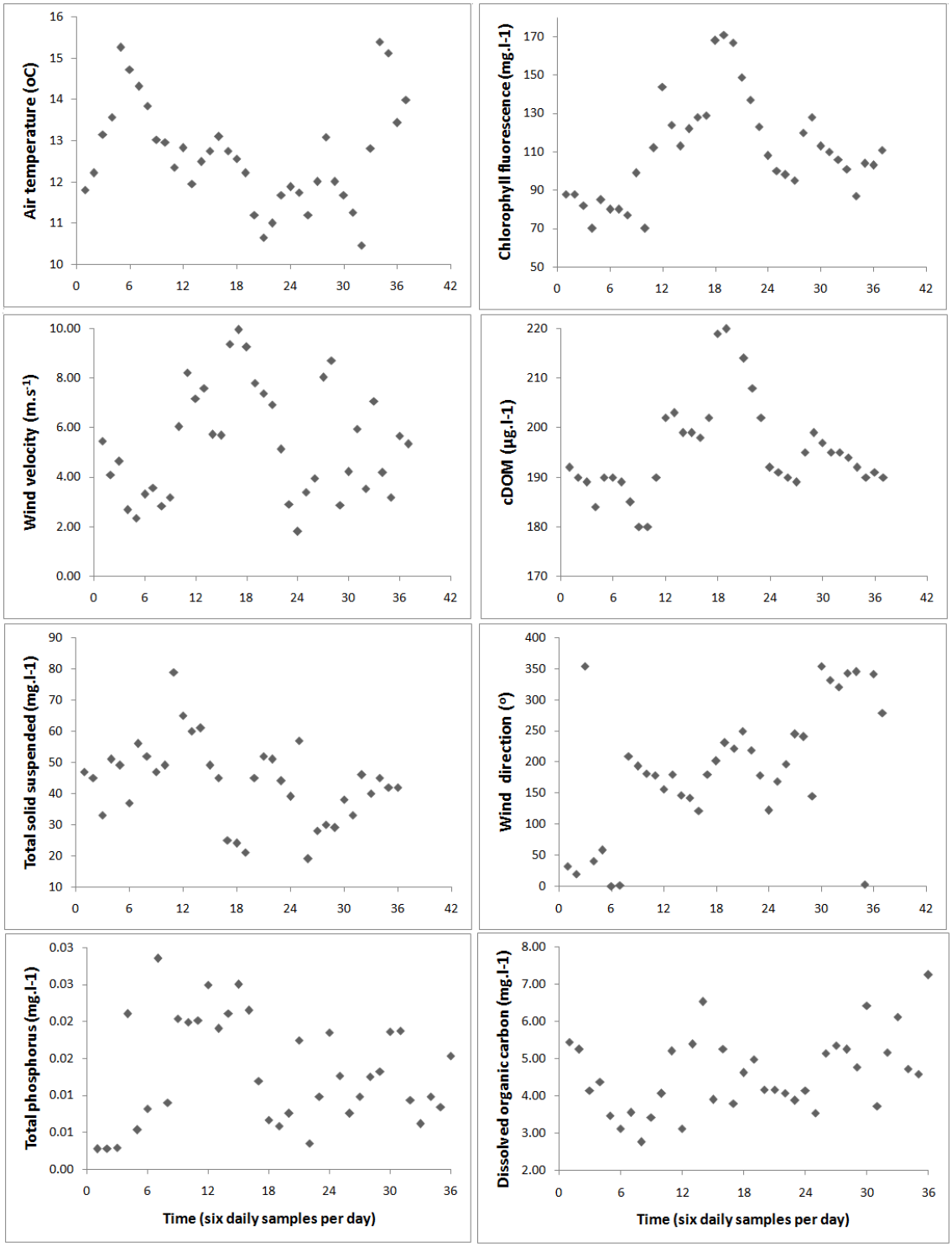

Figure 2 Variation of environmental conditions measured during seven days of six daily sampling along the short-term cold front event, totalizing 42 samples. Except for total solid suspended, total phosphorus and dissolved organic carbon, in which for the first day of sampling we have only two samples, and for the second day sampling, four samples, totalizing 36 samples during seven days.

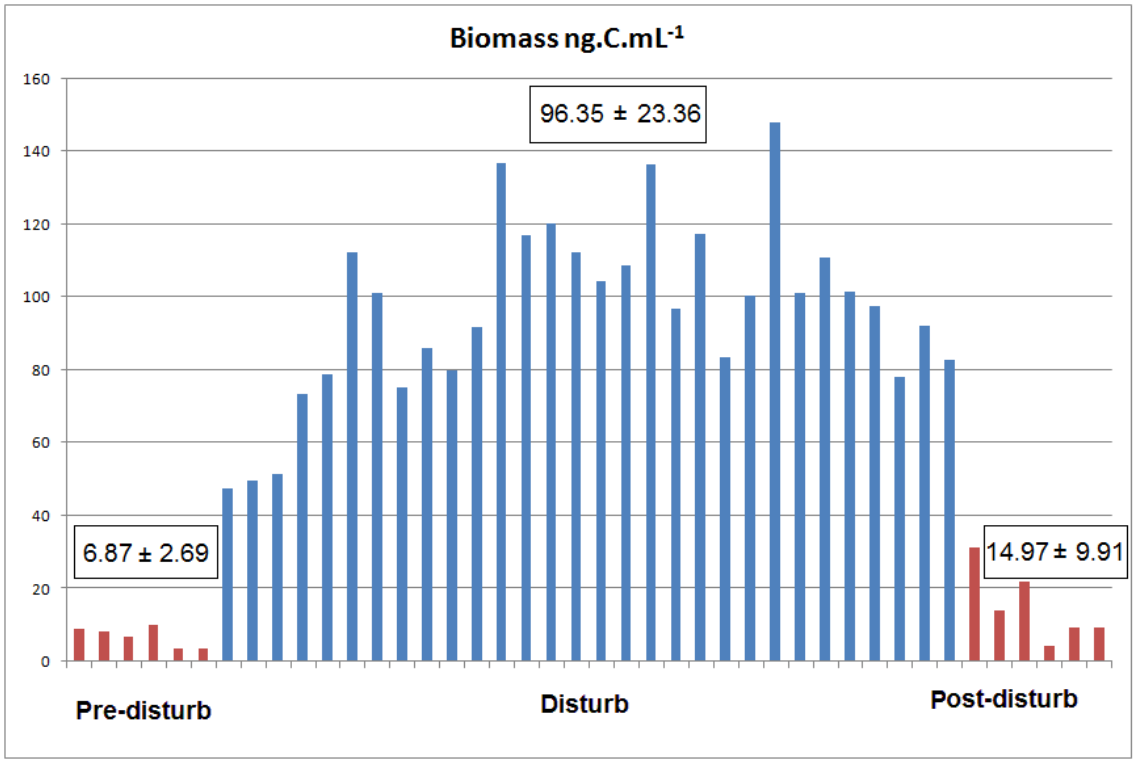

Figure 3. Bacterial biomass concentration values, indicating variation along the cold front (seven days, with six daily samplings), including pre, disturb and post-disturb periods. Numbers in the box are average and standard deviation.

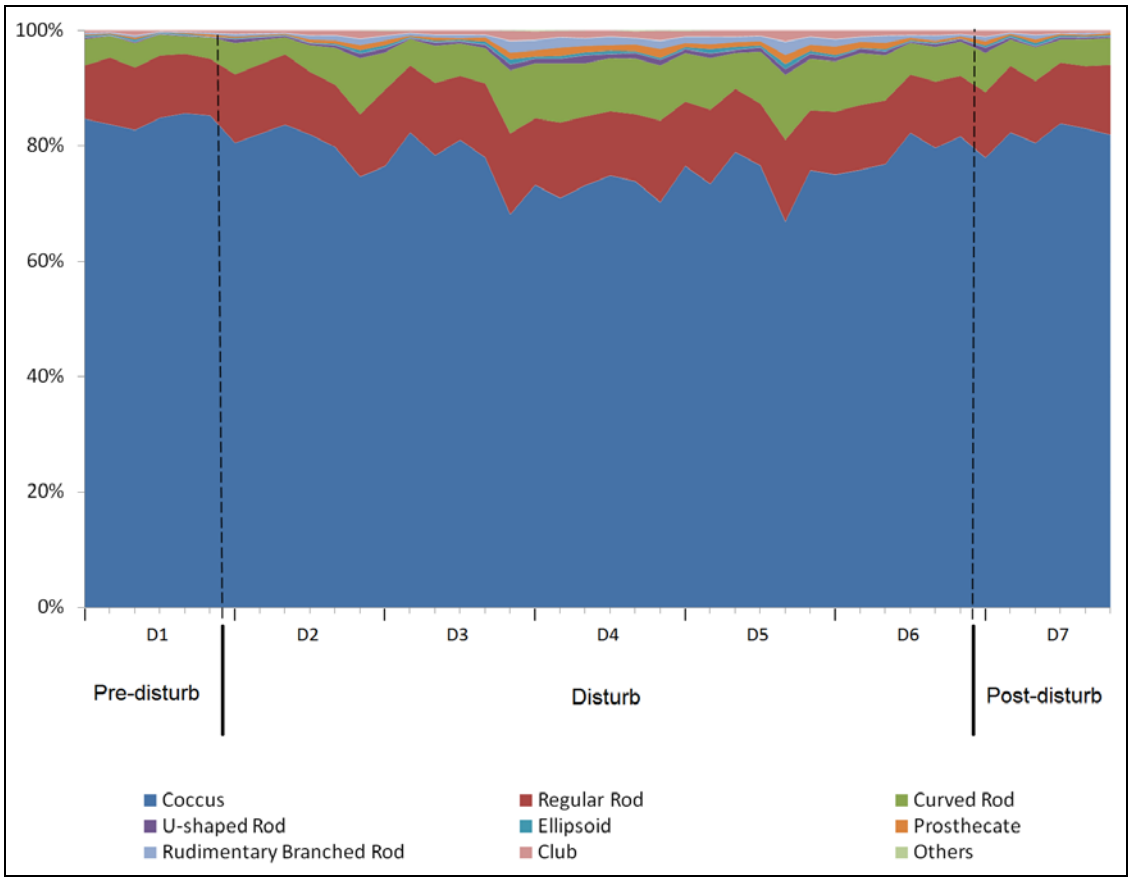

Figure 4 Temporal relative biomass variation of bacterial morphotypes along seven days of short-term cold front event (pre, during and post distrub) in Lake Mangueira.

Table 1 SIMPER analysis of morphotypes comparing the contribution of each and the mean abundance between pre-disturb x disturb, pre-disturb x post-disturb and disturb x post-disturb.

	Contribution	Cumulative %	Mean abundance	Mean abundance
			Pre-disturb	Disturb
Coccus	3.8750	48.98	0.8450	0.7670
Curved Rod	1.9040	73.05	0.0383	0.0757
Regular Rod	0.7416	82.42	0.1050	0.1180
Rudimentary Branched Rod	0.3868	87.31	0.0022	0.0099
Club	0.3315	91.5	0.0039	0.0102
Prosthecate	0.3079	95.4	0.0026	0.0086
U-shaped Rod	0.2537	98.6	0.0014	0.0065
Ellipsoid	0.1017	99.89	0.0024	0.0038
Others	0.0088	100	0.0000	0.0002
			Pre-disturb	Post-disturb
Coccus	1.4870	46.16	0.8450	0.8160
Curved Rod	0.6548	66.47	0.0383	0.0509
Regular Rod	0.4724	81.13	0.1050	0.1120
Club	0.1528	85.87	0.0039	0.0057
Rudimentary Branched Rod	0.1321	89.97	0.0022	0.0034
U-shaped Rod	0.1306	94.03	0.0014	0.0040
Prosthecate	0.1218	97.81	0.0026	0.0047
Ellipsoid	0.0707	100	0.0024	0.0035
Others	0.0000	100	0.0000	0.0000

	Contribution	Cumulative %	Mean abundance Disturb	Mean abundance Post-disturb
Coccus	2.7230	46.79	0.7670	0.8160
Curved Rod	1.4340	71.43	0.0757	0.0509
Regular Rod	0.5214	80.39	0.1180	0.1120
Rudimentary Branched Rod	0.3590	86.56	0.0099	0.0034
Club	0.2663	91.13	0.0102	0.0057
Prosthecate	0.2427	95.3	0.0086	0.0047
U-shaped Rod	0.1737	98.29	0.0065	0.0040
Ellipsoid	0.0908	99.85	0.0038	0.0035
Others	0.0088	100	0.0002	0.0000

Regarding the Spearman correlation, bacterial biomass significantly correlated with air temperature, ChlF, CDOM and WD (Table 2), and showed no significant correlation with TSS, TP, TN, DOC and WV. By means of a redundancy analysis (RDA) (Figure 5 and 6), the increase of biomass occurred during disturb also marked by a slight increase in other morphotypes varieties and small decrement of coccus (Figure 4, 5). This period was influenced by WD and WV, the increase of ChlF and CDOM and decrease of temperature. The pre and post-disturb was marked by lower bacterial biomass, conferred by the greater number of coccus forms in relation to the other morphotypes, as well as higher temperature, lower ChlF and cDOM, besides different wind conditions (Figure 5). In contrast, the OTU relative abundance of the BCC did not showed a clear differentiation between the three different periods of cold front (pre, during and post disturbance), nevertheless wind velocity and direction, cDOM and ChlF were positively related to the majority of samples from the disturb, whereas air temperature was positively related to the majority of samples from pre and post-disturb (Figure 6).

Table 2. Spearman correlation values between biomass and environmental variables along a cold front.

	Biomass ($ng.C.L^{-1}$)	*P-value*
Chlorophyll Fluorescence ($\mu g.L^{-1}$)	0.778	<0.0001***
Air Temperature (^oC)	-0.646	<0.0001***
Wind velocity ($m.s^{-1}$)	0.283	0.069
CDOM ($\mu g.L^{-1}$)	0.731	<0.0001***
Wind direction (^{o})	0.326	0.035*
TSS	0.102	0.551
TP	0.313	0.063
DOC	-0.260	0.125

Legend: significance, * P <0.05; ** P <0.01. *** P <0.001.

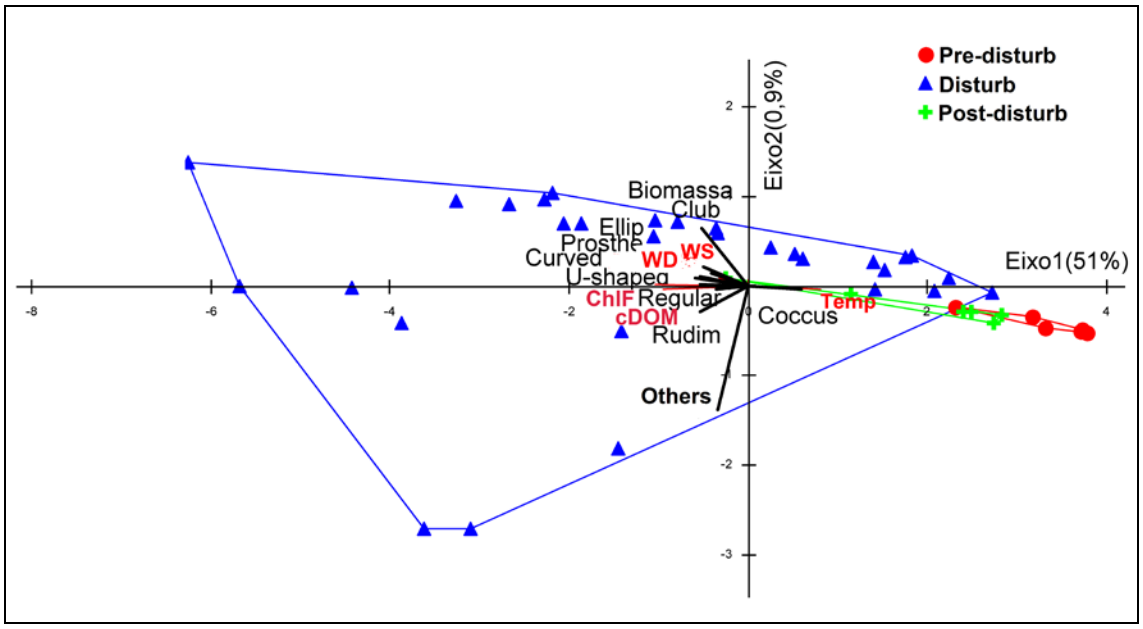

Figure 5 Redundancy analyses (RDA biplot) between bacterial biomass, bacterial morphotypes and environmental variables. Temp: air temperature; WD: wind direction; WS: Wind velocity; ChlF: chlorophyll fluorescence; cDOM: chromophoric dissolved organic matter.

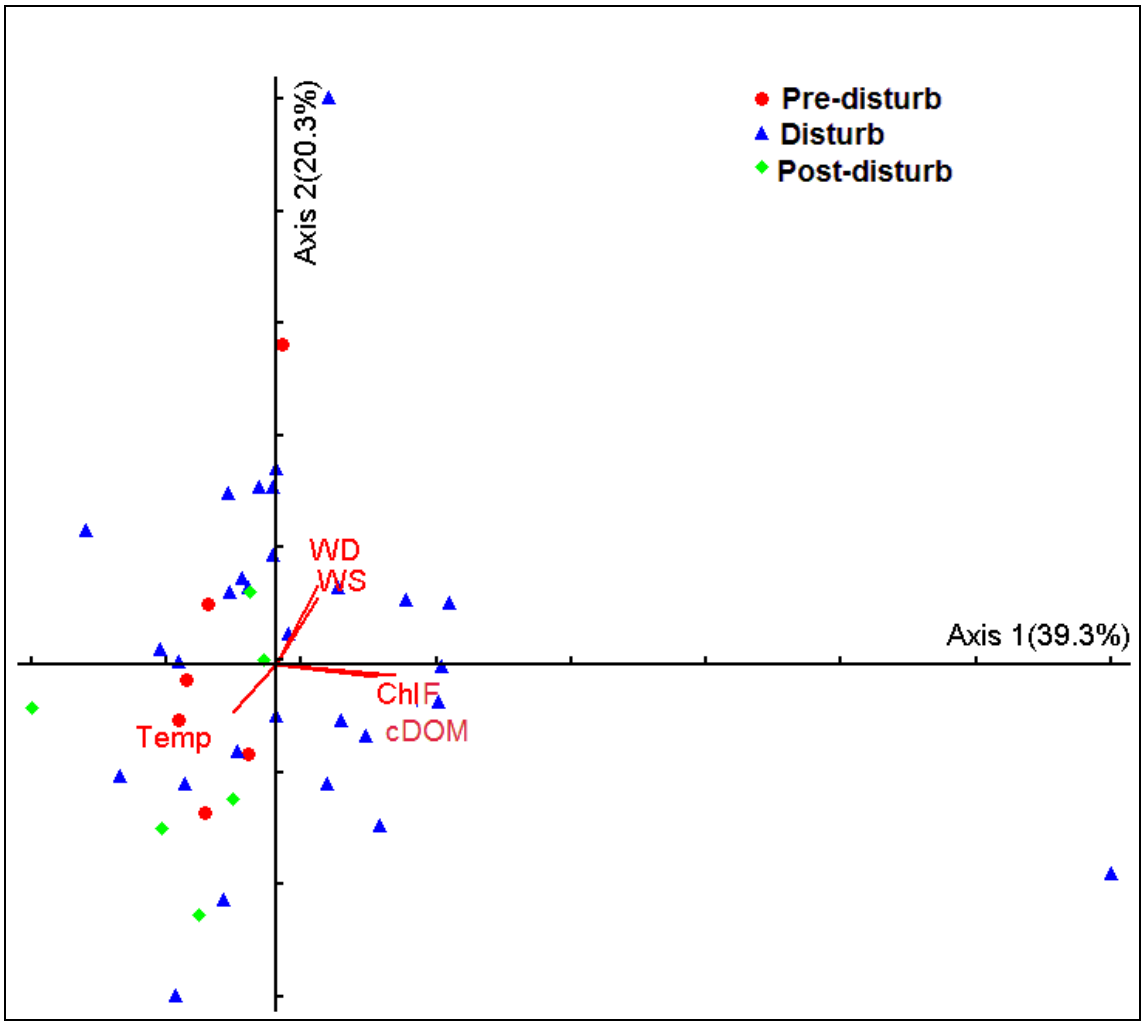

Figure 6 RDA biplot between bacterial composition and environmental variables. Temp: air temperature; WD: wind direction; WS: Wind velocity; ChlF: chlorophyll fluorescence; cDOM: Organic matter dissolved chromophoric.

Discussion

Overall, we found that the bacterial community in terms of biomass and morphotypes varied over the cold front event in response to the change in environmental conditions. However, the BCC did not follow this tendency. In this way, bacterial biomass of Lake Mangueira changed during the disturbance, recovering after the occurrence of the event, suggesting a resilience-type response, as a result of environmental variability along de cold front. The morphtypes also changed during disturbance, but were not immediately

resilient to it. The BCC, in contrast, did not change over all the short-term cold front event, in which variation in OTU relative abundance were randomly.

The response of the bacterial biomass and morphotypes along to the cold front event may be a result of short-term hydrodynamics conditions variation. When the cold fronts reach coastal lakes the colder air passes over lakes and the long fetch, due to prevailing northeast or southeast winds parallel to the main axis of the lake, displaces water, mixing the water column more than usually occurs within shallow lakes (Fragoso *et al*. 2008; Tundisi *et al*. 2010). Hence, the wind-driven environment promotes great resuspension of the sediment in water column, and changes in environmental conditions, as physical, chemical, and in biological descriptors (Cardoso and Motta-Marques 2009), although no significant relationship were observed between TSS, and suspended nutrients. Such changes in the water column may potentially have a major impact on the aquatic microbial community (Shade et al. 2012b), because when the water column mixes, there is thermal and chemical homogenization and such gradients are known to influence the microbial distribution (Weithoff et al., 2000). In Lake Mangueira, the increasing in cDOM and ChlF, for example, and also the recruitment of benthic bacteria in the water column, previously only available in the sediment, were directly or indirectly affected by hydrodynamics, thus contributing for the increasing in biomass and morphotypes variety to the community, but not affecting BCC structure, which remained resistant to the perturbation.

Baho et al. (2012) suggest that the bacterial response to disorder might be likely due to temporal and spatial refuges that facilitate community to resist or recover acting analogous to a regional species pool in a metacommunity. In which higher species diversity may dampen disorders dynamics within a community (Allison, 2004). In this

context, is reasonable to assume that repeated perturbations shape communities to be more resistant or resilient.

The community stability is a response to disturbances, reflecting the sensitivity or insensitivity to a stressor agent. It is recognized, mainly as the resilience or resistance of the community in response to the disturbance (Pimm, 1984). Thus, the community can present mechanisms of resilience and, therefore, the capacity to recover after a disturbance (Allison and Martiny, 2008). The microbial community may also be resistant and thus withstand the disturbances (Pimm, 1984; Allison and Martiny, 2008). Also the microbial composition can be sensitive to disturbances, but not immediately resilient to it, regardless of the taxonomic range of the group or the type of disorder (Allison and Martiny 2008). However, depending on the type of disorder, it is possible to verify distinct behaviors in the community. Jones et al. (2009), for example, observed that the bacterial community exhibited many characteristics of secondary succession, initiated after large infrequent disturbances, but which induced some interesting differences. Allison and Martiny (2008) suggest that as the frequency of disturbances increases, if the composition of the bacterial community is very resilient, it may be less likely to detect compositional change (confounding the stability conferred by high resilience as resistance). However, Shade et al. (2012a) complain that the community's report of the disorder's sensitivity in many studies may be a reflection of the lack of publication of studies in which no changes in composition and/or function were found after disturbance, reflecting a potential trend of the results.

In this study, the bacterial community changed after the disturbance, exhibiting biomass sensitivity immediately to the beginning of the perturbation event, followed by quick post-disturbance recovery, thus being resiliente of the event. Bacterial

morphotypes also immediately changed during the event, but were not promptly resilient. In contrast bacterial composition showed resistant to the perturbation.

In conclusion, these findings have implications for the understanding of bacterial community responses on the effects of environmental disturbances on short-term temporal scales. As far as we know, it was the first time that microbial community (composition, structure, biomass and morphotypes) was investigated for their response to a disturbance event in subtropical coastal shallow lake.

Acknowledgments

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Capítulo 5. Discussão Geral

Os trabalhos desenvolvidos nesta tese avaliaram a dinâmica da comunidade bacteriana em termos de composição, consumo de fontes de carbono e biomassa em múltiplas escalas espaciais e temporais. Esses estudos utilizaram diferentes amostragens e estratégias para avaliar amplamente o efeito das escalas espaciais e temporais, das variáveis ambientais e biológicas (biomassa fitoplanctônica), de paisagem e meteorológicas sobre a comunidade bacteriana. Os estudos foram conduzidos em dois sistemas lacunares distintos: *i*) Sistema Hidrológico do Taim, lagoa Mangueira e *ii*) Sistema Hidrológico do Rio Tramandaí, em 25 lagoas. Ambos os sistemas caracterizam-se por serem rasos, costeiros e de clima subtropical, sobre grande influência da hidrodinâmica devido à velocidade e direção dos ventos (Cardoso e Motta Marques, 2007; Fragoso Jr. *et al.*, 2010), que tem grande efeito sobre a homogeneização da coluna d'água (Tundisi, 1983; Tundisi *et al.*, 2010) o qual tende a promover alta conectividade intra-lagoa.

No primeiro trabalho desenvolvido (Capítulo 2) a CCB e os AF apresentaram dinâmica temporal e espacial, sendo que a dinâmica temporal foi mais significativa que a dinâmica espacial. O padrão temporal verificado pode estar ligado a vários aspectos: *i)* uma dependência do ciclo fenológico de outros componentes do elo microbiano, tais como fitoplâncton e zooplâncton (Kent *et al.*, 2004; Kent *et al.*, 2007), provavelmente como consequência da influência da produção fitoplanctônica sobre o bacterioplâncton, através dos exsudatos de matéria orgânica dissolvida (MOD) (Baines e Pace, 1991; del Giorgio e Cole, 1998; Cotner e Biddanda, 2002; Eiler e Bertilsson, 2004; Tada e Suzuki, 2016; Lima *et al.*, 2016); *ii*) reflexo do decaimento da similaridade ao longo do tempo e do tamanho do ecossistema, conforme relatado para várias espécies plantônicas (Korhonen *et al.*, 2010); além de *iii*) variações de temperatura e de disponibilidade de nutrientes e substratos orgânicos (Dickerson e Williams, 2014).

O padrão espacial, embora mais fraco que o temporal, não foi menos importante, trazendo grandes implicações à importância do papel da heterogeneidade intra-lagoa para o funcionamento do ecossistema e as implicações para a escala apropriada para usar em amostragem de comunidades bacterianas. De maneira geral, foram observados efeitos contrastantes governando a composição e os AF da comunidade intra-lagoa. Em termos composicionais a dinâmica da comunidade bacteriana foi fortemente influenciada pelos filtros ambientais (variáveis ambientais e fitoplanctônicas), reforçando o papel da *species sorting* na composição da comunidade, demonstrando que barreiras de dispersão bacteriana, devido à grande distância entre os extremos norte e sul da lagoa foram sobrepostas pela alta conectividade da lagoa, conferida pela hidrodinâmica (velocidade do vento e mistura da coluna d'água). Já a influência exclusiva do espaço para os AF é um dos primeiros registros de dispersão dos AF, mostrada pela menor escala de variação (~49km) dentro da lagoa. Esse padrão inesperado pode estar sendo impulsionado pela dispersão genética, pelos efeitos indiretos das condições locais sobre taxa bacteriana migrante e/ou as bactérias aderidas ao sedimento.

O segundo trabalho desenvolvido nesta tese, apresentado no capítulo 3, indicou que o padrão de distribuição da diversidade beta bacteriana aquática, independente da escala dentro e entre as 25 lagoas foi principalmente caracterizado pelo *turnover*, indicando que as variações da CCB ocorrem principalmente pela substituição de espécies, invés da perda de espécies. Em geral microorganismos são abundantes, com curto tempo de geração e altas taxas de mutação, características que produzem variação genética, que juntamente com a diversidade fisiológica viabilizam a diversificação, sendo importante para a especiação microbiana (Horner-Devine *et al.*, 2004; Foissner, 2008) e permitindo ampla dispersão e baixa taxa de extinção em escalas locais (Fenchel e Finlay, 2004) Por outro lado, a alta densidade que apresentam tende a favorecer a homogeneização ao longo dos habitats, porém, conforme observado neste estudo,

dissimilaridades na estrutura e composição bacteriana foram influenciadas por filtros ambientais, tais como a heterogeneidade ambiental e heterogeneidade na composição do fitoplâncton. E essa contribuição significativa dos filtros ambientais sobre a diversidade beta bacteriana (β_{Sor}, β_{Sim} and Bray-Curtis dissimilarity), enfatiza a regulação da distribuição da metacomunidade bacteriana por *species sorting* (Chase e Leibold, 2003; Leibold *et al.*, 2004). No entanto, o fator espacial também foi observado, influenciando a distribuição da CCB. Neste caso, porém, esse fator, surpreendentemente, revelou que a diversidade beta dada pela dissimilaridade de Simpson (*turnover*) não apresentou a esperada relação positiva com a distância (i.e distance-decay; Nekola e White 1999; Heino *et al.*, 2013; Heino *et al.*, 2015), pelo contrário, descresceu; enquanto que o padrão aninhado de distribuição apresentou essa relação positiva com a distância espacial. Esse resultado aponta para várias explicações alternativas, dentre elas, ser consequência de interações de respostas ambientais espécie-específica que podem estar espacialmente estruturadas e não foram avaliadas neste estudo. Com relação à variação da diversidade beta intra-lagos entre as lagoas, não foi confirmada variação significativa, além disso, nenhuma variável explicou a variação da diversidade beta melhor do que um modelo nulo sem variáveis.

Já o terceiro estudo, apresentado no capítulo 4 revelou as respostas da comunidade bacteriana (em termos composicionais, estruturais, de biomassa e morfotipos), frente a distúrbio climático de curta duração provocado por frentes frias. Nesse contexto, a estrutura e composição da comunidade bacteriana mostrou-se resistente ao longo de todo o distrúbio. Contrastando essa resposta, a biomassa bacteriana e os morfotipos apresentarammudanças significativas em resposta à entrada da frente, ocorrendo um aumento da biomassa durante o distúrbio, juntamente com um pequeno recrutamento de outras formas de morfotipos, em relação à pequena redução da

contribuição de *coccus* para a comunidades. Cessada a frente fria a biomassa retornou à sua condição original juntamente com o aumento de formas *coccus* e redução das demais formas. Entretanto, a resposta dos morfotipos após a passagem da frente fria não refletiu um retorno à condição original anterior ao distúrbio. A variação desses descritores ao longo da passagem da fente fria pode ser reflexo das alterações das condiçoes ambientais, no que se refere ao revolvimento do fundo, trazendo à coluna d'água bacterias do sedimento que antes não estavam disponíveis.

Esses resultados demonstram a habilidade da comunidade a responder ao distúrbio, seja sofrendo alterações e se recuperando caracterizando-se por ser resiliente ou não sofrendo alterações significativas e se caracterizando por ser resistente ao distúrbio. Esse resultado traz grandes implicações ao estudo da estabilidade de comunidades bacterianas, agregando mais informação a respeito de suas dinâmicas frente a distúrbios.

Finalmente, nossos resultados sugerem como é importante considerar múltiplas escalas espaciais para a compreensão da biogeografia microbiana e escalas temporais para a compreensão da resposta bacteriana frente a distúrbios, uma vez que a maioria dos padrões ecológicos e processo são escala dependentes (Levin, 1992; Logue *et al.*, 2011; Moritz *et al*., 2013; Stein, 2014). Nesse contexto temos que a menor escala de heterogeneidade bacteriana detectada pode estar positivamente relacionada ao tamanho do lago, conforme apontado no capítulo 2, , fortalecendo o papel dos fatores locais na estruturação da CCB em sistemas altamente conectados e surpreendentemente dos fatores espacias na estruturação das características funcionais. Ou como apontado no capítulo 3, que a relação entre a beta diversidade bacteriana e a heterogeneidade ambiental responde a diferentes escalas, nas quais a diversidade beta bacteriana é resultado de *species-sorting* entre lagoas, enquanto que intra-lagos a ausência de

explicação das variáveis ambientais sobre diversidade beta, pode ser o reflexo do efeito de massa (Leibold *et al.*, 2004). E finalmente, no útlimo capítulo 4, de que comunidade bacteriana responde às mudanças do sistema apresentando diferentes respostas de estabilidade: mudança sem recuperação, resiliência e resistência.

Neste contexto, as interações de múltiplos fatores ambientais embutidos nessas múltiplas escalas também se mostraram relevantes para compreender a variabilidade da comunidade bacteriana aquática. A hidrodinâmica das lagoas, por exemplo, é um fator potencial responsável pela maior conectividade dentro e entre lagoas, devido ao longo fetch e a intensidade dos ventos, influenciando as condiçoes ambientais das lagoas e provocando a mistura interna. E nesse estudo a hidrodinâmica, sendo com efeito direto ou indireto sobre as condições ambientais influenciou a composiçao, a estrutura, a função, a diversidade beta bacteriana e a biomassa nas múltiplas escalas estudadas. Outro fator relevante é a paisagem, que tende a provocar efeitos sobre a composição das comunidades. Medidas de conectividade, área do lago, distância do mar, distância entre lagoas são fatores que ajudam a investigar limitações de dispersão. A conectividade, por exemplo, é um fator central para o estudo da ecologia de metacomunidades (Leibold *et al.*, 2004;. Heino, 2013). Considerando que a conectividade em sistemas aquáticos é comumente sinônimo de isolamento, lagos geralmente são ligados entre si por diferentes tipos e graus de conectividade (Guimarães *et al.*, 2014; Heino *et al.*, 2015). Desta forma, a conectividade em lagos pode representar eficiência diferente como filtros de limitação de dispersão, dependendo do organismo estudado. De maneira geral, organismos aquáticos com limitação de motilidade (por exemplo, crustáceo, zooplâncton e peixe) podem ser muito mais afetados por fatores espaciais do que por fatores ambientais. Nesse caso, em sistema de lagoas interligadas, seria de esperar maior riqueza e similaridade na composição de espécies entre lagoas próximas, enquanto que

em sistemas isolados ou com baixa conectividade, seria de esperar baixas taxas de dispersão e colonização, onde a riqueza é o resultado da área da lagoa e as condições ambientais locais (e.g. Heino e Muotka, 2006;. Shurin *et al.*, 2009). No entanto, para os microorganismos, esses tendem a ser mais afetados por fatores ambientais que pelo espaço (Beisner *et al.*, 2006; Jones e McMahon, 2009; Souffreau *et al.*, 2015; Lima *et al.*, 2016).

Neste contexto, as condições físicas e químicas do sistema atuam como um conjunto de filtros para a distribuição da CCB, enfatizando a separação espacial de nicho ecológico. Sugerindo que a autocorrelação espacial da estrutura ambiental controla fortemente a metacomunidade. Assim taxa imigrantes são selecionados por filtros ambientais ao atingirem a lagoa de destino, sendo que lagoas próximas tendem a ser semelhante ambientalmente, abrigando espécies similares, em relação às lagoas mais distantes (Lichstein *et al.*, 2002;. Moritz *et al.*, 2013).

Outro fator igualmente importante é a influência da composição da comunidade fitoplanctônica, definindo padrões na CCB (Fuhrman *et al.*, 1980; del Giorgio e Cole, 1998; Cotner e Biddanda, 2002; Eiler e Bertilsson, 2004; Jones *et al.*, 2009). Tal relação pode ser resultado da influência da produção fitoplanctônica sobre o bacterioplâncton através dos exsudatos fitoplanctônicos (MOD), como também das interações específicas entre fitoplâncton e bacterioplâncton (Baines e Pace, 1991; Kent *et al.*, 2006; Kent *et al.*, 2007). Em vista disso, poderia se esperar que uma composição fitoplanctônica mais variável resultaria em mais tipos de exsudados e, consequentemente, uma maior diversidade bacteriana (Baines e Pace, 1991; Tada e Suzuki, 2016), de modo que a CCB passa a ser afetada pela composição fitoplanctônica tanto quanto pelas condições físico-químicas da lagoa.

Outro fator de relevância para os estudos de comunidade bacteriana, igualmente discutido nos capítulos anteriores, é a limitação dos métodos utilizados para acessar a CCB, através do ARISA (A*utomated Ribossomal Intergenic Spacer Analysis*) e os AF através do consumo de fontes de carbono pelo Biolog Ecoplate™.

Com relação ao ARISA, é importante destacar que esse método, como tantos outros métodos *fingerprinting* são tendenciosos para a detecção das OTUs mais abundantes, pois a técnica utiliza a amplificação do DNA através de PCR, em que apenas espécies com alta frequência de ocorrência (>1-2%; Murray *et al.*, 1996; Nocker *et al.*, 2007) serão principalmente detectadas e até mesmo células mortas e inativas, devido à estabilidade do DNA no ambiente. Embora apresente tais limitações, esse métodos é altamente reprodutível, permitindo detectar diferenças composicionais de aproximadamente 0.05 ou mais através do índice de Sørensen, por exemplo (Jones *et al.*, 2012). De maneira geral, o ARISA pode detectar OTUs com uma única diferença de comprimento de onda quando os fragmentos estão entre 300-1000bp de comprimento. Essa resolução decai para 3-5pb, a medida que os comprimentos aumentam de 1000 para 1500bp (comprimento máximo), isso devido à mancha ou alongamento de bandas que migram através do capilar do sequenciador (Fisher e Triplett, 1999).

Como resultado, nossas análises negligenciam a provável contribuição de numerosos membros raros da comunidade bacteriana aquática, sendo portanto, difícil prever como a inclusão de membros mais raros de comunidades de bactérias aquáticas impactariam os padrões aqui observados, uma vez que espécies raras possuem forte sinal biogeográfico (Galand, 2009).

Com relação ao Biolog Ecoplate™, este ensaio foi utilizado para avaliar os atributos funcionais da comunidade bacteriana através da variação na utilização das fontes de carbono pela comunidade. Essa abordagem foi utilizada somente no capítulo 2

deste trabalho. Esse ensaio oferece uma resposta interessante para o potencial metabólico da comunidade microbiana, permitindo avaliar, simultaneamente, as respostas ao consumo de 31 fontes de carbono distintas. Entretanto, ele não reflete a função da comunidade microbiana global *in situ*, pois os substratos disponíveis nas placas, não são necessariamente encontrados no ambiente de estudo, ou os substratos do sistema aquático podem ser mais complexos do que aqueles no Ecoplate (Smalla *et al.*, 1998, Preston-Mafham *et al.*, 2002). Ainda, esse ensaio poder ser seletivo, uma vez que a avaliação de consumo do substrato se dá pela mudança de coloração, para leitura da absorbância, a qual depende do crescimento de bactérias em altas densidades (> 10^8 células mL^{-1}) (Konopka *et al.*, 1998), não representando todos os membros da comunidade, embora a presença de membros raros possa ser detectada (Haack *et al.*, 1995). Apesar das limitações consideradas, esta técnica permite de forma rápida acessar a variabilidade funcional da comunidade, sendo uma ferramenta valiosa que produz um rico conjunto de dados, ideal para detectar e comparar diferenças específicas na diversidade funcional microbiana ao longo de escalas espaciais e temporais (Zak *et al.*, 1994, Garland, 1997).

Conclusões

De maneira geral, conseguimos responder às perguntas propostas que nortearam esse estudo.

Observamos que a composição da comunidade e seus atributos funcionais variaram temporal e espacialmente. A variabilidade da composição foi explicada principalmente pela atuação de filtros ambientais, tais como as condições físico-químicas e a composição fitoplanctônica, indicando que as barreiras de dispersão pela longa distância de norte a sul da lagoa Mangueira são superadas pela conectividade da lagoa, e que a distribuição da CCB é explicada pelos fatores locais (*species sorting*). Já a atividade bacteriana (AF), foi influenciada exclusivamente pelo espaço, devido provavelmente à dispersão gênica, às bacterias migrantes e em suspensão e/ou aos efeitos indiretos dos fatores locais.

Neste estudo também observou-se que a variabilidade intra-lagoa pode ser um importante componente para a variabilidade geral e o funcionamento do sistema. Portanto, estudos de biogeografia deveriam ser ampliados para incluir tais escalas de variabilidade para explicar padrões ecológicos. Isso sugere a importância em se considerar a escala de amostragem apropriada conforme o ambiente estudado.

Também verificamos que o *turnover* predominou para o padrão da diversidade beta bacteriana tanto intra-lagoas como entre as 25 lagoas. Além disso, a diversidade beta total, dada pela dissimilaridade de Sørensen foi positivamente relacionada à dissimilaridade do fitoplâncton, enquanto que a dissimilaridade de Bray-Curtis e o componente de *turnover* foram positivamente relacionados com a dissimilaridade ambiental e fitoplanctônica. O *turnover* foi negativamente relacionado à distância espacial entre os lagos, enquanto que o componente de aninhamento foi explicado apenas pela distância espacial. Contrariamente à nossa expectativa, nenhuma variável

explicativa medida foi relacionada à diversidade beta bacteriana ou à algum de seus componentes (βsør, βsim, βsne or Bray-Curtis) dentro das lagoas. No geral, a relação da diversidade beta bacteriana à heterogeneidade ambiental entre as lagoas se dá pelo efeito dos fatores locais, enquanto que intra-lagoa pode estar relacionado ao efeito de massa, devido à alta conectividade dentro das lagoas, mascarando os fatores locais.

E finalmente, vimos que a comunidade bacteriana em termos de biomassa apresenta-se sensível ao distúrbio e se passada a perturbação do sistema ela prontamente se recupera, Enquanto que os morfotipos permanecem sensíveis mesmo após cessado o distúrbio. Diferentemente, a composição da comunidade bacteriana mostrou-se insensível ao evento de frente fria, sugerindo que embora sua biomassa e morfotipos alterem, sua estrutura manteve-se resistente ao distúrbio.

Contribuições e Perspectivas

Esta tese de doutorado permitiu verificar os padrões ecológicos da comunidade bacteriana entre múltiplas escalas espaciais e temporais. Os resultados aqui obtidos nos permitiram incrementar o debate sobre quais os fatores responsáveis pelos padrões biogeográficos microbianos, que têm sido considerados importantes estruturadores dessas comunidades. Nesse contexto, sugerimos nesse trabalho que estudos de padrões biogeográficos microbianos devem ser ampliados para incluir múltiplas escalas de análise, desde variações intra-lagoa até entre lagoas, abrangendo distintas variações de distância espacial, atentando para os diferentes fatores que influenciam tais padrões biogeográficos para explicar a biodiversidade e o funcionamento do ecossistema. Além disso, nessa tese conseguimos discutir a dinâmica da comunidade em sistemas perturbados por evento climático e sugerimos que mais estudos envolvendo a resposta bacteriana a perturbações devam ser explorados, incluindo por exemplos sua relação

com outras variáveis biológicas, como o fitoplâncton e ampliando o período de amostragem para incluir mais eventos.

A tese de doutorado aumenta o conhecimento acerca dos fatores que ditam os padrões de distribuição espacial e temporal da comunidade bacteriana aquática. Ressalta a forte influência dos fatores locais (*species sorting*) sobre a composição e estrutura da comunidade. Tanto em ambientes, cujas barreiras de dispersão, em lagoas de grande extensão, são superadas devido à conectividade e à hidrodinâmica, como também entre lagoas com diferentes graus de conectividade. Dentre os fatores locais, destaca-se a influência das condições ambientais e da composição fitoplanctônica na distribuição bacteriana. Também acrescenta a habilidade da comunidade a responder de diferentes formas a perturbações.

Finalmente, este estudo colabora com o desenvolvimento do entendimento de padrões e processos ecológicos relacionados à estrutura e função de comunidades bacterianas aquáticas em lagos rasos costeiros subtropicais. Apesar desses avanços, há ainda para a microbiologia aquática questões que são sugeridas após esse estudo, para incrementarem o entendimento da escala de variação característica das comunidades microbianas aquáticas:

i) Quais os efeitos da composição fitoplanctônica, a heterogeneidade ambiental, a conectividade, a hidrodinâmica, e a distância espacial sobre a biomassa bacteriana nas múltiplas escalas? Terão o mesmo efeito que na composição bacteriana?

ii) Qual a relação espécie-específica ou gênero-específica entre a comunidade bacteriana e fitoplanctônica ao longo de gradientes espaciais e temporais?

iii) Qual a relação da estrutura e função bacteriana ao longo de evento de distúrbio de curta duração?

iv) Como perturbações contínuas (de longa duração) no sistema afetam a comunidade bacteriana, em relação a sua estrutura e função?

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ANEXO

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SHALLOW LAKES

Contrasting factors drive within-lake bacterial community composition and functional traits in a large shallow subtropical lake

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Abstract Lakes are considered as "islands" for assessing microbial biogeography, but bacterial community composition (BCC) and function may vary significantly within lakes, with the roles of scale and connectivity still unclear. This study investigated the spatial and temporal heterogeneity of the BCC (automated ribosomal intergenic spacer analysis) and functional traits (FT, carbon-source utilization), and the contribution of: (i) environmental variables, (ii) phytoplankton, (iii) season, and (iv) space, through variance partitioning in the large and well-mixed Lake Mangueira. The BCC and FT differed in time and space, with BCC being explained by environmental variables and phytoplankton, whereas FT was explained only by space. The smallest scale of variability detected by the BCC and FT (~49 km) was larger than scales found in the other studies, suggesting an effect of lake size (fetch and connectivity). Our results indicate that barriers to bacterial dispersal due to long distances are overcome by high connectivity, reinforcing the role of species sorting for BCC. FT were probably driven by gene dispersal and/or the effects of local conditions on migrant bacterial taxa and resuspended bacteria. Our results highlight the role of within-lake heterogeneity for ecosystem functioning and the implications for the appropriate scale to use in sampling bacterial communities.

Keywords Automated ribosomal intergenic spacer analysis · Ecoplate · Within-lake biogeography · Variance partitioning · Species sorting · Dispersal · Lake Mangueira

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Introduction

Microbes represent a large fraction of the biosphere's diversity (Pace, 1997) and are key players in aquatic ecosystem functioning (Cole, 1999). Consequently, understanding the mechanisms controlling their diversity and function in aquatic ecosystems is of paramount importance.

Lakes are often assumed to have a homogeneous microbial community and are therefore considered as "islands" in most biogeographical analyses that test factors shaping microbial diversity and distribution. Consequently, the factors driving variation among lakes are relatively well-known, and lake microbial communities change rapidly in space and time in response to habitat size (Horner-Devine et al., 2004; Yannarell & Triplett, 2004; Bell et al., 2005; Reche et al., 2005; Green & Bohannan, 2006; Crump et al., 2007). However, increasing evidence for within-lake variability in factors such as physical and chemical conditions (Van der Gucht et al., 2007; Souffreau et al., 2015), biotic interactions (Kent et al., 2006), and trophic status (Yannarell et al., 2003; Wu et al., 2007) suggests that microbial communities within lakes are also heterogeneous. As environmental variables tend to be structured across the landscape in space and time, elucidating the drivers of microbial diversity and function is a key topic for research (Lear et al., 2014). Studies using approaches based on variance partitioning have obtained contrasting results, indicating significant pure effects of the environment (van der Gucht et al., 2007; Logue & Lindström, 2010), space (Lear et al., 2014), or a combination of both environment and space (Langenheder & Ragnarsson, 2007; Schiaffino et al., 2011; Liu et al., 2015; see the thorough discussion by Lindström & Langenheder, 2012).

Several studies have described the temporal and spatial dynamics of bacterial functional traits using patterns of substrate utilization as a proxy for bacterial activity in freshwater (e.g., Grover & Chrzanowski, 2000; Christian & Lind, 2006; Tiquia, 2010), sediment–water interfaces (Christian & Lind, 2007), and the marine environment (Sala et al., 2006). All these studies found that substrate utilization was strongly associated with environmental conditions. In contrast, studies on smaller scales in freshwater ponds (Lear et al., 2014) and small to meso-scales in soil (Bissett et al., 2010) have found either a higher contribution of geographical distance (Lear et al., 2014) or an absence of relationships between function and environmental variables (Bissett et al., 2010). These differences suggest that other factors such as the degree of connectivity, rates of dispersal, and life history of microorganisms can drive bacterial functional traits in space and time (Bissett et al., 2010; Severin et al., 2013; Lear et al., 2014).

Few studies have addressed biogeographical questions within individual lakes, since rates of dispersal and connectivity are typically assumed to be high within lakes. Surprisingly, these studies have found not only an important additional role of within-lake heterogeneity of environmental conditions (Wu et al., 2007; Shade et al., 2008; Tian et al., 2009), but also evidence for barriers to dispersal on scales as small as 20 m in a lake (Lear et al., 2014). In view of such small-scale heterogeneity within lakes, a key question in the study of microbial diversity and function is how to select the most appropriate scale for sampling.

Similarly to large-bodied organisms, microbes exhibit a distance-decay relationship between species composition and geographical distance (see review by Green & Bohannan, 2006), which suggests an increasing role of environmental heterogeneity and barriers to dispersal in larger lakes. However, the relative roles of local factors (i.e., environmental variables) versus space (i.e., barriers to dispersal) in shaping the bacterial community composition and functional traits within a lake remain unknown. We hypothesized that a wind-driven and well-mixed large lake with high connectivity will not present barriers to the dispersal of bacterial taxa and their functional traits, even across larger meso-scales.

In this study, we investigated the spatial and temporal heterogeneity of the bacterial community composition (BCC) and functional traits (FT), as well as the pure effects of lake environmental conditions, phytoplankton biomass, space and time, through variance partitioning of BCC and FT in Mangueira, a large shallow subtropical lake. The sampling design covered the 90-km length of the lake during 1 year, i.e., a biogeographical mesoscale (10–3,000 km; Martiny et al., 2006). We tested whether there were consistent differences among lake areas (Center, South, and North) and seasons (winter, spring, summer, and autumn) in BCC and FT, and also which

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explanatory variables significantly explained individual fractions of the variances of BCC and FT.

Materials and methods

Study area and sampling

Lake Mangueira (Fig. 1) is located in the state of Rio Grande do Sul, southern Brazil (~30°31′22″S 53°07′48″W). It is a large shallow subtropical coastal lake, covering a total area of 820 km^2, 90 km long and 3–10 km wide, with a mean depth of 2.6 m and a maximum depth of 7.0 m. The trophic state ranges from oligotrophic to mesotrophic (Crossetti et al., 2013). Lake Mangueira is bordered by fixed dunes on the Atlantic Ocean side, emergent macrophytes in the northern wetland, and a dense bed of submersed macrophytes on the southern side. The western side is used for agriculture, primarily rice production in former wetlands.

Water samples for environmental and biological variables including chlorophyll *a*, phytoplankton, and bacterial compositional and functional characterization were collected in summer (February 2010), autumn (May 2010), winter (August 2010), and spring (November 2010). In each season, the lake was sampled in an identical fashion for all variables, at 18 sampling points: 6 in the southern, 6 in the center, and 6 in the northern part of the lake (Fig. 1 and Supplementary Table S1).

Environmental variables and chlorophyll *a*

Turbidity (Turb), pH, dissolved oxygen (DO), depth, water temperature, and oxidation–reduction potential (ORP) were measured with a multiparameter probe (YSI 6600). Water transparency (WT) was estimated with a Secchi disk, and the amount of total suspended solids (TSS) was assessed gravimetrically by water evaporation in porcelain dishes (APHA, 1999). Nutrients, including total nitrogen (TN), nitrate (NO_3^-), and soluble reactive phosphorus (SRP) were measured through colorimetric methods, following APHA (1999). Analyses of ammonium (NH_4^-) and total phosphorus (TP) followed Mackereth et al. (1989). A carbon analyzer (Shimadzu VCPH) was used to determine the dissolved organic carbon (DOC) of the fraction that passed through a 450°C pre-combusted glass-fiber filter (0.45-µm mean mesh size). Chlorophyll *a* (Chl*a*) was extracted from GF/F filters with 90% ethanol and measured by spectrophotometry (Jespersen & Christoffersen, 1987). Humic substances (HS) were estimated as the ratio of the absorption coefficients at 250 and 365 nm (Strome & Miller, 1978), using a Varian Cary 1-E spectrophotometer with a quartz cuvette.

The meteorological data (wind velocity and direction; precipitation; nebulosity, i.e., the percentage in tenths of the sky covered by clouds; insolation and evaporation) were obtained from the Santa Vitória do Palmar Meteorological Station (INMET, Rio Grande do Sul), located approximately 23 km from the lake, with data collected three times per day (00:00, 12:00, and 18:00 h). Data were interpolated according to the time when each sampling point was visited.

Phytoplankton biomass

Phytoplankton was counted according to Utermöhl (1958) after sedimentation (Lund et al., 1958). At least 100 specimens of the most frequent species were enumerated (counting error <5%, Lund et al., 1958). Biomass ($mm^3\ l^{-1}$) was estimated through biovolume, according to Hillebrand et al. (1999). For data analysis, the biomass of all phytoplankton taxonomic classes was used.

Bacterial composition and functional traits

The bacterial community composition (BCC) was assessed through amplified ribosomal intergenic spacer analysis (ARISA). Bacterial cells were concentrated by filtration of 250 ml of water from each sample onto cellulose acetate membrane filters (0.22-µm pore size; 47 mm diameter; Sartorius). Total DNA was extracted from the membrane filter using the PowerSoil DNA isolation kit (MO BIO). We used the filter in place of the 0.25 g soil recommended for this method. The region between the 23S and 16S ribosomal RNA genes from the total DNA extracted was amplified using the 6-FAM-labeled universal primer 1406F (5′-TGYACACCGCCCGT-3′) and the bacteria-specific primer 23Sr (5′-GGGTTBCCCCATTCRG-3′) (Fisher & Triplett, 1999; Yannarell et al., 2003). ARISA profiles were analyzed according to Jones & McMahon (2009). Since this method under-represents rare members of the

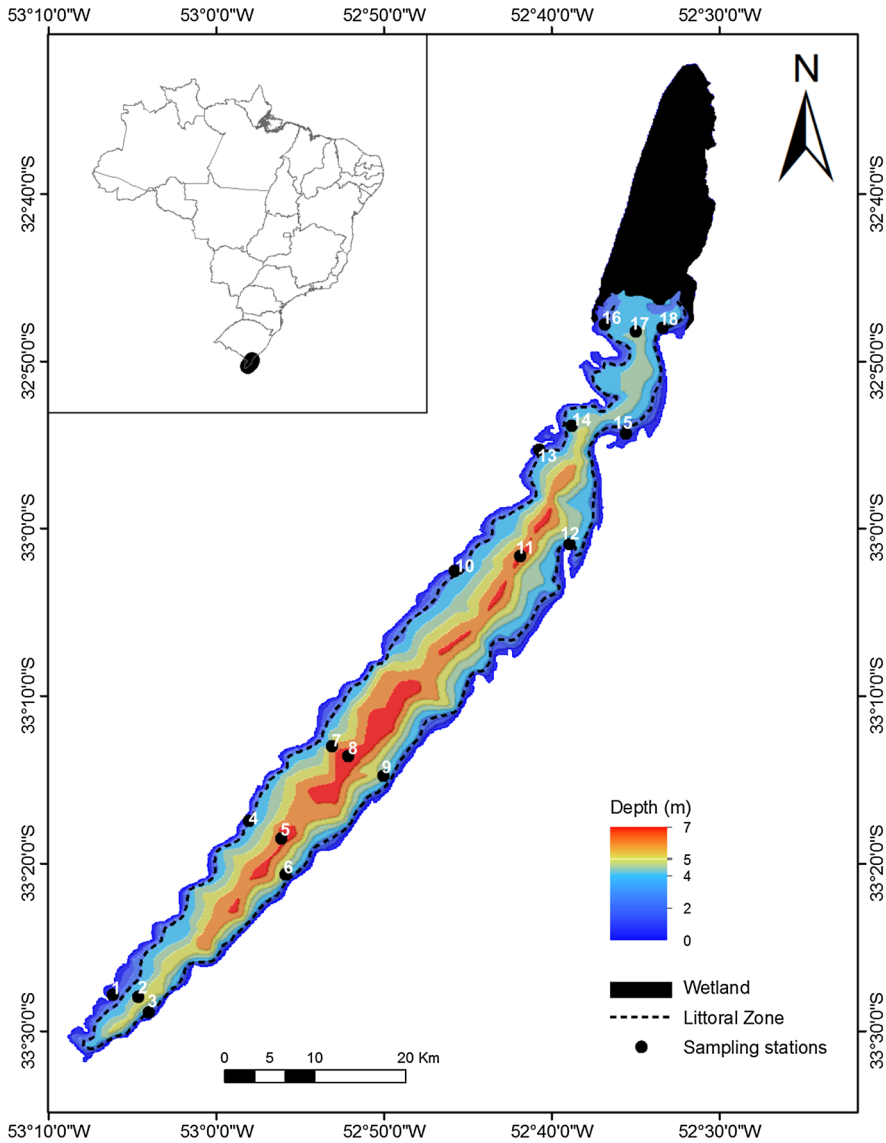

Fig. 1 Sampling points in Lake Mangueira, southern Brazil. South (points 1–6), Center (points 7–12), and North (points 13–18)

community, BCC hereafter refers to the most abundant taxa.

The functional traits of the heterotrophic microbial community were measured as carbon utilization patterns through Biolog Ecoplates® (Hayward, CA, USA). In the field, water samples were pre-filtered through a 20-µm-mesh sieve and placed in 50-ml amber glass bottles to prevent interference from phytoplankton. In the laboratory, samples were inoculated in the plates and incubated at approximately 22°C (Christian & Lind, 2007). The utilization of the substrates was assessed through optical density (OD) measured at 590 nm, with an automated plate reader (SpectraMax 5.0, Molecular Devices), immediately after the wells were filled ($t = 0$ h) and every 24 h for 12 days, to ensure that saturation of carbon utilization was reached in all samples (Salomo et al., 2009).

Statistical analyses

For BCC analyses, we used relative abundance data, including OTUs with > 5% frequency of occurrence (Shade et al., 2008). For functional traits, we corrected the raw absorbance data by subtracting each response well against its own first reading ($t = 0$), to compensate for the intrinsic absorbance of the carbon sources

(Insam & Goberna, 2004). Then, we subtracted the control from the response well, and any negative values were considered zero (no oxidation). The areas under the curve of the three Ecoplate replicates were integrated for each of the 31 carbon sources in order to estimate the curve integration index (CI) (Guckert et al., 1996). The CI is a trapezoidal approximation, and this approach incorporates additional information from the absorbance versus incubation time (lag phases, rates of color development, and maximum absorbance) into a single number. The single value obtained for each substrate was expressed as the percentage area of the total area of the plate (sum of all areas of substrates).

Since the data were nonparametric, we performed PERMANOVAs (PERmutation MANOVAs) for BCC and for FT, and PERANOVAs (PERmutation ANOVAs) for environmental variables (Euclidean distance) and phytoplankton (Bray-Curtis distance) to test for differences overtime (winter, spring, summer, and autumn) and space (South, Center, and North). P-values were adjusted by Bonferroni correction for multiple testing. The OTUs (Bray-Curtis of transformed abundances) and substrates (Euclidean distance) that contributed most to the dissimilarity among groups of factors (time and space) were examined through similarity percentage analysis (SIMPER), with a threshold of >1.5% for OTUs and >5.5% for substrates.

The degree of association between the BCC and FT data was assessed through a Mantel test (Mantel, 1967). This is used to compare two independent dissimilarity (or similarity) matrices describing the same set of entities, and to test whether the correlation is higher than expected by chance (Sokal & Rolf, 1995).

For the variance partitioning of BCC and FT, we first performed redundant analyses (RDA) on each of the four explanatory matrices: environmental variables (physical, chemical, and meteorological variables + chlorophyll *a*); time (using dummy variables to encode for summer, spring, winter, and autumn); phytoplankton (biomass of phytoplankton taxonomic classes); and space (PCNM vectors obtained from latitude and longitude coordinates; Borcard & Legendre, 2002; Dray et al., 2006). The explanatory variables for the models were selected through variation inflation factors (VIFs), which identify collinear constraints. Variables with VIF > 10 were removed from the RDAs (Gross, 2003). The significance of the global RDAs was tested by permutation (P value ≤ 0.05, 1,000 iterations) and the significant RDAs were then submitted to variation partitioning through partial redundancy analysis (pRDA) (Borcard et al., 1992; Legendre & Legendre, 1998) to evaluate the significance of the contribution of each matrix after the effect of the others was removed. The BCC data were treated using Hellinger transformation prior to the analyses (Ramette, 2007). The PER(M)ANOVA analyses were performed in PAST software, the SIMPER analysis was carried out in PRIMER v.6.1.9 (Primer-E Ltd, Plymouth, UK), and the Mantel test, RDAs, pRDAs, and graphical presentations were performed in the software Rstudio (R Core Team, 2013) using the vegan package (Oksanen et al., 2013).

Results

Temporal and spatial differences

Environmental variables, chlorophyll a, and phytoplankton

The PERANOVA revealed differences among seasons for several variables. Most of them were higher in summer (chl*a*, TP, and pH) or in summer and in another season: temperature, ORP, and insolation (summer and spring); TSS, TN, and HS (summer and autumn). Other variables were higher in other seasons: SRP (autumn and spring); DO and water transparency (winter); nitrate (winter and spring); precipitation, wind velocity (WV), and nebulosity (autumn). DOC was lowest in spring. Biomass of most phytoplankton groups was higher in spring (Cyanobacteria and Zygnemaphyceae) or in spring and autumn (Bacillariophyceae, Chlorophyceae). There was no seasonal variation for Dinophyceae, Chrysophyceae, and Euglenophyceae. Among all groups, Cyanobacteria dominated overall and was mainly represented by *Aphanocapsa* spp., *Chroococcus* spp. and *Planktolyngbya* spp. (Tables 1, 2).

Spatial differences were also detected by PERANOVA. The North stations were generally shallower, with lower water transparency and higher turbidity, TP, NH_4^+, and nebulosity; while the South stations were generally deeper, with higher water transparency, lower turbidity, and chlorophyll *a* concentrations. The Center

Table 1 Mean (standard deviation) of environmental variables and chlorophyll *a* across seasons and locations for Lake Mangueira

	Season				Location		
	Summer	Autumn	Winter	Spring	South	Center	North
Temp (°C)	22.75 (1.05)^{a}	17.79 (0.34)^{b}	11.66 (0.44)^{c}	22.16 (0.66)^{a}	18.18 (4.39)^{a}	18.63 (4.61)^{a}	19.27(4.54)^{a}
DO (mg l^{-1})	9.09 (0.43)^{a}	9.33 (0.19)^{a}	11.45 (0.31)^{b}	8.61 (0.16)^{c}	9.53(1.21)^{a}	9.65 (1.18)^{a}	9.61 (0.99)^{a}
ORP (mV)	170.37 (13.83)^{a}	137.74 (17.61)^{b}	96.01 (15.38)^{c}	166.19 (9.29)^{a}	145.62 (38.29)^{a}	142.64 (33.66)^{a}	141.37 (26.08)^{a}
Depth (m)	2.92 (1.53)^{a}	2.74 (1.52)^{a}	3.12 (1.68)^{a}	3.30 (1.64)^{a}	3.8 (1.47)^{a}	3.30 (2.04)^{ab}	2.36 (0.76)^{b}
WT (m)	1.16 (0.26)^{a}	0.94 (0.31)^{a}	1.63 (0.62)^{b}	1.08 (0.25)^{a}	1.41 (0.47)^{a}	1.12 (0.47)^{ab}	1.05 (0.34)^{b}
TSS (mg l^{-1})	14.50 (4.17)^{a}	14.31 (6.57)^{a}	9.71 (3.22)^{b}	10.06 (5.48)^{b}	10.71 (4.42)^{a}	13.06 (5.24)^{a}	12.78 (6.43)^{a}
Turb (NTU)	4.90 (2.11)^{a}	6.33 (2.81)^{a}	5.18 (2.14)^{a}	4.08 (3.07)^{a}	3.70 (1.78)^{a}	5.58 (2.15)^{b}	6.12 (3.26)^{b}
TP (mg l^{-1})	0.04 (0.01)^{a}	0.02 (0.01)^{b}	0.03 (0.02)^{b}	0.03 (0.01)^{b}	0.02 (0.01)^{a}	0.03 (0.01)^{ab}	0.03 (0.01)^{b}
SRP (μg l^{-1})	10.10 (2.02)^{a}	12.76 (5.06)^{ab}	11.54 (2.25)^{a}	15.46 (3.67)^{b}	11.30 (3.58)^{a}	12.96 (2.85)^{a}	13.21 (5.04)^{a}
TN (mg l^{-1})	0.48 (0.12)^{a}	0.43 (0.07)^{a}	0.25 (0.03)^{b}	0.22 (0.13)^{b}	0.35 (0.12)^{a}	0.32 (0.14)^{a}	0.37 (0.18)^{a}
NH_4^+ (mg l^{-1})	0.05 (0.11)^{a}	0.03 (0.02)^{a}	0.03 (0.02)^{a}	0.13 (0.09)^{a}	0.03 (0.03)^{ab}	0.03 (0.02)^{a}	0.06 (0.09)^{b}
Nitrate (mg l^{-1})	0.07 (0.05)^{a}	0.06 (0.01)^{a}	0.11 (0.02)^{b}	0.13 (0.09)^{b}	0.10 (0.06)^{a}	0.08 (0.05)^{a}	0.10 (0.07)^{a}
DOC (mg l^{-1})	2.07 (1.19)^{ab}	2.72 (0.86)^{a}	2.68 (0.81)^{a}	1.96 (0.55)^{b}	2.46 (1.01)^{a}	2.02 (0.92)^{a}	2.59 (0.78)^{a}
HS (nm)	8.06 (1.88)^{a}	8.18 (1.31)^{a}	4.33 (0.68)^{b}	5.12 (0.91)^{c}	7.14 (2.76)^{a}	6.34 (1.82)^{a}	5.84 (1.47)^{a}
chl*a* (μg l^{-1})	6.20 (2.01)^{a}	3.79 (1.58)^{bc}	4.36 (1.35)^{b}	2.95 (1.09)^{c}	3.52 (1.45)^{a}	4.81 (1.97)^{b}	4.66 (2.15)^{ab}
pH	8.54 (0.12)^{a}	8.13 (0.05)^{b}	8.04 (0.09)^{c}	8.01 (0.10)^{c}	8.19 (0.17)^{a}	8.17 (0.21)^{a}	8.28 (0.31)^{a}
WD (°)	S (S)^{a}	SSE (NE-WSW)^{ab}	SE (S-E)^{b}	SE (SE)^{ab}	SE (S-ENE)^{a}	S (SO-ESE)^{a}	SSE (SSW-ESE)^{a}
WV (m s^{-1})	1.75 (0.39)^{a}	7.78 (1.17)^{b}	1.94 (1.03)^{a}	3.17 (0.77)^{c}	3.44 (3.31)^{a}	3.46 (2.15)^{a}	4.17 (2.24)^{a}
Nebulosity (tenths)	1.58 (1.20)^{a}	9.50 (0.79)^{b}	3.00 (3.68)^{ac}	6.33 (3.28)^{c}	3.13 (4.31)^{a}	5.48 (4.10)^{ab}	6.87 (2.38)^{b}
Precip (mm)	0.00 (0.00)^{a}	9.19 (5.82)^{b}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.53 (0.93)^{a}	2.94 (5.65)^{a}	3.57 (6.15)^{a}
Insol (h)	10.80 (0.00)^{a}	0.55 (0.45)^{b}	5.44 (1.30)^{c}	5.60 (0.00)^{ac}	5.85 (3.96)^{a}	5.51 (3.67)^{a}	5.43 (3.72)^{a}

Different letters indicate significance at $P < 0.05$

Temp water temperature, *DO* dissolved oxygen, *ORP* oxidation–reduction potential, *depth* local depth, *WT* water transparency, *TSS* total suspended solids, *Turb* turbidity, *TP* total phosphorus, *SRP* soluble reactive phosphorus, *TN* total nitrogen, *NH4+* ammonium, *DOC* dissolved organic carbon, *HS* humic substances ratio (Abs 250:365 nm), *chla* chlorophyll *a*, *WD* wind direction, *WV* wind velocity, *Precip* precipitation, *Insol* insolation

Table 2 Mean (standard deviation) of biomass (mg l^{-1}) of major classes of phytoplankton across seasons and locations for Lake Mangueira

	Season				Location		
	Summer	Autumn	Winter	Spring	South	Center	North
Bacillariophyceae	0.10 (0.33)^{a}	0.11 (0.09)^{b}	0.02 (0.02)^{c}	0.15 (0.15)^{b}	0.09 (0.10)^{a}	0.10 (0.28)^{a}	0.09 (0.14)^{a}
Chlorophyceae	0.21 (0.24)^{a}	0.43 (0.31)^{bc}	0.29 (0.08)^{b}	0.96 (0.83)^{c}	0.33 (0.31)^{a}	0.53 (0.61)^{a}	0.57 (0.64)^{a}
Chrysophyceae	0.00 (0.00)^{a}	0.01 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.01)^{a}	0.00 (0.00)^{a}
Cyanobacteria	4.31 (2.56)^{a}	4.86 (1.34)^{a}	6.00 (2.13)^{a}	9.67 (4.66)^{b}	4.95 (2.23)^{a}	6.26 (3.93)^{a}	7.48 (4.00)^{a}
Dinophyceae	0.02 (0.04)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.01 (0.04)^{a}	0.00 (0.00)^{a}	0.01 (0.04)^{a}	0.01 (0.03)^{a}
Euglenophyceae	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.01)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
Zygnemaphyceae	0.01 (0.01)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{b}	0.02 (0.03)^{c}	0.00 (0.01)^{a}	0.01 (0.02)^{a}	0.01 (0.02)^{a}

Different letters indicate significance at $P < 0.05$

Table 3 Differences among seasons and locations, assessed by PERMANOVA for BCC and FT in Lake Mangueira

	BCC			Functional traits		
	F	R^2	*P*	*F*	R^2	*P*
Temporal	6.271	0.22	0.0001*	3.432	0.13	0.0001*
Summer versus Autumn	4.216	–	0.0414*	2.052	–	0.048*
Summer versus Winter	8.708	–	0.0006*	3.315	–	0.0006*
Summer versus Spring	5.205	–	0.003*	3.254	–	0.0006*
Autumn versus Winter	6.719	–	0.0006*	5.087	–	0.0006*
Autumn versus Spring	6.892	–	0.0006*	4.473	–	0.0012*
Winter versus Spring	6.135	–	0.0006*	3.091	–	0.0066*
Spatial	2.314	0.06	0.01*	2.126	0.06	0.001*
South versus Center	1.178	–	0.8394	1.193	–	0.7305
South versus North	3.267	–	0.0129*	3.183	–	0.0012*
Center versus North	2.272	–	0.1155	2.045	–	0.0279*

* Significant at $P < 0.05$

– not available

stations were more similar to the North stations in some variables, and to the South stations in others. For phytoplankton, no spatial differences were observed (Tables 1, 2).

Bacterial community composition (BCC) and functional traits (FT)

A total of 121 operational taxonomic units (OTUs) were detected by ARISA; 116 of them had >5% of frequency of occurrence. Most of them were shared over time (78%) or locations (95%), but when taking into account the abundance of the OTUs, the PERMANOVA indicated that the temporal variation was more important than the spatial variation ($R^2 = 0.22$ and $R^2 = 0.06$, respectively). All seasons were different from each other, whereas only South × North displayed consistent differences in BCC (Table 3).

A similar response was found for FT, with all 31 carbon sources being consumed in all samples; however, the degree of utilization varied over time and along the spatial scale. There were consistent differences among all seasons ($R^2 = 0.13$), while for the spatial variation ($R^2 = 0.06$) only South x Center did not differ significantly (Table 3).

Based on the SIMPER analysis and the arbitrary cutoffs chosen, the OTUs 399, 403, 404, 438, 511, 520, 546, 547, 634, 650, 654, and 669, and the

substrates phenylethylamine (G4), 2-hydroxy benzoic acid (C3), γ-hydroxybutyric acid (E3), glucose-1-phosphate (G2), α-D-lactose (H1), α-ketobutyric acid (E3), i-erythritol (C2), glycogen (F1), and L-phenylalanine (C4) made stronger contributions to the dissimilarity among seasons (see Supplementary Table S2 and Supplementary Figure S1 for details). Regarding spatial differences, the OTUs 399, 403, 404, 511, 546, 547, 634, and 654 and the carbon sources phenylethylamine (G4), i-erythritol (C2), glucose-1-phosphate (G2), γ-hydroxybutyric acid (E3), and 2-hydroxybenzoic acid (C3), most notably G4 and C3, contributed most to the dissimilarity among sites (see Supplementary Table S3 and Supplementary Figure S2 for details).

Although BCC and FT showed similar temporal and spatial patterns, they were not significantly correlated (Mantel test, $r = -0.05258$, $P = 0.71$), suggesting that even though they shared the same pattern, they responded to different drivers.

Variance partitioning of BCC and FT

All explanatory matrices were significant for BCC when separate RDAs were run, and hence were included in the variance partitioning. However, when tested for their isolated effects through pRDA, only the matrices for the environmental variables and the phytoplankton biomass were significant. These matrices explained 7.3 and 4.7%, respectively, of the total inertia, and the residual variance was very high (63.6%) (Table 4). The contributions of the environmental variables to the BCC pattern after the removal of the other explanatory matrices were in decreasing order of importance: I) First RDA axis: TP, SRP, turbidity, TN, chl*a*, and depth (negatively); II) Second RDA axis: WT, NH_4^+, WD, pH, nitrate, TSS, and evaporation (negatively) and HS, nebulosity, ORP, WV, and DOC (positively) (Fig. 2). The contribution of phytoplankton classes to the BCC patterns after the removal of the other explanatory matrices were in decreasing order of importance: I) First RDA axis: Zygnemaphyceae and Bacillariophyceae (negatively) and Euglenophyceae (positively); II) Second RDA axis: Dinophyceae, Cyanobacteria, Chlorophyceae, and Chrysophyceae (negatively) (Fig. 3).

For bacterial FT, all explanatory matrices run separately were significant except the phytoplankton matrix, which therefore was not included in the

Table 4 RDA and variation partitioning (pRDA) of the bacterial community composition (BCC) among four explanatory matrices in Lake Mangueira

Explanatory matrix	Description	RDA		Variation partitioning (pRDA)			
		R^2 adj	*P*	R^2 adj	*P*	%
Selected environmental variables	TSS, Turb, TP, SRP, TN, NH_4^+, Nitrate, Chl*a*, DOC, Depth, WT, pH, ORP, HS, WD, WV, Nebulosity, Evap	0.271	0.005*	0.073	0.017*	7.3
Spatial	PCNM Vectors	0.040	0.036*	0.005	0.410	0.5
Temporal	Dummy variables for summer, autumn, winter and spring	0.169	0.005*	0.017	0.140	1.8
Phytoplankton	Biomass of Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cyanobacteria, Dinophyceae, Euglenophyceae and Zygnemaphyceae	0.115	0.005*	0.047	0.031*	4.7
				Shared	0.221	22.1
				Residual	0.636	63.6
				Total	1.000	100

* Significant at $P < 0.05$

Abbreviations as in Table 1

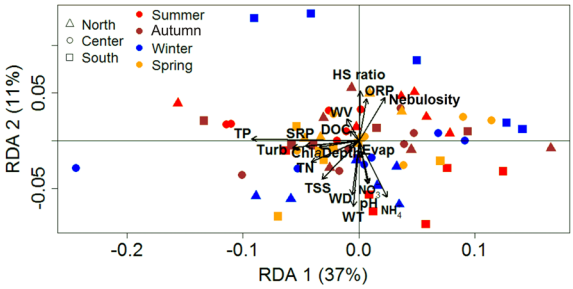

Fig. 2 Biplot of the pRDA of the BCC constrained by the environmental variables matrix, after removing the effect of all other explanatory matrices

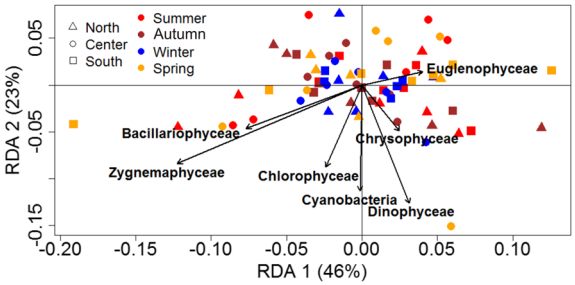

Fig. 3 Biplot of the pRDA of the BCC constrained by the phytoplankton major classes' biomass matrix, after removing the effect of all other explanatory matrices

variance partitioning. The pRDA revealed that only the spatial matrix (PCNMs) was significant after the effect of the other explanatory matrices was removed. Around 81.5% of the total inertia remained unexplained by the explanatory matrices tested (Table 5).

Discussion

Our results revealed a strong spatial and temporal heterogeneity in Lake Mangueira for environmental variables, chlorophyll *a*, and phytoplankton taxonomic classes. Similarly, the BCC and FT showed strong temporal heterogeneity, while the spatial analysis showed differences along the north–south axis. When considering differences in BCC and FT as assessed by PERMANOVA, the effects of environmental variables and distance are confounded, and it is therefore necessary to compare their individual effects on the dynamics of BCC and FT. The pRDAs revealed that local factors (environment and phytoplankton) were the significant factors explaining the BCC dynamics, whereas unexpectedly, the FT seemed to be driven by dispersal constraints.

Although 95% of OTUs were found in all three parts of the lake and 78% in all seasons of the year, the

Table 5 RDA and variation partitioning (pRDA) of the bacterial functional traits (FT) among four explanatory matrices in Lake Mangueira

Explanatory matrix	Description	RDA			Variation partitioning (pRDA)		
		R^2 adj	*P*		R^2 adj	*P*	%
Selected environmental variables	TSS,Turb, TP, SRP, TN, NH_4^+, Nitrate, Chl*a*, DOC, Depth, WT, pH, ORP, HS, WD, WV, Nebulosity, Evap	0.118	0.005*		0.032	0.160	3.2
Spatial	PCNM Vectors	0.042	0.015*		0.062	0.005*	6.2
Temporal	Dummy variables for summer, autumn, winter and spring	0.093	0.005*		0.0004	0.500	0.0
Phytoplankton	Biomass of Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cyanobacteria, Dinophyceae, Euglenophyceae and Zygnemaphyceae	0.021	0.130		–	–	–
				Shared	0.090		9.1
				Residual	0.815		81.5
				Total	1.000		100.00

Significant at $P < 0.05$; Abbreviations as in Table 1

BCC differed significantly from the northern to the southern areas of the lake and across all seasons, suggesting that the majority of freshwater bacterial taxa are not confined to a subset of regions on the geographical or temporal scales. Our results suggest that ubiquitous taxa are prevalent in bacterial communities, and support previous observations of high percentages of OTUs occurring across sampling sites (85, 76, and 77.6% of all OTUs) found by Van der Gucht et al. (2007), Souffreau et al. (2015), and Lear et al. (2014), respectively. Even though the ARISA method is highly reproducible (Jones et al., 2012), it is biased toward the detection of the most abundant OTUs, a limitation of all fingerprinting methods (Jones et al., 2012). It is possible that the inability to detect a pure spatial effect for the BCC stemmed from the failure to detect rare members of the bacterial community, since rare OTUs may display strong biogeographical signals and significantly impact the results of the analysis if they are included (Yannarell & Triplett, 2004; Galand et al., 2009).

Assessing within-lake heterogeneity is a critical issue when considering the appropriate scale for sampling microbial communities. Such small-scale variability was recognized early, and Palmer et al. (1967) reported patchiness in the distribution of bacterial plate counts at scales of approximately 20 m. More recently, a significant variability of within-lake BCC has been reported via molecular and culture-independent methods (e.g., Wu et al., 2007), and scales as short as 10 m (Jones et al., 2012) to <20 m (Lear et al., 2014) have proved to be significant for dispersal of bacterial OTUs. Although the scale of heterogeneity seems to be positively related to lake size, within-lake variation in BCC occurs across all types of morphometry (Table 6). Another important factor controlling variability in BCC is the degree of connectivity, which depends on the mixing regime, wind (velocity, direction, and fetch), the presence of physical barriers such as islands, embayments, or constrictions (Yannarell & Triplett, 2004; Lear et al., 2014) and lake heterogeneity (e.g., presence of macrophytes) (Wu et al., 2007; Lear et al., 2014).

Given the high connectivity of Lake Mangueira due to its wind-driven and well-mixed nature, barriers to dispersal are expected to be weak or nonexistent, and this is reflected in the smallest scale of heterogeneity in BCC detected (~49 km), which is the largest yet found in comparison to other studies (Table 6). Assuming that the fetch increases with lake size, we hypothesize that the relatively large scale of variability in BCC in Lake Mangueira is derived from its long fetch. The significant differences between the northern and southern parts of the lake can be explained by (i) the long fetch due to prevailing northeast or southeast winds parallel to the main axis of the lake, which controls sediment resuspension between these two areas (Fragoso et al., 2008), and (ii) differences in the composition of dominant macrophytes (mostly emergent in the northern wetlands and mostly submersed in the southern part), which are known to affect BCC in many lakes (Wu et al., 2007; Lear et al., 2014) including Lake Mangueira (They et al., 2010).

The temporal patterns of BCC and FT were stronger than the spatial patterns. Even though patterns in time are not as often explored as patterns in space for BCC and FT, the few studies available are in agreement with our results. Stronger temporal variation of BCC was reported for a large estuarine area, where temperature changes had a stronger seasonal influence than the spatial dynamics (Kan et al., 2007). Alternative explanations may include a dependence on the phenological cycle of other components of the microbial loop such as phytoplankton and zooplankton (Kent et al., 2004). Considering that Lake Mangueira is large, this pattern is in agreement with the positive relationship between the decay in similarity over time and ecosystem size found for several aquatic species, including planktonic ones (Korhonen et al., 2010). Similarly, FT (assessed as substrate utilization) have been reported to vary on a seasonal basis in lakes, associated with patterns of stratification (Christian & Lind, 2007), temperature, and the availability of nutrients and organic substrates (Dickerson & Williams, 2014).

The higher explanatory power of local factors, versus dispersal, has often been emphasized in studies that addressed BCC variation over geographical scales (Beisner et al., 2006; van der Gucht et al., 2007, Sommaruga & Casamayor, 2008; Souffreau et al., 2015). Other studies have found that both environmental and spatial factors explain bacterioplankton community dynamics (Schiaffino et al., 2011), or even that the interaction of environmental conditions, region, time, and landscape influence BCC (Yannarell & Triplett, 2005). Our study supports the view that the bacterioplankton community structure is primarily the

Table 6 Comparison of studies that have addressed within-lake heterogeneity in BCC through molecular approaches

Number of systems studied	Size (km^2)	Ecosystem	Mean depth (m)	Max depth (m)	Sampling layout	No. sampling points	Smallest scale identified	Reference
1	820	Large, shallow subtropical lake	2.6	7.0	18 sampling sites, from ~ 1.9 to ~ 90 km apart	18	49 km*	This study
2	0.00245 0.0028 0.0039	Shallow, small ponds	0.5 (all)	0.5 (all)	7 × 7 m grids	33 35 53	20 m	Lear et al. (2014)
3	0.005 39.38	Small and shallow lake × Large and deep lake	1.7 12.8	2.5 25.3	32 sites	32 32	10 m	Jones et al. (2012)
1	2338	Large, shallow subtropical lake	1.9**	< 3.0	3 sampling sites 100 and 1000 m apart, located inside 6 sampling areas	3	>1 km	Wu et al. (2007)
13	0.005–39.38	Small to large, shallow to deep temperate lakes	NA	2.5–35.7	3 stations in each basin - equilateral triangle centered on the deepest point. The variability within lakes was determined at two scales: 10 m (station level) and 100 m (basin level)	90	100 m	Yannarell & Triplett (2004)

* Considering the smallest difference between pairs of northern and southern sampling stations

** Qin, B., 2008. Lake Taihu, China: Dynamics and Environmental Change. Springer. 356 pp

NA not available

result of species-sorting selection (Chase & Leibold, 2003; Leibold et al., 2004), which is in accordance with the high rates of dispersal expected for Lake Mangueira.

In Lake Mangueira, the significant environmental variables that contributed most to the RDAs explaining the BCC (total phosphorus, PO_4^{-3}, NH_4^{+}, water transparency, turbidity, and water temperature) varied seasonally and spatially as a result of water withdrawal for agricultural irrigation and nutrient inputs (i.e., low-watershed nutrient loads in winter and high loads in summer) (Fragoso et al., 2008; Rodrigues et al., 2015). These inorganic nutrients are essential and often limiting for bacterial growth (Carlsson & Caron, 2001; Caron, 1994), whereas water transparency and turbidity are inversely linked, and along with temperature (Lindström et al., 2005) can be an important source of variation of the BCC in lakes (e.g., Yannarell & Triplett, 2005).

In addition, the significance of the share of BCC variance explained by the biomass of phytoplankton taxonomic classes is in accordance with the influence of phytoplankton on bacterioplankton diversity and metabolism (Kamjunke et al., 1997; del Giorgio & Cole, 1998; Höfle et al., 1999; Cotner & Biddanda, 2002; Eiler & Bertilsson, 2004; Horner-Devine et al., 2003; Kent et al., 2007). Phytoplankton also competes with bacteria for nutrients (Cotner & Biddanda, 2002) and positively influences bacteria through exudation of dissolved organic matter, which can fuel as much as half of the carbon required for bacterial production (Baines & Pace, 1991), determining patterns of BCC (Jones et al., 2009). Since phytoplankton production tends to be more important in larger lakes (Vadeboncoeur et al., 2008), especially in the pelagic zone, algae are expected to significantly influence BCC in Lake Mangueira. The biomass of large groups such as Cryptophytes and Chrysophytes has been found to be correlated with BCC in lakes (Lindström, 2000); however, since a wide metabolic repertoire in terms of exudates of organic carbon is expected for these broad classes of phytoplankton, it is impossible to link each specific algal class to the results found. Bacterivory by phytoplankton is another possibility, since mixotrophy is higher in more oligotrophic lakes (Saad et al., 2013) and prevalent in members of Dinophyceae and Chrysophyceae, among others (see review by Stoecker, 1998). Notably, in some systems mixotrophy may represent as much as 50% of the total bacterivory by flagellates (Unrein et al., 2007).

A similar pattern of spatial and temporal variation was found for FT, even though all substrates were oxidized in all seasons and locations, and the BCC and FT were not significantly correlated. This lack of correlation between BCC and FT has also been found by Lear et al. (2014) for individual ponds, and by Comte & del Giorgio (2010), who reported that even though BCC and FT were not directly correlated, their rates of change along environmental gradients did correlate. Several explanations such as those summarized by Lear et al. (2014) can be considered: (i) the inability of migrant bacteria to grow, thereby contributing to BCC but not to FT; (ii) the contribution of Archaea or picoeukaryotes to the substrate utilization pattern; and (iii) the potentially higher functional redundancy due to the generic metabolic pathways assessed by this study.

To our knowledge, this is the first evidence for dispersal of functional traits in bacterial communities, as shown by the smallest scale of variation found for FT ($\sim$ 49 km). This unexpected dispersal-driven FT may be explained by: i) indirect effect of local factors, ii) exclusion of significant explanatory variables, and iii) horizontal gene transfer. Local factors act by modulating the FT due to the interaction of local conditions and migrating bacteria. For example, Severin et al. (2013) found that the ecosystem functioning (using bacterial secondary production as a proxy) was well explained by the functional traits of bacterial communities along a dispersal-rate gradient, with the receiving local environment being critical for the final effects on the functional traits of the migrant cells. Hence, the adaptive success and subsequent likelihood that immigrant bacteria will contribute their original functional traits to the receiving community are inversely proportional to the difference in the local conditions (i.e., distance). In terms of potentially significant explanatory variables excluded, the contribution of attached (sediment) bacteria stands out. Since the wind velocity differed significantly across seasons and the wind often resuspends the sediment in shallow lakes, we believe that sediment bacteria may have had a role in the FT patterns found. This was confirmed by the significant correlation of wind velocity with 4 of 9 substrates that were indicated by SIMPER as the most important for differences among seasons (not shown). Additionally, attached bacteria may be richer in oxidized Ecoplate® substrates compared to free-living bacteria (Lyons & Dobbs,

2012). A further indication that other important variables may not have been included in the models tested is the large proportion of unexplained variation, since it is inherently impossible to control field conditions and/or to measure all relevant environmental variables. Last, horizontal gene transfer may be another factor that is unaccounted for in this study, as gene flow can occur even when phylogenetic dispersal is highly limited (Parnell et al., 2010). In this case, we may have detected a signal of gene dispersal, which unfortunately cannot be confirmed through patterns of substrate utilization.

In summary, this study showed significant differences in bacterioplankton community composition and function over spatial and temporal gradients in a shallow lake, with temporal differences being stronger than spatial ones. Compared to previous studies, the smallest scale of variability detected in BCC and FT was large ($\sim$49 km). Thus, our results suggest that the smallest scale of heterogeneity detected may be positively related to lake size. However, at least part of the results was likely due to the long fetch of Lake Mangueira, which may be responsible for higher connectivity through mixing. Variance partitioning revealed, however, that the bacterial community composition and functional traits were driven by environmental conditions and spatial factors, respectively, and our results reinforce the role of local factors in structuring the BCC in highly connected systems. Regardless of whether the spatial differences found were driven by environmental factors or dispersal, the results have important implications for sampling design and for understanding the dynamics of freshwater microbial communities. Specifically, spatial and temporal differences in microbial communities within a lake may be comparable to differences among lakes. The current results are in line with a growing number of other studies (summarized in Table 6), which together highlight within-lake variability as an important component of the total variability of the landscape. Thus, biogeographical studies should be extended to include scales of variability within lakes for explaining biodiversity and ecosystem function.

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