INSTITUTO FEDERAL
DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA
Minas Gerais
Campus Bambuí

Marcos de Paula Júnior

Análise espacial e temporal das comunidades microbianas do sedimento de leito do Rio São Francisco na região da Serra da Canastra em Minas Gerais

Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais – IFMG Bambuí

Agosto / 2020

Marcos de Paula Júnior

Análise espacial e temporal das comunidades microbianas do sedimento de leito do Rio São Francisco na região da Serra da Canastra em Minas Gerais

Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Sustentabilidade e Tecnologia Ambiental do Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais - IFMG, como parte dos requisitos para obtenção do título de Mestre em Sustentabilidade e Tecnologia Ambiental.

Área de concentração: Ciências Ambientais

Linha de Pesquisa: Ecologia Aplicada

Orientador: prof. Dr. Gustavo Augusto Lacorte

Coorientadora: prof. Dra. Amanda Soriano Araújo Barezani

MINISTÉRIO DA EDUCAÇÃO
SECRETARIA DE EDUCAÇÃO PROFISSIONAL E TECNOLÓGICA
INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA DE MINAS GERAIS
Campus Bambuí
Diretoria Geral
Diretoria de Inovação, Pesquisa e Pós-graduação
Coordenadoria de Pós-Graduação
Av. Professor Mário Werneck, 2590 - Bairro Buritis - CEP 30575-180 - Belo Horizonte - MG
37 3431 4900 - www.ifmg.edu.br

FICHA DE APROVACAO

Mestrando: **Marcos de Paula Júnior.**

Orientador: Prof. Dr. **Gustavo Augusto Lacorte.**

Dissertação de Mestrado, intitulada **"Análise espacial e temporal das comunidades microbianas do sedimento de leito do rio São Francisco na região da Serra da Canastra em Minas Gerais"**, de autoria do mestrando em Sustentabilidade e Tecnologia Ambiental**, Marcos de Paula Júnior,** aprovado pela Banca Examinadora de Defesa, em 28/08/2020, com a média de pontuação dos baremas de **84,5 pontos.**

Bambuí (MG), 28 de agosto de 2020.

Documento assinado eletronicamente por **Ronaldo dos Reis Barbosa**, **Assistente em Administração**, em 30/08/2020, às 09:00, conforme art. 1º, III, "b", da Lei 11.419/2006.

Documento assinado eletronicamente por **Gustavo Augusto Lacorte**, **Professor**, em 02/09/2020, às 11:18, conforme art. 1º, III, "b", da Lei 11.419/2006.

Documento assinado eletronicamente por **Ricardo Sousa Cavalcanti**, **Professor**, em 03/09/2020, às 23:49, conforme art. 1º, III, "b", da Lei 11.419/2006.

Documento assinado eletronicamente por **Amanda Soriano Araujo Barezani**, **Professora**, em 04/09/2020, às 08:31, conforme art. 1º, III, "b", da Lei 11.419/2006.

Documento assinado eletronicamente por **Paulo dos Santos Pompeu**, **Usuário Externo**, em 08/09/2020, às 07:46, conforme art. 1º, III, "b", da Lei 11.419/2006.

A autenticidade do documento pode ser conferida no site https://sei.ifmg.edu.br/consultadocs informando o código verificador **0627354** e o código CRC **09FA2EEE.**

P324a Paula Júnior, Marcos de.
Análise espacial e temporal das comunidades microbianas do sedimento de leito do Rio São Francisco na região da Serra da Canastra em Minas Gerais. / Marcos de Paula Júnior. – Bambuí, 2020.
109 f.: il.; color.

Orientador: Prof. Dr. Gustavo Augusto Lacorte.
Dissertação (Mestrado) - Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais – Campus Bambuí, MG, Curso Mestrado Profissional em Sustentabilidade e Tecnologia Ambiental, 2020.

1. Bacia hidrográfica. 2. Comunidades microbianas. 3. Bioindicadores. I. Lacorte, Gustavo Augusto. II. Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais – Campus Bambuí, MG. III. Título.

CDD 579

DEDICATÓRIA

À minha filha e aos meus pais, dedico.

AGRADECIMENTOS

Primeiramente às mutações genéticas e ao crossing over por me fazer único entre bilhões.

À Charles Darwin, pela inspiração nos anos iniciais dos meus estudos.

À minha coorientadora Dra. Amanda, pelos puxões de orelha.

Ao meu orientador Dr. Gustavo, pelo empenho e paciência.

Ao Thiagão, companheiro é companheiro!!!

À Daphne, é “nóis” mano!!!

À minha namorada Feliciane, pelo apoio e compreensão.

À turma da carona.

À minha família.

Aos técnicos dos laboratórios.

Aos estagiários de IC.

Ao Ronaldo, secretário do mestrado do IFMG.

Ao meu amigo Ney Martins, pelo apoio nas coletas.

À toda equipe do mestrado do IFMG.

Aos meus amigos.

A Mary Caroline pelo apoio psicológico.

E a todos que de alguma forma se envolveram nesta conquista.

BIOGRAFIA

Discente: Marcos de Paula Júnior

Filiação: Marcos de Paula e Gislene de Oliveira Rabelo e Paula

Naturalidade: Itaúna Estado:

MG Data de nascimento: 07/11/1982

Informações escolares:

- Ensino Médio: Escola Estadual de Itaúna
- Curso Superior: Universidade de Itaúna, Ciências Biológicas, Trabalho de conclusão de curso: Bioindicadores de qualidade de Água
- Curso de Especialização: PUC/MG Microbiologia

Experiência profissional: Lecionou para cursos técnicos e para Ensino Fundamental e Médio de escolas regulares e, também, escolas indígenas no estado do Pará.

RESUMO

Uma bacia hidrográfica forma-se através dos desníveis de terreno e por onde as águas pluviais escoam entre estes até o curso d'água principal, ou se infiltram formando lençóis freáticos e nascentes. Os ecossistemas naturais e as atividades antrópicas dependem dos serviços ambientais que são prestados pela bacia hidrográfica. Entretanto os rios sofrem com diversos impactos antrópicos, os quais trazem mudanças físico-químicas nas águas, o que reflete no uso e ocupação da terra. A Bacia Hidrográfica do São Francisco possui 639.219 km² de área de drenagem que tem uso múltiplo de suas águas para as mais diversas atividades, além de receber grande impacto antrópico. As principais fontes de poluição do Rio São Francisco são a indústria alimentícia, a extração de calcário, a agricultura, a pecuária, o esgotamento doméstico e os indícios de desmatamento de matas ciliares. A UPGRH, a que é denominada Bacia Hidrográfica do Alto São Francisco (SF1), sofre diversos tipos de pressões antrópicas que impactam diretamente na qualidade ambiental dos ecossistemas aquáticos. A SF1 é de maior importância para o monitoramento da qualidade ambiental, pois abrange as nascentes e seus primeiros afluentes cujo monitoramento periódico é feito com métodos tradicionais. Neste estudo, foram utilizadas as comunidades microbianas do sedimento do leito do rio para determinar a qualidade ambiental no SF1 sob três diferentes tipos de impactos antrópicos: (1) atividades de ecoturismo, (2) ocupação urbana e (3) atividades agrícolas tendo a nascente como controle. Diante disso, as comunidades microbianas do sedimento do leito do dia sofrem com essas ações e atividades antrópicas e tem suas estruturas modificadas, podendo assim ser utilizadas em estudos de qualidade ambiental. No primeiro artigo (capítulo 1), aborda-se a utilização de estrutura taxonômica e funcional de comunidades microbianas para determinar a influência dos usos da terra em sua comunidade e funcionalidade, onde se encontra grupos microbianos, candidatos a bioindicadores, organismos que devido a sua sensibilidade, podem indicar alterações no meio ambiente pela sua presença ou ausência. Comunidades microbianas do sedimento do leito do rio refletem em sua composição o uso da terra e podem ser ótimos bioindicadores No segundo artigo (capítulo 2), avalia-se, de forma temporal e espacial, a influência do uso da terra nas comunidades microbianas de sedimento de leito do rio São Francisco e, no capítulo 3, mostrou-se como os resultados podem ser utilizados em uma nova metodologia de análise de água usando a estrutura das comunidades como fonte de dados para os impactos antrópicos nos corpos d'água.

Palavras-chave: Bacia hidrográfica - Comunidades microbianas - Bioindicadores

ABSTRACT

A hydrographic basin is formed through the unevenness of the terrain and through which the rainwater flows between them until the main watercourse, or infiltrates forming water tables and springs. Natural ecosystems and human activities depend on the environmental services that are provided by the river basin. However, rivers suffer from several anthropic impacts, which bring physical-chemical changes in the waters, which reflects in the use and occupation of the land. The São Francisco Hydrographic Basin has 639,219 km² of drainage area that has multiple uses of its waters for the most diverse activities, in addition to receiving great anthropic impact. The main sources of pollution on the São Francisco River are the food industry, limestone extraction, agriculture, livestock, domestic sewage and evidence of deforestation of riparian forests. The UPGRH, which is called the Upper São Francisco Hydrographic Basin (SF1), suffers from several types of anthropogenic pressures that directly impact on the environmental quality of aquatic ecosystems. SF1 is of the greatest importance for monitoring environmental quality, as it covers springs and their first tributaries whose periodic monitoring is done with traditional methods. In this study, microbial communities from the riverbed sediment were used to determine the environmental quality in SF1 under three different types of anthropic impacts: (1) ecotourism activities, (2) urban occupation and (3) agricultural activities with the source as a control. Therefore, the microbial communities of the sediment of the day bed suffer from these anthropic actions and activities and have their structures modified, thus being able to be used in environmental quality studies. In the first article (chapter 1), the use of taxonomic and functional structure of microbial communities is addressed to determine the influence of land uses in their community and functionality, where microbial groups, candidates for bioindicators, organisms that due to their sensitivity, may indicate changes in the environment due to their presence or absence. Microbial communities of river bed sediment reflect land use in their composition and can be excellent bioindicators In the second article (chapter 2), the influence of land use on microbial sediment communities is evaluated in a temporal and spatial way. of the São Francisco riverbed and, in chapter 3, it was shown how the results can be used in a new methodology of water analysis using the structure of the communities as a source of data for anthropic impacts on water bodies..

Keywords: Hydrographic basin; Microbial communities; Bioindicators

LISTA DE ABREVIAÇÕES

CONAMA - Conselho Nacional do Meio Ambiente

PAs - protected areas

NP - National Park

ASV - Amplicon sequence variant

BZ - Buffer Zone

PCoA - Principal Coordinate Analysis

KO - metabolic function

MMA - Ministério do Meio Ambiente

PCR - Polymerase Chain Reaction

OTU - operational taxonomic unit

DNA – Ácido Desoxirribonucleico

SISGEN – Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado.

UPGRH - Unidade de Planejamento e Gestão de Recursos Hídricos

OD - Oxigênio Dissolvido

IFMG – Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais

IGAM – Instituto de Gestão de Águas de Minas Gerais

LISTA DE ILUSTRAÇÕES

CAPÍTULO 1

CAPÍTULO 2

APÊNDICE

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

SUMÁRIO

PREFÁCIO

Esta dissertação está organizada em quatro partes : uma introdução, que apresenta o tema e os assuntos abordados nos artigos para a compreensão do contexto, como conceitos de bioindicadores e a descrição da área estudada; capítulo 1, o artigo "Using sediment microbial communities to assess the buffer zone effectiveness of a freshwater protected area placed at an intensely impacted ecoregion from Brazilian Savanna", submetido à revista Microbiology and Environmental Microbiology Reports , que tem como objetivo apresentar os resultados e a utilização da estrutura taxonômica e funcional das comunidades bacterianas de sedimentos como ferramenta de diagnóstico de qualidade ambiental ; capítulo 2, artigo submetido à revista Journal of Soils and Sediments , "Spatial distribution of sediment bacterial communities from São Francisco River headwaters is influenced by human land-use activities and seasonal climate shifts", que contém a avaliação de forma temporal e especial das comunidades do sedimento do leito do rio São Francisco e a influência do uso da terra nessas comunidades; capítulo 3, em que há o desenvolvimento de uma metodologia para complementar as análises clássicas físico-químicas de análise de água que foi enviado ao Sr. Hidelbrando Canabrava Rodrigues Neto**,** Secretário Executivo de meio ambiente do estado de Minas Gerais, que encaminhou ao Instituto Mineiro de Gestão das Águas (Igam) para estudo de implementação. Embora estes capítulos sejam distintos, eles têm uma relação entre si, tanto que contribuem para a compreensão do sistema de comunidades microbianas do sedimento do leito do rio São Francisco na sua cabeceira.

1. INTRODUÇÃO

Segundo BARELLA (2001), uma bacia hidrográfica pode ser definida como um conjunto de terras delimitadas por divisores topográficos localizados nas partes mais altas do relevo, drenadas por um rio principal e seus afluentes, onde as águas pluviais, ou escoam superficialmente formando os riachos e rios, ou infiltram no solo para formação do lençol freático e de nascentes. No interior da bacia hidrográfica, os desníveis dos terrenos orientam os rumos da água sempre da posição mais alta para a mais baixa do relevo. A parcela da água das chuvas que se abate sobre a área de uma bacia, chamada de precipitação efetiva, transforma-se em escoamento superficial e escoamento subterrâneo no seu interior e, por meio da rede hidrográfica, ou rede de drenagem, que é formada por diversos cursos d'água, formam o rio principal da bacia, que recebe a contribuição dos seus afluentes e dos rios que deságuam nesses últimos, que são chamados subafluentes.

A manutenção dos ecossistemas naturais bem como das atividades antrópicas depende dos serviços ambientais que são prestados pelos elementos de uma bacia hidrográfica (MEA, 2003). Segundo SCHULER et al. (2017), somente os rios prestam diversos tipos de serviços ambientais estruturados em cinco categorias: (1) suprimento de água para uso extrativo - abastecimento público, agricultura, indústria, etc; (2) suprimento de água sem consumo - produção de energia hidrelétrica, recreação, transporte, pesca; (3) mitigação de danos relacionados ao ciclo da água – inundações, salinização de solos em regiões áridas; (4) serviços culturais – manutenção de valores estéticos, espirituais, históricos, educacionais e turísticos; (5) serviços hidrológicos de suporte aos ecossistemas naturais.

Por outro lado, os rios são coletores naturais de água das paisagens, o que reflete o uso e ocupação do solo de sua respectiva bacia de drenagem, pois eles sofrem diversos impactos antrópicos ao longo de seu percurso como despejamentos domésticos, rurais e industriais, lixiviação de insumos agrícolas, assoreamento de seu leito, desmatamento da mata ciliar dentre outros (SUTTI et al., 2016). Vale ressaltar que o lançamento de poluentes pode ser de natureza química, desde fosfatos (atividades agropecuárias) a metais pesados (atividades industriais), mas também de contaminantes biológicos (esgotamento doméstico) como vírus, bactérias e outros organismos patogênicos (LÓPEZ-ARCHILLA, 2001; JONES et al., 2018).

Essas atividades antrópicas podem trazer grandes impactos aos rios, dentre eles mudanças na físico-química das águas. Alterações na temperatura, OD (oxigênio dissolvido), pH, salinidade, adição de metais pesados, sólidos dissolvidos entre outros, o que influencia diretamente nas comunidades de organismos de um rio (GIBBONS et al 2014). Segundo SOARES (2017), o lançamento de despejos industriais e domésticos, que são ricos em matéria orgânica, fósforo e nitrogênio, são as principais fontes de poluição antrópica em áreas urbanas e na zona rural devido ao uso excessivo de agrotóxicos e fertilizantes que podem lançar metais pesados, através de lixiviação, para os cursos d'água.

Devido à esse cenário, em 2005, o poder público por meio do Conselho Nacional do Meio Ambiente, CONAMA, regulamentou o lançamento de efluentes nos ambientes aquáticos e deixou claro a necessidade de se investigar e monitorar a presença de substâncias nos sedimentos e na biota aquática bem como restringir o lançamento de substâncias que podem, nas águas, levar ao surgimento de características capazes de causar efeitos letais ou alteração de comportamento, reprodução ou fisiologia da vida (CONAMA, 2005).

A partir dessa regulamentação, tornou-se obrigatório o monitoramento dos ambientes aquáticos no território nacional, sendo de extrema necessidade estabelecer métodos de avaliação de parâmetros de qualidade ambiental, que podem ser definidos como as principais variáveis do ambiente que afetam o bem-estar dos organismos que o compõe, especialmente, os humanos (HORBERRY, 1984). Em ecossistemas aquáticos, os parâmetros de qualidade ambiental a serem avaliados podem ser de natureza abiótica ou biótica, estruturados pelos seus compartimentos: coluna d'água e suas subfases e sedimentos (ZAGATTO & BERTOLETTI, 2006).

Os parâmetros abióticos são os mais utilizados na avaliação da qualidade dos ambientes aquáticos e envolvem a aferição de parâmetros físicos (temperatura, sabor e odor, cor, turbidez, teor de sólidos e condutividade elétrica) e parâmetros químicos (pH, alcalinidade, dureza, demanda química/bioquímica de oxigênio, matéria orgânica, oxigênio dissolvido, concentração de compostos químicos e indicadores diversos). Já os parâmetros bióticos envolvem, principalmente, a verificação da carga de organismos patogênicos (coliformes totais, coliformes fecais, protozoários e ovos de helmintos) e mais recentemente a interpretação das modificações estruturais das comunidades de organismos sensíveis às perturbações ambientais chamados organismos bioindicadores de qualidade ambiental (ZAMBONI, 1993).

A aferição desses principais parâmetros levou a criação de índices de qualidade que são utilizados como referenciais para os órgãos gestores dos recursos hídricos no Brasil (Agência Nacional de Águas – ANA e Instituto Mineiro de Gestão de Águas – IGAM).

O Índice de Qualidade das Águas – IQA reflete a contaminação das águas em decorrência da matéria orgânica e fecal, sólidos e nutrientes e sumariza os resultados de nove parâmetros cujos valores do índice variam entre zero e 100 e os níveis de qualidade são classificados como Muito Ruim ($0 \leq IQA \leq 25$), Ruim ($25 < IQA \leq 50$), Médio ($50 < IQA \leq 70$), Bom ($70 < IQA \leq 90$) e Excelente ($90 < IQA \leq 100$). A Contaminação por Tóxicos – CT avalia a presença de 13 substâncias tóxicas nos corpos de água cujos resultados das análises laboratoriais são comparados com os limites definidos nas classes de enquadramento dos corpos de água pelos órgãos gestores. O Índice de Estado Trófico (IET) tem por finalidade classificar corpos de água em diferentes graus de trofia, ou seja, avaliar a qualidade da água quanto ao enriquecimento por nutrientes e seu efeito relacionado ao crescimento excessivo de algas (eutrofização). Como decorrência do processo de eutrofização, o ecossistema aquático passa da condição de oligotrófico e mesotrófico para eutrófico ou mesmo hipereutrófico. Para a classificação desse índice, são adotados os seguintes estados de trofia: Ultraoligotrófico ($IET \leq 47$), Oligotrófico ($47 < IET \leq 52$), Mesotrófico ($52 < IET \leq 59$), Eutrófico ($59 < IET \leq 63$), Supereutrófico ($63 < IET \leq 67$) e Hipereutrófico ($IET > 67$) (IGAM, 2017).

Segundo ZAMBONI (1993), as análises físico-químicas, que, em 1980, predominaram como base dos padrões da legislação sobre qualidade ambiental, pois sozinhas não são suficientes para prever o impacto dos mesmos aos organismos aquáticos, uma vez que quaisquer substâncias ou compostos químicos podem iniciar uma infinidade de interações entre si e com os constituintes do meio, que poderão resultar nas mais diferentes formas de ação sobre as comunidades biológicas a elas expostas. Por outro lado, as comunidades biológicas refletem a integridade ecológica total dos ecossistemas, visto que integram os efeitos dos diferentes fatores causais e fornecem uma medida agregada dos eventuais efeitos adversos (BARBOUR et al.,1999). As comunidades biológicas de ecossistemas aquáticos são formadas por organismos que apresentam adaptações evolutivas a determinadas condições ambientais e apresentam limites de tolerância a diferentes alterações das mesmas (ALBA-TERCEDOR, 1996).

Em teoria, qualquer organismo que viva em um dado ambiente pode ser utilizado para monitorar sua qualidade. Na prática, os grupos mais utilizados para avaliar a qualidade da água de rios apresentam características básicas como abundância, elevado número de espécies que são, relativamente, fáceis de coletar e identificar além de apresentarem ampla distribuição geográfica (BUSS et al., 2003). Entretanto, os bioindicadores mais eficientes são aqueles com sensibilidade suficiente para diferenciar entre fenômenos naturais, como por exemplo, mudanças de estação e ciclos de chuva-seca e estresses de origem antropogênica, os quais são relacionados a fontes de contaminação pontuais ou difusas. Quanto mais sensíveis forem as comunidades, mais pronunciadas serão as respostas ecológicas dos organismos aquáticos bioindicadores de qualidade de água (CALLISTO et al., 2001). Grupos taxonômicos específicos têm sido selecionados (protozoários, ciliados, algas, macro-invertebrados bentônicos e peixes) e utilizados em diferentes métodos de avaliação ambiental (ROSENBERG & RESH, 1993).

Os grupos bacterianos dificilmente vivem isolados em seu ambiente natural, porque, sempre, interagem entre si, com outros seres vivos e com fatores abióticos (pH, temperatura, dentre outros), formando comunidades complexas no ambiente que habitam (SHAPIRO & DWORKIN, 1997; RUDI et al., 2007). Além disso, as bactérias são os seres vivos mais abundantes da Terra, já que são encontradas em qualquer tipo de ambiente com grande diversidade. Esses atributos colocam os grupos bacterianos como um forte candidato a organismos bioindicadores.

Contudo a utilização de bactérias como bioindicadores nem sempre foi utilizada devido à dificuldade de isolamento e identificação, pois, até a década de 1980, a classificação e identificação de bactérias baseavam-se em comparações fenotípicas, que incluíam características morfológicas, fisiológicas, metabólicas e químicas das células. Todavia, com a recente popularização do uso de técnicas de sequenciamento de nova geração, que é associado à utilização da variação no gene de RNA ribossômico 16S (rRNA), como marcador molecular de caracterização de linhagens filogenéticas de bactérias, a classificação e a identificação de grupos de Bacteria e Archea (chamadas abordagens metagenômicas) tornou-se mais acessível e tem fornecido informações consideráveis sobre a relação taxonômica, papel ecológico e a evolução de espécies de procariotos encontradas nas amostras ambientais, sem a necessidade de isolamento e cultivo (RAPPÉ &GIOVANNONI, 2003; TRINGE & HUGENHOLTZ , 2008).

Dessarte é possível, através de abordagens metagenômicas, obter uma rápida e confiável caracterização completa da diversidade e estrutura de comunidades microbianas a partir de qualquer amostra ambiental através procedimentos laboratoriais, o que torna os microrganismos alternativas viáveis para estudos de qualidade ambiental e com resultados consistentes (TAO et al., 2019; HUANG et al., 2019; WANG et al., 2018; CHEN et al., 2018; RASSOL & XIAO, 2018; XIE et al., 2017; WANG et al., 2016). Adicionalmente, estudos recentes têm recomendado o uso de comunidades bacterianas de sedimento do leito dos rios como indicadoras em detrimento às presentes na coluna d'água, uma vez que (1) diversidade microbiana nos sedimentos são, consideravelmente, mais altas do que as dos corpos de água (ZINGER et al., 2011) , (2) as comunidades microbianas do sedimento desempenham um papel crítico como recicladores de nutrientes para o ecossistema aquático (HUANG et al., 2015); e (3) os sedimentos de sistemas fluviais pouco profundos são altamente sensíveis a mudanças nas condições ambientais (WANG et al., 2018).

A área de estudo: Sub-região SF1 (ou Sub-região Canastra) da bacia do Rio São Francisco

A Bacia Hidrográfica do Rio São Francisco é considerada uma das 12 Regiões Hidrográficas brasileiras definidas pelo Conselho Nacional de Recursos Hídricos – CNRH, por meio da Resolução nº 32, de 15 de outubro de 2003. Ela possui 639.219 km² de área de drenagem (cerca de 8% do País), que abrange 521 municípios em sete unidades da Federação (Bahia, Minas Gerais, Pernambuco, Alagoas, Sergipe, Goiás, e Distrito Federal), tendo como uma das características marcantes o uso múltiplo de suas águas para as mais diversas atividades: abastecimento populacional, irrigação, geração de energia, navegação, saneamento, pesca e aquicultura, atividades turísticas e de lazer (LOPES et al., 2002).

Devido à sua extensão e aos diferentes ambientes que percorre, essa Região Hidrográfica está dividida em quatro regiões fisiográficas, que constituem as Sub unidades na base do CNRH: São Francisco Alto (desde as suas nascentes até a região de Pirapora-MG) , São Francisco Médio (da região de Pirapora até o reservatório de Remanso –BA) , São Francisco Sub-Médio (entre os reservatórios de Remanso e Paulo Afonso –BA) e São Francisco Baixo (de Paulo Afonso até sua foz) (MMA, 2006).

Dentre as quatro regiões, a do Alto São Francisco destaca-se pelo alto grau de impacto antrópico que, historicamente, vem sofrendo. Essa região apresenta uma taxa de urbanização de 93%, que inclui a região metropolitana de Belo Horizonte, que apresenta como principais atividades econômicas a indústria, a mineração, a pecuária e a geração de energia (MMA, 2006).

Esse alto grau de impacto antrópico tem se refletido nos níveis de poluição que o Rio São Francisco e seus afluentes sofrem nessa região, visto que, em 2002, o Instituto Mineiro de Gestão das Águas identificou as principais fontes de poluição para região do Alto São Francisco: indústria alimentícia, extração de calcário, agricultura, pecuária (em especial a suinocultura), o esgotamento doméstico além de existir registros de desmatamento ilegal de matas ciliares para produção de carvão vegetal. Todo este cenário reflete, também, na saúde dos ecossistemas aquáticos da bacia do São Francisco, de forma que, desde a Sub-bacia São Francisco 1, que abriga as nascentes, o rio e seus afluentes sofre degradações com sérios impactos sobre as águas. A maioria dos pequenos povoados da zona rural que margeiam a bacia nos rios. Ademais há registros de despejos de garimpos, mineradoras e indústrias que contaminam as águas com metais pesados. Isso porque, desde a década de 70, houve uma intensa ocupação agrícola na região, com áreas destinadas tanto à agricultura quanto às pastagens que, consequentemente, levam ao lançamento de fertilizantes e agrotóxicos nos corpos d’água (MMA, 2006).

A região do Alto São Francisco possui uma área de 100 mil km², que politicamente, foi dividida em 10 Unidades de Planejamento e Gestão de Recursos Hídricos (UPGRH). Dentre essas unidades, a UPGRH denominada Bacia Hidrográfica do Alto São Francisco (SF1) ou “Unidade Canastra” com uma área total de 14.203 km² (passando por 29 municípios do centro-oeste mineiro) abrange as nascentes do Rio São Francisco e seus primeiros principais afluentes: na margem direita, o ribeirão Sujo, o ribeirão dos Patos, o rio São Miguel, o rio São Domingos, o ribeirão da Usina e o ribeirão Santa Luzia, já pela margem esquerda o rio Samburá, o ribeirão Arrudas, o rio Bambuí, o rio São Mateus e o rio Veados (IGAM, 2017). De maneira similar às demais UPGRHs do Alto São Francisco, a Unidade SF1 sofre diversos tipos de pressões antrópicas que impactam diretamente na qualidade ambiental dos ecossistemas aquáticos: esgotamento sanitário de pequenas áreas urbanas e propriedades rurais que margeiam seus afluentes, contaminação por atividades mineradoras, contaminação por fertilizantes defensivos agrícolas oriundos de áreas cultiváveis e efluentes de produção pecuária, como mostrado pelo histórico dos relatórios de monitoramento de qualidade das águas da Unidade SF1 de 2007 a 2018, cujos resultados apontaram os seguintes parâmetros fora dos padrões estabelecidos, em algumas amostragens como manganês, sólidos em suspensão totais, turbidez, Escherichia coli, sulfeto, demanda bioquímica de oxigênio, fósforo total, cianeto livre, fenóis totais, chumbo total e zinco total. Esses parâmetros, segundo a ANA e o IGAM, refletem impactos de atividades antrópicas de indústrias de alimentos, extração de calcário, agricultura, pecuária e esgotamento doméstico (IGAM, 2002, ANA, 2003).

2. OBJETIVOS

2.1. OBJETIVO GERAL

Analisar, de forma espacial e temporal, a comunidade microbiana do sedimento do leito do rio São Francisco na região da cabeceira do rio (Alto São Francisco) e a elaboração de uma nova metodologia de análise de água que leva, em consideração, a composição e a diversidade da comunidade microbiana, a fim de complementar as análises físico-químicas clássicas de água.

2.2. OBJETIVOS ESPECÍFICOS

1. Estimar a diversidade e composição das comunidades bacterianas dos pontos amostrais selecionados como área de controle (sem impacto) e áreas impactadas (ecoturismo, ocupação urbana e atividades agrícolas);
2. Verificar a existência de associação entre os parâmetros de diversidade (α-diversidade e β-diversidade) e os tipos impactos;
3. Verificar a presença de grupos bacterianos dominantes relacionados a um tipo específico de impacto;
4. Verificar se existem diferenças significativas nos parâmetros de diversidade (α-diversidade e β-diversidade) das comunidades bacterianas encontradas entre as estações seca e chuvosa;
5. Elaborar uma nova metodologia de análise de água com os dados coletados e bioindicadores microbianos.

Referências

ABIA, Akebe Luther King et al. Quantitative microbial risk assessment (QMRA) shows increased public health risk associated with exposure to river water under conditions of riverbed sediment resuspension. **Science of the Total Environment**, v. 566, p. 1143-1151, 2016.

ALBA-TERCEDOR, J. 1996. Macroinvertebrados acuaticos y calidad de lãs águas de los rios. Actas del IV Simposio del agua en Andalucia (SIAGA), v. p. 203-213.

BARBOUR, Michael T. et al**. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish**. Washington, DC: US Environmental Protection Agency, Office of Water, 1999.

BARELLA, W.; PETRERE JR., M.; SMITH, W. S.; MONTAG, L. F. A. As relações entre as matas ciliares, os rios e os peixes. In: RODRIGUES, R. R. e LEITÃO FILHO, H. F. Matas ciliares: Conservação e recuperação. EDUSP, 2ª ed., São Paulo, p.187-207, 2001.

BOLYEN, Evan et al. **QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science**. PeerJ Preprints, 2018.

BUSS, Daniel Forsin; BAPTISTA, Darcílio Fernandes; NESSIMIAN, Jorge Luiz. Bases conceituais para a aplicação de biomonitoramento em programas de avaliação da qualidade da água de rios. **Cadernos de Saúde Pública**, v. 19, p. 465-473, 2003.

CALLISTO, Marcos et al. Macroinvertebrados bentônicos como ferramenta para avaliar a saúde de riachos. **Revista Brasileira de Recursos Hídricos**, v. 6, n. 1, p. 71-82, 2001.

CHAPMAN, Toni A. et al. Comparison of virulence gene profiles of Escherichia coli strains isolated from healthy and diarrheic swine. **Appl. Environ. Microbiol.**, v. 72, n. 7, p. 4782-4795, 2006.

CHEN, Yong et al. Long-term and high-concentration heavy-metal contamination strongly influences the microbiome and functional genes in Yellow River sediments**. Science of the Total Environment**, v. 637, p. 1400-1412, 2018.

CONAMA (Conselho Nacional do Meio Ambiente). 2005. Ministério do Meio Ambiente. Resolução n° 357, de 17 de março de 2005.

GIBBONS, Sean M. et al. Human and environmental impacts on river sediment microbial communities. **PloS one**, v. 9, n. 5, p. e97435, 2014.

GILBERT, Jack A.; JANSSON, Janet K.; KNIGHT, Rob. The Earth Microbiome project: successes and aspirations. **BMC biology**, v. 12, n. 1, p. 69, 2014.

HORBERRY, John. Status and application of environmental impact assessment for development. In: **International Seminar on Environmental Impact Assessment for Development: DSE/UNEP**. Conservation for Development Centre, 1984.

HUANG, Wei et al. Comparison among the microbial communities in the lake, lake wetland, and estuary sediments of a plain river network. **MicrobiologyOpen**, v. 8, n. 2, p. e00644, 2019.

HUANG, Wei et al. Effect of ionic strength on phosphorus sorption in different sediments from a eutrophic plateau lake. RSC Advances, v. 5, n. 97, p. 79607-79615, 2015.

INSTITUTO MINEIRO DE GESTÃO DAS ÁGUAS. **Monitoramento da Qualidade das Águas Superficiais no Estado de Minas Gerais - Relatório Trimestral - 4° Trimestre de 2017**. Belo Horizonte: Igam, 2017.

INSTITUTO MINEIRO DE GESTÃO DE ÁGUAS. **Portal dos Comitês**. Disponível em: < http://comites.igam.mg.gov.br/comites-estaduais-mg/sf1-cbh-alto-sao-francisco>. Acesso em 04 de jun. de 2019.

INSTITUTO MINEIRO DE GESTÃO DE ÁGUAS. **Portal InfoHidro**. Disponível em: . Acesso em 14 de ago. de 2017.

JONES, Chance E. et al. Persistence of bacterial pathogens, antibiotic resistance genes, and enterococci in tidal creek tributaries. **Environmental pollution**, v. 240, p. 875-883, 2018.

LOPES, J. E. G.; BARROS, M. T. L.; BRANDÃO, J. L. B. Projeto de gerenciamento integrado das atividades desenvolvidas em terra na bacia do São Francisco subprojeto

4.4: determinação de subsídios para procedimentos operacionais dos principais reservatórios da bacia do São Francisco. Brasília: ANA/GEF/PNUMA/OEA, 2002.

LÓPEZ-ARCHILLA, Ana Isabel; MARÍN, Il; AMILS, R. Microbial community composition and ecology of an acidic aquatic environment: the Tinto River, Spain. **Microbial ecology**, v. 41, n. 1, p. 20-35, 2001.

MASTERS, Nicole et al. Escherichia coli virulence genes profile of surface waters as an indicator of water quality. **Water research**, v. 45, n. 19, p. 6321-6333, 2011.

MINISTÉRIO DO MEIO AMBIENTE. 2006. Caderno da Região Hidrográfica do São Francisco. Ministério do Meio Ambiente, Secretaria de Recursos Hídricos. Brasília: MMA, 2006. 148 p.

QUAST, Christian et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. **Nucleic acids research**, v. 41, n. D1, p. D590-D596, 2013.

RANJBAR, Reza; KHAMESIPOUR, Faham; HOSSEINI, Sorayya. Prevalence and distribution of E. coli and its virulence genes from different water sources in Alborz province, Iran. **Journal of Biochemical Technology**, v. 7, n. 2, p. 1116-1122, 2017.

RAPPÉ, Michael S.; GIOVANNONI, Stephen J. The uncultured microbial majority**.** **Annual Reviews in Microbiology**, v. 57, n. 1, p. 369-394, 2003.

RASOOL, Atta; XIAO, Tangfu. Response of microbial communities to elevated thallium contamination in river sediments. **Geomicrobiology journal**, v. 35, n. 10, p. 854-868, 2018.

ROSENBERG, D.; RESH, V. Freshwater biomonitoring and macroinvertebrates 1993. 1993.

RUDI, K. et al. Use of multivariate statistics for 16S rRNA gene analysis of microbial communities. **International journal of food microbiology**, v. 120, n. 1, p. 95-99, 2007.

SCHULER, Azeneth Eufrausino et al. Serviços ambientais hídricos. **Embrapa Solos-Capítulo em livro técnico (INFOTECA-E)**, 2017.

SHAPIRO, James A. et al. (Ed.). Bacteria as multicellular organisms . Oxford, UK:: Oxford University Press, 1997

SOARES, Juliane Alessandra Cavalieri et al. Efeitos antrópicos sobre a qualidade dos sedimentos do rio Toledo (PR, Brasil): análises físico-químicas e ecotoxicológicas. 2017.

STACKEBRANDT, EaBMG; GOEBEL, Brett M. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. **International journal of systematic and evolutionary microbiology**, v. 44, n. 4, p. 846-849, 1994.

SUTTI, Bruno Otero et al. Nitrogênio e matéria orgânica em dois rios com diferentes graus de impactos no sistema estuarino de Santos (São Paulo, Brasil). Brazilian Journal of Aquatic Science and Technology, v. 20, n. 1, p. 93-104, 2016.

TAO, Ke et al. Patterns of bacterial and archaeal communities in sediments in response to dam construction and sewage discharge in Lhasa River. **Ecotoxicology and environmental safety**, v. 178, p. 195-201, 2019.

TRINGE, Susannah G.; HUGENHOLTZ, Philip. A renaissance for the pioneering 16S rRNA gene. **Current opinion in microbiology**, v. 11, n. 5, p. 442-446, 2008.

WANG, Lan et al. Shift in the microbial community composition of surface water and sediment along an urban river. **Science of the Total Environment**, v. 627, p. 600-612, 2018.

WANG, Peng et al. Characteristics of aquatic bacterial community and the influencing factors in an urban river. **Science of The Total Environment**, v. 569, p. 382-389, 2016.

XIE, Yuwei et al. Environmental DNA metabarcoding reveals primary chemical contaminants in freshwater sediments from different land-use types. **Chemosphere**, v. 172, p. 201-209, 2017.

ZAGATTO, P. A.; BERTOLETTI, E. Ecotoxicologia aquática. **Princípios e Aplicações. Rima Editora, São Carlos. 478p**, 2006.

ZAMBONI, A.J. & ABESSA, D.M.S. 2002. Tríade da Qualidade de Sedimentos. Cap.20. p. 233-243. In: Métodos em Ecotoxicologia Marinha: Aplicações no Brasil. Nascimento, I.A., SOUSA, E.C.P. & NIPPER, M. (Eds.). São Paulo: Editora Artes Gráficas. 262p.

ZAMORA-MUNOZ, C.; ALBA-TERCEDOR, J. Bioassessment of organically polluted Spanish rivers, using a biotic index and multivariate methods. **Journal of the North American Benthological Society**, v. 15, n. 3, p. 332-352, 1996.

ZINGER, Lucie et al. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. **PloS one**, v. 6, n. 9, p. e24570, 2011.

3. CAPÍTULO 1

Manuscrito submetido à revista Environmental Microbiology and Environmental Microbiology Reports

Using sediment microbial communities to assess the buffer zone effectiveness of a freshwater protected area placed at an intensely impacted ecoregion from Brazilian Savanna

Marcos de Paula Jr., Thiago Augusto da Costa e Silva, Amanda Soriano Araújo Barezani, Gustavo Augusto Lacorte

Molecular Biology Lab, Department of Science and Languages, Instituto Federal de Minas Gerais – Bambuí Campus, Bambuí, Minas Gerais State, Brazil;

Corresponding author: Gustavo Augusto Lacorte; e-mail: gustavo.lacorte@ifmg.edu.br;

ORCID iD: 0000-0002-6866-348X; Phone number: +55 37 3431-5600.

Summary

Here, we applied the taxonomic and functional structure of sediment bacterial communities as a diagnosing tool to assess the effectiveness of Serra da Canastra National Park buffer zone in reducing the impacts of surrounding land-use activities on freshwater ecosystems from São Francisco River headwaters, a protected area from one of the highest impaired ecoregions from Brazilian Savanna. Our findings showed that sediment communities from Serra da Canastra NP buffer zone presented diversity and composition patterns, for both taxonomic and functional dimensions, within the expected for an adequate buffer zone: the diversity at buffer zone communities was similar to the core of protected area as well the variety at buffer zone presented higher diversity levels than the bacterial diversity found at communities from non-protected areas. We still verified a clear similarity at both taxonomic and functional composition between populations from the core protected area and buffer zone as well that there was a significant turnover from buffer zone to non-protected areas sediment communities. Our findings also support that bacterial community assessments from river bed sediments constitutes a useful tool for

diagnosis and monitoring of ecosystem health in areas of vulnerable freshwater environments and should be incorporated into water quality programs.

Introduction

Freshwater ecosystems (in rivers, wetlands, and lakes) support vast biodiversity and provide environmental goods and services of critical importance to human populations everywhere (Collen et al., 2014; Saunders et al., 2012; Carpenter et al., 2009). In the last decades, freshwater ecosystems have been severely impaired by human activities such as flow alteration, overharvest, contamination, invasive species, direct habitat alteration, and climate change (Abell et al., 2007). Brazil has a large number of freshwater ecosystems, many of which have high biodiversity and endemism (Azevedo-Santos et al., 2017; Agostinho et al., 2005). However, many of these ecosystems have been disrupted by human activities, and the adoption of conservation has become urgent. The creation of protected areas (PAs) has been highlighted as an essential and recurrent strategy for the preservation of the threatened environment in Brazil (Casarim et al., 2020; Azevedo-Santos et al., 2018). However, in most populous Brazilian regions, PAs are created in mosaics of habitats, and their existence results in only a smaller area under protection within larger, unprotected ecosystems (Rodrigues et al., 2004; Powell et al., 2000). Because of this scenario, the National Environment Council (CONAMA, Portuguese acronym) defined a 10km buffer zone around protected areas, in which human activities are subjected to specific rules and restrictions in an attempt to minimize negative impacts on land-use activities on protected areas (Gonçalves et al., 2009). Briefly, buffer zones around parks/reserves are perimetral areas designed to maintain ecological integrity and to ensure community participation in biodiversity conservation (Lamichhaane et al., 2019). The ecological functions of buffer zones include: (i) the enhanced preservation of species with high mobility or ecological relevance; (ii) their functioning as physical barriers to human encroachment; (iii) reduction of the edge effect; and (iv) enhancement of the environmental services provided by the protected area (Shafer, 1999; Sayer, 1991). Although the ecological roles of buffer zones are well established, few studies have investigated the effectiveness of buffer zones in terms of their ecological buffering functions (Astri et al., 2020).

Recent pieces of evidence have revealed that during the intensification of land-use process contaminants are absorbed by the fine particles of sediments and interact with local microbial communities, causing long-term impacts on biological organization of the communities and leading to changes in both diversity and composition of the microbial communities (Saxena et al., 2015; Xie et al;. 2016; Xie et al., 2017; Huang et al., 2019). Briefly, the use of microorganisms as bioindicators for aquatic ecosystems is based on the premise that microbial diversity is a key factor affecting the biological quality of ecosystems due to its role in nutrients recycling, the degradation of pollutants, and in the stability of ecosystems. Thus changes on taxonomic diversity and composition of microbial communities is a suitable indicator of perturbations within freshwater ecosystems (Zhang et al., 2019; Yang et al., 2019; Adhikari et al., 2019; Liu et al., 2018; Wang et al., 2018; Huang et al., 2017).

The Cerrado biome is considered one of the world's hotspots for biodiversity conservation because of its richness and high levels of endemism (Silva and Bates, 2002). However, Cerrado biome lands also host some of the most intensive agricultural and livestock activities in the world (Rada, 2013). Recently, Cerrado biome lands were classified in 19 ecoregions based on the range of environmental factors and human activities to produce an integrated spatial framework for landscape management (Sano et al., 2019). One of the three most endangered Cerrado ecoregions by agricultural practices is Upper São Francisco, which consists of the Cerrado land areas included at first the São Francisco River sub-basin, named Upper São Francisco sub-basin (Figure 1) (Sano et al., 2019). Although there is a high demand for protected areas, just over 3% Upper São Francisco ecoregion is composed by protected areas (PAs), and Serra da Canastra National Park is one of the key protected areas of this ecoregion because this National Park was created to preserve the São Francisco River headwaters from surrounding activities (ICM-BIO, 2020). This protected area is managed with regulations and restrictions for land-use at its Buffer Zone in an attempt to reduce and mitigate human impacts through orderly human occupation and sustainable development (Casarim et al., 2020).

In this brief ecological case study, we applied Illumina amplicon sequencing of the 16S rRNA V4 region to estimate the diversity and composition of bacterial communities and use the variation in the structure of these bacterial communities as bioindicators of freshwater ecosystem health to assess the effectiveness of Serra da Canastra NP Buffer zone in reducing the impacts of surrounding land-use activities on freshwater ecosystems from São Francisco River headwaters, a protected area from one of the highest impaired ecoregions from Brazilian Savanna. Here, we hypothesize that whether Serra da Canastra NP Buffer zone is effective in its key role in reducing the impacts of surrounding anthropogenic activities on freshwater ecosystems, the bacterial diversity of São Francisco River sediment sampled at the Buffer Zone should be higher than the bacterial diversity found in bacterial communities from non-protected areas as well the composition of bacterial communities from Buffer Zone samples should be more similar to that found at PA core sediment samples than bacterial communities from non-protected areas.

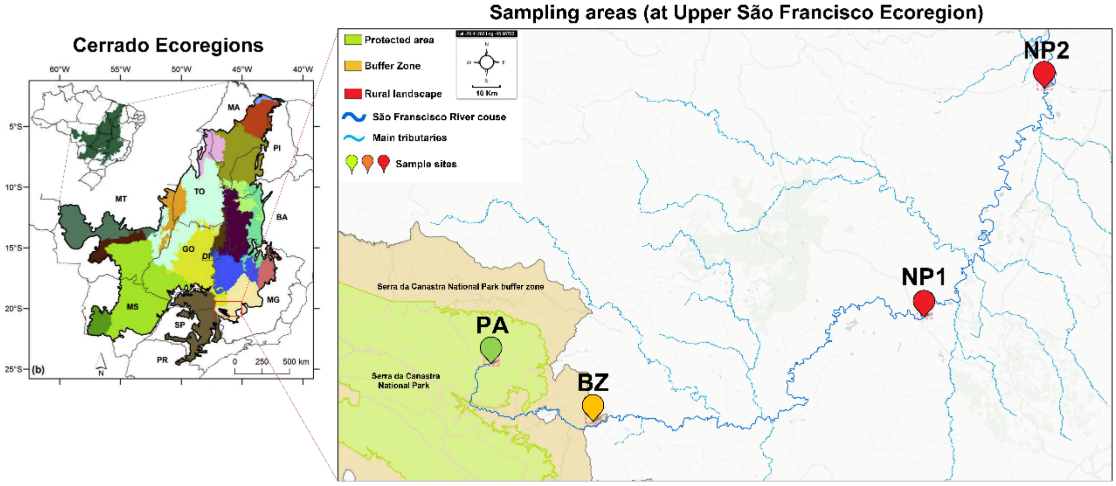


Figure 1. Study sites located at Serra da Canastra National Park (PA), Serra da Canastra buffer zone (BZ) and non-protected areas from Upper São Francisco River Cerrado Ecoregion.

Results

The high-throughput sequencing generated a total of 211,884 sequence reads (ranging from 13,233 to 21,557), with the DADA2 approach recovering 1,919 ASVs. The rarefaction curves indicated the saturation stage for all samples, suggesting that sequencing depth was sufficient to cover most of the taxonomic diversity for all accessed microbial communities (Figure S1). The classic Shannon diversity index calculated for each group of sediment samples revealed that the bacterial diversity at Protected Area

(PA), which corresponds to the São Francisco River spring waters, is lower than the bacterial diversity found at Buffer Zone (BZ) and that Shannon diversity at Non-protected areas (NPs) decreased significantly concerning BZ samples (Figure 2, Table S1). However, when we considered the phylogenetic diversity index, which takes into account the richness of evolutionary branches present in accessed communities, the phylogenetic diversity at Protected Area and Buffer Zone is similar and also higher than the phylogenetic diversity found at non-protected areas. We could not observe significant differences in both diversity indices when comparing the two sampled Non-protected areas (Wilcoxon test p-values = 0.06 and 0.27 for Shannon and Faith diversity indices, respectively).

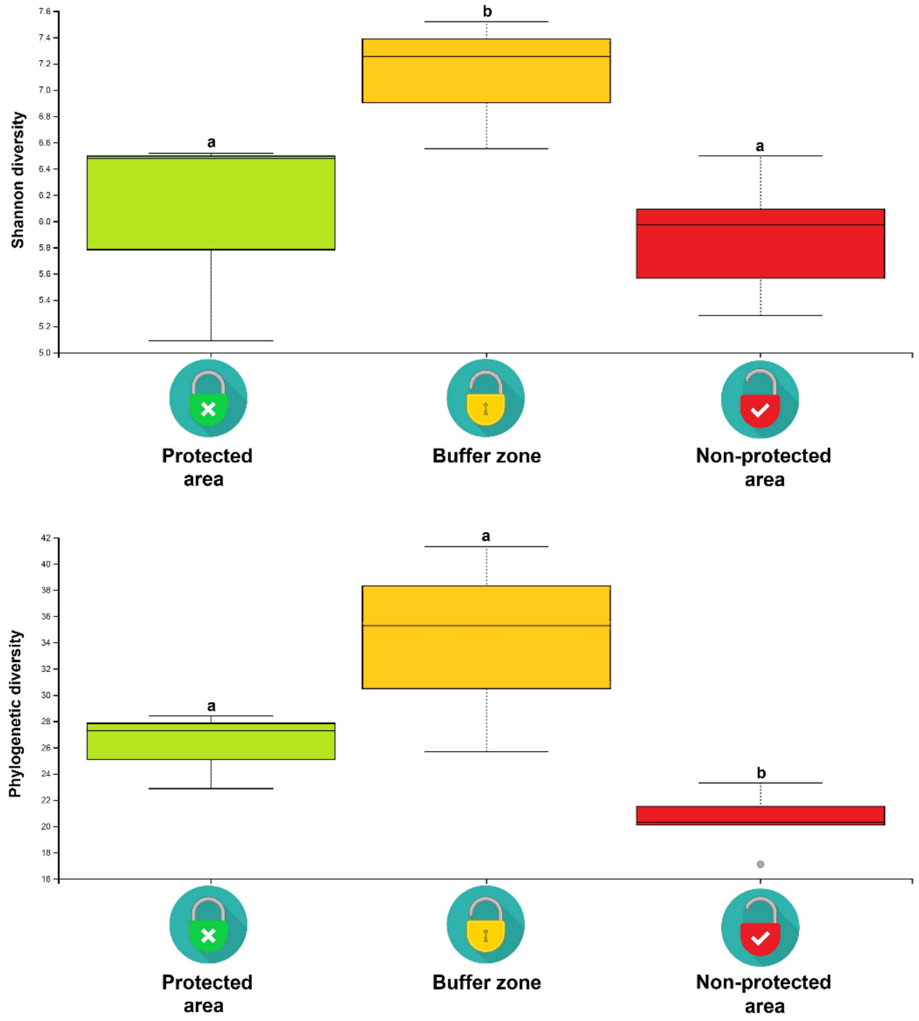


Figure 2. Comparison of Shannon and Phylogenetic diversity indices for sediment bacterial communities from Protected (PA), Buffer Zone (BZ) and Non-protected sampled areas.

The plots of Principal Coordinate Analysis (PCoA), presented similar patterns of bacterial community composition (beta-diversity) for all applied metrics, with the two principal axes explaining 65,2% (Bray-Curtis), 43,4% (Unifrac Unweighted) and 68,4% (Unifrac Weighted) of the total variation in the microbial community structure (Figure 3). Bacterial communities samples were clustered in the ordination plot in two groups. Bacterial communities composed one group from PA and BZ samples, while communities from NP areas composed the other group. These two clusters were supported by PERMANOVA tests for all three metrics adopted to calculate the dissimilarity matrices among sediment samples (Figure S2, Table S2). In addition, we could not verify significant differences on taxonomic composition among sediment samples from the two NP areas (Bray-Curtis $P_{PERM} = 0.10$, Unifrac Unweighted $P_{PERM} =$ 0.09, Unifrac Weighted $P_{PERM} = 0.11$).

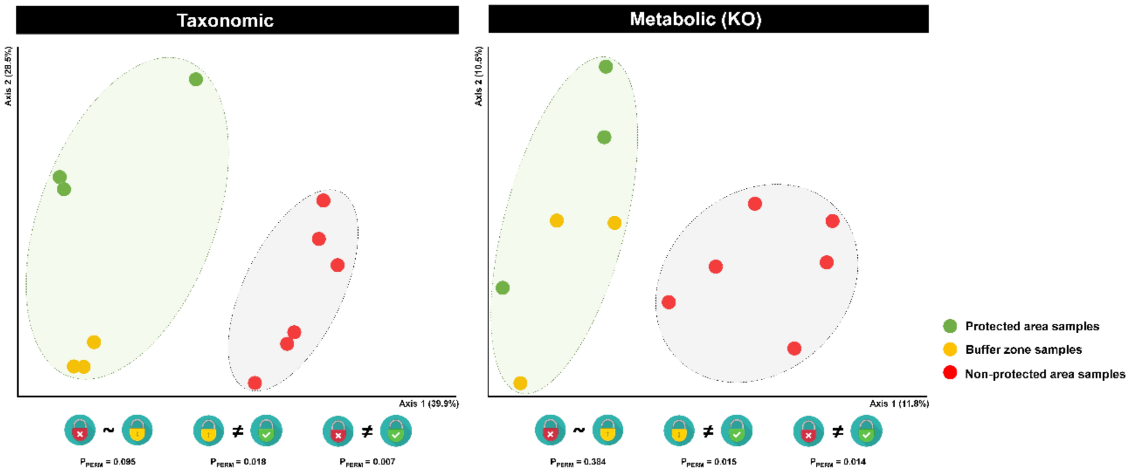


Figure 3. Principal coordinates analysis (PCoA) plots of Unifrac Weighted (left plot) and Bray-Curtis (right plot) for taxonomic and KO dissimilarity matrices, respectively.

Based on the trained classifier implemented in QIIME2, the dominant phyla (with relative abundance > 1% in all samples) were Proteobacteria (mainly Gammaproteobacteria), Firmicutes, Verrucomicrobia, Acidobacteria, Cyanobacteria, Actinobacteria, Bacterioidetes, Planctomycetes and Cloriflexi (Figure 4). While Proteobacteria, Firmicutes, Planctomycetes, and Bacterioidetes phyla were ubiquitous, the distribution of the other dominant bacterial species varied among the groups: Acidobacteria was more frequent at PA samples; Actinobacteria and Cloriflexi species were more abundant at NP samples, and Verrucomicrobia and Cyanobacteria were less frequent at NP areas. At the general level, we found 37 dominant bacterial genera in the sediment samples (relative abundance> 3% at least one sediment sample), and most of them presented a typical spatial distribution that could be classified into bright patterns (Figure 5).

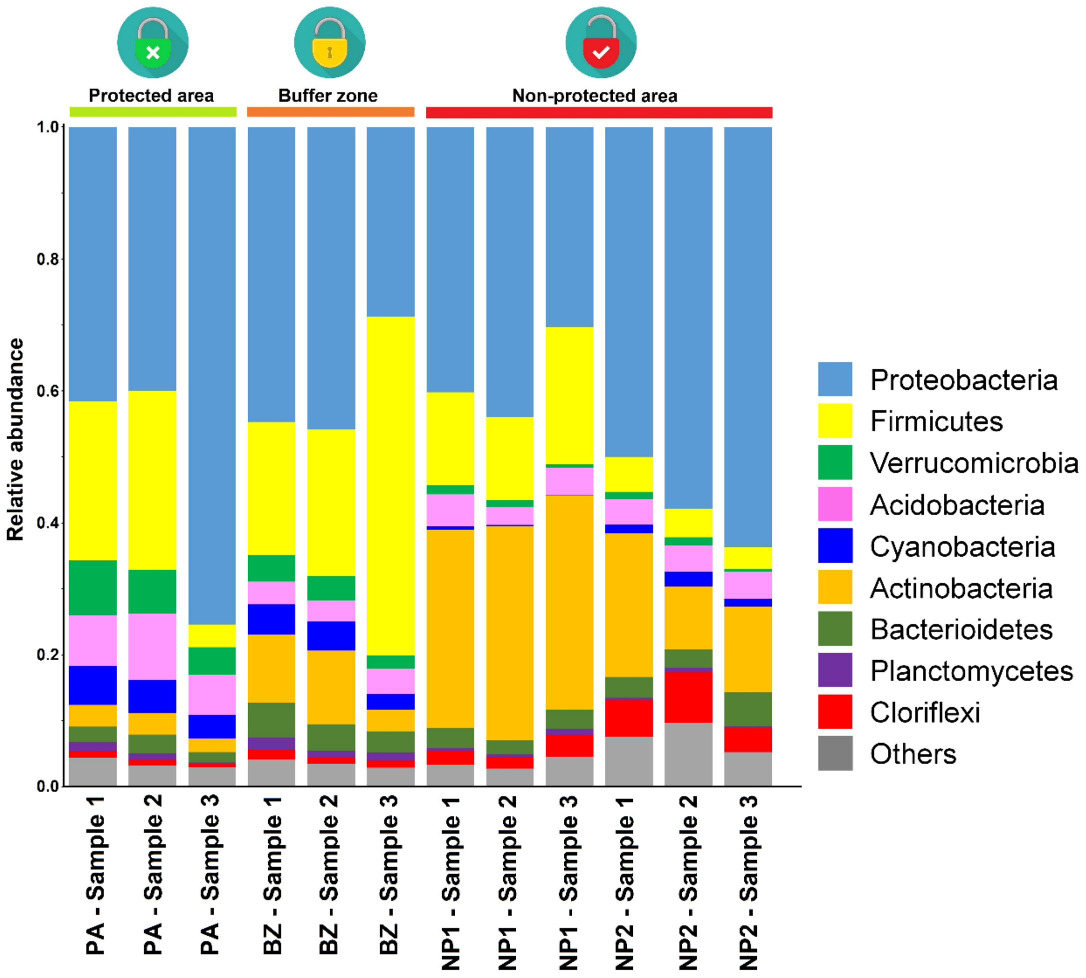


Figure 4. Relative abundances of dominant bacterial phyla in sediment samples.

Five dominant genera that showed greater abundance in PA samples (*Paraburkholderia, Streptococcus, Acidothermus, Reseiarcus,* and *Pseudomonas*), while four genera (*Anaeromyxobacter*, *Crenothrix*, *Rombustia*, and *three Clostridium* lineages) were practically absent at PA sediment samples. We also found ten dominant genera that were more abundant at BZ samples and other 11 dominant genera whose distribution was associated with NP samples.

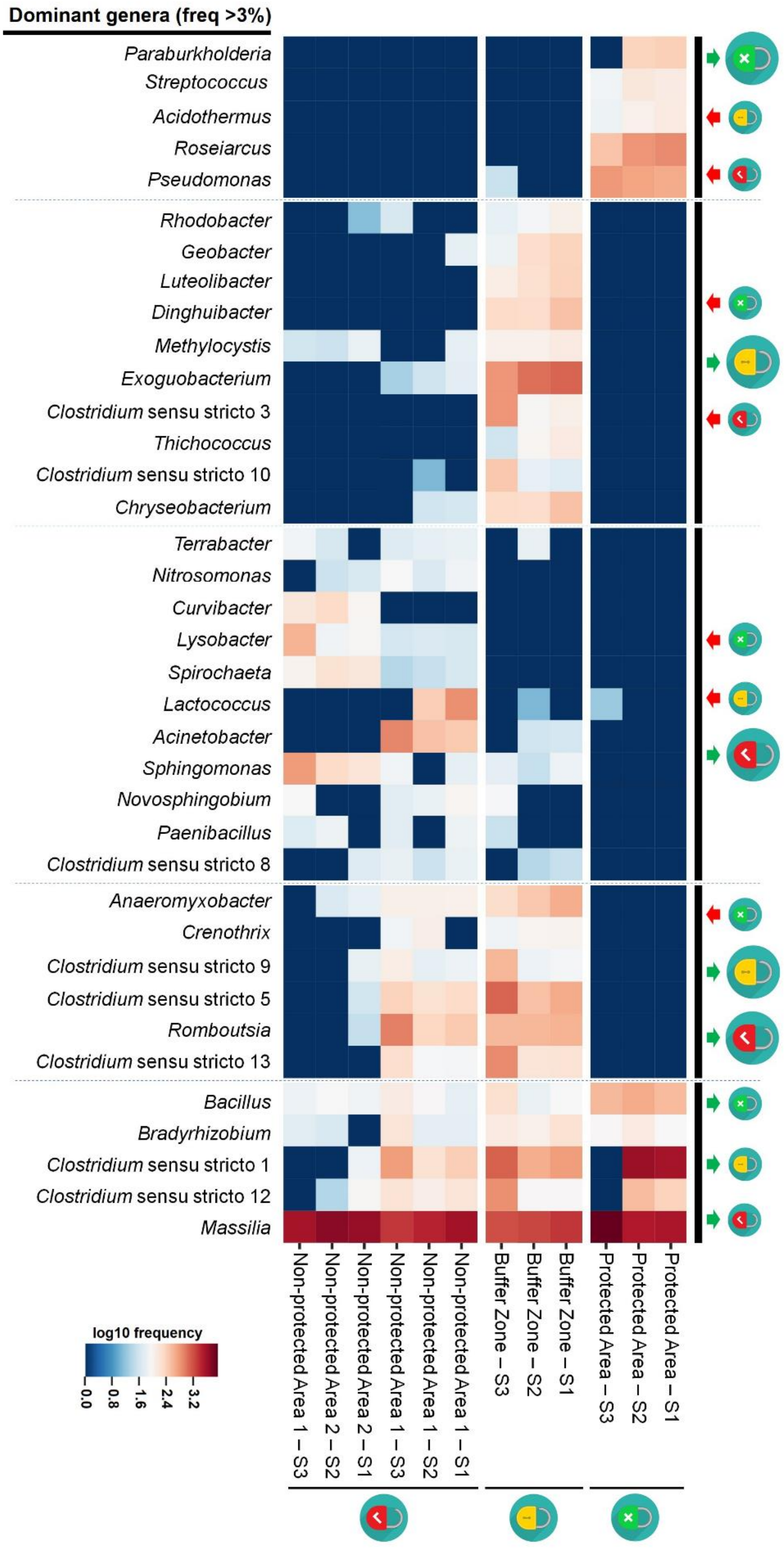


Figure 5. Heatmap analysis of dominant genera found at sediment samples.

Our PICRUSt2 prediction approach applied for our sediment samples revealed that though we found significant differences in taxonomic diversity between BZ and NP sediment samples, the overall KO diversity levels among PA, BZ, and NP samples were similar (Table 1). However, when we structured the KO diversity at secondary levels of KEGG Pathway Hierarchy (KEGG Level 2), we could verify that diversity of metabolic functions (KOs) related to carbohydrates, Cofactors, Lipid metabolism was significantly lower for NP samples. In contrast, KO diversity related to Xenobiotics degradation was higher for NP samples (Table 1). Similarly to taxonomic composition patterns, our comparative analysis of KO composition from sediment bacterial communities revealed that the KO composition of bacterial communities from PA and BZ samples was similar. Still, the KO composition of both types of the environment was significantly different from KO composition patterns found for NP samples (Figure 3). Considering the analysis of KO composition structured by secondary levels of KEGG Pathway Hierarchy, only six of the 20 categories did not show the same variation pattern found at overall analysis: Glycan metabolism, Metabolism of terpenoids and polyketides, Transcription, Translation, Membrane transport, and Cell growth and death.

Table 1. Significance values of comparative tests among KO diversity and KO composition estimated for the sample groups structured by Secondary level of KEGG Pathway Hierarchy

Pairwise comparison		Overall	Amino acid metabolism	Carbohydrate metabolism	Cell community	Cell cycle	Cell motility	Cofactor and vitamin metabolism	Drug resistance	Energy metabolism	Folding. sorting and degradation	Glycan metabolism	Lipid metabolism	Membrane transport	Nucleotide metabolism	Replication and repair	Secondary metabolites biosynthesis	Signal transduction	Terpenoid metabolism	Transcription	Translation	Xenobiotics degradation
KO Diversity* (Shannon diversity)																						
BZ	PA	0.51	0,28	0,83	0,13	0,83	0,51	**0,05**	0,51	0,83	0,51	0,28	0,28	0,83	0,83	0,83	0,05	0,28	0,05	0,13	0,05	0,51
	NP	0.12	0,30	**0,02**	**0,02**	0,44	0,80	**0,02**	0,20	0,80	0,80	**0,02**	0,80	0,44	0,80	1,0	0,12	0,44	0,07	1,0	0,30	1,0
PA	NP	0.20	0,07	0,07	**0,04**	0,61	0,44	**0,02**	0,20	0,80	0,80	**0,02**	0,20	0,80	0,61	0,80	0,30	**0,02**	**0,02**	0,20	0,80	0,61
KO Composition (Bray-Curtis)**																						
BZ	PA	0.39	0,44	0,70	0,80	0,22	0,40	0,31	0,22	0,29	0,19	0,10	0,31	0,22	0,48	0,70	0,71	0,22	0,10	0,23	0,30	0,89
	NP	**0.01**	**0,02**	**0,02**	**0,02**	**0,04**	0,16	**0,02**	**0,04**	**0,06**	0,11	**0,03**	0,17	0,82	**0,04**	**0,01**	0,87	**0,03**	**0,02**	0,07	**0,03**	**0,01**
PA	NP	**0.01**	**0,01**	**0,01**	**0,01**	**0,03**	**0,02**	**0,01**	**0,02**	0,41	**0,02**	**0,01**	0,07	**0,01**	**0,01**	**0,01**	**0,05**	**0,01**	**0,02**	**0,02**	**0,01**	**0,02**

*Kruskal-Wallis test p-values for estimated Shannon diversity vectors. ** PERMANOVA test p-values for estimated Bray-Curtis matrices. BZ = Buffer zone sediment samples; NP = Non-protected areas sediment samples; PA = Protected Area sediment samples.

Discussion

Here, to assess whether the Serra da Canastra NP buffer zone is effective in its crucial role of reducing human activities impacts at the surrounding of freshwater environments from the São Francisco River headwaters, we assume that two conditions regarding the bacterial diversity of sediment communities should have complied: first, the diversity at Buffer Zone communities should be similar to the core of protected area; and second, the diversity at BZ should present higher diversity levels than the bacterial diversity found at NP communities since these areas are under the impact of agricultural activities. Our estimates of taxonomic diversity revealed these two conditions were met. First, we found that the bacterial diversity levels between BZ and PA sediment communities were similar when we considered phylogenetic diversity. When we consider Shannon diversity, BZ diversity was higher than PA. This increase at taxonomic diversity from PA to BZ communities was also verified by Casarim et al. (2020) when comparing the fish diversity. It could be explained by the fact that the PA area corresponds to the São Francisco River springs, composed by a high elevated area with steep slopes, low temperature, and rocky bottoms, that contribute to less diverse aquatic ecosystems (Hansen and Defries, 2007). Besides, our results for both Shannon and Phylogenetic diversity indices showed that taxonomic diversity is significantly less at NP communities. Since 1997, water quality in the Upper San Francisco River sub-basin has been monitored by state water management bureau (IGAM, Portuguese acronym) and recent studies that analyzed the historical series of this monitoring revealed that in areas intensely impacted by land-use, the water quality parameters of the São Francisco River, including the locations sampled here, have altered significantly, reaching quality levels below the minimum parameters established by local legislation (Dantas et al., 2020; Costa et al .., 2007).

Another parameter that we adopted to assess the effectiveness of the Serra da Canastra NP buffer zone was the maintenance of bacterial taxa composition at sediment communities from PA to BZ. Our results showed that there was a clear similarity at taxonomic composition between PA and BZ communities as well it was verified that there was a significant turnover at bacterial sediment communities from BZ to NP sampled areas. Typical inputs derived from farming practices (fertilizers, pesticides, and livestock residues) that occur at NP sampled areas could be creating selective local pressures capable of becoming a bacterial composition of NP sediment communities different from the other sampled areas. Several studies that have shown that higher concentrations of organic and inorganic nutrients associated with agricultural activities may alter microbial communities and their functions on river stream and sediment (Marti and Balcázar, 2014;

Lu and Lu, 2014; Drury et al., 2013; Kalia and Gosal 2011; Wakelin et al., 2008). Considering the dominant bacterial phyla, we found that some phyla presented abundance associated with occurrence area, especially if the area presented land-use restrictions, in which Actinobacteria and Chloriflexi phyla occurred in greater abundance at NP areas while Verrucomicrobia and Cyanobacteria phyla were less frequent at NP areas. Several studies also found similar results, in which some of them presented correlation of these phyla with an increase at nitrogenous and phosphorous nutrients, whose origin was related to agricultural or urban inputs runoff (Abia et al., 2018; Wu et al., 2017 ; Xie et al., 2016; Hu et al., 2014). In addition, the heatmap generated from the abundance of dominant bacterial genera (freq.> 3%) revealed that some genera presented their abundance associated with the sampling area, such as *Roseiarcus* and *Paraburkholderia* genera for PA area, *Exoguobacterium* and *Luteolibacter* genera for BZ area as well *Spirochaeta* and *Sphingomonas* genera for NP areas. The differential distribution of these genera is likely related to the different environmental conditions of riverbed sediment from each sampled area, in which some bacterial species are favored due to their metabolic characteristics, such as *Sphingomonas* species which are specialized at aromatic compounds metabolism that are often found at impaired freshwater environments (Basta et al., 2005; Rosenberg et al., 2013). Here, we consider that the identification of ASVs with a high correlation with a specific freshwater environment (revealed by our differential abundance results) constitutes the first step for the validation of these bacterial taxa as ecological indicators. However, additional evidence of metabolic characteristics of these taxa, their ecological roles in each ecosystem as well the verification of the same patterns of variation in similar systems is required for the complete validation of these bacterial taxa as health indicators of freshwater environments (Zeglin, 2015; Bouchez et al., 2016).

In addition to taxonomic structure analyses of sediment communities (diversity and composition), we performed a functional analysis of the structure of the communities by prediction of metabolic functions (KOs) using the PiCRUSt2 approach. Our PiCRUSt2 results showed that the reduction at taxonomic diversity levels observed for NPs concerning BZ communities was not reflected at overall KO diversity, but only in specific metabolic categories that are sensitive to environmental disturbances such as carbohydrate and lipid metabolism, in which presented lower levels at NP communities.

Alternatively, we found that diversity of KOs related to xenobiotic metabolism was more significant in sediment communities from NP areas, which may be related to chemical input runoff events that are more intense at sampled NP areas (Costa et al., 2007). Considering that biological communities with greater taxonomic diversity tend to exhibit greater diversity of ecological functions and more possible ecological equivalents for essential functions, resulting in an increased resilience of the community (Kirchman, 2012; Sigee, 2005), our results of functional diversity for the BZ sediment communities also indicate that the Serra da Canastra NP buffer zone has effectively accomplished its role of contributing to the maintenance of the freshwater ecosystems from São Francisco River headwaters. On the other hand, our results also make an alert to the risk for freshwater ecosystems deterioration at accessed NP areas. In addition, we verified that the turnover at bacterial composition from BZ to NP communities was reflected at KO composition, which can represent significant changes at ecosystem services provided by these communities to maintain the local freshwater ecosystems since sediment bacterial communities are involved in ecosystem vital roles such as biomass production, biogeochemical cycles with assimilation and mineralization of chemical elements as well biodegradation and bioremediation of pollutants (Craft et al., 2002; Winter et al., 2007; Gibbons et al., 2014; Lliro et al., 2014; Sorokin et al., 2014).

Recent studies have shown that the Cerrado ecoregions are under different types and degrees of anthropic impact (mainly agricultural and livestock production) and the neglect in facing this environmental conflict has already led to noticeable consequences in the provision of ecosystem services by the Cerrado environments that are under the anthropic impact (Resende et al., 2019; Rada, 2013; Martinelli et al., 2010). In this context, PAs have a fundamental role in maintaining the ecosystems present in the Cerrado ecoregions, especially those that are under the high impacts, such as Upper São Francisco (Sano et al., 2019). Most of the PAs created in these areas of intense land use represents a small area of permanent preservation of ecosystems whose limits go beyond the PA area and extend to non-protected areas (Rodrigues et al., 2018; Rodrigues et al., 2004; Powell et al., 2000). This scenario enhances the requirement of PA buffer zones effectiveness for the maintenance of threatened ecosystems. It reinforces the need for continuous monitoring of buffer zone effectiveness in reducing human activities impacts in ecosystems harbored at protected areas, as Serra da Canastra NP. Besides, management actions proposed for both core PA areas and associated buffer zones are biased towards the conservation of terrestrial ecosystems so that activities specifically designed freshwater ecosystems maintenance tends to be undervalued (Casarim et al., 2020; Azevedo-Santos et al., 2019).

Moreover, the current approach adopted to freshwater quality monitoring in Brazil (CONAMA Resolution 357/2005, Brasil, 2005) was based on the Water Quality Index (WQI) that was initially developed by the National Sanitation Foundation in the United States of America to estimate the quality of water human consumption purposes (NSF, 2020). Due to the fact that the parameters used to estimate the WQI index were not originally designed to assess the health of freshwater ecosystems, it is reasonable to consider that new parameters are needed to diagnose and monitor more efficiently the health of freshwater ecosystems in Brazil. Similarly to other studies, our findings showed satisfactorily that the use of patterns of variation in the diversity and structure of the microbial communities of the riverbed sediment could be useful for diagnosis and monitoring of the health of freshwater ecosystems for areas whose human land-use is entirely (PAs) or partially restricted (buffer zones) with a purpose to guarantee the quality of fragile and vulnerable freshwater environments or even be incorporated into regular water quality programs of river basins since the analysis of microorganisms from riverbed sediment have been shown efficient for detecting chronic anthropogenic impacts in freshwater ecosystems.

Experimental Procedures

Study sites and sampling

In general, our approach adopted to assess the effectiveness of Serra da Canastra NP buffer zone in reducing the negative effects of anthropogenic activities on the health of freshwater ecosystems, using sediment communities as bioindicators, consisted of comparing the structure (taxonomic and functional) of sediment bacterial communities from the São Francisco River main course sampled at four well-defined areas (Figure 1): the core protected area, within the Serra da Canastra National Park (PA samples); within the buffer zone area of Serra da Canastra NP (BZ samples); and within two non-protected areas from Upper São Francisco Cerrado ecoregion and mapped by the TerraClass Cerrado 2013 project (MMA, 2015) as areas of intense agricultural activity (NP1 and

NP2 samples). Three collection sites were established for each sampling area, totaling 12 sediment collection sites. For each collection site, 50g of surface riverbed sediment was collected with a sterile bottle, at a distance of approximately 5 m from the river border or at the middle point of the mainstream. After sampling, the bottles were stored in a portable icebox and transferred into the laboratory within 12 hours. All samples were collected in 2017 during the dry season. The access to genetic material related to this study was adequately registered in the official database of Brazilian genetic patrimony – SISGEN – with access number A07CD46.

DNA extraction, PCR amplification, and high-throughput sequencing

Total genomic DNA was extracted from the sediment samples using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The samples were processed right after they come from the collection step. DNA concentration were estimated using a NanoDrop Spectrophotometer (Thermo Scientific, CA, USA) and stored at -20ºC for further analysis.

Microbial diversity of each sediment sample was accessed by high-throughput sequencing of the amplified V3-V4 region of the 16S rRNA gene by using primers 314F and 806R, following the guidelines established by the Earth Microbiome Project (Gilbert et al., 2014). PCR amplifications were performed in triplicates using customized primers containing both Illumina adapters and distinct barcode sequences so that each amplified DNA sample included a different combination of barcodes in order to distinguish the libraries after the sequencing step (Gilbert et al., 2014). All PCR reactions were carried out using the optimized PCR reaction mix OneTaq® Hot Start Quick-Load® 2X Master Mix with GC Buffer (New England Biolabs), with the following thermocycling PCR program: 94°C for 3 min; 25 cycles of 94°C for 45 s/50°C for 60s/72°C for 90 s; 72ºC for 10 min. The final PCR reactions were cleaned up using AMPure XP beads (Beckman Coulter, Brea, CA) and quantified with Picogreen dsDNA assays (Invitrogen, USA). In an attempt to normalize the sequencing step, equal amounts of PCR products from each sample (50 ng/sample) were pooled. Pooled PCR was subjected to electrophoresis on 1% agarose gel for purification by isolation of the PCR bands (300-500 bp) using a sterile razor. Pooled PCR bands were purified from agarose gel with NucleoSpin™ Gel and PCR Clean-up Kit (Macherey-Nagel™, Germany) and quantified by a Qubit®2.0 Fluorometer

(Thermo Scientific, CA, USA). Pooled PCR was subjected to paired-end sequencing (2x250 bp) on a MiSeq platform (Illumina, San Diego, CA, USA) with a MiSeq Reagent Kit V2 (500 cycles) from CEFAP Facility (São Paulo University, São Paulo, Brazil). All sequencing data generated in this study can be accessed from GenBank Database at Bio project PRJNA611749.

Bioinformatic analysis

The amplified 16S rRNA gene sequences were processed using the QIIME 2 pipeline version 2017.11 (Caporaso et al., 2010; QIIME 2 Development Team, 2017). Plugin "demux" was used to visualize interactive quality plots and check read quality. Plugin "DADA2" (Callahan et al., 2016) was subsequently applied to remove primers, truncate poor-quality bases based on the interactive plots, dereplicate, identify chimeras, and to merge paired-end reads. The representative sequences of ASVs were taxonomically assigned with a Naïve Bayes Classifier. They were trained with the "feature-classifier" plugin using the 16S rRNA gene database at 99% similarity to the SILVA database (v.132) as reference (Quast et al., 2013). Exploratory and statistical data analyses were performed at the ASV level (ASV frequency tables) since the ASV approach is a higher-resolution equivalent of the operational taxonomic unit (OTUs), delineated by 100% sequence similarity (Callahan et al., 2017; Porter and Hajibabaei, 2018). Functional prediction analysis from bacterial groups recovered by QIIME2 was performed in software Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 - PICRUSt2 (Douglas et al., 2020). We used the pipeline "qiime picrust2 full-pipeline" optimized to run in the QIIME2 environment in which generated frequency tables of predicted functional orthologs (KOs) entries of the KEGG database (Kanehisa and Goto, 2000) as output, for each sediment sample.

Statistical analysis

For taxonomic data analyses, we used QIIME2 to generate alpha- and beta-diversity vectors after rarefaction of the samples to 5000 sequences based on rarefaction curves generated previously (Figure S1). For alpha-diversity analyses, Observed ASVs, Shannon's diversity, Faith's phylogenetic diversity, and Pielou's evenness indices were calculated, and comparative Kruskal-Wallis tests were conducted for PA, BZ and NP comparisons, using R statistical software (R version 3.3.2). Compositional patterns (Beta-diversity) was estimated by Bray-Curtis and Unifrac (Weighted and Unweighted) dissimilarity metrics. Pairwise tests were performed with Permutational Multivariate Analysis of Variance (PERMANOVA), and the clustering patterns were visualized using Principal Coordinate Analysis plots (PCoAs), using QIIME2 applications. The relative abundance of most abundant phyla (frequency >1%) was presented in frequency bar plots, summarized by the sample groups (PA, BZ, and NP). Also, the most abundant genera (which compose > 3% of the reads at least in one sample) were used to generate a heatmap, including all sediment samples. Kruskal-Wallis tests were performed to compare the frequency of dominant taxa (phyla or genera) among the sample groups.

Here, we adopt two approaches to analyze the functional PICRUSt2 outputs: an overall and a structured approach. At the whole approach, each KO entry was considered as a feature to generate Shannon diversity indices and Bray-Curtis dissimilarity matrices. At the formal approach, Shannon diversity vectors and Bray-Curtis dissimilarity matrices were calculated for each Secondary level of KEGG Pathway Hierarchy independently. We perform pairwise tests between bacterial communities accessed in sample groups (Kruskal-Wallis tests). Similarly, PERMANOVA pairwise tests were calculated from Bray-Curtis dissimilarity matrices. All comparative analyses were computed using QIIME2.

Acknowledgments

Instituto Federal de Minas Gerais granted this work - Edital de Pesquisa Aplicada no. 169/2015 and by Fundação de Amparo à Pesquisa de Minas Gerais - FAPEMIG (MPR-01008-16).

References

Abell, R., Allan, J. D., and Lehner, B. (2007) Unlocking the potential of protected areas for freshwaters. Biol Conserv 134: 48-63. https://doi.org/10.1016/j.biocon.2006.08.017

Abia, A. L. K., Alisoltani, A., Keshri, J. and Ubomba-Jaswa, E. (2018) Metagenomic analysis of the bacterial communities and their functional profiles in water and sediments of the Apies River, South Africa, as a function of land use. Sci Total Environ 616: 326-334. https://doi.org/10.1016/j.scitotenv.2017.10.322

Adhikari, N. P., Liu, Y., Liu, K., Zhang, F., Adhikari, S., Chen, Y. and Liu, X. (2019) Bacterial community composition and diversity in Koshi River, the largest river of Nepal. Ecol Indic 104: 501-511. https://doi.org/10.1016/j.ecolind.2019.05.009

Agostinho, A. A., Thomaz, S. M. and Gomes, L. C. (2005) Conservation of the biodiversity of Brazil's inland waters. Conserv Biol 19: 646–652. https://doi.org/10.1111/j.1523-1739.2005.00701.x

Atsri, K. H., Abotsi, K. E., Kokou, K., Dendi, D., Segniagbeto, G. H., Fa, J. E., and Luiselli, L. (2020) Ecological challenges for the buffer zone management of a West African National Park. J Environ Plan Manag 63: 689-709. https://doi.org/10.1080/09640568.2019.1603844

Azevedo-Santos, V. M., Fearnside, P. M., Oliveira, C. S., Padial, A. A., Pelicice, F. M., Lima, D.P. , et al. (2017) Removing the abyss between conservation science and policy decisions in Brazil. Biodivers Conserv 26: 1745-1752. http://dx.doi.org/10.1007/s10531-017-1316-x

Azevedo-Santos, V. M., Frederico, R. G., Fagundes, C. K., Pompeu, P. S., Pelicice, F. M., Padial, A. A., et al. (2019). Protected areas: A focus on Brazilian freshwater biodiversity. Divers Distrib 25: 442-448. https://doi.org/10.1111/ddi.12871

Basta, T., Buerger, S. and Stolz, A. (2005) Structural and replicative diversity of large plasmids, from sphingomonads that degrade polycyclic aromatic compounds and xenobiotics. Microbiology 151: 2025-2037. https://doi.org/10.1099/mic.0.27965-0

Bouchez, T., Blieux, A. L., Dequiedt, S., Domaizon, I., Dufresne, A., Ferreira, S., et al., (2016) Molecular microbiology methods for environmental diagnosis. Environ Chem Lett 14: 423-441. https://doi.org/10.1007/s10311-016-0581-3

Brasil (2005) Ministério do Desenvolvimento Urbano e Meio Ambiente. Conselho Nacional do Meio Ambiente - CONAMA. (2005) Resolução nº 357, de 17 de março de 2005. Diário Oficial da República Federativa do Brasil, Brasília, DF, 18 mar. 2005.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13: 581. https://doi.org/10.1038/nmeth.3869

Callahan, B. J., McMurdie, P. J., and Holmes, S. P. (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 11: 2639. https://doi.org/10.1038/ismej.2017.119

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108:S1 4516-4522. https://doi.org/10.1073/pnas.1000080107

Carpenter, S. R., Mooney, H. A., Agard, J., Capistrano, D., DeFries, R. S., Díaz, S., et al. (2009) Science for managing ecosystem services: Beyond the Millennium Ecosystem Assessment. PNAS 106: 1305-1312. https://doi.org/10.1073/pnas.0808772106

Casarim, R., Caldeira, Y. M., and Pompeu, P. S. (2020) Representativeness of national parks in protecting freshwater biodiversity: A case of Brazilian savanna. Ecol Freshw Fish. https://doi.org/10.1111/eff.12547

Collen, B., Whitton, F., Dyer, E. E., Baillie, J. E., Cumberlidge, N., Darwall, W. R., et al. (2014) Global patterns of freshwater species diversity, threat and endemism. Global Ecol Biogeogr 23: 40-51. https://doi.org/10.1111/geb.12096

Costa, E. P., Pinto, C. C., Soares, A. L. C., Melo, L. D. V. and Oliveira, S. C. (2017) Evaluation of violations in water quality standards in the monitoring network of São Francisco River Basin, the third largest in Brazil. Environ Monit Assess 189: 2–16. https://doi.org/10.1007/s10661-017-6266-y

Craft, J. A., Stanford, J. A. and Pusch, M. (2002) Microbial respiration within a floodplain aquifer of a large gravel-bed river. Freshw Biol 47:51-261. https://doi.org/10.1046/j.1365-2427.2002.00803.x

Dantas, M.S., Oliveira, J.C., Pinto, C.C. and Oliveira, S.C. (2020) Impact of fecal contamination on surface water quality in the São Francisco River hydrographic basin in Minas Gerais, Brazil. J Water Health 18: 48-59. https://doi.org/10.2166/wh.2019.153

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., et al. (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38: 685–688. https://doi.org/10.1038/s41587-020-0548-6

Drury, B., Rosi-Marshall, E. and Kelly, J.J. (2013) Wastewater treatment effluent reduces the abundance and diversity of benthic bacterial communities in urban and suburban rivers. Appl Environ Microbiol 79: 1897-1905. https://doi.org/10.1128/AEM.0352712

Gibbons, S.M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., et al. (2014) Human and environmental impacts on river sediment microbial communities. PLoS One 9: e97435. https://doi.org/10.1371/journal.pone.0097435.

Gilbert, J.A., Jansson, J.K. and Knight, R. (2014) The Earth Microbiome project: successes and aspirations. BMC Biol 12: 69. https://doi.org/10.1186/s12915-014-0069-1

Goncalves, C., Lima, L., Lintomen, B., Casella, P. and Berlinck, C. (2009) Buffer zone: Creation or delimitation? Nat Conserv 7: 130–135.

Hansen, A. J. and DeFries, R. (2007) Ecological mechanisms linking protected areas to surrounding lands. Ecol Appl 17: 974–988. https://doi.org/10.1890/05-1098

Hu, A., Yang, X., Chen, N., Hou, L., Ma, Y. and Yu, C. P. (2014) Response of bacterial communities to environmental changes in a mesoscale subtropical watershed, Southeast China. Sci Total Environ 472: 746-756. https://doi.org/10.1016/j.scitotenv.2013.11.097

Huang, W., Chen, X., Jiang, X. and Zheng, B.H. (2017) Characterization of sediment bacterial communities in plain lakes with different trophic statuses. MicrobiologyOpen 6: e503. https://doi.org/10.1002/mbo3.503

Huang, W., Chen, X., Wang, K., Chen, J., Zheng, B. and Jiang, X. (2019) Comparison among the microbial communities in the lake, lake wetland, and estuary sediments of a plain river network. MicrobiologyOpen 8: e00644. https://doi.org/10.1002/mbo3.644

ICMBio (2020) Instituto Chico Mendes de Conservação da Biodiversidade. Retrieved from http://www.icmbio.gov.br/porta l/planosmanejo

Kalia, A. and Gosal, S. K. (2011) Effect of pesticide application on soil microorganisms. Arch Agron Soil Sci 57: 569-596. https://doi.org/10.1080/03650341003787582

Kanehisa, M. and Goto, S. (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 28: 27-30. https://doi.org/10.1093/nar/28.1.27

Kirchman, D. L. (2018) Processes in microbial ecology. Oxford University Press.

Lamichhane, B. R., Persoon, G. A., Leirs, H., Poudel, S., Subedi, N., Pokheral, et al. (2019) Contribution of buffer zone programs to reduce human-wildlife impacts: the case of the Chitwan National Park, Nepal. Hum Ecol 47: 95-110. https://doi.org/10.1007/s10745-019-0054-y

Liu, J., Chen, X., Shu, H. Y., Lin, X. R., Zhou, Q. X., Bramryd, et al. (2018) Microbial community structure and function in sediments from e-waste contaminated rivers at Guiyu area of China. Environ Pollut 235: 171-179. https://doi.org/10.1016/j.envpol.2017.12.008

Lliro, M., Inceoglu, O., Garcia-Armisen, T., Anzil, A., Leporcq, B., Pigneur, L., et al. (2014) Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. PLoS One 9:e1–27. https://doi.org/10.1371/journal.pone.0116145

Lu, X.-M. and Lu, P-Z. (2014) Characterization of bacterial communities in sediments receiving various wastewater effluents with high-throughput sequencing analysis. Microb Ecol 67: 612-623. https://doi.org/10.1007/s00248-014-0370-0

Marti, E. and Balcázar, J.L. (2014) Use of pyrosequencing to explore the benthic bacterial community structure in a river impacted by wastewater treatment plant discharges. Res Microbiol 165: 468-471. https://doi.org/10.1016/j.resmic.2014.04.002

Martinelli, L. A., Naylor, R., Vitousek, P. M. and Moutinho, P. (2010) Agriculture in Brazil: impacts, costs, and opportunities for a sustainable future. Curr Opin Env Sust 2: 431-438. https://doi.org/10.1016/j.cosust.2010.09.008

MMA. (2015) Mapeamento do uso e cobertura do Cerrado: Projeto TerraClass Cerrado. Available at: http://www.mma.gov.br/images/arquivo/80049/Cerrado/publicacoes/Livro%20EMBRAPA-WEB-1-TerraClass%20Cerrado.pdf, Accessed date: 20 June 2020.

NSF. (2020) National Sanitation Foundation - NSF. Water Quality Index (WQI). http://www.nsf.org/consumer/earth_day/wqi.asp (access 10 June 2020).

Porter, T. M. and Hajibabaei, M. (2018) Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. Mol Ecol 27: 313-338. https://doi.org/10.1111/mec.14478

Powell, G. V. N., Barborak, J. and Rodriguez, S. M. (2000) Assessing representativeness of protected natural areas in Costa Rica for conserving biodiversity - A preliminary gap analysis. Biol Conserv 93: 35–41. https://doi.org/10.1016/S0006-3207(99)00115-9

QIIME 2 Development Team. (2017) QIIME 2. https://docs.qiime2.org.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41: D590–D596. https://doi.org/10.1093/nar/gks1219

Rada, N. (2013) Assessing Brazil's Cerrado agricultural miracle. Food Policy 38: 146-155.

Resende, F. M., Cimon-Morin, J., Poulin, M., Meyer, L. and Loyola, R. (2019) Consequences of delaying actions for safeguarding ecosystem services in the Brazilian Cerrado. Biol Conserv 234: 90-99. https://doi.org/10.1016/j.biocon.2019.03.009

Rodrigues, A. S. L., Andelman, S. J., Bakarr, M. I., Boitani, L., Brooks, T. M., Cowling, R. M. et al. (2004) Effectiveness of the global protected area network in representing species diversity. Nature 428: 640–643. https://doi.org/10.1038/natur e02422

Rodrigues, A. A., Bustamante, M. M. C. and Sano, E. (2018) As far as the eye can see: Scenic view of Cerrado National Parks. Perspect Ecol Conser 16: 31–37. https://doi.org/10.1016/j.pecon.2017.11.004

Rosenberg, E., Edward, E.-I.-C., Delong, F., Lory, S., Stackebrandt, E. and Thompson, F. (2013) The Prokaryotes: Applied Bacteriology and Biotechnology. Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-31331-8

Sano, E. E., Rodrigues, A. A., Martins, E. S., Bettiol, G. M., Bustamante, M. M., Bezerra, et al. (2019) Cerrado ecoregions: A spatial framework to assess and prioritize Brazilian savanna environmental diversity for conservation. J Environ Manage 232: 818-828. https://doi.org/10.1016/j.jenvman.2018.11.108

Saunders, D. L., Meeuwig, J. J., and Vincent, A. C. J. (2002) Freshwater protected areas: strategies for conservation. Conserv Biol 16: 30-41. https://doi.org/10.1046/j.1523-1739.2002.99562.x

Saxena, G., Marzinelli, E.M., Naing, N.N., He, Z.L., Liang, Y.T., Tom, L., et al. (2015) Ecogenomics reveals metals and land-use pressures on microbial communities in the waterways of a megacity. Environ Sci Technol 49: 1462-1471. https://doi.org/10.1021/es504531s

Sayer, J. (1991) Rainforest buffer zones: guidelines for protected area managers. International Union for Conservation of Nature and Natural Resources.

Shafer, C. L. (1999) US national park buffer zones: historical, scientific, social, and legal aspects. Environ manage 23: 49. https://doi.org/10.1007/s002679900167

Sigee, D. (2005) Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment. John Wiley & Sons.

Silva, J.M.C. and Bates, J.M. (2002) Biogeographic patterns and conservation in the South American Cerrado: a tropical savanna hotspot. Bio Sci 52: 225–234. https://doi.org/10.1641/0006-3568(2002)052[0225:BPACIT]2.0.CO;2.

Sorokin, D.Y., Berben, T., Melton, E.D., Overmars, L., Vavourakis, C.D. and Muyzer, G. (2014) Microbial diversity and biogeochemical cycling in soda lakes. Extremophiles 18: 791–809. https://doi.org/10.1007/s00792-014-0670-9

Wakelin, S.A., Colloff, M.J. and Kookana, R.S. (2008) Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. Appl Environ Microb 74: 2659-2668. https://doi.org/10.1128/AEM.02348-07

Wang, L., Zhang, J., Li, H., Yang, H., Peng, C., Peng, Z. and Lu, L. (2018) Shift in the microbial community composition of surface water and sediment along an urban river. Sci Total Environ 627: 600-612. https://doi.org/10.1016/j.scitotenv.2018.01.203

Winter, C., Hein, T., Kavka, G., Mach, R.L. and Farnleitner, A.H. (2007) Longitudinal changes in the bacterial community composition of the Danube River: a whole-river approach. Appl Environ Microb 73: 421–431. https://doi.org/10.1128/AEM.01849-06

Wu, H., Li, Y., Zhang, J., Niu, L., Zhang, W., Cai, W. and Zhu, X. (2017) Sediment bacterial communities in a eutrophic lake influenced by multiple inflow-rivers. Environ Sci Pollut R 24: 19795–19806. https://doi.org/10.1007/s11356-017-9602-4

Xie, Y., Wang, J., Wu, Y., Ren, C., Song, C., Yang, J., et al. (2016) Using in situ bacterial communities to monitor contaminants in river sediments. Environ Pollut 212: 348-357. https://doi.org/10.1016/j.envpol.2016.01.031

Xie, Y., Wang, J., Yang, J., Giesy, J. P., Yu, H. and Zhang, X. (2017) Environmental DNA metabarcoding reveals primary chemical contaminants in freshwater sediments from different land-use types. Chemosphere 172: 201-209. https://doi.org/10.1016/j.chemosphere.2016.12.117

Yang, Y., Li, S., Gao, Y., Chen, Y. and Zhan, A. (2019) Environment-driven geographical distribution of bacterial communities and identification of indicator taxa in Songhua River. Ecol Indic 101: 62-70. https://doi.org/10.1016/j.ecolind.2018.12.047

Zeglin, L.H. (2015) Stream microbial diversity in response to environmental changes: review and synthesis of existing research. Front Microbiol 6: 454. https://doi.org/10.3389/fmicb.2015.00454

Zhang, L., Zhao, T., Wang, Q., Li, L., Shen, T. and Gao, G. (2019) Bacterial community composition in aquatic and sediment samples with spatiotemporal dynamics in large, shallow, eutrophic Lake Chaohu, China. *J Freshwater Ecol* 34: 575-589. https://doi.org/10.1080/02705060.2019.1635536

Supplementary materials

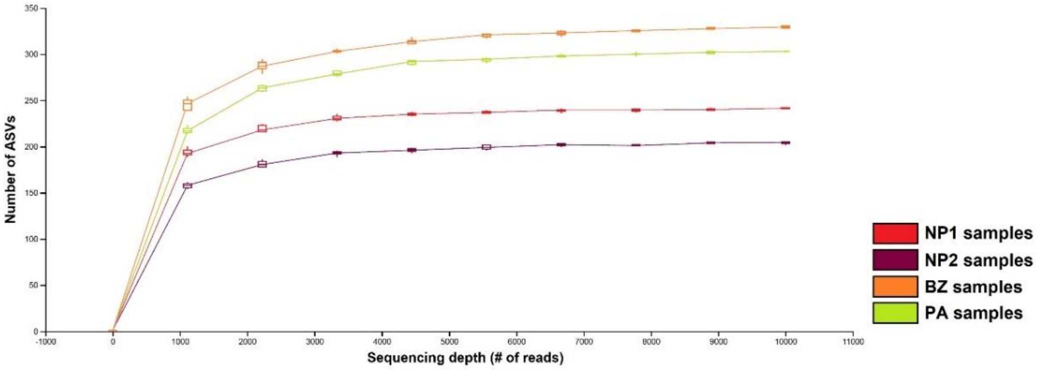


Figure S1. Rarefaction curves of ASVs structured by the sampling sites.

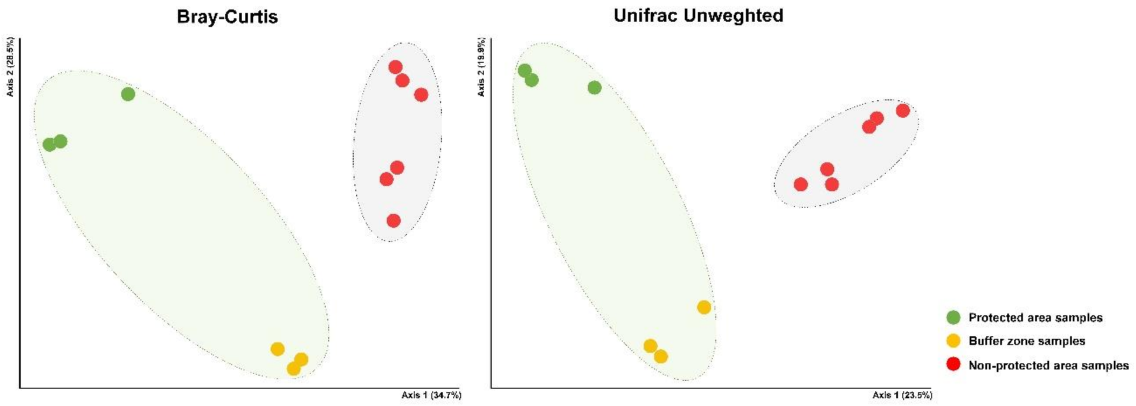


Figure S2. Principal coordinates analysis (PCoA) plots using Bray-Curtis and Unifrac Unweighted dissimilarity matrices for sediment samples.

Table S1. Pairwise Kruskal-Wallis tests (p-values) for taxonomic alpha-diversity indices among sampled groups

Sample group 1	Sample group 2	Observed ASVs	Shannon	Evenness	Phylogenetic diversity
BZ	**PA**	0.275	0.060	**0.050**	0.275
	NP	0.071	**0.020**	**0.020**	**0.020**
PA	**NP**	0.302	0.606	0.796	**0.039**

Table S2. P_{PERM} scores of PERMANOVA tests for three taxonomic dissimilarity metrics among sampled groups

Sample group 1	Sample group 2	Bray-curtis	Unifrac Unweighted	Unifrac Weighted
BZ	**PA**	0.101	0.095	0.095
	NP	**0.005**	**0.011**	**0.018**
PA	**NP**	**0.016**	**0.014**	**0.007**

4. CAPÍTULO 2

Manuscrito submetido à revista Journal of Soils and Sediments

Spatial distribution of sediment bacterial communities from São Francisco River headwaters is influenced by human land-use activities and seasonal climate shifts

Marcos de Paula Jr., Thiago Augusto da Costa e Silva, Amanda Araújo Barezani Soriano, Gustavo Augusto Lacorte

Molecular Biology Lab, Department of Science and Languages, Federal Institute of Minas Gerais – Bambuí Campus, Bambuí, Minas Gerais State, Brazil;

Corresponding author: Gustavo Augusto Lacorte; e-mail: gustavo.lacorte@ifmg.edu.br; ORCID iD: 0000-0002-6866-348X; Phone number: +55 37 3431-5600.

Abstract

Purpose

São Francisco River headwaters section represents a typical forest-rural-urban landscape from the Brazilian Cerrado biome which constitutes a suitable biological system to study impacts of human practices on riverbed ecosystems. Here, spatial analysis of sediment microbial distribution from São Francisco River headwaters section was conducted in order to accomplish two major goals: (i) to investigate whether the diversity and composition of bacterial communities accessed in riverbed sediments vary in response to distinct land-use activities and whether the response patterns may be influenced by local seasonal climate changes; and (ii) to estimate if the diversity patterns of metabolic functions vary in a similar way to taxonomic patterns.

Materials and methods

The study area corresponds to the São Francisco River headwaters micro basin which is placed in an elevated region from Southeast Brazil named “Canastra Ridge”. This region compounds a typical fragmented and human-impacted landscape from the Brazilian Cerrado biome. Nine sediment sampling sites were selected covering the four defined land-use zones: Spring zone, Tourism zone, Rural zone and Urban zone. For each sampling site, three riverbed sediment samples were accessed at two collection rounds corresponding the two well-defined seasons of Brazilian Cerrado biome: Wet and Dry seasons. Microbial diversity of each sediment sample was accessed by high-throughput sequencing of the amplified V3-V4 region of the 16S rRNA gene and functional prediction analysis from bacterial groups recovered by QIIME2 was performed in PICRUSt2.

Results

Our results revealed that variation in bacterial diversity of sediment communities was associated with changes in land-use practices, but also the mode in which the bacterial communities respond to these anthropogenic changes were influenced by the seasonal component. PICRUSt2 results showed that the sediment communities in which presented significant variation in taxonomic diversity also presented changes in the diversity of predicted metabolic functions and the most changes in the composition of predicted metabolic functions were verified in protected/unprotected transition zones.

Conclusion

Our findings contributed with new evidence about the impact of typical land-use practices conducted in countryside landscapes from developing countries on riverbed bacterial communities, both in their taxonomic and functional structure. The way in which the anthropogenic impacts influence bacterial communities is highly associated with the seasonal component, in which the effects become more severe during periods of greater rainfall in which the nutrient runoff events intensify.

Keywords: Sediment; River; Bacterial communities; 16S rDNA; Land-use; Season

Introduction

Rivers are freshwater environments that accomplish essential environmental services such local climate regulation, water resources stockpile, wastewater purification, fishery maintenance and other critical functions for maintaining human health, industrial and agricultural production (Gutknecht et al., 2006; Ansola et al., 2014; Wu et al., 2015). Ecologically, rivers are lotic ecosystems that represent a continuum of abiotic and biotic elements in both space and time along with the flow from headwaters to the river mouth. In addition to stream continuum, rivers also consist of a water column lying over a bed of sediments with also a vertical succession of biotic communities (Sigee, 2005). Rivers are intimately associated with the surrounding land and other connected water bodies (Cottrell et al., 2005; Liu et al., 2018). Expressly in developing countries, as human activities progress through the river basin land areas, river ecosystems have experienced more impacts of anthropogenic activities, mainly associated with inputs from fertilizer runoff of agricultural activities, dumping of industrial pollutants and urban wastewater launching (Williamson et al. 2008; Vörösmarty et al., 2010; Oribhabor et al. 2013). Because of the increasing loading of nutrients, heavy metals, and other contaminants, the health of river ecosystems has been seriously impaired with the loss of biodiversity and major effects on ecosystem functions and environmental services (Weijters et al., 2009; Villéger et al., 2011).

At the bottom of river environments, riverbed sediments represent both a sink and source for nutrient cycles in freshwater ecosystems and recent evidence has depicted that sediment collect inputs from a variety of sources associated with human activities in river basins such as nutrients, heavy metals and sulfide pollution (Taylor and Owens, 2009; Keestra et al., 2012; Vignesh et al. 2014; Wang et al., 2016). Microbes constitute the major biomass of river sediments and play vital roles in these ecosystems. They are involved in biomass production, biogeochemical cycles with assimilation and mineralization of chemical elements as well biodegradation and bioremediation of pollutants (Craft et al., 2002; Winter et al., 2007; Gibbons et al., 2014; Lliro et al., 2014; Sorokin et al., 2014). Nevertheless, when the environmental conditions of the sediment-water interface change with modifications on pH level, redox potential, oxygen concentration, organic matter content and input of xenomolecules, the metabolism of microbial populations are affected and changes in microbial communities structure and functioning can be altered (Savio et al., 2015; Liu et al., 2018; Wang et al., 2018). So, is expected that sediment microbial communities are highly sensitive to changes in water physicochemical properties, especially changes influenced by land-use practices as well as variations in hydrological features caused by seasonal climatic fluctuations (Staley et al., 2013; Zeglin, 2015; Hu et al.,2017; Wang et al., 2017).

The São Francisco River is the largest river located entirely within Brazil whose basin area represents 7.5% of the Brazilian territory. The river course flows northwards 2700 km long from the Southeast region to the Atlantic Ocean in the Northeast region, encompassing seven Brazilian states and 507 municipalities (almost 10% of all municipalities in the country) (CBHSF, 2016a). Water use of the São Francisco River basin is structured in 90% for agriculture irrigation, 5% for livestock, 3% for urban supply, and 2% for industrial purposes. Furthermore, economic activities developed at São Francisco River basin areas contributes to almost 6% of Brazilian Gross Domestic Product (GDP) and the most the activities (72% of the total) is concentrated at the first part of the basin named Upper São Francisco River sub-basin (ANA, 2015; CBHSF, 2016b).

The São Francisco River headwaters are located in an elevated region of Minas Gerais State named "Canastra Ridge". This region represents a Cerrado biome landscape with two well-defined seasons: the dry season, which includes South hemisphere's autumn and winter, is characterized by warm temperatures (8-15°C) and low rainfall rates (30 mm/month, reaching up to 5mm in June-July); and the Wet season, including spring and summer, is characterized by higher average temperatures (24-31 °C) and higher rainfall rates (200 mm/month) (Silva et al, 2008). Moreover, this first section of the São Francisco River basin is composed of a mosaic of patches with distinct anthropogenic land-uses such as ecotourism, agriculture, livestock, urban areas, and a conservation unit which harbors the springs of São Francisco River. All these characteristics label São Francisco River headwaters as a typical forest-rural-urban landscape which constitutes a suitable biological system to study impacts of human practices on riverbed ecosystems as well how the seasonal climate component could influence the process.

Here, spatiotemporal analysis of sediment microbial distribution from São Francisco River headwaters section was conducted using Illumina 16S rRNA-V4 region amplicon sequencing in order to accomplish two major goals: (i) to investigate whether the diversity and composition of bacterial communities accessed in riverbed sediments vary in response to distinct land-use activities and whether the response patterns may be influenced by local seasonal climate changes; and (ii) to estimate if the diversity patterns of metabolic functions, predicted by PICRUSt2 approach, to accessed bacterial communities vary in a similar way to taxonomic patterns. The answers to these issues improve our knowledge about the impacts of anthropogenic practices in typical headwater environments placed in countryside areas of developing countries.

Materials and methods

Description of sampling sites and collection procedures

The study area corresponds to the São Francisco River headwaters micro basin which is placed in an elevated region (800-1200 m high) from Southeast Brazil (20º00'-20º30' S; 46º15'-47º00' W) named “Canastra Ridge”. This region compounds a typical fragmented and human-impacted landscape from the Brazilian Cerrado biome (Figure 1B). The sampling sites were selected along the São Francisco River headwaters region representing four anthropogenic activity zones - Spring, Tourism, Rural, and Urban - identified by main land-use activities (Figure 1A). The Spring zone corresponds to a protected area within the conservation unit, named Serra da Canastra National Park, which harbors several springs and small water streams with restricted access. The Tourism zone refers to another portion of the conservation unit, but intensely used for ecotourism activities such as swimming and eco-adventure sports. The Rural zone corresponds to areas adjacent to the conservation unit, composed of rural properties that impact the river with agricultural (corn, coffee, pasture, soy fields) and livestock activities. The Urban zone refers to the urban perimeter of the first city encompassed by the São Francisco River with recent records of violations in water quality parameters due to urban sewage run-off (Dantas et al., 2020).

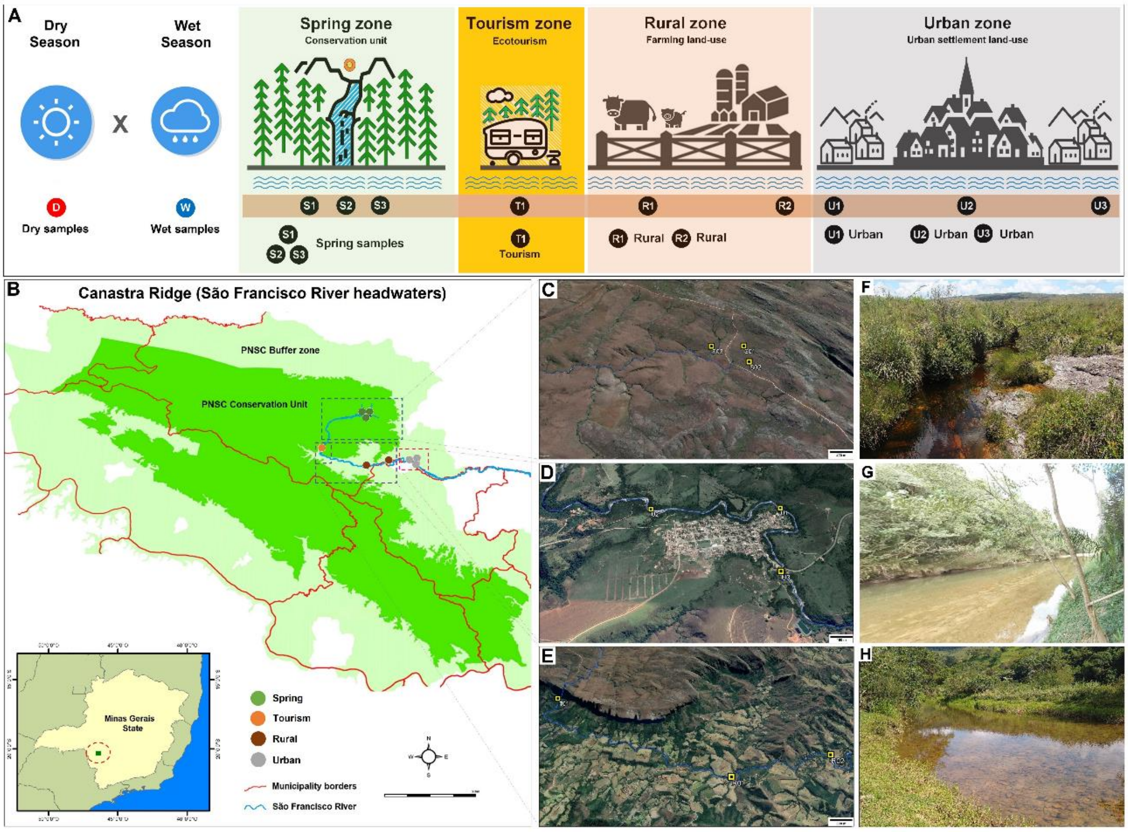


Figure 1. Schematic diagram of study design and sampling. Study design (A); São Francisco River headwaters placed at Canastra Ridge region (B); Google Earth images from sampled zones: Spring (C), Urban (D), Tourism + Rural (E); Collection sites: Spring (F), Urban (G), Rural (H).

Nine sediment sampling sites were selected covering the four defined land-use zones: Spring zone (S1, S2, and S3 sites, Figure 1C); Tourism zone (T1 site, Figure 1E); Rural zone (R1 and R2 sites, Figure 1E); and Urban zones (U1, U2, and U3 sites, Figure 1D). For each sampling site, three riverbed sediment samples (about 50g) was collected with sterile bottles, at a linear distance of 10 meters from each other and at a distance of approximately 5 m from the river border or at the middle point of the mainstream when the river was less than 5 meters wide. After sampling, the bottles were stored in a portable icebox and transferred into the laboratory within 12 hours. Two collection rounds, at the same collecting points, were carried out, so that the first round occurred in the summer of 2018 (Wet season) and the other collecting round occurred in winter of 2018 (Dry season). The access to genetic material related to this study was properly registered in the official database of Brazilian genetic patrimony – SISGEN – with access number A07CD46.

DNA extraction, PCR amplification, and high-throughput sequencing

Total genomic DNA was extracted from the sediment samples using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The samples were processed right after they come from the collection step. DNA concentration was estimated using a NanoDrop Spectrophotometer (Thermo Scientific, CA, USA) and stored at -20ºC for further analysis.

Microbial diversity of each sediment sample was accessed by high-throughput sequencing of the amplified V3-V4 region of the 16S rRNA gene by using primers 314F and 806R, following the guidelines established by the Earth Microbiome Project (Gilbert et al., 2014). PCR amplifications were performed in triplicates using customized primers containing both Illumina adapters and distinct barcode sequences so that each amplified DNA sample contained a different combination of barcodes in order to distinguish the libraries after the sequencing step (Gilbert et al., 2014). All PCR reactions were carried out using the optimized PCR reaction mix OneTaq® Hot Start Quick-Load® 2X Master Mix with GC Buffer (New England Biolabs), with the following thermocycling PCR program: 94°C for 3 min; 25 cycles of 94°C for 45 s/50°C for 60s/72°C for the 90s; 72ºC for 10 min. The final PCR reactions were cleaned up using AMPure XP beads (Beckman Coulter, Brea, CA) and quantified with Picogreen dsDNA assays (Invitrogen, USA). In an attempt to normalize the sequencing step, equal amounts of PCR products from each sample (50 ng/sample) were pooled. Pooled PCR was subjected to electrophoresis on 1% agarose gel for purification by isolation of the PCR bands (300-500 bp) using a sterile razor. Pooled PCR bands were purified from agarose gel with NucleoSpin™ Gel and PCR Clean-up Kit (Macherey-Nagel™, Germany) and quantified by a Qubit®2.0 Fluorometer (Thermo Scientific, CA, USA). Pooled PCR was subjected to paired-end sequencing (2x250 bp) on a MiSeq platform (Illumina, San Diego, CA, USA) with a MiSeq Reagent Kit V2 (500 cycles) from CEFAP Facility (São Paulo University, São Paulo, Brazil).

Bioinformatic analysis

The amplified 16S rRNA gene sequences were processed using the QIIME 2 pipeline version 2017.11 (Caporaso et al., 2010; QIIME 2 Development Team, 2017). Plugin "demux" was used to visualize interactive quality plots and check read quality.

Plugin “DADA2” (Callahan et al., 2016) was subsequently applied to remove primers, truncate poor-quality bases based on the interactive plots, dereplicate, identify chimeras, and to merge paired-end reads. The representative sequences of ASVs were taxonomically assigned with a Naïve Bayes Classifier and were trained with the “feature-classifier” plugin using the 16S rRNA gene database at 99% similarity to SILVA database (v.132) as reference (Quast et al., 2013). Exploratory and statistical data analyses were performed at the ASV level (ASV frequency tables) since the ASV approach is a higher-resolution equivalent of the operational taxonomic unit (OTUs), delineated by 100% sequence similarity (Callahan et al., 2017; Porter and Hajibabaei, 2018). All sequencing data generated in this study can be accessed from GenBank Database at Bioproject PRJNA639954.

Functional prediction analysis from bacterial groups recovered by QIIME2 was performed in software Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 - PICRUSt2 (Douglas et al., 2020). We used the pipeline “qiime picrust2 full-pipeline” optimized to run in QIIME2 environment in which generated frequency tables of predicted MetaCyc pathways of BioCyc Metabolic Pathway Database (Caspi et al., 2018) and predicted functional orthologs (KOs) and Enzymes (ECs) entries of KEGG database (Kanehisa and Goto, 2000) as output, for each sediment sample.

Statistical analysis

The taxonomic and functional data of each sediment sample were organized into two hierarchical levels: firstly we assigned the samples to two major groups referring to the seasons (Wet and Dry groups). Subsequently, we created within Wet and Dry groups, sub-groups corresponding to each land-use activity zone (Spring, Tourism, Rural, and Urban sub-groups). Comparative analyses among land-use groups were performed for each seasonal group (Wet and Dry) independently. In addition, for each land-use group, a comparative analysis was performed between samples collected in Dry and Wet seasons.

For taxonomic data analyses, we used QIIME2 to generate alpha- and beta-diversity vectors after rarefaction of the samples to 5000 sequences based on rarefaction curves generated previously (Figure S1). For alpha-diversity analyses, Observed ASVs, Shannon’s diversity, Faith’s phylogenetic diversity, and Pielou’s evenness indices were calculated, and comparative Wilcoxon pairwise tests were performed for Wet vs. Dry comparisons and Kruskal-Wallis tests were conducted for land-use group comparisons, using R statistical software (R version 3.3.2). Compositional patterns (Beta-diversity) was estimated by Bray-Curtis and Unifrac (Weighted and Unweighted) dissimilarity metrics. Pairwise tests were performed with Permutational Multivariate Analysis of Variance (PERMANOVA) and the clustering patterns were visualized using Principal Coordinate Analysis plots (PCoAs). The relative abundance of most abundant phyla (frequency >1%) were presented in frequency bar plots, summarized by the sampling sites and structured by the seasonal groups (Wet and Dry). Also, the most abundant families (which compose > 3% of the reads at least in one sample) were used to generate a heatmap including all sediment samples. Wilcoxon pairwise tests were performed to compare the frequency of dominant taxa (phyla or families) estimated for Dry and Wet samples.

Here, we adopt two approaches to analyze the functional PICRUSt2 outputs: a general and a structured approach. At the general approach, each entry (MetaCyc pathway, KO, or EC) was considered as a feature to generate Shannon diversity indices and Bray-Curtis dissimilarity matrices. At the structured approach, Shannon diversity vectors and Bray-Curtis dissimilarity matrices were calculated for each Secondary level of KEGG Pathway Hierarchy independently. We perform pairwise tests between bacterial communities accessed in Dry and Wet seasons (Wilcoxon tests) as well among bacterial communities accessed at the four land-use activity groups (Kruskal-Wallis tests). Similarly, PERMANOVA pairwise tests were calculated from Bray-Curtis dissimilarity matrices. KO frequency data structured by Secondary level of KEGG were used to perform Kruskal-Wallis pairwise tests among Land-use groups and the results were presented in frequency bar plots by season.

Results

The high-throughput sequencing of sediment samples generated a total of 1,282,331 16S sequencing reads, with a median frequency of reads per sample of 22,726, recovering 13,130 ASVs with an average of 352 ASVs per sample. The rarefaction curves indicated the saturation stage at sampling sites, suggesting that current sequencing depth was sufficient to cover most of the bacterial community diversity (Figure S1).

The estimates of alpha diversity indices revealed significant variation on diversity levels for sediment bacterial communities accessed throughout the spatial sampling with two distinct patterns associated with Wet and Dry season (Figure 2, Table S1). For Dry samples, the diversity levels increased from Spring to Tourism zone, decreased into Rural zone, and remain stable in the Urban zone. At Wet season, diversity levels were similar between Spring and Tourism zone and increased in the Rural zone and remain in the Urban zone. Considering the seasonal variation in bacterial diversity of sediment communities from each Land-use zone accessed, we found that Spring and Rural zones showed lower levels of diversity when the communities were accessed at Dry season (Table 1).

Table 1. Season alpha-diversity indices* comparisons structured by land-use groups.

*Data corresponds the means of calculated indices for samples accessed in each zone of land-use activities observed in the study area, with standard errors in brackets. Comparisons significantly different according Wilcoxon test are depicted in bold.

Alpha-diversity parameter	**Season**	**Land-use groups**			
		Spring	Tourism	**Rural**	Urban
Observed ASVs	Dry	319 (56)	402 (6)	294 (57)	362 (145)
	Wet	363 (99)	306 (78)	429 (22)	364 (140)
	Wilcoxon p-value	0.08	0.1	**0.004**	0.89
Shannon diversity index	Dry	7.62 (0.31)	8.28 (0.05)	7.6 (0.4)	7.86 (0.77)
	Wet	7.99 (0.59)	7.72 (0.47)	8.39 (0.11)	8.03 (0.69)
	Wilcoxon p-value	**0.05**	0.1	**0.004**	0.67
Pielou evenness index	Dry	0.92 (0.02)	0.96 (0.01)	0.93 (0.02)	0.93 (0.03)
	Wet	0.95 (0.02)	0.94 (0.01)	0.96 (0.01)	0.95 (0.02)
	Wilcoxon p-value	**0.03**	0.1	**0.01**	0.12
Faith phylogenetic diversity index	Dry	30.41 (3.25)	37.66 (1.07)	30.48 (5.32)	35.7 (8.1)
	Wet	31.43 (7.00)	29.33 (4.73)	35.32 (2.66)	31.05 (7.06)
	Wilcoxon p-value	0.67	0.1	0.13	0.37

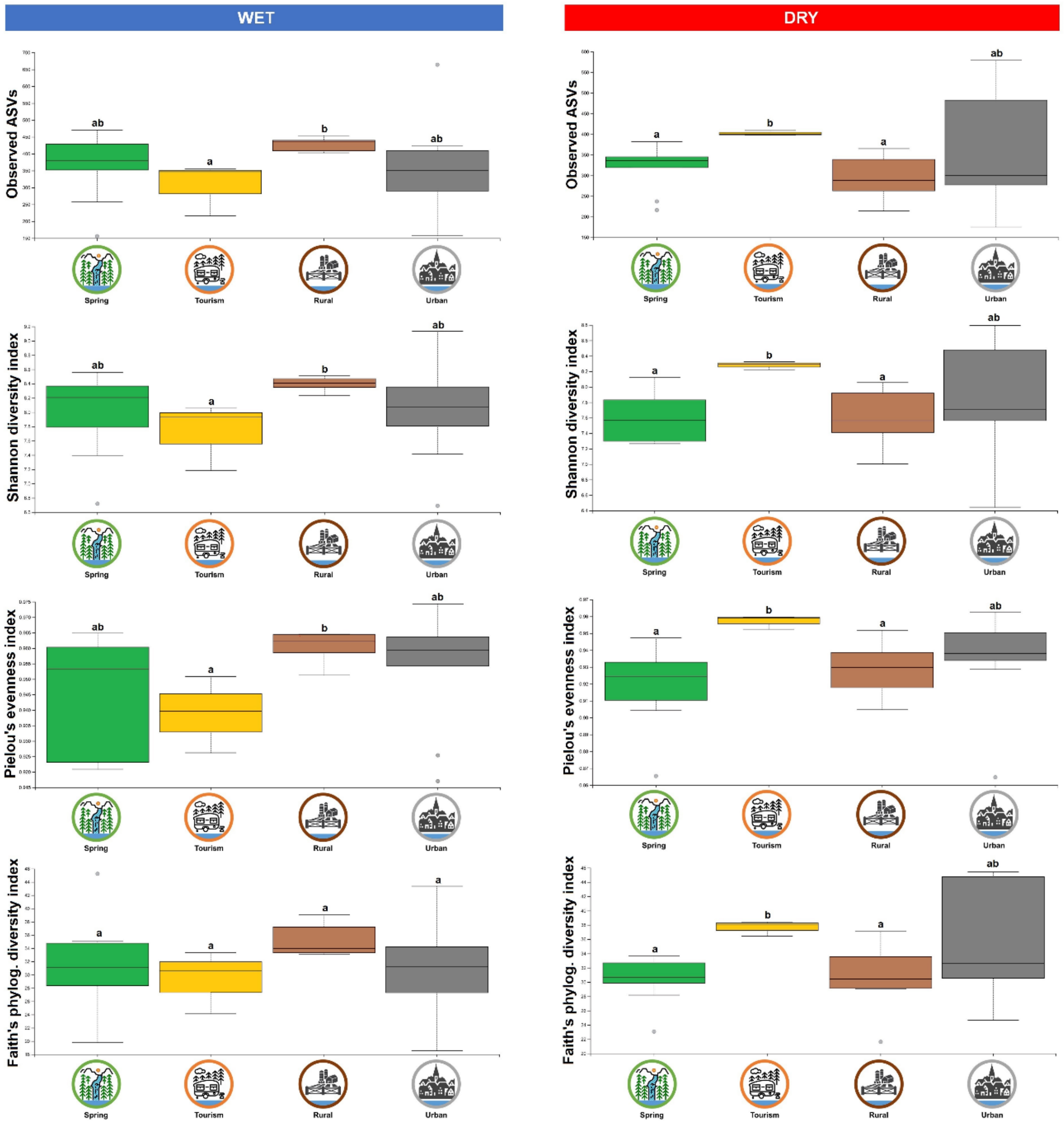


Figure 2. Taxonomic diversity indices of the sediment samples from the four land-use zones organized by season.

Alternatively, the diversity levels of the bacterial communities from Tourism and Urban zones remained unchanged between the seasonal sampling. In addition, we verified the same. Based on our estimates of taxonomic composition, we verified that sediment samples showed significant differences in taxonomic composition among all Land-use zones, both at Dry and Wet seasons (Table 2, Figure S2). We also observed significant differences in the taxonomic composition of bacterial communities from the same Land-use zone but sampled at Dry and Wet seasons (Table S2). Equivalent dissimilarity patterns were found for the three metrics used to calculate the similarity matrices (Bray-Curtis, Unifrac Unweighted, and Weighted) (Figure S2).

Table 2. Pairwise PERMANOVA tests p-values for taxonomic clustering by land-use groups (Weighted Unifrac metric)

		Spring	Tourism	Rural	Urban	
Spring	***	***	**0.028**	**0.002**	**0.001**	
Tourism		**0.013**	***	**0.028**	**0.005**	**Wet**
Rural	**Dry**	**0.003**	**0.02**	***	**0.015**	
Urban		**0.001**	**0.005**	**0.008**	***	***

The taxonomic characterization of bacterial communities from sediment samples revealed the presence of some dominant taxa. At the phylum level, we observed 11 dominant phyla whose abundances contributed to 93-88% of the total (Figure 3). Four of these phyla were the most abundant in all sediment samples: Proteobacteria (46% to 27%), Acidobacteria (21% to 4%); Planctomycetes (20% to 4%) and Verrucomicrobia (17 to 6%). Alternatively, the phyla Cyanobacteria and Bacterioidetes were found more frequently in sediment samples from Dry season (Wilcoxon test p = 0.003 and p = 0.001, respectively). Only Spirochaetes phylum showed a differential abundance among the zones, which showed greater abundance in Urban zone samples.

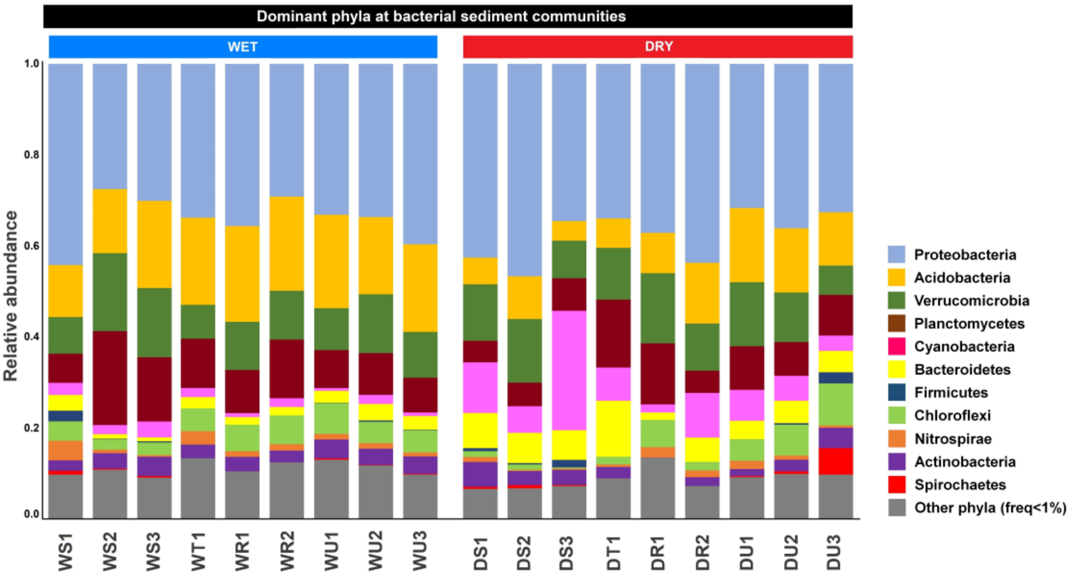


Figure 3. Relative abundances of dominant phyla in sediment communities from each sampling site organized by season.

At the family level, we found 36 dominant bacterial families in the sediment samples, which presented similar distribution between Wet and Dry samples, but that presented a typical spatial distribution that could be classified into three clear patterns (Figure 4). The first pattern represents a group of 13 dominant families that showed greater abundance in zones protected by the conservation unit (Spring and Tourism). The second pattern includes 13 other dominant families whose distribution was not associated with any land-use zone. The third pattern, which represents the distribution of 10 dominant families, presented low abundance in protected zones and high abundance in a zone with no restrictions for land-use activities (Rural and Urban zones). In addition, we found that Gallionellaceae (β-Proteobacteria) and Spirochaetaceae (Spirochaetes) families showed specifically greater abundance in the Spring and Urban zones, respectively.

Our PICRUSt2 prediction approach applied for our sediment samples presented different results in some patterns of functional diversity when we compared the two available databases used by the program (BioCyc and KEGG databases) (Table S4). Because of these discrepancies, subsequent functional analyses were conducted using only KEGG database entries (KOs), since we considered that the KEGG database is more robust and more widely used than the BioCyc database. Considering the variation in KO diversity found among samples from the Land-use zones, we also verified two KO variation patterns associated with the seasonal component (Figure 5). At the Dry pattern, KOs diversity decreases from Spring to Tourism zone, increases from Tourism to Rural zone, and decreases again in the Urban zone. At the Wet pattern, there are no well-marked shifts in KO diversity between the adjacent zones, but with a descending trend over the Land-use zones, which Urban zone communities presented the lowest KO diversity levels. No variation at estimated EC diversity was found between the zones in both Wet and Dry samples. Considering the seasonal variation, we found variation in KOs diversity only for sediment communities accessed at Spring and Rural zones, with higher KO diversity estimated for Wet season samples (Figure 5, Table S5).

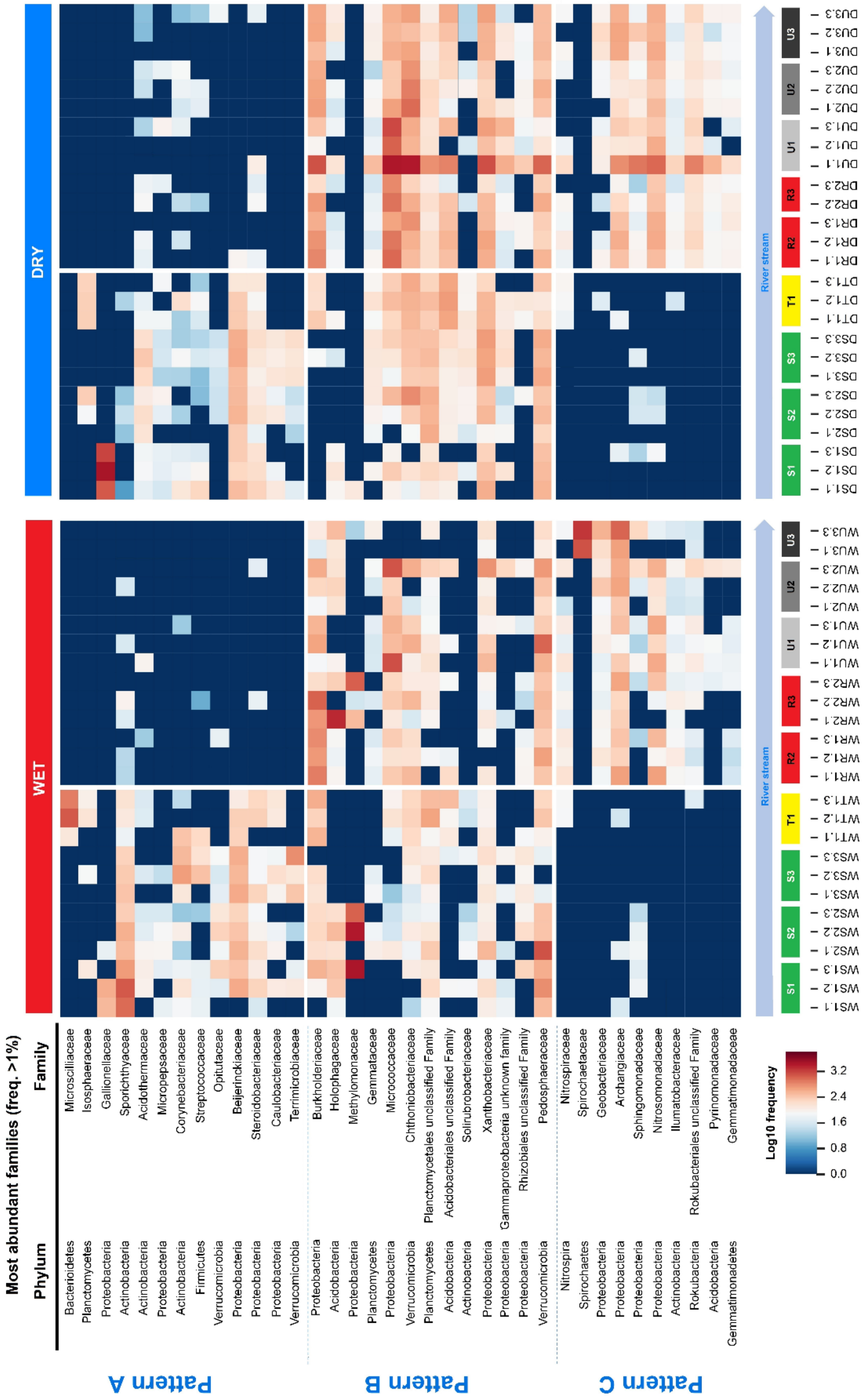


Figure 4. Heatmap diagram of dominant family abundances from each sediment sample organized by season.

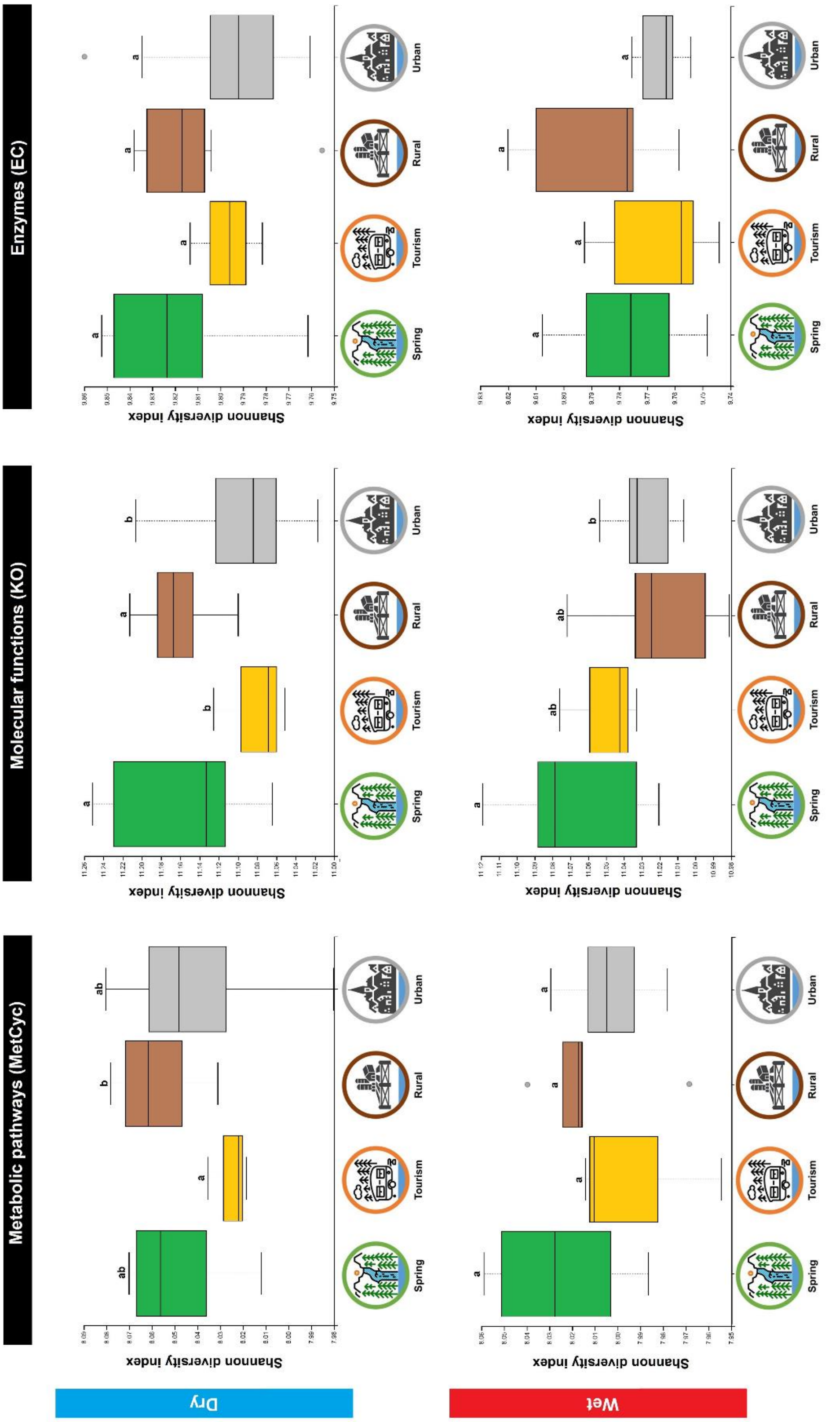


Figure 5. Diversity of metabolic functions predicted by PICRUSt2 for bacterial communities from each land-use zone organized by season.

Bacterial communities from Spring and Tourism zones showed similar KO composition at Dry season, but dissimilar patterns at Wet season. Alternatively, Rural and Urban bacterial communities exhibited different KO composition at Dry season, but similar at Wet season. In addition, the KO composition of Spring and Rural communities showed significant differences when we compared samples accessed at Dry and Wet seasons, while for Tourism and Urban samples the KO composition remained similar between the seasons (Table S6).

Table 3. Pairwise PERMANOVA tests (P_{PERM} scores for Bray-curtis metric) for metabolic function clustering by land-use groups

	Pairwise test		**Functional parameter**		
			Pathways	**KOs**	**Enzymes**
Dry season	Spring	Tourism	0.231	**0.052**	**0.024**
		Rural	0.262	0.166	0.178
		Urban	**0.007**	**0.013**	**0.005**
	Rural	Tourism	**0.031**	**0.008**	**0.007**
		Urban	**0.027**	**0.013**	**0.045**
	Urban	Tourism	**0.016**	0.106	**0.023**
			Pathways	**KOs**	**Enzymes**
Wet season	Spring	Tourism	0.155	0.385	0.202
		Rural	**0.002**	**0.002**	**0.001**
		Urban	**0.001**	**0.001**	**0.001**
	Rural	Tourism	**0.021**	**0.014**	**0.019**
		Urban	0.325	0.481	**0.042**
	Urban	Tourism	**0.007**	**0.008**	**0.01**

Our approach of structuring the predicted KO diversity at secondary levels of KEGG Pathway Hierarchy (KEGG Level 2) revealed that the most abundant KEGG Level 2 categories in sediment samples were related to basic metabolic functions such as amino acid, carbohydrate and cofactor/vitamin metabolism; protein synthesis (translation); and membrane transport (Figure 6). However, some categories related to anthropogenic impacts have also present among the most abundant categories such as "Drug resistance" and "Xenobiotic degradation and metabolism".

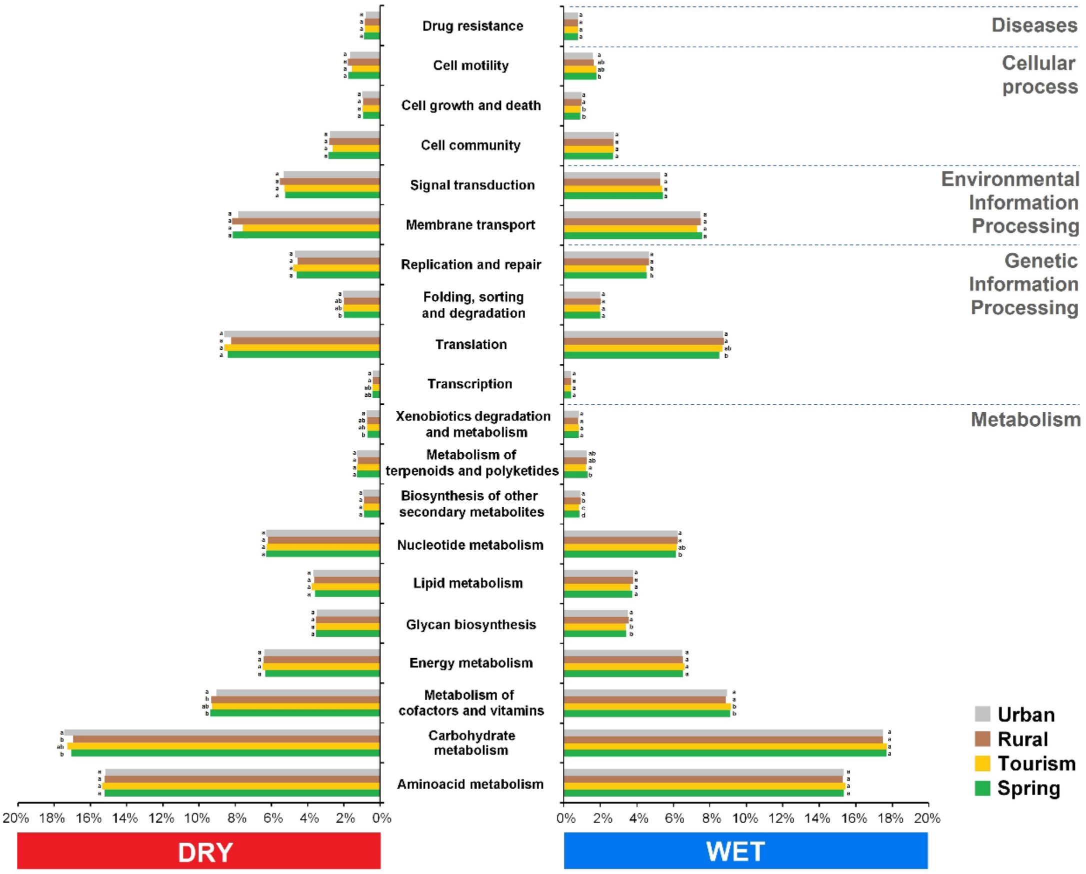
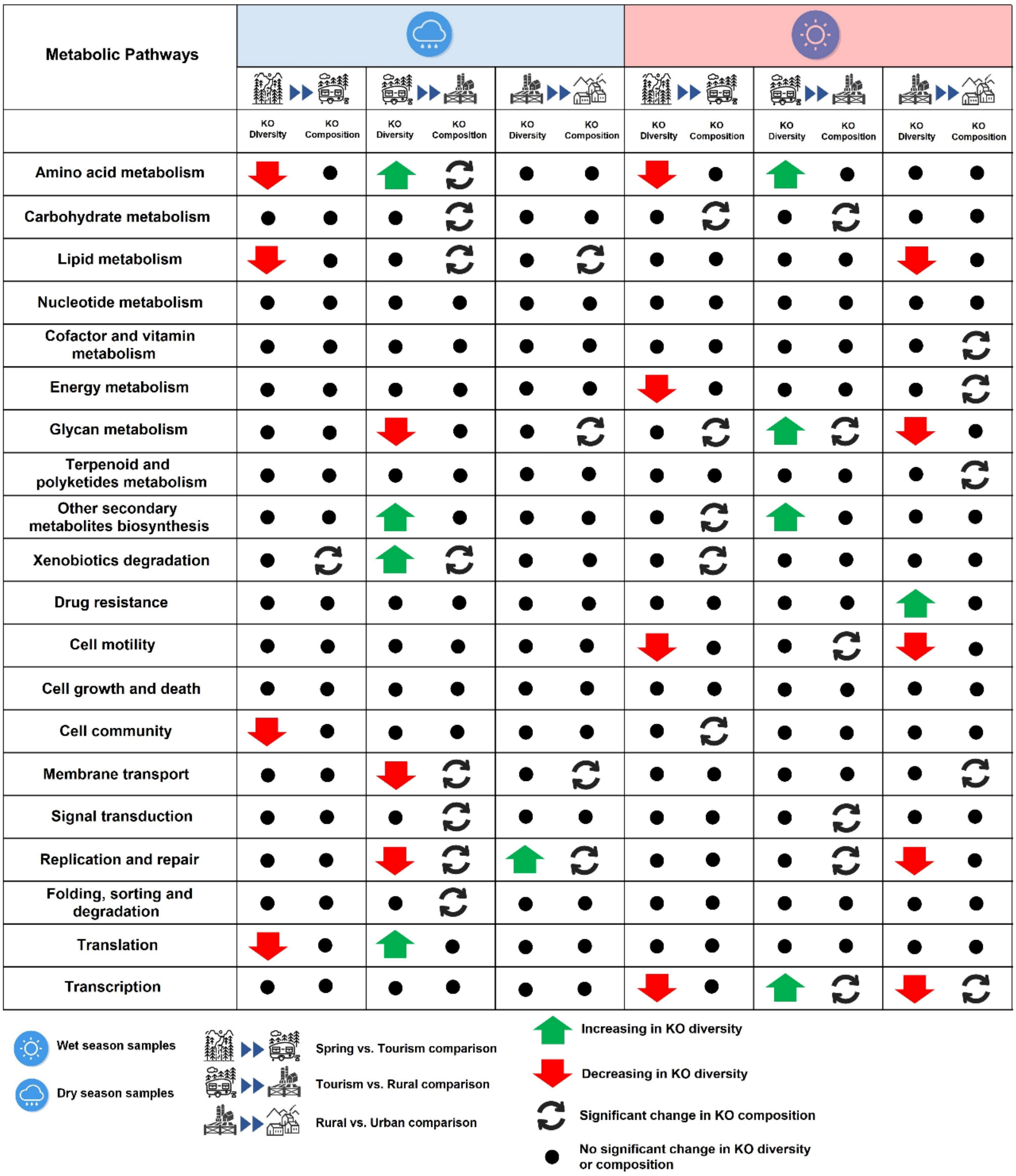


Figure 6. Relative abundance of predicted metabolic functions classified by Secondary Level of KEGG Pathway hierarchy and organized by season. Functional groups with less than < 1% relative abundance were not included.

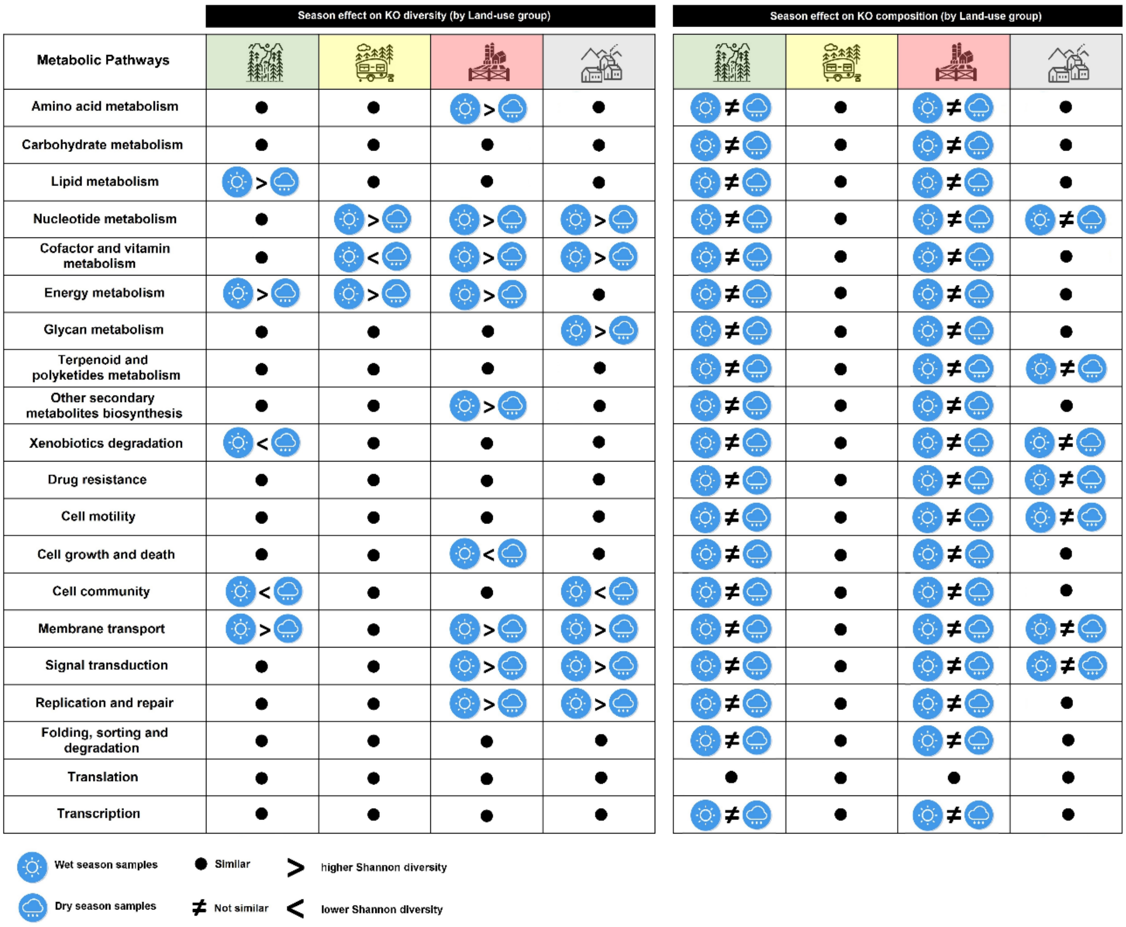
We could not verify significant KO diversity changes between adjacent Land-use zones for 11 of the 20 KEGG Level 2 categories (55%) in both seasons. For the other categories, KO diversity between adjacent zones varied differently between Wet and Dry samples (Figure 7, Table S7). At Dry samples, the most of KO diversity shifts were verified in Rural-Urban comparisons, while at Wet samples, most of KO diversity shifts were verified in Tourism-Rural comparisons. In addition, seven of the 20 KEGG Level 2 categories showed no changes in KO diversity when we compared samples from the same land-use zone collected at different seasons (Figure 8, Table S8). The Rural zone presented the highest number of KEGG Level 2 categories with changes in KO diversity between seasons, with the highest KO diversity levels for samples accessed at Dry season (except for “Cell growth and death” category).



Metabolic Pathways	Dry season samples						Wet season samples					
	Spring vs. Tourism comparison		Tourism vs. Rural comparison		Rural vs. Urban comparison		Spring vs. Tourism comparison		Tourism vs. Rural comparison		Rural vs. Urban comparison	
	KO Diversity	KO Composition	KO Diversity	KO Composition	KO Diversity	KO Composition	KO Diversity	KO Composition	KO Diversity	KO Composition	KO Diversity	KO Composition
Amino acid metabolism	⬇	●	⬆	🔄	●	●	⬇	●	⬆	●	●	●
Carbohydrate metabolism	●	●	●	🔄	●	●	●	🔄	●	🔄	●	●
Lipid metabolism	⬇	●	●	🔄	●	🔄	●	●	●	●	⬇	●
Nucleotide metabolism	●	●	●	●	●	●	●	●	●	●	●	●
Cofactor and vitamin metabolism	●	●	●	●	●	●	●	●	●	●	●	🔄
Energy metabolism	●	●	●	●	●	●	⬇	●	●	●	●	🔄
Glycan metabolism	●	●	⬇	●	●	🔄	●	🔄	⬆	🔄	⬇	●
Terpenoid and polyketides metabolism	●	●	●	●	●	●	●	●	●	●	●	🔄
Other secondary metabolites biosynthesis	●	●	⬆	●	●	●	●	🔄	⬆	●	●	●
Xenobiotics degradation	●	🔄	⬆	🔄	●	●	●	🔄	●	●	●	●
Drug resistance	●	●	●	●	●	●	●	●	●	●	⬆	●
Cell motility	●	●	●	●	●	●	⬇	●	●	🔄	⬇	●
Cell growth and death	●	●	●	●	●	●	●	●	●	●	●	●
Cell community	⬇	●	●	●	●	●	●	🔄	●	●	●	●
Membrane transport	●	●	⬇	🔄	●	🔄	●	●	●	●	●	🔄
Signal transduction	●	●	●	🔄	●	●	●	●	●	🔄	●	●
Replication and repair	●	●	⬇	🔄	⬆	🔄	●	●	●	🔄	⬇	●
Folding, sorting and degradation	●	●	●	🔄	●	●	●	●	●	●	●	●
Translation	⬇	●	⬆	●	●	●	●	●	●	●	●	●
Transcription	●	●	●	●	●	●	⬇	●	⬆	🔄	⬇	🔄

Figure 7. KO diversity and composition comparisons between adjacent zones, structured by Secondary Level of KEGG Pathway hierarchy and organized by season.

Considering KO composition changes by KEGG Level 2, we observed that the largest number of categories with significant changes in their KO composition was found for Tourism-Rural comparison, representing a transition from a protected zone to a land-use zone with no legal protection (Figure 7, Table S9). On the other hand, the transition from Spring to Tourism zones showed different patterns in KO composition for Dry and Wet samples, in which changes were observed in only one category (Xenobiotics degradation and metabolism) at Wet sampling whereas we identified five categories with significant changes at Dry sampling. Seasonal changes in KO composition were verified for all, except for the "Translation" category (Figure 8, Table S10). The Spring and Rural zones presented the largest number of categories with changes (19 of 20) and the Tourism zone presented no seasonal changes in KEGG Level 2 categories.



	Season effect on KO diversity (by Land-use group)				Season effect on KO composition (by Land-use group)			
Metabolic Pathways	🌲	🚐	🏭	🏡	🌲	🚐	🏭	🏡
Amino acid metabolism	●	●	☼>☁	●	☼≠☁	●	☼≠☁	●
Carbohydrate metabolism	●	●	●	●	☼≠☁	●	☼≠☁	●
Lipid metabolism	☼>☁	●	●	●	☼≠☁	●	☼≠☁	●
Nucleotide metabolism	●	☼>☁	☼>☁	☼>☁	☼≠☁	●	☼≠☁	☼≠☁
Cofactor and vitamin metabolism	●	☼<☁	☼>☁	☼>☁	☼≠☁	●	☼≠☁	●
Energy metabolism	☼>☁	☼>☁	☼>☁	●	☼≠☁	●	☼≠☁	●
Glycan metabolism	●	●	●	☼>☁	☼≠☁	●	☼≠☁	●
Terpenoid and polyketides metabolism	●	●	●	●	☼≠☁	●	☼≠☁	☼≠☁
Other secondary metabolites biosynthesis	●	●	☼>☁	●	☼≠☁	●	☼≠☁	●
Xenobiotics degradation	☼<☁	●	●	●	☼≠☁	●	☼≠☁	☼≠☁
Drug resistance	●	●	●	●	☼≠☁	●	☼≠☁	☼≠☁
Cell motility	●	●	●	●	☼≠☁	●	☼≠☁	☼≠☁
Cell growth and death	●	●	☼<☁	●	☼≠☁	●	☼≠☁	●
Cell community	☼<☁	●	●	☼<☁	☼≠☁	●	☼≠☁	●
Membrane transport	☼>☁	●	☼>☁	☼>☁	☼≠☁	●	☼≠☁	☼≠☁
Signal transduction	●	●	☼>☁	☼>☁	☼≠☁	●	☼≠☁	☼≠☁
Replication and repair	●	●	☼>☁	☼>☁	☼≠☁	●	☼≠☁	●
Folding, sorting and degradation	●	●	●	●	☼≠☁	●	☼≠☁	●
Translation	●	●	●	●	●	●	●	●
Transcription	●	●	●	●	☼≠☁	●	☼≠☁	●

☼ Wet season samples
☁ Dry season samples
● Similar
≠ Not similar
\> higher Shannon diversity
< lower Shannon diversity

Figure 8. KO diversity and composition comparisons between Wet and Dry samples for each land-use zone structured by Secondary Level of KEGG Pathway hierarchy and organized by season.

Discussion

The first goal of this work was to generate appropriate evidence, using 16S metabarcoding approaches, to investigate whether the taxonomic and functional structure of sediment bacterial communities from São Francisco River headwaters are influenced by local landscape components, specifically the occurrence of typical land-use practices and seasonal climatic changes. Several studies have been conducted recently with the purpose to understand the impact of seasonal component on bacterial communities of river bed sediments (Zhang et al., 2019; Liu et al., 2019; Roberto et al., 2018). However, the vast majority of these studies have a limited application to Savanna environments due local particularities of seasonal climate.

Our findings revealed that variation in bacterial diversity of sediment communities was associated with changes in land-use practices, but also the mode in which the bacterial communities respond to these anthropogenic changes were influenced by the seasonal component so that sediment communities from Spring and Rural zones showed susceptible to seasonal changes (with higher bacterial diversity at Wet season) while the bacterial communities from Tourism and Rural zones maintained the taxonomic diversity between the seasons (Figure 2). The taxonomic composition of accessed sediment communities presented a significant association with the land-use zones, exhibiting different patterns of bacterial composition for each land-use zone. In addition to the land-use effect, we also verified that all sediment bacterial communities when accessed at different seasons presented distinct taxonomic composition.

The high seasonality of the Cerrado biome causes regular natural shifts in hydrological characteristics of the headwaters section of the São Francisco River basin (Costa et al., 2017). At Spring and Tourism zones, the nutrient inputs from land-use activities could be considered a minor factor of influence since these zones are located in protected areas within a conservation unit. However, the differential susceptibility to seasonal shifts presented to Spring zone communities might be related to the fact of in the Spring zone the water column is remarkably lower than at Tourism zone which makes Spring sediment environments more vulnerable to seasonal variations in water temperature, light penetration and sediment revolving activity by streamflow. Recent studies have shown that natural seasonal variations in water temperature, streamflow, turbidity, and light penetration are critical selective forces capable of causing structural changes in microbial communities from freshwater ecosystems, including riverbed sediments (Roberto et al., 2018; Duarte et al., 2016; Chiaramonte et al., 2013; Fazi et al., 2013; Zhang et al., 2012; Boyero et al., 2011; Zoppini et al., 2010; Moss et al., 2006).

São Francisco River headwaters is typical countryside landscape from a developing country, which rural activities (agriculture and livestock) that occur through the river course surroundings have influence in water quality due to the runoff of fertilizers and livestock residues from farmed land to the river (Dantas et al, 2020). It is plausible that seasonal changes in the structure of Rural zone communities be related to higher nutrient inputs from farming practices by rainfall runoff which is intensified at Wet season. Moreover, these typical inputs derived from farming practices (fertilizers, pesticides, and livestock residues) could be creating local selective pressures capable of becoming a bacterial composition of Rural zone sediment different from the other zones. Several studies that have shown that higher concentrations of organic and inorganic nutrients associated with agricultural activities may alter microbial communities and their functions on river stream and sediment (Marti and Balcázar, 2014; Lu and Lu, 2014; Drury et al., 2013; Kalia and Gosal 2011; Wakelin et al., 2008). Here, we verified that Urban zone sediment communities presented a different bacterial composition from the other sampled zones. Dantas et al. (2020) observed that the water of the São Francisco River in this urban zone presented recurring events of violations in quality standards due to sewage inputs. The sewage inputs tend to stimulate the growth of microorganisms which are likely to be physiologically adapted to this environment and also introduces microbiological contamination to the river. These factors add new components in freshwater ecosystems that lead to a new stabilizing process for bacterial communities that can result in significant changes in their composition (Drury et al., 2013; Crump et al., 2007; Lozupone et al., 2007; Read et al., 2015; Székely et al., 2013).

Similarly to other studies that characterized the composition of the sediment communities from different freshwater ecosystems, we also observed that Proteobacteria, Acidobacteria, Planctomycetes, and Verrucomicrobia phyla are dominant in sediment environments, which reinforces the evidence that this type of freshwater environment is remarkably favorable to members of these phyla (Huang et al, 2019; Zhang et al., 2019; Wang et al., 2018; Abbia et al., 2018; Roberto et al., 2018). Here, we verified that for only two dominant phyla, Cyanobacteria and Bacteroidetes, the abundance was influenced by season, in which were found more frequently in sediment samples from Dry season (Figure 3). Recent studies have evidenced that Cyanobacteria abundance is negative related to nitrate concentrations and that Bacteroidetes abundance is associated with cyanobacterial blooms so that the abundance of these two phyla is positively correlated in freshwater environments (Zhang et al., 2019; Wu et al., 2017; Wei et al. 2014). It is possible that the lower abundance of these two phyla at Wet season could be related to increasing at nitrate levels that occur at the São Francisco headwaters section associated with an increase in rainfall during the Wet season (Costa et al., 2017).

Our results of the spatiotemporal distribution of dominant families (relative frequency > 3%) revealed that the distribution of these families presented no significant association with the seasonal component, but the most of these families presented the abundance associated with the sample zone, more specifically with the protection status of the zone. Some of the dominant families classified as "Pattern A" (Figure 4), which are more abundant in protected environments (Spring and Tourism zones) and almost absent in non-protected zones (Rural and Urban) were reported for the first time in sediment environments under the low anthropogenic impact, such as Isosphaeraceae, Steroidobacteriaceae, and Terrimicrobiaceae. Still, some "Pattern A" families have already been reported in sediments from spring sediments or low-impacted areas such as Sporichthycaceae, Beijerinkiaceae, Streptococcaceae, and Corinebacteriaceae (Febria et al., 2015; Kolb and Horn, 2012; Jani et al., 2018). On the other hand, some dominant bacterial families (Pattern "C", Figure 4) were practically absent in protected environments and remarkably abundant in environments impacted by land-use practices such as Archangiaceae and Geobacteriaceae already reported in sediments from environments impacted by untreated wastewater as well Nitrosomonadaceae, Nitrospiraceae and Gammatimonadaceae identified in environments with high concentrations of nitrogenous nutrients and other fertilizing compounds (Wu et al., 2019; Huang et al., 2018; Zhang et al., 2019, Zhang et al., 2020). We consider that these dominant families (classified as Pattern A and C) constitute potential candidates as bioindicators of freshwater environmental health to be incorporated in River basin water monitoring programs.

Our second goal was to estimate whether observed shifts in the taxonomic structure of the sediment bacterial communities occurred in response to land-use transitions and seasonal changes could also be related to changes in predicted metabolic functions inherent to accessed bacterial populations from sediment communities. The results of our PICRUSt2 prediction approach showed that the sediment communities which presented significant variation in taxonomic diversity (Spring and Rural zone samples), also presented changes in the diversity of predicted metabolic functions for those communities, whereas those communities that maintained their taxonomic diversity levels (from Tourism and Urban zones), functional diversity were also maintained. These findings reinforce the classic theory of the maintenance of the ecological community, which is also valid for microbial communities, which establishes that biological communities with greater taxonomic diversity tend to exhibit greater diversity of ecological functions and more possible ecological equivalents for essential functions, resulting in an increased resilience of the community (Kirchman, 2012; Sigee, 2005). Furthermore, when we compare the diversity of functions predicted by the secondary category between communities in adjacent zones, we found that, at dry season, changes in diversity occurred in different KEGG Level 2 categories across the land-use zone transitions (Figure 7). However, at Wet season, in which nutrient runoff events from soil to the river are more intensive, the most of changes in metabolic diversity occurred at the transition from protected environments to unprotected rural environments. In addition, when we compared the variation in the diversity of metabolic functions from the sediment communities between seasons, we could verify that the most categories with significant variation were concentrated in communities from unprotected zones (Rural and Urban zones) and that the diversity of metabolic functions decrease at Wet season (Figure 8).

We could observe sediment communities presented distinct taxonomic composition among all accessed land-use zones, in both seasons. However, when we compared the compositional patterns of metabolic functions between sediment communities, the differences in functional composition were associated with patterns of taxonomic diversity, whose functional shifts were more pronounced at sediment communities of Spring and Rural zones (Figure 8). On the other hand, when we compare the composition of metabolic functions of communities from adjacent zones, we verified a similar scenario that we observed to the variation in the diversity of metabolic functions in which the most of functional shifts were verified in protected/unprotected zones transition, at Wet season. We consider that these results represent more evidence of the impact of nutrients inputs derived from land-use practices on freshwater environments, including now the sediments. In addition, at countryside landscapes dominated by rural practices and small urban settlements with inefficient sewage treatment systems, the impact of these land-use activities is intensified when there are seasonal periods of intense rainfall. The spring zone sediment communities showed no variation in the composition of metabolic functions between seasons. It is possible that this functional resilience to seasonal changes could be related to the fact of the core of microbial communities contains representatives with versatile physiologies, capable of performing a wide array of biogeochemical cycles (Roberto et al., 2018; Evans and Hofmann, 2012).

Conclusion

Our findings contributed with new evidence about the impact of typical land-use practices conducted in countryside landscapes from developing countries on riverbed bacterial communities, both in their taxonomic and functional structure. Taking into account that the maintenance of whole freshwater ecosystems is supported by ecological services provided by sediments such as degradation of molecules, biogeochemical cycles, and biomass production and these ecological roles in riverbed sediments are majority played by microorganisms, we consider the development of anthropogenic activities without effective monitoring of sediment ecosystem health can damage the freshwater ecosystems functioning as well the environmental services provided to human society by the river basins. In addition, the way in which anthropogenic impacts influence bacterial communities is highly associated with the seasonal component, in which the effects become more severe during periods of greater rainfall in which the nutrient runoff events intensify. We consider that our findings contributed with new evidence about the temporal dynamics of bacterial communities from riverbed sediment ecosystems placed in Savanna biomes whose temperature and rainfall varies dramatically between two seasons as in Cerrado biome in which the Rio São Francisco headwaters represents.

Acknowledgements

This work was granted by Instituto Federal de Minas Gerais - Edital de Pesquisa Aplicada no. 169/2015 and by Fundação de Amparo à Pesquisa de Minas Gerais - FAPEMIG (MPR-01008-16).

References

Abia, A. L. K., Alisoltani, A., Keshri, J., Ubomba-Jaswa, E., 2018. Metagenomic analysis of the bacterial communities and their functional profiles in water and sediments of the Apies River, South Africa, as a function of land use. Science of The Total Environment 616, 326-334. https://doi.org/10.1016/j.scitotenv.2017.10.322

ANA. Agência Nacional de Águas, 2015. Water Resources in Brazil: Brazilian Hydrographic Regions. Available at: http://www.snirh.gov.br/portal/snirh/centrais-de-conteudos/conjuntura-dos-recursos-hidricos/conjuntura_informe_2015.pdf. (Accessed May 18 2020). (Document in Portuguese).

Ansola, G., Arroyo, P., Miera, L.E.S., 2014. Characterisation of the soil bacterial community structure and composition of natural and constructed wetlands. Science of the Total Environment 473, 63-71. https://doi.org/10.1016/j.scitotenv.2013.11.125

Boyero, L., Pearson, R.G., Gessner, M.O., Barmuta, L.A., Ferreira, V., Graça, M.A.S., Dudgeon, D., Boulton, A.J., Callisto, M., Chauvet, E., Helson, J.E., Bruder, A., Albariño, R.J., Yule, C.M., Arunachalam, M., Davies, J.N., Figueroa, R., Flecker, A.S., Ramírez, A., Death, R.G., Iwata, T., Mathooko, J.M., Mathuriau, C., Gonçalves, J.F., Moretti, M.S., Jinggut, T., Lamothe, S., M'Erimba, C., Ratnarajah, L., Schindler, M.H., Castela, J., Buria, L.M., Cornejo, A., Villanueva, V.D., West, D.C., 2011. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. Ecology letters 14 (3), 289-294. https://doi.org/10.1111/j.1461-0248.2010.01578.x

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., Holmes, S. P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods 13 (7), 581. https://doi.org/10.1038/nmeth.3869

Callahan, B. J., McMurdie, P. J., Holmes, S. P. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal 11 (12), 2639. https://doi.org/10.1038/ismej.2017.119

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J.,Walters,W.A.,Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high throughput community sequencing data. Nature Methods 7, 335e336. https://doi.org/10.1038/nmeth.f.303

Caspi, R., Billington, R., Fulcher, C. A., Keseler, I. M., Kothari, A., Krummenacker, M., Latendresse, M., Midford, P. E., Ong, Q., Paley, S., Subhraveti, P., Karp, P.D. 2018. The MetaCyc database of metabolic pathways and enzymes. Nucleic acids research, 46 (D1), D633-D639. https://doi.org/10.1093/nar/gkx935

CBHSF. Comitê de Bacia Hidrográfica do São Francisco (2016a). Plano de Recursos Hídricos da Bacia Hidrográfica do Rio São Francisco 2016–2025. RF3 – Resumo executivo. Available at: https://cbhsaofrancisco.org.br/documentacao/plano-de-recursos-hidricos-2016-2025/. (Accessed May 18 2020). (Document in Portuguese).

CBHSF. Comitê de Bacia Hidrográfica do São Francisco (2016b). Plano de Recursos Hídricos da Bacia Hidrográfica do Rio São Francisco 2016–2025. RP3 Development Scenarios and Forecasts of the São Francisco river Basin. Available at: https://cbhsaofrancisco.org.br/documentacao/plano-de-recursos-hidricos-2016-2025/. (Accessed May 18 2020). (Document in Portuguese).

Chiaramonte, J.B., Roberto, M. do C., Pagioro, T.A., 2013. Seasonal dynamics and community structure of bacterioplankton in upper paraná river floodplain. Microbial Ecology 66, 773-783. https://doi.org/10.1007/s00248-013-0292-2

Costa, E. P., Pinto, C. C., Soares, A. L. C., Melo, L. D. V. , Oliveira, S. C. 2017. Evaluation of violations in water quality standards in the monitoring network of São Francisco River Basin, the third largest in Brazil. Environmental Monitoring Assessment 189 (11), 2–16. https://doi.org/10.1007/s10661-017-6266-y

Cottrell, M.T., Waidner, L.A., Yu, L., Kirchman, D.L., 2005. Bacterial diversity of metagenomic and PCR libraries from the Delaware River. Environmental Microbiology 7, 1883–1895. https://doi.org/10.1111/j.1462-2920.2005.00762.x

Craft, J. A., Stanford, J. A., Pusch, M., 2002. Microbial respiration within a floodplain aquifer of a large gravel-bed river. Freshwater Biology 47 (2), 251-261. https://doi.org/10.1046/j.1365-2427.2002.00803.x

Crump, B. C., Adams, H. E., Hobbie, J. E., Kling, G. W., 2007. Biogeography of bacterioplankton in lakes and streams of an arctic tundra catchment. Ecology 88 (6), 1365-1378. https://doi.org/10.1890/06-0387

Dantas, M.S., Oliveira, J.C., Pinto, C.C., Oliveira, S.C. 2020. Impact of fecal contamination on surface water quality in the São Francisco River hydrographic basin in Minas Gerais, Brazil. Journal of Water and Health 18 (1), 48-59. https://doi.org/10.2166/wh.2019.153

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. Nature Biotechnology 38, 685–688. https://doi.org/10.1038/s41587-020-0548-6

Drury, B., Rosi-Marshall, E., Kelly, J.J., 2013. Wastewater treatment effluent reduces the abundance and diversity of benthic bacterial communities in urban and suburban rivers. Applied Environmental Microbiology 79, 1897-1905. https://doi.org/10.1128/AEM.0352712

Duarte, S., Cássio, F., Ferreira, V., Canhoto, C., Pascoal, C., 2016. Seasonal variability may affect microbial decomposers and leaf decomposition more than warming in streams. Microbial Ecology 72, 263-276. https://doi.org/10.1007/s00248-016-0780-2.

Evans, T.G., Hofmann, G.E., 2012. Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. Philosophical Transations of the Royal Society B 367, 1733-1745. https://doi.org/10.1098/rstb.2012.0019

Fazi, S., Vázquez, E., Casamayor, E.O., Amalfitano, S., Butturini, A., 2013. Stream hydrological fragmentation drives bacterioplankton community composition. PLoS One 8, 1-10. https://doi.org/10.1371/journal.pone.0064109.

Febria, C. M., Hosen, J. D., Crump, B. C., Palmer, M. A., Williams, D. D. 2015. Microbial responses to changes in flow status in temporary headwater streams: a cross-system comparison. Frontiers in microbiology 6, 522. https://doi.org/10.3389/fmicb.2015.00522

Gibbons, S.M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., Hooker, J., Madsen, R., Coleman, M.L., Gilbert, J.A., 2014. Human and environmental impacts on river sediment microbial communities. PLoS One 9, e97435. https://doi.org/10.1371/journal.pone.0097435.

Gilbert, J.A., Jansson, J.K., Knight, R. 2014. The Earth Microbiome project: successes and aspirations. BMC Biology. 12, 69. https://doi.org/10.1186/s12915-014-0069-1

Gutknecht, J.L., Goodman, R.M., Balser, T.C., 2006. Linking soil process and microbial ecology in freshwater wetland ecosystems. Plant and Soil, 289 (1-2), 17-34. https://doi.org/10.1007/s11104-006-9105-4

Hu, A., Ju, F., Hou, L., Li, J., Zhi, X., Wang, H., Mulla, S.I., Sun, Q., Burgmann, H., Yu, C-P., 2017. Strong impact of anthropogenic contamination on the co-occurrence patterns of a riverine microbial community. Environmental Microbiology 19, 4933–5009. https://doi.org/10.1111/1462-2920

Huang, W., Chen, X., Wang, K., Chen, J., Zheng, B., Jiang, X., 2019. Comparison among the microbial communities in the lake, lake wetland, and estuary sediments of a plain river network. MicrobiologyOpen 8 (2), e00644. https://doi.org/10.1002/mbo3.644

Huang, S., Chen, C., Jaffé, P.R., 2018. Seasonal distribution of nitrifiers and denitrifiers in urban river sediments affected by agricultural activities. *Science of The Total Environment*, *642*, 1282-1291. https://doi.org/10.1016/j.scitotenv.2018.06.116

Jani, K., Ghattargi, V., Pawar, S., Inamdar, M., Shouche, Y., Sharma, A., 2018. Anthropogenic activities induce depletion in microbial communities at urban sites of the river Ganges. Current microbiology 75 (1), 79-83. https://doi.org/10.1007/s00284-017-1352-5

Kalia, A., & Gosal, S. K., 2011. Effect of pesticide application on soil microorganisms. Archives of Agronomy and Soil Science 57 (6), 569-596. https://doi.org/10.1080/03650341003787582

Kanehisa, M., Goto, S., 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Research 28 (1), 27-30. https://doi.org/10.1093/nar/28.1.27

Keesstra, S. D., Geissen, V., Mosse, K., Piiranen, S., Scudiero, E., Leistra, M., van Schaik, L., 2012. Soil as a filter for groundwater quality. Current Opinion in Environmental Sustainability 4 (5), 507-516. https://doi.org/10.1016/j.cosust.2012.10.007

Kirchman, D. L., 2018. Processes in microbial ecology. Oxford University Press.

Kolb, S., Horn, M. A. 2012. Microbial CH4 and N2O consumption in acidic wetlands. Frontiers in microbiology 3, 78. https://doi.org/10.3389/fmicb.2012.00078

Liu, J., Chen, X., Shu, H. Y., Lin, X. R., Zhou, Q. X., Bramryd, T., Shu, W-S, Huang, L. N. 2018. Microbial community structure and function in sediments from e-waste contaminated rivers at Guiyu area of China. Environmental Pollution 235, 171-179. https://doi.org/10.1016/j.envpol.2017.12.008

Liu, Y., Tong, T., Li, B., Xie, S., 2019. Dynamics of bacterial communities in a river water treatment wetland. Annals of microbiology 69 (6), 637-645. https://doi.org/10.1007/s13213-019-01454-x

Lliro, M., Inceoglu, O., Garcia-Armisen, T., Anzil, A., Leporcq, B., Pigneur, L., Viroux, L., Darchambeau, F., Descy, J., Servais, P., 2014. Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. PLoS One 9, 1–27. https://doi.org/10.1371/journal.pone.0116145

Lozupone, C.A., Hamady, M., Kelley, S.T., Knight, R., 2007. Quantitative and qualitative β-diversity measures lead to different insights into factors that structure microbial communities. Applied and Environmental Microbiology 73 (5), 1576–1585. https://doi.org/10.1128/AEM.01996-06

Lu, X.-M., Lu, P-Z., 2014. Characterization of bacterial communities in sediments receiving various wastewater effluents with high-throughput sequencing analysis. Microbial Ecology 67, 612-623. https://doi.org/10.1007/s00248-014-0370-0

Marti, E., Balcázar, J.L., 2014. Use of pyrosequencing to explore the benthic bacterial community structure in a river impacted by wastewater treatment plant discharges. Research in Microbiology. 165, 468-471. https://doi.org/10.1016/j.resmic.2014.04.002

Moss, J.A., Nocker, A., Lepo, J.E., Snyder, R.A., 2006. Stability and change in estuarine biofilm bacterial community diversity. Applied and Environmental Microbiology 72, 5679-5688. https://doi.org/10.1128/AEM.02773-05

Oribhabor, B.J., Udoidiong, O.M., Udoh, D.F., Akpan, B.E., 2013. Evaluation of the ecological impact of human settlement on the water quality of Lower Cross River, Nigeria. Journal of Ecosystems, 840295. https://doi.org/10.1155/2013/840295

Porter, T. M., Hajibabaei, M. 2018. Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. Molecular Ecology. 27 (2), 313-338. https://doi.org/10.1111/mec.14478

QIIME 2 Development Team. 2017. QIIME 2. https://docs.qiime2.org

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41, D590–D596. https://doi.org/10.1093/nar/gks1219

Read, D.S., Gweon, H.S., Bowes, M.J., Newbold, L.K., Field, D., Bailey, M.J., Griffiths, R.I., 2015. Catchment-scale biogeography of riverine bacterioplankton. ISME Journal 9 (2), 516. https://doi.org/10.1038/ismej.2014.166

Roberto, A. A., Van Gray, J. B., Leff, L. G. 2018. Sediment bacteria in an urban stream: spatiotemporal patterns in community composition. Water Research. 134, 353-369. https://doi.org/10.1016/j.watres.2018.01.045

Savio, D., Sinclair, L., Ijaz, U.Z., Parajka, J., Reischer, G.H., Stadler, P., Blaschke, A.P., Blöschl, G., Mach, R.L., Kirschner, A.K.T., Farnleitner, A.H., Eiler, A., 2015. Bacterial diversity along a 2600 km river continuum. Environmental Microbiology 17 (12), 4994-5007. https://doi.org/10.1111/1462-2920.12886

Sigee, D., 2005. Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment. John Wiley & Sons.

Silva, F.A.M., 2008. Clima do bioma Cerrado. *In:* Agricultura tropical: quatro décadas de inovações tecnológicas, institucionais e políticas, p. 93-148. (Book chapter in Portuguese).

Sorokin, D.Y., Berben, T., Melton, E.D., Overmars, L., Vavourakis, C.D., Muyzer, G., 2014. Microbial diversity and biogeochemical cycling in soda lakes. Extremophiles 18, 791–809. https://doi.org/10.1007/s00792-014-0670-9

Staley, C., Unno, T., Gould, T.J., Jarvis, B., Phillips, J., Cotner, J.B., Sadowsky, M.J., 2013. Application of Illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River. Journal of Applied Microbiology 115, 1147-1158. https://doi.org/10.1111/jam.12323

Székely, A.J., Berga, M., Langenheder, S., 2013. Mechanisms determining the fate of dispersed bacterial communities in new environments. ISME Journal 7, 61-71. https://doi.org/10.1038/ismej.2012.80

Taylor, K.G., Owens, P.N., 2009). Sediments in urban river basins: a review of sediment–contaminant dynamics in an environmental system conditioned by human activities. Journal of soils and sediments 9 (4), 281-303. https://doi.org/10.1007/s11368-009-0103-z

Vignesh, S., Dahms, H.U., Emmanuel, K.V., Gokul, M.S., Muthukumar, K., Kim, B.R., James, R.A. 2014. Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India. Environmental monitoring and assessment 186 (3), 1875-1887. https://doi.org/10.1007/s10661-013-3501-z

Villéger, S., Blanchet, S., Beauchard, O., Oberdorff, T., Brosse, S., 2011. Homogenization patterns of the world’s freshwater fish faunas. Proceedings of the National Academy of Sciences 108 (44), 18003-18008. https://doi.org/10.1073/pnas.1107614108

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. Nature 467 (7315), 555. https://doi.org/10.1038/nature09440

Wakelin, S.A., Colloff, M.J., Kookana, R.S., 2008. Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. Applied and Environmental Microbiology 74, 2659-2668. https://doi.org/10.1128/AEM.02348-07

Wang, P., Chen, B., Yuan, R., Li, C., Li, Y., 2016. Characteristics of aquatic bacterial community and the influencing factors in an urban river. Science of the Total Environment 569, 382-389. https://doi.org/10.1016/j.scitotenv.2016.06.130

Wang, P., Wang, X., Wang, C., Miao, L., Hou, J., Yuan, Q., 2017. Shift in bacterioplankton diversity and structure: influence of anthropogenic disturbances along the Yarlung Tsangpo River on the Tibetan Plateau, China. Scientific Reports 7 (1), 12529. https://doi.org/10.1038/s41598-017-12893-4

Wang, P., Zhao, J., Xiao, H., Yang, W., Yu, X., 2018. Bacterial community composition shaped by water chemistry and geographic distance in an anthropogenically disturbed river. Science of Total Environment 655, 61–69. https://doi.org/10.1016/j.scitotenv.2018.11.234

Wei, Z., Xinlong, B., Limin, Z., Anqi, W., Yongzhen, P., 2014. Population dynamics of nitrifying bacteria for nitritation achieved in Johannesburg (JHB) process treating municipal wastewater. Bioresource Technology 162, 30–37. https://doi.org/10.1016/j.biortech.2014.03.102

Weijters, M.J., Janse, J.H., Alkemade, R., Verhoeven, J.T., 2009. Quantifying the effect of catchment land use and water nutrient concentrations on freshwater river and stream biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems 19 (1), 104-112. https://doi.org/10.1002/aqc.989

Williamson, C.E., Dodds, W., Kratz, T.K., Palmer, M.A., 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment. 6, 247–254. https://doi.org/10.1890/070140

Winter, C., Hein, T., Kavka, G., Mach, R.L., Farnleitner, A.H., 2007. Longitudinal changes in the bacterial community composition of the Danube River: a whole-river approach. Applied and Environmental Microbiology 73, 421–431. https://doi.org/10.1128/AEM.01849-06

Wu, H., Zeng, G., Liang, J., Guo, S., Dai, J., Lu, L., Wei, Z., Xu., P., Li, F., Yuan, Y., He, X., 2015. Effect of early dry season induced by the Three Gorges Dam on the soil microbial biomass and bacterial community structure in the Dongting Lake wetland. Ecological Indicators 53, 129-136. https://doi.org/10.1016/j.ecolind.2015.01.041

Wu, H., Li, Y., Zhang, J., Niu, L., Zhang, W., Cai, W., Zhu, X., 2017. Sediment bacterial communities in a eutrophic lake influenced by multiple inflow-rivers. Environmental Science and Pollution Research 24, 19795–19806. https://doi.org/10.1007/s11356-017-9602-4

Wu, J., Xu, Z., Li, H., Li, P., Wang, M., Xiong, L., Zhang, J., 2019. Long-term effect of water diversion and CSOs on the remediation of heavy metals and microbial community in river sediments. Water Science and Technology 79 (12), 2395-2406. https://doi.org/10.2166/wst.2019.242

Zeglin, L.H., 2015. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. Frontiers in Microbiology 6, 454. https://doi.org/10.3389/fmicb.2015.00454

Zhang, M., Yu, N., Chen, L., Jiang, C., Tao, Y., Zhang, T., Chen, J., Xue, D., 2012. Structure and seasonal dynamics of bacterial communities in three urban rivers in China. Aquatic Science 74, 113-120. https://doi.org/10.1007/s00027-011-0201-z

Zhang, L., Zhao, T., Wang, Q., Li, L., Shen, T., & Gao, G. (2019). Bacterial community composition in aquatic and sediment samples with spatiotemporal dynamics in large, shallow, eutrophic Lake Chaohu, China. Journal of Freshwater Ecology 34 (1), 575-589. https://doi.org/10.1080/02705060.2019.1635536

Zhang, S., Wang, J., & Hao, X. (2020). Fertilization accelerates the decomposition of microplastics in mollisols. Science of The Total Environment 722, 137950. https://doi.org/10.1016/j.scitotenv.2020.137950

Zoppini, A., Amalfitano, S., Fazi, S., Puddu, A., 2010. Dynamics of a benthic microbial community in a riverine environment subject to hydrological fluctuations (Mulargia River, Italy). Hydrobiologia 657, 37-51. https://doi.org/10.1007/s10750-010-0199-6

Supplementary material

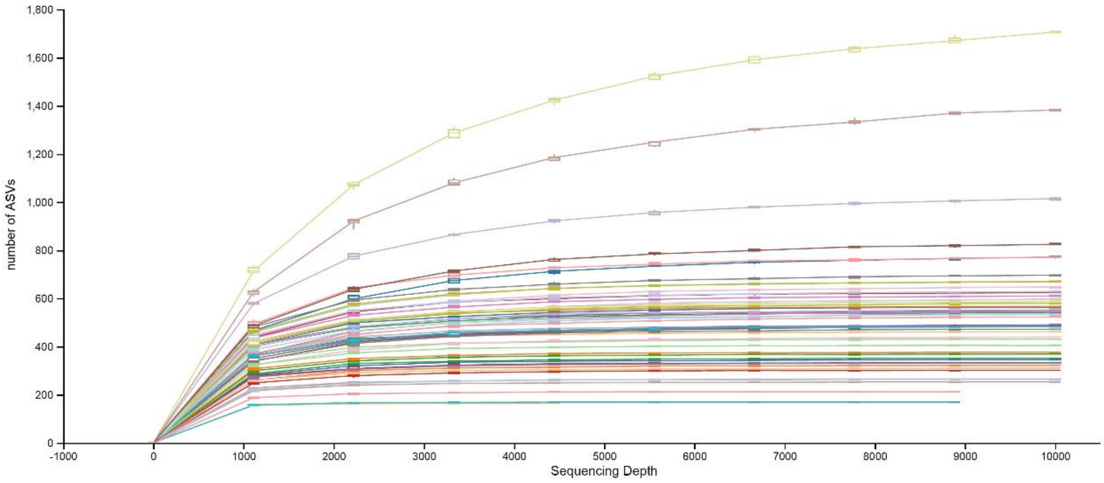


Figure S1. Rarefaction curves of observed ASVs for sediment samples from São Francisco River headwaters.

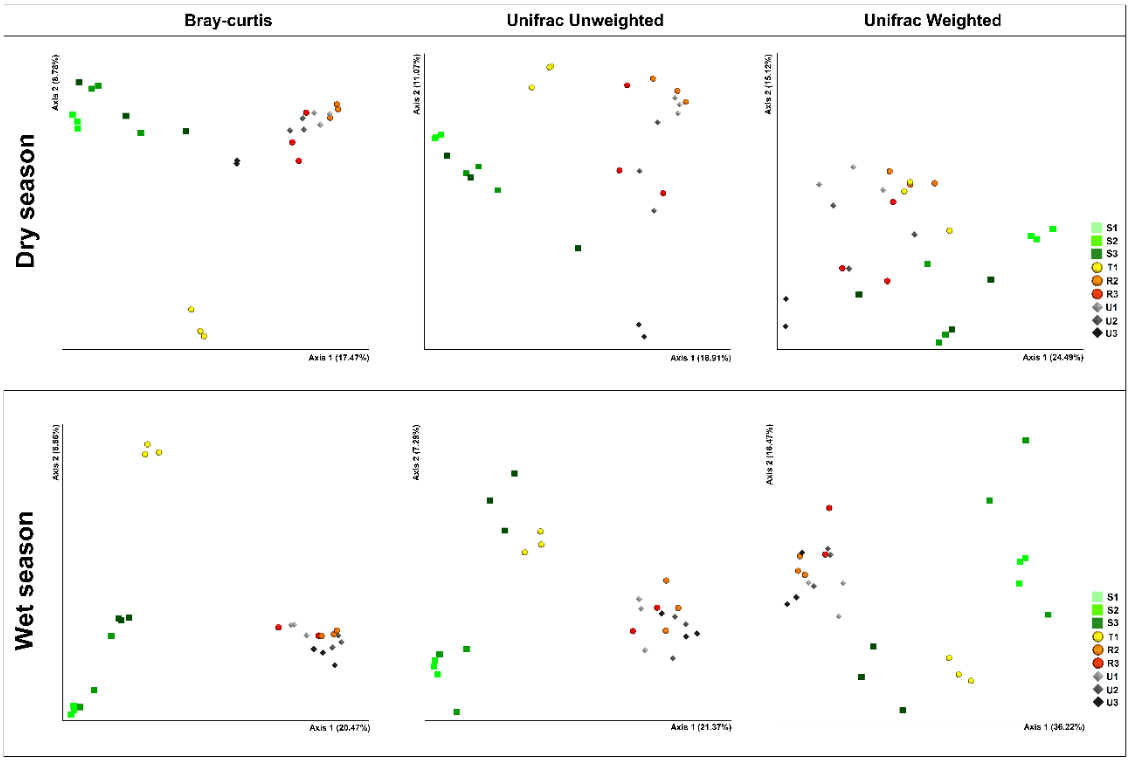


Figure S2. Principal Coordinate Analysis (PCoA) plots of the dissimilarity (Bray-Curtis, Unweighted and Weighted metrics) in bacterial community composition organized by season.

Table S1. Pairwise Kruskal-Wallis tests (p-values) for taxonomic alpha-diversity indices by land-use groups

Dry season					
		Observed ASVs	**Shannon**	**Evenness**	**Phylogenetic diversity**
Spring	**Tourism**	0.012	0.012	0.012	0.012
	Rural	0.047	1.000	0.479	1.000
	Urban	0.923	0.335	0.083	0.248
Rural	**Tourism**	0.020	0.020	0.020	0.038
	Urban	0.438	0.366	0.245	0.245
Urban	**Tourism**	0.540	0.540	0.102	0.540
Wet season					
		Observed ASVs	**Shannon**	**Evenness**	**Phylogenetic diversity**
Spring	**Tourism**	0.165	0.229	0.517	0.517
	Rural	0.125	0.095	0.161	0.125
	Urban	0.596	0.894	0.353	0.964
Rural	**Tourism**	0.025	0.025	0.025	0.101
	Urban	0.061	0.125	0.738	0.205
Urban	**Tourism**	0.517	0.309	0.165	0.517

Table S2. P_{PERM} scores of PERMANOVA tests for three taxonomic dissimilarity between seasonal groups (Wet vs. Dry) by land-use zone

Land-use zone	Pairwise test	Bray-curtis	Unifrac Unweighted	Unifrac Weighted
Spring	Wet *vs.* Dry	0.001	0.001	0.001
Tourism		0.001	0.01	0.03
Rural		0.001	0.001	0.001
Urban		0.001	0.001	0.001

Table S3. P_{PERM} scores of PERMANOVA tests for three taxonomic dissimilarity metrics among land-use groups by season

Dry season				
		Bray-curtis	Unifrac Unweighted	Unifrac Weighted
Spring	**Tourism**	0.005	0.008	0.013
	Rural	0.001	0.001	0.003
	Urban	0.001	0.001	0.001
Rural	**Tourism**	0.013	0.01	0.02
	Urban	0.011	0.027	0.008
Urban	**Tourism**	0.006	0.013	0.005
Wet season				
		Bray-curtis	Unifrac Unweighted	Unifrac Weighted
Spring	**Tourism**	0.01	0.004	0.028
	Rural	0.001	0.002	0.002
	Urban	0.001	0.001	0.001
Rural	**Tourism**	0.018	0.024	0.028
	Urban	0.004	0.003	0.015
Urban	**Tourism**	0.006	0.006	0.005

Table S4. Picrust metabolic functions diversity analysis. Pairwise Kruskal-Wallis tests (p-values) for Shannon diversity indices by land-use groups

Dry season				
		Pathways (MetaCyc)	**Molecular functions (KO)**	**Enzymes (EC)**
Spring	**Tourism**	0.079	0.079	0.166
	Rural	0.239	0.906	0.289
	Urban	0.630	0.034	0.102
Rural	**Tourism**	0.039	0.039	0.197
	Urban	0.366	0.053	0.302
Urban	**Tourism**	0.221	0.683	0.540
Wet season				
		Pathways (MetaCyc)	**Molecular functions (KO)**	**Enzymes (EC)**
Spring	**Tourism**	0.229	0.405	0.166
	Rural	0.548	0.072	0.289
	Urban	0.102	0.037	0.102
Rural	**Tourism**	0.101	0.180	0.197
	Urban	0.317	0.548	0.302
Urban	**Tourism**	0.926	0.165	0.540

Table S5. Pairwise Kruskal-Wallis tests (p-values) for Shannon diversity indices between seasonal groups (Wet vs. Dry) by land-use zone

Land-use zone	Pairwise test	**Pathways (MetaCyc)**	**Molecular functions (KO)**	**Enzymes (EC)**
Spring	Wet *vs.* Dry	0.057	**0.001**	0.122
Tourism		0.275	0.512	0.827
Rural		**0.017**	**0.018**	**0.007**
Urban		0.290	0.248	0.068

Table S6. Picrust P_{PERM} scores of PERMANOVA tests for dissimilarity between seasonal groups (Wet vs. Dry) by land-use zone

Land-use zone	Pairwise test	**Pathways (MetaCyc)**	**Molecular functions (KO)**	**Enzymes (EC)**
Spring	Wet *vs.* Dry	0.001	0.001	0.003
Tourism		0.095	0.301	0.396
Rural		0.006	0.006	0.007
Urban		0.021	0.397	0.083

Table S7. Shannon diversity tests (Wilcoxon p-values) between KEGG Pathway categories calculated from each Land-use group of sediment samples

	Pairwise comparison		Amino acid metabolism	Carbohydrate metabolism	Cell community	Cell cycle	Cell motility	Cofactor and vitamin metabolism	Drug resistance	Energy metabolism	Folding. sorting and degradation	Glycan metabolism	Lipid metabolism	Membrane transport	Nucleotide metabolism	Replication and repair	Secondary metabolites biosynthesis	Signal transduction	Terpenoid metabolism	Transcription	Translation	Xenobiotics degradation
Dry season	Spring	Tourism	0.205	0.125	0.125	0.947	0.386	0.162	0.947	0.072	0.841	0.205	0.096	**0.039**	0.096	0.641	0.317	0.947	0.841	0.549	0.463	0.841
		Rural	**0.053**	0.297	0.101	0.881	0.180	0.297	0.180	0.101	0.180	**0.025**	0.881	0.655	0.655	0.881	**0.053**	0.655	0.881	**0.053**	0.655	0.101
		Urban	0.770	0.770	0.242	0.306	**0.040**	0.464	**0.040**	0.079	0.884	**0.008**	**0.028**	0.188	0.079	**0.019**	0.143	1.000	0.770	**0.019**	0.884	0.464
	Tourism	Rural	**0.033**	0.644	0.518	0.926	**0.021**	0.926	0.229	**0.021**	0.079	0.116	0.166	0.309	0.926	0.309	0.166	0.926	0.518	**0.052**	0.782	0.116
		Urban	0.441	**0.054**	**0.007**	0.211	**0.001**	0.773	**0.021**	0.386	0.336	0.083	**0.007**	**0.007**	0.386	0.124	0.700	0.847	0.290	**0.007**	0.336	0.211
	Rural	Urban	**0.066**	0.102	**0.041**	0.307	0.540	0.683	0.221	0.838	0.221	1.000	0.102	**0.041**	0.683	**0.025**	0.414	0.683	0.838	0.683	1.000	**0.025**
Wet season	Spring	Tourism	0.947	0.947	0.257	0.739	0.841	0.641	0.162	0.257	0.205	0.096	**0.014**	0.096	0.841	**0.028**	**0.020**	0.317	0.739	**0.020**	0.386	0.549
		Rural	0.655	0.655	0.881	0.456	0.456	0.456	0.456	0.180	0.101	**0.053**	0.297	**0.053**	0.456	**0.025**	**0.025**	0.456	0.101	0.101	**0.025**	**0.025**
		Urban	0.125	0.841	0.841	0.386	0.641	0.947	0.641	0.096	0.386	0.947	0.549	0.947	0.549	**0.053**	0.162	0.162	0.317	0.549	0.947	0.463
	Tourism	Rural	0.079	0.782	**0.033**	0.782	0.518	0.229	0.518	0.926	0.166	0.926	**0.013**	0.309	0.782	0.644	0.644	0.309	0.079	0.782	**0.033**	0.079
		Urban	0.070	0.691	0.058	0.895	0.825	0.508	0.310	0.965	0.453	**0.019**	**0.024**	**0.019**	0.965	0.965	**0.031**	**0.038**	0.566	**0.031**	0.354	0.965
	Rural	Urban	**0.013**	0.926	0.166	0.782	0.405	0.518	0.782	0.405	0.116	**0.021**	0.116	**0.021**	0.926	0.405	0.079	0.405	0.166	0.166	**0.021**	**0.052**

Table S8. Season alpha-diversity indices* comparisons for KEGG Pathway categories structured by land-use groups

Level 2 KEGG Pathways	Wet vs. Dry Wilcoxon test (p-value)			
	Spring	**Tourism**	**Rural**	**Urban**
Amino acid metabolism	0.027	0.700	0.091	0.359
Carbohydrate metabolism	0.027	0.825	0.829	0.227
Cell community	0.013	0.077	0.012	0.700
Cell cycle	0.258	1.000	0.222	0.267
Cell motility	0.001	0.100	0.012	0.060
Cofactor and vitamin metabolism	0.042	1.000	0.012	0.083
Drug resistance	0.001	0.100	0.012	0.092
Energy metabolism	0.010	0.200	0.008	0.102
Folding. sorting and degradation	0.077	0.077	0.012	0.245
Glycan metabolism	0.756	0.700	0.012	0.146
Lipid metabolism	0.004	0.100	0.008	0.229
Membrane transport	0.005	1.000	0.016	0.385
Nucleotide metabolism	0.002	0.100	0.012	0.008
Replication and repair	0.215	0.077	0.036	0.469
Second. metabolites biosynthesis	0.063	0.077	0.151	0.135
Signal transduction	0.112	0.700	0.530	0.531
Terpenoid metabolism	0.824	0.643	0.141	0.500
Transcription	0.050	0.164	0.012	0.497
Translation	0.003	0.100	0.049	0.101
Xenobiotics degradation	0.027	0.400	0.463	0.773

Table S9. Bray-Curtis dissimilarity tests (P_{PERM}) between KEGG Pathway categories calculated from each Land-use group of sediment samples

	Pairwise comparison		Amino acid metabolism	Carbohydrate metabolism	Cell community	Cell cycle	Cell motility	Cofactor and vitamin metabolism	Drug resistance	Energy metabolism	Folding. sorting and degradation	Glycan metabolism	Lipid metabolism	Membrane transport	Nucleotide metabolism	Replication and repair	Secondary metabolites biosynthesis	Signal transduction	Terpenoid metabolism	Transcription	Translation	Xenobiotics degradation
Dry season	Spring	Tourism	0.095	**0.051**	**0.031**	0.188	0.122	0.646	0.179	0.202	0.056	**0.01**	0.129	0.389	0.229	0.448	**0.053**	0.07	0.128	0.118	0.226	**0.021**
		Rural	0.473	0.46	**0.008**	0.087	0.102	0.182	0.494	0.517	0.693	0.421	0.575	0.466	0.292	0.486	0.450	0.130	0.770	0.443	0.600	0.288
		Urban	**0.003**	**0.001**	**0.014**	**0.003**	**0.001**	**0.013**	**0.048**	**0.001**	**0.034**	**0.003**	**0.001**	**0.031**	**0.003**	0.221	**0.011**	**0.001**	**0.004**	**0.001**	0.728	**0.003**
	Tourism	Rural	0.373	**0.024**	0.067	0.501	**0.051**	0.414	0.077	0.264	0.262	**0.024**	0.646	0.167	0.474	**0.052**	0.227	**0.022**	0.325	**0.038**	0.413	0.454
		Urban	**0.049**	**0.008**	0.28	0.445	0.152	0.320	0.079	**0.028**	0.14	**0.025**	0.157	0.066	0.733	**0.049**	**0.021**	0.235	0.109	0.598	0.616	**0.023**
	Rural	Urban	0.252	0.261	0.125	0.121	0.189	**0.028**	0.062	**0.016**	0.113	0.093	0.156	**0.036**	0.076	0.127	0.197	0.116	**0.012**	**0.015**	0.346	0.113
Wet season	Spring	Tourism	0.991	0.304	0.601	0.396	0.124	0.294	0.613	0.503	0.228	0.665	0.153	0.061	0.944	0.595	0.488	0.481	0.567	0.281	0.848	**0.043**
		Rural	**0.002**	**0.004**	**0.003**	**0.030**	0.796	**0.009**	0.081	0.06	**0.008**	**0.009**	**0.029**	**0.007**	**0.028**	**0.001**	**0.004**	**0.002**	**0.008**	0.057	0.645	**0.001**
		Urban	**0.012**	**0.004**	0.089	**0.001**	0.596	**0.004**	**0.025**	**0.023**	**0.005**	**0.001**	**0.003**	**0.001**	0.079	0.462	**0.003**	**0.001**	**0.003**	**0.05**	0.51	**0.001**
	Tourism	Rural	**0.050**	0.587	0.223	0.448	0.082	0.516	0.273	0.086	**0.021**	0.218	**0.017**	**0.025**	0.817	**0.024**	0.218	**0.025**	0.145	0.161	0.715	**0.021**
		Urban	0.491	0.225	0.519	**0.052**	**0.012**	0.383	0.565	0.243	**0.016**	**0.032**	**0.004**	**0.004**	0.722	**0.048**	0.287	**0.014**	0.281	0.456	0.094	**0.005**
	Rural	Urban	0.138	0.614	0.154	0.858	0.811	0.145	0.402	0.974	0.503	**0.029**	**0.013**	**0.004**	0.066	**0.01**	0.37	0.234	0.539	0.716	0.554	0.139

Table S10. Table SX.Bray-curtis dissimilarity tests (P_{PERM}) between KEGG Pathway categories

Level 2 KEGG Pathways	**Spring**	**Tourism**	**Rural**	**Urban**
Amino acid metabolism	**0.001**	0.106	**0.009**	0.07
Carbohydrate metabolism	**0.001**	0.102	**0.014**	0.105
Cell community	**0.002**	0.08	**0.012**	0.601
Cell cycle	**0.001**	0.108	**0.015**	0.007
Cell motility	**0.002**	0.087	**0.016**	**0.040**
Cofactor and vitamin metabolism	**0.004**	0.112	**0.006**	0.439
Drug resistance	**0.001**	0.101	**0.008**	**0.050**
Energy metabolism	**0.001**	0.098	**0.007**	0.301
Folding. sorting and degradation	**0.001**	0.386	**0.036**	0.329
Glycan metabolism	**0.002**	0.223	**0.008**	0.536
Lipid metabolism	**0.001**	0.108	**0.029**	0.092
Membrane transport	**0.006**	0.083	**0.005**	**0.052**
Nucleotide metabolism	**0.001**	0.207	**0.009**	**0.009**
Replication and repair	**0.001**	0.089	**0.013**	0.417
Second. metabolites biosynthesis	**0.03**	0.092	**0.008**	0.192
Signal transduction	**0.003**	0.196	**0.006**	**0.038**
Terpenoid metabolism	**0.003**	0.293	**0.011**	**0.018**
Transcription	**0.003**	0.187	**0.052**	0.08
Translation	0.262	0.572	0.449	0.555
Xenobiotics degradation	**0.001**	0.525	**0.010**	**0.004**

5. APÊNDICE – Produção Técnica

Produto Técnico: Proposta de nova metodologia de análises de corpos d'água encaminhada à Secretaria de Meio Ambiente do Estado de Minas Gerais.

Sugestão de aplicação da metodologia metabarcode como ferramenta para otimizar os resultados da análise de água padrão feitas pelo IGAM em cursos d'água em Minas Gerais

Pesquisa realizada no IFMG/Bambuí – Laboratório de Biologia Molecular

Marcos de Paula Júnior RA 0028233, Thiago Augusto da Costa Silva RA 0028234 (CRBio 087571/04-D) – Alunos do Mestrado Profissional em Sustentabilidade e Tecnologia Ambiental

Orientador: Gustavo Augusto Lacorte - Biólogo - Doutor em Genética e Evolução - CRBio 657236/04-D

Introdução

Os ecossistemas de água doce sustentam uma enorme biodiversidade e fornecem bens e serviços ambientais de importância crítica para as populações humanas. Nas últimas décadas, a saúde destes ecossistemas fluviais está sendo gravemente ameaçada por atividades humanas como a implantação de grandes projetos de irrigação, a alteração dos cursos de água naturais para construção de barragens e a poluição e eutrofização da água por descargas industriais, agrícolas e urbanas. O desafio de aliar as necessidades humanas à um mínimo impacto para ecossistemas de água doce requer a adoção de estratégias sustentáveis de gestão da água, sendo que a avaliação e monitoramento da sua qualidade constituem uma das etapas de seu gerenciamento. As abordagens de avaliação e monitoramento atuais são baseadas em análises de indicadores ecológicos (físicos, químicos e biológicos) desenvolvidos para detectar e medir mudanças nas condições ecológicas da água doce.

Por serem altamente sensíveis a estressores ambientais, os indicadores biológicos têm sido cada vez mais utilizados nos programas de gestão. Os grupos taxonômicos clássicos utilizados como bioindicadores são macrorganismos como macro invertebrados aquáticos, peixes, aves aquáticas e plantas. Devido à dificuldade de isolamento e identificação por cultura pelos métodos de microbiologia clássica, microrganismos não eram utilizados como bioindicadores de água doce. Seu uso seria vantajoso uma vez que a maioria da biomassa aquática é formada por grupos microbianos, além deles serem altamente sensíveis a distúrbios físicos, químicos e bióticos no ambiente.

Com a consolidação, nos últimos anos, de métodos moleculares independentes de cultura para identificação microbiana foi possível identificar os grupos microbianos de maneira rápida, tornando o uso de microrganismos como bioindicadores uma alternativa atraente e viável. Nesse contexto, o uso do sequenciamento massivo de fragmentos amplificados de DNA (genes ribossômicos considerados marcadores taxonômicos) derivados de amostras de ambientes fluviais poderiam ser usados para determinar a diversidade taxonômica e composição das comunidades microbianas destes ambientes.

Nos ecossistemas fluviais, água e sedimentos são as opções disponíveis para amostragem, mas a diversidade nos sedimentos é consideravelmente maior que as comunidades microbianas da água em decorrência da corrente. Sedimentos são altamente sensíveis a mudanças nas condições ambientais, tanto oriundas de atividades antropogênicas, quanto por desastres naturais (Wang et al., 2018). Como componentes importantes da migração e transformação de elementos na água do rio, os sedimentos servem como fonte em ciclos de nutrientes nos ecossistemas aquáticos que são metabolizados pelos microrganismos da comunidade do sedimento (Tao et al., 2019). Evidências recentes (Tao et al., 2019, Wang et al., 2018) revelaram que, durante a intensificação do processo de uso da terra, contaminantes são absorvidos pelas finas partículas de sedimentos e interagem com as bactérias microbianas locais causando impactos a longo prazo sobre a organização biológica das comunidades.

Microrganismos de sedimentos podem indicar a saúde dos ecossistemas das bacias hidrográficas por causa de seus papéis vitais na conversão de energia, degradação de poluentes, ciclagem biogeoquímica de nutrientes e transformação e migração de elementos (Tao et al 2019). As características dos sedimentos refletem não apenas a qualidade da água, mas também o status trófico e a função ecológica dos rios, porque os sedimentos contêm milhares de microrganismos, que interagem com a matéria orgânica e afetam diretamente os ecossistemas (Wang et al., 2018). Uma mudança na comunidade microbiana pode ser uma resposta rápida às mudanças ambientais e esta alteração de diversidade taxonômica e composição das comunidades microbianas é um indicador adequado de perturbações dentro de um ecossistema, sugerindo que as comunidades microbianas são bons indicadores ambientais (Xie et al., 2016).

Diante do exposto, este documento propõe uma nova metodologia de análise de corpos d'água, baseada nos resultados promissores de sua utilização na determinação das comunidades microbianas com potencial para uso como bioindicadores de qualidade ambiental de amostras do sedimento do leito do Rio São Francisco. Sugere-se, assim, que a metodologia metabarcode seja aplicada como ferramenta para otimizar os resultados da análise de água padrão feitas pelo IGAM em cursos d'água em Minas Gerais.

Metodologia

O DNA das amostras de sedimentos (0,25 mg/amostra) foi extraído utilizando o Kit comercial DNeasy PowerSoil Kit (Qiagen), seguindo as instruções do fabricante incluindo apenas um período de incubação à 95°C por 2 minutos para favorecer a extração de DNA de bactérias gram-positivas. A qualidade do DNA extraído foi verificada por eletroforese de uma alíquota da amostra em gel de agarose a 1,5% corado com corante não-mutagênico. A concentração do DNA extraído foi estimada por espectrofotometria utilizando o aparelho NanoDrop (ThermoFischer).

A caracterização da composição das comunidades procarióticas típicas de cada amostra foi realizada através do sequenciamento da região hipervariável V4 do gene ribossomal rDNA 16S, utilizando os oligoiniciadores 515F (5'-GTGYCAGCMGCCGCGGTAA-3') e 806R (5'-GTGYCAGCMGCCGCGGTAA-3') adicionados de adaptadores que permitem o sequenciamento simultâneo de muitas amostras por placa de sequenciamento, através da presença de uma sequência de nucleotídeos exclusiva de cada oligoiniciador (chamada "barcode") e sequências de ligação ao cartucho da plataforma Illumina MiSeq (GILBERT; JANSSON; KNIGHT, 2014).

A reação de PCR foi realizada em triplicata utilizando as condições padrões do sistema de amplificação otimizado OneTaq® Hot Start Quick-Load® 2X Master Mix with GC Buffer (New England Biolabs) num volume total de 25 µL, com concentração final de cada primer de 5 µM. As condições de amplificação foram: 1X(94ºC/3'), 25X(94ºC/45s, 50ºC/60s, 72ºC/90s), 1X(72ºC/10m, 04ºC/∞).Os amplicons produzidos em triplicata foram combinados e purificados utilizando o sistema Agencourt® AMPure ® XP (Beckman Coulter). A quantificação dos amplicons foi realizada utilizando o kit Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher/Invitrogen cat. no. P11496) seguindo as instruções do fabricante.

Os amplicon purificados foram reunidos num único tubo (pool de bibliotecas) em quantidades equitativas de amplicons (normalização das bibliotecas) de modo a não favorecer o sequenciamento de alguma amostra em detrimento às demais. As amostras foram submetidas ao sequenciamento utilizando a plataforma Illumina MiSeq no centro multiusuário de sequenciamento CEFAP, localizado no Instituto de Ciências Biomédicas da Universidade de São Paulo (ICB/USP). O sequenciamento seguiu as condições padrão do sistema Illumina MiSeq V2 de 500 ciclos, esquema de sequenciamento de "pontas pareadas" (paired-end) equitativo (2 x 250), no qual o fragmento alvo é sequenciado no sentido direto (do primeiro nucleotídeo em diante) e também no sentido reverso (a partir do último nucleotídeo em direção ao primeiro).

Todo o tratamento dos dados gerados no sequenciamento foi realizado utilizando a plataforma bioinformática de análise dados de microbiomas gerados por sequenciamento de nova geração QIIME2TM (BOLYEN et al, 2018) e seguindo os parâmetros sugeridos pelo The Earth Microbiome Project (GILBERT; JANSSON; KNIGHT, 2014). As sequências brutas (chamadas reads) de cada amostra sequenciada (tratada como biblioteca de reads) foram separadas do arquivo contendo todas as reads através do barcode identificador. A qualidade de todas as reads foi avaliada manualmente e as extremidades das reads (regiões de baixa qualidade) foram cortadas. Após este primeiro tratamento, as reads de cada biblioteca, geradas no sequenciamento direto e reverso, foram combinadas gerando reads únicas. Estas reads foram filtradas novamente, eliminando-se reads quimeras e as de baixa qualidade.

Cada uma das diferentes reads remanescente do processo de curadoria, chamadas agora de ASVs (amplicon sequencing variants) foram contabilizadas por amostra. Para atribuir cada ASV a um grupo taxonômico ou OTU (operational taxonomic unit), foi gerado um arquivo contendo todas as ASVs para ser confrontado com o banco de referência taxonômica para as comunidades de procariotos SILVA 1.32 (QUAST et al., 2012) e cada OTU foi definida pelas ASVs que apresentassem similaridade mínima de

97% com alguma sequência presente no banco (STACKEBRANDT & GOEBEL, 1994). Ao final desta análise foi gerada uma tabela (chamada OTU table) contento a lista de OTUs presentes em cada amostra e seu grupo procariótico correspondente classificado de Domínio à até o nível taxonômico mais refinado possível. Além da tabela, uma matriz relacionando quais OTUs estão presentes e sua respectiva abundância em cada biblioteca/amostra. Foi realizada também uma estimativa das relações filogenéticas entre as OTUs atribuídas.

Resultados e discussão

Utilizando a metodologia metabarcode de análise de corpos d'água descrita acima, determinou-se as comunidades microbianas de amostras do sedimento do leito do rio São Francisco, coletadas em três áreas distintas e devidamente caracterizadas como: área controle na nascente do rio, área de baixo impacto antrópico (área tampão) e área de alto impacto antrópico.

A primeira análise foi feita considerando a prevalência de filos bacterianos, como demonstrado no esquema da figura 1, que evidencia a abundância relativa de filos bacterianos nas áreas amostradas, bem como a porcentagem de cada filo em relação ao ambiente. Percebe-se que bactérias pertencentes ao filo *Cyanobacteria*, por exemplo, estão presentes em maior quantidade em ambientes sem impacto e com pouco impacto antrópico e, de comportamento oposto, as bactérias do filo *Actinobacteria* estão presentes em abundância em ambientes com alto impacto antrópico.

Grandes alterações ambientais geradas pela ação do homem parecem favorecer o crescimento de bactérias pertencentes aos filos *Cloriflexi* e *Actinobacteria*, já que eles aparecem em uma porcentagem consideravelmente mais alta nestes ambientes que em ambientes com baixo ou nenhum impacto antrópico. Assim, os dois filos poderiam ser utilizados como bioindicadores de ambientes com alto grau de impacto antrópico. De maneira oposta, bactérias pertencentes aos filos *Cyanobacteria* e *Verrucomicrobia* aparecem em uma porcentagem bem mais baixa em ambientes com alto impacto antrópico em relação aos ambientes onde a ação do homem não causou ou causou mínimo impacto. Como a porcentagem de bactérias pertencentes ao filo *Acidobacteria* no ambiente controle prevaleceu sobre sua porcentagem nos dois outros ambientes amostrados, este filo poderia ser um bioindicador para ambientes sem impacto antrópico.

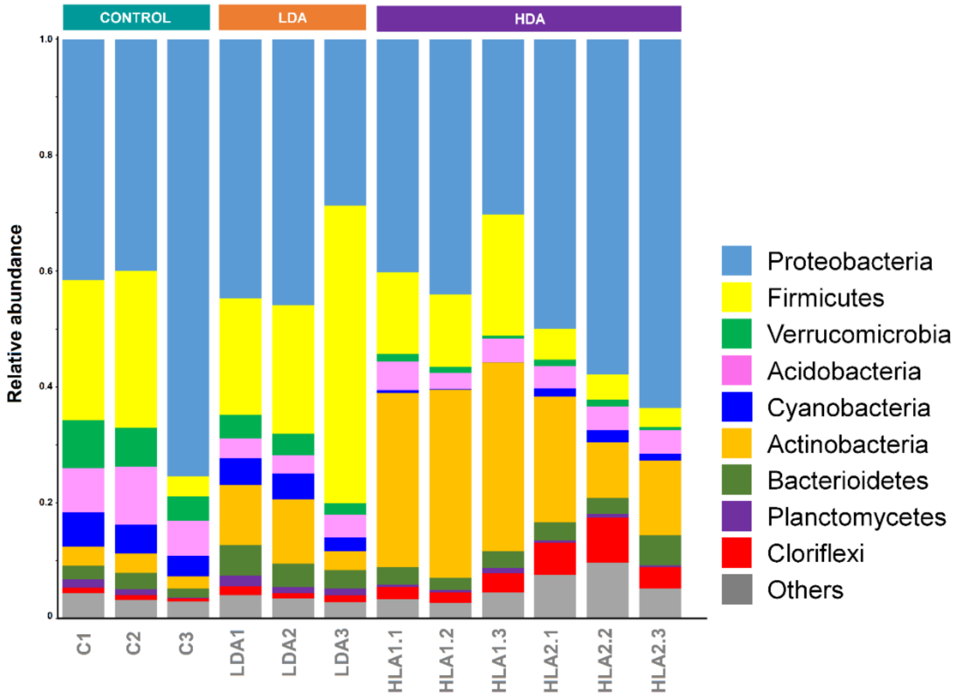


Figura 1. Gráfico de porcentagem de cada filo das comunidades bacterianas em relação ao ambiente. Nota-se a abundância de diferentes filos em cada um dos ambientes amostrados, onde as amostras C1, C2 e C3 são referentes a área controle; as amostras LDA 1, LDA2 e LDA3, a área de baixo impacto antrópico e HLA1.1, HLA1.2, HLA1.3,HLA2.1, HLA2.2 e HLA2.3 a área de alto impacto.

A composição taxonômica no nível de família das comunidades bacterianas em amostras de sedimentos de três regiões é mostrada na Figura 2. Famílias, como *Micrococcaceae* e *Anaerolinaeceae*, estão presentes em maior frequência em ambientes de alto impacto antrópico e a família *Bacillales* em ambientes de baixo impacto antrópico. Já no ambiente controle, as famílias *Pseudomonadaceae* e *Acidobacteriales* surgem em uma frequência bem maior que nos demais ambientes. Famílias como *Burkholderiaceae* e *Xanthobacteriace* parecem não ter predileção por nenhum dos ambientes amostrados, estando presente em todos eles, refletindo sua ampla capacidade adaptativa. Alguns grupos são abundantes em certos pontos amostrais, não aparecendo em outros, sugerindo que, naquele ponto, possa estar ocorrendo uma pressão seletiva que os favorece em relação aos demais, descartando-os como possíveis bioindicadores.

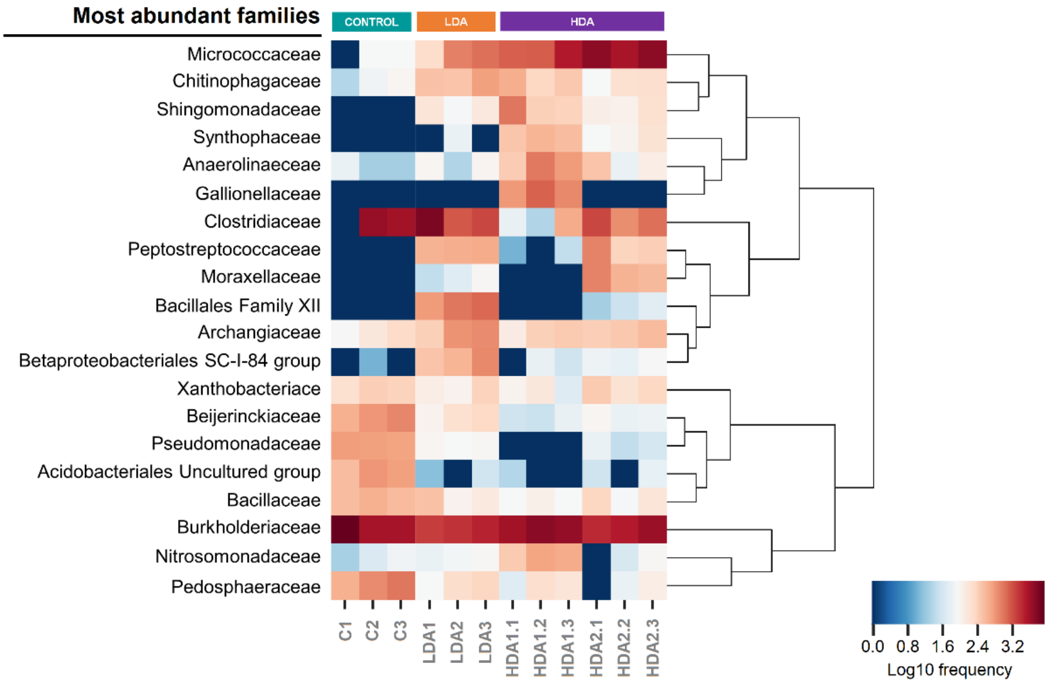


Figura 2. Mapa de calor mostrando a frequência das famílias nos ambientes amostrados, onde as amostras C1, C2 e C3 são referentes a área controle; as amostras LDA 1, LDA2 e LDA3, a área de baixo impacto antrópico e HLA1.1, HLA1.2, HLA1.3,HLA2.1, HLA2.2 e HLA2.3 a área de alto impacto

Na análise de composição de gênero, observou-se que em cada ambiente amostrado, haviam bons candidatos a organismos bioindicadores de qualidade ambiental. No ambiente controle, por exemplo, foi identificado cinco gêneros bacterianos dominantes (*Paraburkholderia*, *Streptococcus*, *Acidothermus*, *Reseiarcus e Pseudomonas*), enquanto quatro gêneros (*Anaeromyxobacter*, *Crenothrix*, *Rombustia e Clostridium*) estavam praticamente ausentes nas amostras de sedimentos deste ambiente. Também se observou os dez gêneros dominantes (*Parabuburkholderia*, *Streptococcus*, *Acidothermos*, *Roseiarcus*, *Pseudomonas*, *Bacillus*, *Bradyrhizobium*, *Clostridium* sensu stricto 1, *Clostridium* sensu stricto 12 *e Massilia*) que eram mais abundantes nas amostras de ambiente com leve impacto ambiental e outros 11 gêneros dominantes (*Curvibacter*, *Lysobacter*, *Spirochaeta*, *Sphingomonas*, *Clostridium* sensu stricto 9, *Clostridium* sensu stricto 5, *Romboutsia*, *Clostridium* sencsu stricto 13, *Clostridium* sensu stricto 1, *Clostridium* sensu stricto 12 *e Massilia*) cuja distribuição foi associada às amostras de ambientes de alto impacto ambiental.

Em síntese, foram observadas diferenças significantes nas comunidades das três áreas amostradas, cujo potencial de bioindicação de vários grupos bacterianos foram encontrados, tanto em relação ao filo, quanto à família e ao gênero.

Conclusão

Essas análises revelam, satisfatoriamente, que o uso de padrões de variação na diversidade e estrutura das comunidades microbianas do sedimento do leito do rio pode ser útil para a bioindicação da qualidade do ambiente fluvial. Portanto, considera-se que esta metodologia constitui uma ferramenta útil para diagnóstico e monitoramento de áreas cujo uso humano da terra é total ou parcialmente restrito, como áreas de preservação de nascentes ou áreas tampão, garantindo a qualidade de ambientes de água doce frágeis e vulneráveis .

O método metabarcode oferece vantagens em comparação com os métodos clássicos, como, por exemplo, uma abordagem mais moderna que mostra a dinâmica ambiental pelos grupos microbianos. Ao aplicar esse método nos monitoramentos regulares o IGAM, pode-se, dessa forma, melhorar a qualidade de seus relatórios técnicos e enriquecê-los com novos parâmetros de análises biológicas.

Thiago Augusto da Costa Silva

Marcos de Paula Júnior

Thiago Augusto da Costa Silva

Gustavo A. Lacorte

Gustavo Augusto Lacorte

Referências

1. Adhikari, N. P., Liu, Y., Liu, K., Zhang, F., Adhikari, S., Chen, Y., & Liu, X. (2019). Bacterial community composition and diversity in Koshi River, the largest river of Nepal. Ecological indicators, 104, 501-511.
2. Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... & Bai, Y. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature biotechnology, 37, 852-857.
3. Faust, K., & Raes, J. (2012). Microbial interactions: from networks to models. Nature Reviews Microbiology, 10, 538-550.
4. Gilbert, J. A., Jansson, J. K., & Knight, R. (2014). The Earth Microbiome project: successes and aspirations. BMC biology, 12, 69.
5. Guo, J., Zhao, L., Lu, W., Jia, H., & Sun, Y. (2016). Bacterial communities in water and sediment shaped by paper mill pollution and indicated bacterial taxa in sediment in Daling River. Ecological indicators, 60, 766-773.
6. Huang, W., Chen, X., Jiang, X., & Zheng, B. (2017). Characterization of sediment bacterial communities in plain lakes with different trophic statuses. MicrobiologyOpen, 6, e00503.
7. Huang, W., Chen, X., Wang, K., Chen, J., Zheng, B., & Jiang, X. (2019). Comparison among the microbial communities in the lake, lake wetland, and estuary sediments of a plain river network. MicrobiologyOpen, 8, e00644.
8. Huang, W., Chen, X., Wang, K., Chen, J., Zheng, B., & Jiang, X. (2019). Comparison among the microbial communities in the lake, lake wetland, and estuary sediments of a plain river network. MicrobiologyOpen, 8, e00644.
9. INSTITUTO MINEIRO DE GESTÃO DAS ÁGUAS. Monitoramento da Qualidade das Águas Superficiais no Estado de Minas Gerais - Relatório Trimestral - 4° Trimestre de 2017. Belo Horizonte: Igam, 2017
10. INSTITUTO MINEIRO DE GESTÃO DE ÁGUAS. Portal InfoHidro. Disponível em: <http://portalinfohidro.igam.mg.gov.br/>. Acesso em 14 de ago. de 2017
11. Karimi, B., Maron, P. A., Boure, N. C. P., Bernard, N., Gilbert, D., & Ranjard, L. (2017). Microbial diversity and ecological networks as indicators of environmental quality. Environmental chemistry letters, 15, 265-281.

12. Kurilkina, M. I., Zakharova, Y. R., Galachyants, Y. P., Petrova, D. P., Bukin, Y. S., Domysheva, V. M., ... & Likhoshway, Y. V. (2016). Bacterial community composition in the water column of the deepest freshwater Lake Baikal as determined by next-generation sequencing. *FEMS microbiology ecology*, *92*(7).
13. Liu, J., Chen, X., Shu, H. Y., Lin, X. R., Zhou, Q. X., Bramryd, T., ... & Huang, L. N. (2018). Microbial community structure and function in sediments from e-waste contaminated rivers at Guiyu area of China. Environmental Pollution, 235, 171-179.
14. Lopes, J. E. G., Barros, M. T. L., & Brandão, J. L. B. (2002). Projeto de gerenciamento integrado das atividades desenvolvidas em terra na bacia do São Francisco-subprojeto 4.4: determinação de subsídios para procedimentos operacionais dos principais reservatórios da bacia do São Francisco. Brasília: ANA. GEF/PNUMA/OEA.
15. MINISTÉRIO DO MEIO AMBIENTE. 2006. Caderno da Região Hidrográfica do São Francisco. Ministério do Meio Ambiente, Secretaria de Recursos Hídricos. Brasília: MMA, 2006. 148 p
16. Niu, L., Li, Y., Wang, P., Zhang, W., Wang, C., Li, J., & Wu, H. (2018). Development of a microbial community-based index of biotic integrity (MC-IBI) for the assessment of ecological status of rivers in the Taihu Basin, China. Ecological Indicators, 85, 204-213.
17. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research, 41, D590-D596.
18. Stackebrandt, E., & GOEBEL, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. International journal of systematic and evolutionary microbiology, 44, 846-849.
19. Staebe, K., Botes, M., Madlala, T., Oberholster, P. J., & Cloete, T. E. (2018). Microbial community diversity as a potential bioindicator of AMD and steel plant effluent in a channelled valley bottom wetland. Water, Air, & Soil Pollution, 229, 397.

20. Tao, K., Liu, Y., Ke, T., Zhang, Y., Xiao, L., Li, S., ... & Hu, T. (2019). Patterns of bacterial and archaeal communities in sediments in response to dam construction and sewage discharge in Lhasa River. Ecotoxicology and environmental safety, 178, 195-201.
21. Wang, L., Zhang, J., Li, H., Yang, H., Peng, C., Peng, Z., & Lu, L. (2018). Shift in the microbial community composition of surface water and sediment along an urban river. Science of the Total Environment, 627, 600-612.
22. Wang, L., Zhang, J., Li, H., Yang, H., Peng, C., Peng, Z., & Lu, L. (2018). Shift in the microbial community composition of surface water and sediment along an urban river. Science of the Total Environment, 627, 600-612.
23. Wang, P., Chen, B., Yuan, R., Li, C., & Li, Y. (2016). Characteristics of aquatic bacterial community and the influencing factors in an urban river. Science of the Total Environment, 569, 382-389.
24. Wei, J., Gao, J., Wang, N., Liu, Y., Wang, Y., Bai, Z., ... & Zhuang, G. (2019). Differences in soil microbial response to anthropogenic disturbances in Sanjiang and Momoge Wetlands, China. FEMS microbiology ecology, 95, fiz110.
25. Xie, Y., Wang, J., Wu, Y., Ren, C., Song, C., Yang, J., ... & Zhang, X. (2016). Using in situ bacterial communities to monitor contaminants in river sediments. Environmental Pollution, 212, 348-357.
26. Zhang, L., Zhao, T., Wang, Q., Li, L., Shen, T., & Gao, G. (2019). Bacterial community composition in aquatic and sediment samples with spatiotemporal dynamics in large, shallow, eutrophic Lake Chaohu, China. Journal of Freshwater Ecology, 34, 575-589.
27. Zinger, L., Gobet, A., & Pommier, T. (2012). Two decades of describing the unseen majority of aquatic microbial diversity. Molecular ecology, 21, 1878-1896.

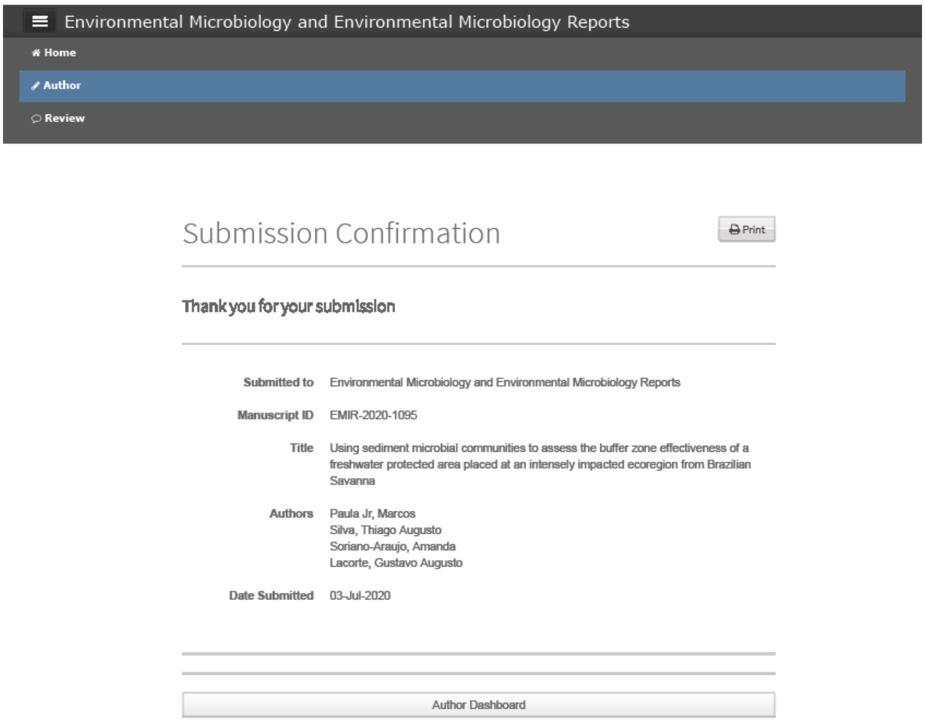
6. Considerações Finais

Diante dos dados apresentados nesta dissertação, conclui-se que o presente estudo é de grande interesse para a comunidade científica, uma vez que descreve como a comunidade microbiana do sedimento de leito do rio São Francisco, em sua cabeceira, comporta-se nos diferentes usos da terra nos ambientes estudados, além de servir de base para estudos similares. Além disso, demonstra como essas comunidades microbianas podem ser utilizadas como bioindicadores da qualidade de ambientes fluviais, apresentando-as como uma nova ferramenta de análise de corpos hídricos.

ANEXOS

1- Cópia do comprovante de submissão do manuscrito à revista Environmental Microbiology and Environmental Microbiology Reports (Capítulo 1 - **Using sediment microbial communities to assess the buffer zone effectiveness of a freshwater protected area placed at an intensely impacted ecoregion from Brazilian Savanna**)

/



Submission Confirmation

Thank you for your submission

Submitted to Environmental Microbiology and Environmental Microbiology Reports

Manuscript ID EMIR-2020-1095

Title Using sediment microbial communities to assess the buffer zone effectiveness of a freshwater protected area placed at an intensely impacted ecoregion from Brazilian Savanna

Authors Paula Jr, Marcos
Silva, Thiago Augusto
Soriano-Araujo, Amanda
Lacorte, Gustavo Augusto

Date Submitted 03-Jul-2020

2- Cópia do comprovante de submissão do manuscrito à revista Journal of Soils and Sediments (Capítulo 2 - **Spatial distribution of sediment bacterial communities from São Francisco River headwaters is influenced by human land-use activities and seasonal climate shifts**)

Journal of Soils and Sediments

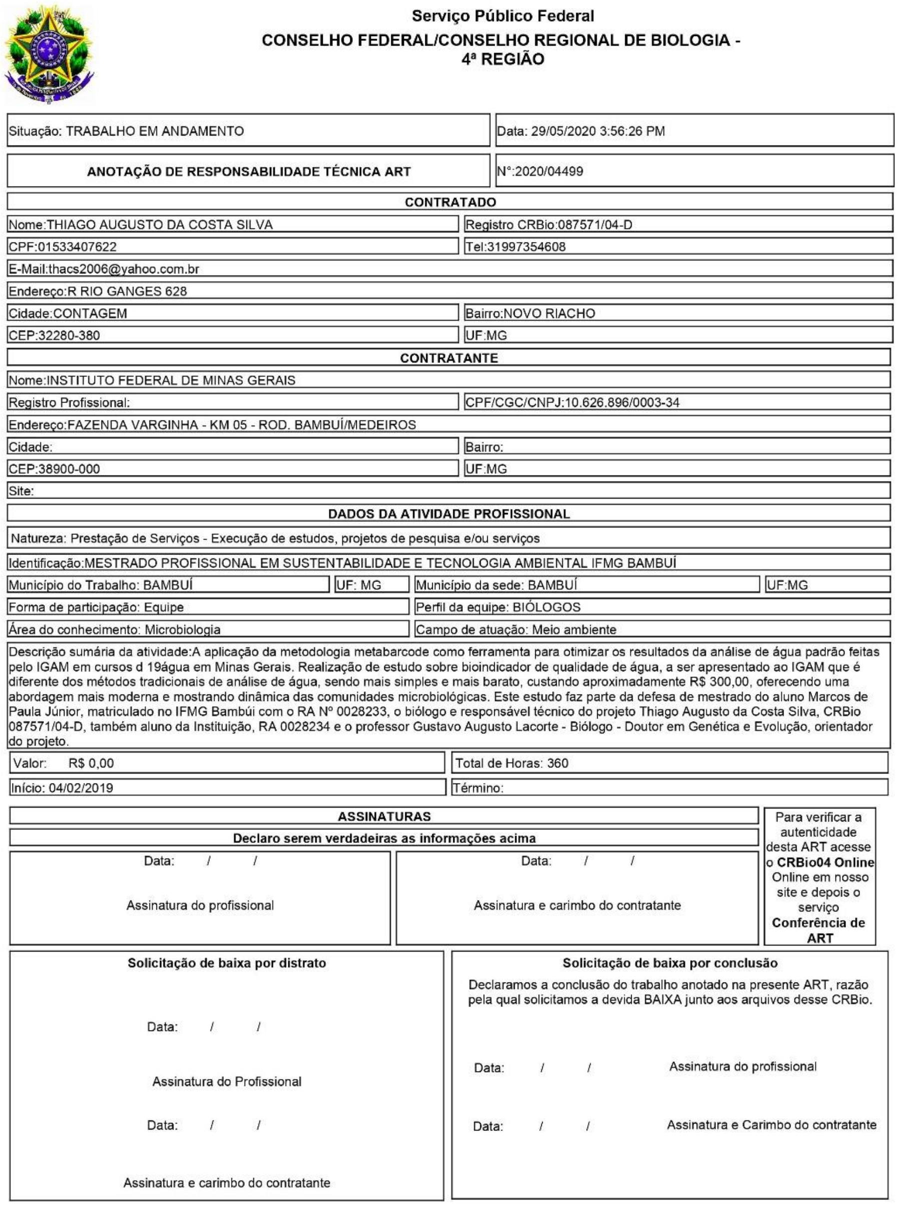
Spatial distribution of sediment bacterial communities from São Francisco River headwaters is influenced by human land-use activities and seasonal climate shifts

--Manuscript Draft--

Manuscript Number:		
Full Title:	Spatial distribution of sediment bacterial communities from São Francisco River headwaters is influenced by human land-use activities and seasonal climate shifts	
Article Type:	Research Article	
Section/Category:	Sediments	
Corresponding Author:	Gustavo Augusto Lacorte Instituto Federal de Minas Gerais - Campus Bambuí Bambui, MG BRAZIL	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Instituto Federal de Minas Gerais - Campus Bambuí	
Corresponding Author's Secondary Institution:		
First Author:	Marcos Paula Jr	
First Author Secondary Information:		
Order of Authors:	Marcos Paula Jr	
	Thiago Augusto Costa e Silva	
	Amanda Soriano-Araújo	
	Gustavo Augusto Lacorte	
Order of Authors Secondary Information:		
Funding Information:	Fundação de Amparo à Pesquisa do Estado de Minas Gerais (MPR-01008-16)	Dr. Gustavo Augusto Lacorte
	PRPPG-IFMG (Edital de Pesquisa Aplicada no. 169/2015)	Dr. Gustavo Augusto Lacorte
Abstract:	Riverbed sediments are freshwater environments that contain thousands of bacterial populations that support sediment ecosystems with a vast array of metabolic functions and organized in communities whose structure is highly influenced by local disturbances. São Francisco River headwaters section represents a typical forest-rural-urban landscape from the Brazilian Cerrado biome which constitutes a suitable biological system to study impacts of human practices on riverbed ecosystems. Here, a spatial analysis of sediment microbial distribution from São Francisco River headwaters section was conducted using Illumina 16S rRNA-V4 region amplicon sequencing in order to accomplish two major goals: (i) to investigate whether the diversity and composition of bacterial communities accessed in riverbed sediments vary in response to distinct land-use activities and whether the response patterns may be influenced by local seasonal climate changes; and (ii) to estimate if the diversity patterns of metabolic functions, predicted by PICRUSt2 approach, to accessed bacterial communities vary in a similar way to taxonomic patterns. Our findings revealed that variation in bacterial diversity of sediment communities was associated with changes in land-use practices , but also the mode in which the bacterial communities respond to these anthropogenic changes were influenced by the seasonal component . PICRUSt2 results showed that the sediment communities in which presented significant variation in taxonomic diversity also presented changes in the diversity of predicted metabolic functions and the most changes in the composition of predicted metabolic functions were verified in protected/unprotected transition zones . Our findings contributed with new evidence about the impact of typical land-use	

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

3- Cópia da Anotação de Responsabilidade Técnica – ART, emitida pelo Conselho Regional de Biologia – 4ª Região, tornando válida a Proposta de utilização de nova metodologia de análises de corpos d’água encaminhada à Secretaria de Meio Ambiente do Estado de Minas Gerais (Apêndice - **Sugestão aplicação da metodologia metabarcode como ferramenta para otimizar os resultados da análise de água padrão feitas pelo IGAM em cursos d’água em Minas Gerais)**



Serviço Público Federal

CONSELHO FEDERAL/CONSELHO REGIONAL DE BIOLOGIA - 4ª REGIÃO

Situação: TRABALHO EM ANDAMENTO

Data: 29/05/2020 3:56:26 PM

ANOTAÇÃO DE RESPONSABILIDADE TÉCNICA ART

Nº:2020/04499

CONTRATADO

Nome:THIAGO AUGUSTO DA COSTA SILVA

Registro CRBio:087571/04-D

CPF:01533407622

Tel:31997354608

E-Mail:thacs2006@yahoo.com.br

Endereço:R RIO GANGES 628

Cidade:CONTAGEM

Bairro:NOVO RIACHO

CEP:32280-380

UF:MG

CONTRATANTE

Nome:INSTITUTO FEDERAL DE MINAS GERAIS

Registro Profissional:

CPF/CGC/CNPJ:10.626.896/0003-34

Endereço:FAZENDA VARGINHA - KM 05 - ROD. BAMBUÍ/MEDEIROS

Cidade:

Bairro:

CEP:38900-000

UF:MG

Site:

DADOS DA ATIVIDADE PROFISSIONAL

Natureza: Prestação de Serviços - Execução de estudos, projetos de pesquisa e/ou serviços

Identificação:MESTRADO PROFISSIONAL EM SUSTENTABILIDADE E TECNOLOGIA AMBIENTAL IFMG BAMBUÍ

Município do Trabalho: BAMBUÍ

UF: MG

Município da sede: BAMBUÍ

UF:MG

Forma de participação: Equipe

Perfil da equipe: BIÓLOGOS

Área do conhecimento: Microbiologia

Campo de atuação: Meio ambiente

Descrição sumária da atividade:A aplicação da metodologia metabarcode como ferramenta para otimizar os resultados da análise de água padrão feitas pelo IGAM em cursos d 19água em Minas Gerais. Realização de estudo sobre bioindicador de qualidade de água, a ser apresentado ao IGAM que é diferente dos métodos tradicionais de análise de água, sendo mais simples e mais barato, custando aproximadamente R$ 300,00, oferecendo uma abordagem mais moderna e mostrando dinâmica das comunidades microbiológicas. Este estudo faz parte da defesa de mestrado do aluno Marcos de Paula Júnior, matriculado no IFMG Bambúi com o RA Nº 0028233, o biólogo e responsável técnico do projeto Thiago Augusto da Costa Silva, CRBio 087571/04-D, também aluno da Instituição, RA 0028234 e o professor Gustavo Augusto Lacorte - Biólogo - Doutor em Genética e Evolução, orientador do projeto.

Valor: R$ 0,00

Total de Horas: 360

Início: 04/02/2019

Término:

ASSINATURAS

Declaro serem verdadeiras as informações acima

Data: / /

Assinatura do profissional

Data: / /

Assinatura e carimbo do contratante

Para verificar a autenticidade desta ART acesse o **CRBio04 Online** Online em nosso site e depois o serviço **Conferência de ART**

Solicitação de baixa por distrato

Data: / /

Assinatura do Profissional

Data: / /

Assinatura e carimbo do contratante

Solicitação de baixa por conclusão

Declaramos a conclusão do trabalho anotado na presente ART, razão pela qual solicitamos a devida BAIXA junto aos arquivos desse CRBio.

Data: / /

Assinatura do profissional

Data: / /

Assinatura e Carimbo do contratante

4- Cópia do e-mail enviado ao Sr. Hidelbrando Canabrava Rodrigues Neto**,** Secretário Executivo de meio ambiente do estado de Minas Gerais com a proposta **Sugestão de aplicação da metodologia metabarcode como ferramenta para otimizar os resultados da análise de água padrão feitas pelo IGAM em cursos d'água em Minas Gerais:**

15/07/2020 Gmail - Fwd: Proposta de metodologia para otimizar os resultados da análise de água

Gmail **Marcos de Paula Júnior <prof.marcosbiologo@gmail.com>**

Fwd: Proposta de metodologia para otimizar os resultados da análise de água
1 mensagem

Hidelbrando Canabrava Rodrigues Neto <hidelbrando.neto@meioambiente.mg.gov.br> 23 de junho de 2020 18:05
Para: prof.marcosbiologo@gmail.com
Cc: Renata Batista Ribeiro <renata.ribeiro@meioambiente.mg.gov.br>, Gabinete do Igam <gabinete.igam@meioambiente.mg.gov.br>

Prezado,

confirmo recebimento e, considerando o tema do estudo, informo que encaminhei seu e-mail ao Instituto Mineiro de Gestão das Águas (Igam).

Atenciosamente,
Hidelbrando Canabrava Rodrigues Neto
Secretaria Executiva - Secretário Executivo
31 3915.1897 - secretario.executivo@meioambiente.mg.gov.br

Sistema Estadual de Meio Ambiente e Recursos Hídricos - Sisema
Secretaria de Estado de Meio Ambiente e Desenvolvimento Sustentável - Semad
www.meioambiente.mg.gov.br

De: "Marcos de Paula Junior" <prof.marcosbiologo@gmail.com>
Para: "Hidelbrando Canabrava Rodrigues Neto" <hidelbrando.neto@meioambiente.mg.gov.br>
Enviadas: Terça-feira, 23 de junho de 2020 17:52:48
Assunto: Fwd: Proposta de metodologia para otimizar os resultados da análise de água

Bom dia
Excelentíssimo Secretario de Meio Ambiente do Estado de Minas Gerais
Segue em anexo estudo realizado por Marcos de Paula Júnior, Thiago Augusto da Costa Silva e Dr. Prof. Gustavo Augusto Lacorte, para o Mestrado Profissional em Sustentabilidade e Tecnologia Ambiental - IFMG/Bambuí. O presente estudo mostra que a utilização de tecnologia metabarcode pode otimizar os resultados das analises de água feitas no estado e assim garantir uma melhor gestão de tais recursos. Peço que analise e considere nosso estudo.
Em anexo segue o estudo.
No aguardo
Atenciosamente
Marcos de Paula Júnior

2 anexos

ArtDocumento (1).pdf
75K

A aplicação da metodologia metabarcode como ferramenta para otimizar os resultados da análise de água padrão feitas pelo IGAM em cursos d'água em Minas Gerais.pdf
484K

https://mail.google.com/mail/u/2?ik=587c1f6169&view=pt&search=all&permthid=thread-f%3A1670325322324301962&simpl=msg-f%3A1670325322324301… 1/1