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Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre

TESE

RESERVATÓRIOS NO SEMIÁRIDO E SUA AVALIAÇÃO ECOLÓGICA:

Dos padrões ambientais e biológicos à elaboração de ferramenta de suporte à reabilitação

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Daniele Jovem da Silva Azevêdo

Belo Horizonte

Julho, 2018

RESERVATÓRIOS NO SEMIÁRIDO E SUA AVALIAÇÃO ECOLÓGICA: *Dos padrões ambientais e biológicos à elaboração de ferramenta de suporte à reabilitação*

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Trabalho de Tese apresentado junto ao Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, como parte dos requisitos para a obtenção do título de Doutor pela Universidade Federal de Minas Gerais.

Orientador: Prof. Dr. José Fernandes Bezerra Neto

Coorientador (a) Brasileiro (a):

Prof. Drª Joseline Molozzi (Universidade Estadual da Paraíba, Departamento de Biologia/ Programa de Pós-Graduação em Ecologia e Conservação)

Coorientador (a) Internacional:

Prof. Drª Maria João de Medeiros Brazão Lopes Feio (MARE - Marine and Environmental Sciences Centre & Faculty of Sciences and Technology, Department of Life Sciences, University of Coimbra – Portugal)

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RESUMO

Os reservatórios representam águas de transição entre rios e lagos e têm como principal finalidade a retenção de água para suprimento de demandas humanas (geração de energia, abastecimento, dessedentação animal, irrigação, lazer). Apesar de artificiais, os reservatórios são importantes elementos na paisagem, sendo ecossistemas que possibilitam a conservação da biota aquática, especialmente em regiões em que eventos de seca são frequentes (ex. regiões áridas e semiáridas) e a malha hidrográfica é predominantemente representada por rios intermitentes. Os reservatórios podem ser ecossistemas de água doce ainda mais importantes no futuro, pois de acordo com a perspectiva de mudanças climáticas, há previsões de aumento na intermitência das bacias hidrográficas. Desde os anos 70, a construção de barragems tem crecimento consideravelmente em todo o mundo, número que atualmente chega a 700 barragens/ano. Esse dado é preocupante e ao mesmo tempo desafiador, pois o desenvolvimento não planejado de diversas atividades humanas ao entorno desses ecossistemas, exige o acompanhamento permanente do seu estado de saúde, ou estado ecológico, além da proposição de medidas de reversão do estado de degradação. O principal objetivo dessa tese foi analisar características ecológicas de reservatórios no semiárido (padrões ambientais, estruturais e funcionais das comunidades de macroinvertebrados) para classificação do estado ecológico, bem como, elaborar uma ferramenta multivariada e emprega-la para simulações de reabilitação desses corpos hídricos. Nossa base de dados foi obtida a partir de seis reservatórios localizados no Nordeste brasileiro, em região semiárida. Esses reservatórios pertencem a duas bacias hidrográficas: bacia do rio Piranhas-Assu (estado do Rio Grande do Norte) e bacia do rio Paraíba (estado da Paraíba). As amostragens foram realizadas em quatro períodos: Junho, Setembro e Dezembro de 2014 e Março de 2015. O período de amostragem foi classificado como atípico, com seca intensa e prolongada, o que ainda direcionou o nosso olhar para análise dos padrões ambientais e bióticos frente a eventos extremos. O conjunto de dados bióticos demonstrou que a comunidade de macroinvertebrados é dominada em muitos locais por uma espécie de molusco invasor (*Melanoides tuberculata*). Verificou-se que a diversidade beta entre as bacias hidrográficas é principalmente mantida pela substituição das espécies (*turnover*), mas que no período mais seco, quando as condições ambientais são mais limitantes, a perda de espécies (*nestedness*) é um fator similarmente importante para o padrão de distribuição da diversidade, o que torna as comunidades mais pobres e dominadas por espécies tolerantes (como *Melanoides tuberculata*). A variação da diversidade ao longo do tempo também se reflete na alteração do padrão funcional das comunidades. De fato, ao longo do período seco há perda da riqueza funcional e aumento da uniformidade funcional, indicando homogeneização biótica. Adicionalmente, analisando a variação dos gradientes tróficos, observamos altos níveis de trofia (ambientes hipereutróficos) e de carbono orgânico dissolvido (COD). Locais com maior nível de trofia (durante o período mais seco) foram aqueles com menores valores COD. Sob estas condições, o carbono orgânico autóctone indica ser uma importante fonte para o COD e assim pode favorecer o processo de eutrofização. Todos os resultados obtidos demonstram que alto nível de degradação é uma realidade, e por isso nosso maior desafio foi propor medidas de suporte para reversão da baixo estado ecológico. Para isso, elaboramos um modelo preditivo que indica as comunidades esperadas em determinadas condições ambientais e. Adicionalmente, testamos o efeito de medidas de mitigação em diferentes cenários de reabilitação. Os resultados são promissores e demonstram que a alteração de variáveis relacionadas a eutrofização (ex. fósforo, transparência e clorofila-*a*) sozinhas já resultam em mudanças do estado ecológico (com melhoria ambiental e biótica), incluindo a expansão de taxa sensíveis (Ephemeroptera e Trichoptera). Os resultados obtidos a partir desta tese reunem informações sobre a flutuação dos padrões estruturais e funcionais das comunidades sob uma condição de perturbação intensa e fornece importantes *insights* para o biomonitoramento de reservatórios e suas respectivas bacias hidrográficas. A baixa qualidade ecológica em reservatórios no semiárido é preocupante e ações para a reversão do estado de degradação são necessárias. Aqui, demos um passo promissor em direção as ações de melhoria e assim, estudos futuros podem ser desenvolvidos com esta metodologia, considerando diferentes níveis de melhorias a partir de outros condicionantes de impactos.

Palavras-chave: macroinvertebrados bentônicos, espécies exóticas, diversidade beta, atributos funcionais, carbono orgânico dissolvido, modelos preditivos.

ABSTRACT

The reservoirs represent transitional waters between rivers and lakes and have as main goal the water storage to supply the human demands (e.g., electric power generation, cities supply, irrigation or recreation). Although artificial, the reservoirs are important elements on landscape, being ecosystems that support biota conservation, especially in regions where drought events are frequent (as arid and semi-arid regions) and its hydrographic networks are predominantly represented by intermittent rivers. The reservoirs may be freshwater ecosystems even more important in future, as according the climate changes predictions, watersheds will increase their intermittency. In fact, the number of dams is growing in the entire world, at a rate of 700 dams/year. This data is worrying and challenging at the same time, as the development of several human activities associated to these ecosystems, requires the monitoring their health status, or ecological status, and the elaboration of rehabilitation measures to improve their degradation status. Thus, the main aim this thesis was analyze ecological characteristics of reservoirs (environmental, structural and functional patterns) to classified their ecological status, as well as to elaborate a multivariate tool to simulate the effect of rehabilitation measures in macroinvertebrate communities. Our dataset was obtained from six reservoirs on Brazilian Northeast, semi-arid region. These reservoirs belong to two watersheds: Piranhas-Assu river (Rio Grande do Norte state) and Paraíba river (Paraíba state). The samplings were performed during four periods: June, September and December (2014) and March (2015). This period was classified as atypical, with an intense and prolonged drought, which led to the analysis of the effects of extreme events. Our results had demonstrated the high degradation level of reservoirs, including the in the increase of densities of an invader mollusk (*Melanoides tuberculata*). We found that the diversity among sites at watersheds is mainly maintained by random substitution (turnover), however in the driest period, when the environmental conditions are most limiting, the species loss (nestedness) is a factor with similar importance for the distribution pattern of diversity, resulting in poorer communities dominated by tolerant species (as *Melanoides tuberculata*). The diversity variation over time is reflected on changes in communities functional patterns. Indeed, along the drought period there was a loss in functional richness and increasing of functional evenness, indicating biotic homogenization. Additionally, analyzed the variation of trophic gradients we observed high trophic levels and also of dissolved organic carbon (DOC). Sites with highest trophic level (during the drier period) were those with lower DOC values. Under these conditions, the autochthonous DOC indicates be an important source to DOC in ecosystem and favor the eutrophication process. All results obtained show that the poor ecological status is a reality and therefore our largest challenge is to propose useful measures to reverse the low ecological condition. Therefore, we elaborated a predictive model to simulate the effect of rehabilitation measures in macroinvertebrate communities and tested its applicability the study sites. The results are promising and demonstrate that a change in variables associated to eutrophication alone (e.g., phosphorus, transparency and chlorophyll-a) results in changes in the ecological status (environmental and biotic improvement) of reservoirs, including expansion of sensible taxa. The obtained results from of this thesis gathers information on the fluctuation of structural and functional patterns of the communities under intense perturbation condition and provides important insights to biomonitoring of reservoirs and its watersheds. The low ecological quality in semi-arid reservoirs is worrisome and actions to the reversal from degradation status are need. Here, we given a promising step in direction to these actions, and thus future studies may be develop with this method, considering different levels of improvement from others impact factors.

Keywords: benthic macroinvertebrates, non-native species, beta-diversity, functional traits, dissolved carbon organic, predictive models.

APRESENTAÇÃO

Para a avaliação do estado ecológico e elaboração da ferramenta multivariada de suporte à reabilitação, analisamos uma série de descritores ambientais: parâmetros físicos e químicos da água, descritores do habitat, descritores da paisagem, e biológicos. No que diz respeito aos aspectos biológicos, analisamos a composição taxonômica, abundância, diversidade e atributos funcionais ("functional traits") de macroinvertebrados bentônicos. Selecionamos esta comunidade, devido a sua importância nos processos de transferência de energia e ciclagem da matéria nos ecossistemas.

Primeiramente, nosso objetivo foi caracterizar os gradientes ambientais e inventariar a biodiversidade existente. Ao concluir o inventário da biodiversidade, percebemos que a comunidade de macroinvertebrados, em muitos dos locais amostrados, era dominada por uma espécie de molusco exótico (*Melanoides tuberculata* - Müller, 1974). Com isso, buscamos entender a importância de fatores intrínsecos (resistência biótica) e extrínsecos (variáveis abióticas e de distúrbio) para o grau de invasão desse molusco. Isto culminou na elaboração do primeiro capítulo da tese. Este capítulo agrega informações importantes, especialmente para discussão da dinâmica de invasores em reservatórios e suas bacias hidrográficas.

A partir do primeiro capítulo, foram estabelecidos os gradientes ambientais e a distribuição da comunidade entre esses gradientes. Ficamos intrigamos em saber como a diversidade da comunidade é distribuída em diferentes escalas espaciais. Deste modo, analisamos a diversidade local (diversidade alfa), regional (diversidade gama) e as dissimilaridades entre os locais (diversidade beta). Mas queríamos ir além, respondendo a seguinte questão: qual o principal mecanismo de manutenção da beta-diversidade (*turnover* ou aninhamento) em ecossistemas artificiais sob condição de seca supra-sazonal? A busca de resposta para tal questão, resultou na elaboração do segundo capítulo. Nesse, mostramos que o *turnover* é o principal mecanismo de manutenção da beta-diversidade, mas que também há um aumento do aninhamento das espécies durante o período mais seco. Estes resultados indicam que em um cenário de mudança do clima, onde os períodos de seca serão ainda mais intensos e frequentes, a perda de espécies pode ser maior que a substituição, resultando em comunidades empobrecidas e com dominância de espécies tolerantes.

E o padrão functional da comunidade, como se apresenta frente a condição de distúrbio intenso? Essa questão norteou a elaboração do terceiro capítulo, onde estudamos um conjunto de oito atributos funcionais (28 categorias) em larvas de mosquitos (Dípteros). Selecionamos esse grupo por ser numericamente representativo frente a diferentes gradientes ambientais, além de ser um grupo diverso em reservatórios. Assim, o principal objetivo foi avaliar a flutuação atributos funcionais de Diptera durante período de seca extrema. Nossos resultados mostram que houve mudanças dos atributos funcionais das comunidades durante o período estudado, com alternância na dominância das categorias. Além disso, a variação dos atributos funcionais esteve associada a diferentes variáveis ambientais ao longo da seca prolongada, apontando seu potencial indicador. Um resultado importante neste capítulo, é que de fato há redução na riqueza funcional e aumento na uniformidade funcional, indicando que a perda da qualidade ambiental (decorrente da redução do volume hídrico) seleciona espécies com atributos resistentes e promove homogeneização da comunidade.

Até aqui, todos os resultados obtidos demonstram a baixa qualidade ecológica dos reservatórios estudados desde o início do estudo. Intrigados com a baixa qualidade ecológica analisamos a dinâmica da matéria orgânica dissolvida, através da quantidade e qualidade do carbono orgânico dissolvido (DOC), como indicador biogeoquímico das principais fontes que "alimentam" o processo de eutrofização. Buscamos assim entender se a concentração e a qualidade do carbono orgânico dissolvido podem ser utilizadas para avaliação do nível de degradação em reservatórios (assim como métricas tradicionais), com indicativo das principais fontes de nutrientes nos sistemas. Este capítulo traz informações relevantes, pois encontramos altos níveis de DOC, com valores incomuns aos registrados na literatura, incluindo reservatórios eutróficos. Além disso, o DOC autóctone representa uma importante fração do carbono nesses ecossistemas, com acentuada redução ao longo do período seco. Isso é ainda mais preocupante se considerarmos que essas variações podem representar mudanças importantes para o ciclo do carbono especialmente com aumento da emissão de gases de efeito estufa para atmosfera.

Diante do alto nível de degradação dos corpos hídricos, fomos desafiados a analisar alternativas de suporte para reabilitação do atual estado ecológico dos reservatórios e suas bacias hidrográficas. Assim, o último capítulo dessa tese teve como objetivo elaborar um modelo preditivo multivariado, que possa ser usado para simular medidas de reabilitação ecológica dos reservatórios. Este capítulo agrega importantes aspectos para ecologia aplicada, já que foi utilizada uma ferramenta baseada no uso simultâneo de três técnicas de *machine learning* (HYDRA) e a partir dela foi desenvolvido um modelo *dirty-water.* Neste tipo de modelo, ao contrário daqueles baseados na Condição de Referência, são usados na sua construção locais com vários graus de perturbação e variáveis preditivas que traduzem o stress ambiental. Registramos uma alta acurácia do modelo, o que indica ser uma ferramenta útil para medir o efeito de reabilitação em reservatórios e suas bacias hidrográficas. Com o modelo preditivo elaborado, simulamos dois cenários de melhoria da qualidade da água, considerando o efeito do tratamento de águas residuais urbanas a partir da manipulação de variáveis associadas a eutrofização. Nestas simulações encontramos resultados promissores para o contexto de reabilitação de ecossistemas aquáticos. Observamos que no maior nível de melhoria é previsto o aumento da riqueza, inclusive a expansão na cobertura de taxa sensíveis (como Trichoptera Polycentropodidae). Os resultados também mostram que os locais com maior nível de degradação melhoram, mas que as melhorias são previstas até para locais com menor nível de impacto. A partir dos resultados apresentados nesse capitulo, medidas mais direcionadas e eficientes para a reabilitação de corpos aquáticos podem ser propostas, a partir de testes *a priori*.

Os capítulos que compõe essa tese reúnem um conjunto de respostas, que em alguns casos fugiram do padrão esperado, mas que aumentam nossa compreensão sobre a dinâmica de ecossistemas aquáticos, em particular os sistemas de transição (reservatórios). Em um futuro relativamente próximo, os reservatórios podem representar os ecossistemas aquáticos predominantes, tendo em vista que devido ao aumento da temperatura global, são previstas alterações na distribuição e intensidade de precipitação, ampliando a problemática da escassez hídrica para a escala mundial. Uma das questões importantes a referir é que ainda falta muito para avançarmos em políticas de manejo dos corpos hídricos e ainda mais em medidas de reabilitação. No entanto, os resultados gerados a partir dessa tese constituem mais um passo que apontam na direção de ferramentas eficientes, as quais possam auxiliar cientistas e gestores nos processos de avaliação da saúde e reabilitação de corpos hídricos.

1. INTRODUÇÃO GERAL

Que desafios motivaram este estudo?

Há séculos a humanidade desenvolve atividades associadas diretamente aos ecossistemas aquáticos. O crescimento exponencial da população mundial tem elevado a demanda de utilização desses ecossistemas. A intensa construção de barragens representa uma das maiores ameaças antropogênicas, pois resulta em alterações ao ecossistema aquático natural, de lótico em lêntico, com consequentes alterações nos aspectos hidromorfológicos, resultando em um quadro de degradação. Além disso, devido aos usos do solo e da água que aparecem associados aos ecossistemas aquáticos, há riscos potenciais para declínio ou extinção de espécies. Associada às pressões antropogênicas, extremos climáticos também configuram fatores de alteração da dinâmica dos ecossistemas aquáticos, sendo mais um risco para a perda de funções e serviços ecossistêmicos. Assim, acompanhar a saúde desses ecossistemas representa o primeiro passo para estabelecer a condição ecológica local e uma base para a tomada de decisão em casos de reabilitação.

O emprego de indicadores ecológicos constitui uma ferramenta útil e subsidia informações relevantes para detecção de impactos (naturais ou antrópicos), mesmo que sutis. A partir dessas informações, os ecossistemas podem ser avaliados e classificados de acordo com seu estado ecológico (estado de saúde). Isso de fato é importante, pois quando há o conhecimento acerca da saúde ecossistêmica, o grande desafio é estabelecer medidas eficientes para reabilitação frente um cenário de degradação.

Por isso, no presente trabalho agregamos análises de aspectos ecológicos (ambientais e biológicos) e aplicados (ferramenta de suporte à reabilitação), buscando empreender esforços para ampliar o conhecimento acerca de ferramentas de avaliação do estado ecológico e para simulação de cenários de reabilitação em ecossistemas aquáticos. Acreditamos que a partir dos resultados obtidos será possível gerar respostas para questões de manejo e gestão a longo prazo.

Contextualização teórica

Em um quadro de mudanças climáticas, os ecossistemas aquáticos estão diretamente sujeitos aos efeitos dos extremos de cheia e seca. Além dos impactos naturais, as populações humanas têm ocupado áreas mais próximas desses ecossistemas, o que tem elevado o nível de pressão antrópica (Lee et al. 2012; IPCC, 2014; Tan & Gan 2015; Azevêdo et al., 2017). Por esse conjunto de fatores, os ecossistemas aquáticos estão entre os mais ameaçados do mundo, aumentando o desafio dos pesquisadores em prever as mudanças na dinâmica natural e suas consequências para a biodiversidade, funções e serviços ecossistêmicos (Kim et al. 2013; Daneshvar et al. 2016).

Os indicadores ecológicos são comumente empregados para a avaliação mais ampla da saúde dos ecossistemas (Cáceres et al., 2010; Heink & Kowarik, 2010). Jørgensen (2005), faz uma analogia para exemplificar a importância dos indicadores ecológicos, quando diz: "os paralelos dos indicadores com a avaliação da saúde humana são bem óbvias". Assim, os indicadores ecológicos seriam os "sintomas" de saúde, os quais possibilitam inferir acerca das "doenças" que caracterizam o estado dos ecossistemas.

Selecionar indicadores viáveis para uma avaliação precisa do estado de saúde, entretanto, não é uma tarefa fácil, sobretudo, devido a ampla gama de indicadores existentes. Em virtude dessa limitação, Noss (1990) aponta características que os indicadores ideais devem apresentar: i) sensibilidade às alterações ambientais; ii) distribuição geográfica ampla; iii) subsidiar informação sobre uma variedade de estressores (naturais ou antrópicos); iv) ter independência do tamanho da amostra; v) facilidade de coleta e de avaliação; vi) baixo custo de coleta e medição; vii) e potencial indicador de alterações ecossistêmicas significativas. Um dos maiores desafios dos ecólogos é selecionar indicadores que reunam todas estas características, o que parece ser algo inatingível. Como alternativa podemos considerar que a integração entre diferentes indicadores pode fornecer informações complementares e mais robustas para a avaliação da saúde dos ecossistemas.

A análise de indicadores ou características ecológicas e de seus padrões de flutuação (tempo-espaço) são base para avaliação da saúde ecossistêmica, o que nos possibilita inferir sobre as relações de causa-efeito e classificar o melhor e o pior estado de saúde (Xu et al., 1999). As características ecológicas estão associadas aos aspectos fundamentais dos ecossistemas: composição, estrutura e função, mensurados a partir de espécies/populações, comunidades/ecossistemas e paisagem (Dale & Beyeler, 2001). A composição está relacionada a variedade de elementos que compõem os ecossistemas, incluindo as espécies. A estrutura, retrata como esses elementos estão organizados entre gradientes ambientais e nas diferentes escalas. E por fim, a função está associada a processos ecossistêmicos e aos componentes associados (processos demográficos, história de vida, biomassa, substituição de espécies, além dos processos de distúrbios naturais ou induzidos) (Franklin et al., 1981; Noss, 1990).

Particularmente nos ecossistemas aquáticos, durante muitos anos, a avaliação da saúde ecológica baseava-se exclusivamente em parâmetros químicos (ex. concentração de nutrientes na coluna de água, principalmente, nitrogênio e fósforo), físicos (ex. transparência da água) (McMurtry et al., 1989) e biológicos (concentração de clorofila-*a*) (Kolkwitz, 1950), mas na maioria dos casos, estas medidas permitiam uma avaliação instantânea, restringindo o conhecimento para avaliações à longo prazo ou da complexidade do ecossistema (Bere and Tundisi, 2010). No século XX, a ausência e a presença de espécies passaram a ser utilizadas como indicadores da saúde (Hall & Grinnell, 1919; Grinnell, 1928), mas só a partir dos anos 80-90 se deu o uso mais regular desses indicadores (Karr, 1981; Moyle and Randall, 1998; Harig and Bain, 1998; European Commission, 2000; Carignan and Villard, 2002; Zampella et al., 2006).

A busca para a definição da saúde ecossistêmica não é algo recente. Desde a *Conference on the Human Environment* ou mais conhecida como Conferência de Estocolmo (1972, Suécia), foram levantadas discussões a respeito de como os ecossistemas são alterados em resposta aos estressores antrópicos. Anos mais tarde, a discussão foi ampliada durante a *Conference on Environment and Development* (Rio-92), sendo o conceito de saúde inclusive utilizado para elaboração da agenda internacional de manejo ecossistêmico (Lu et al., 2014). Considerando que saúde do ecossistema está associada a manutenção da integridade, isto é, que funções e processos flutuam dentro de um limite homeostático, mantendo a auto-organização ao longo do tempo e frente a perturbações (Rapport, 1998). Assim, no melhor estado de saúde, o ecossistema exibe capacidade de manter o equilíbrio biótico e funcional (Nõges & Nõges, 2006).

Mais recentemente, o conceito de saúde ecossistêmica tem sido substituído pelo chamado estado ecológico, o qual é definido como a qualidade estrutural e funcional dos ecossistemas (Moss et al., 2003; Heiskanen et al., 2004). Nos ecossistemas aquáticos, o estado ecológico é avaliado através das comunidades biológicas e das características físicas, químicas, morfológicas e hidrológicas das suas massas de água (ex. lagos, rios, águas de transição, ecossistemas artificiais ou corpos de água fortemente modificados) (Solimini et al., 2006; Nõges et al., 2009).

Comumente, a classificação do estado ecológico é realizada em comparação à condições de referência (Reynoldson et al., 1997; Kelly et al., 2009; Hawkins et al., 2010) e originalmente esse conceito, tem sido relacionado a um estado “pristino”, ou seja, às condições de ausência de perturbação ou qualquer tipo de influência humana, situação praticamente inexistente nos dias atuais. Por isso, o conceito também pode ser empregado quando se refere a melhor condição existente em uma região sob forte influência antropogênica. Stoddard et al. (2006) classificam a condição de referência de ecossistemas aquáticos em 4 categorias: Condição de referência histórica - conjunto de dados históricos que fornecem informações sobre as condições dos ecossistemas pré-distúrbio; Condição minimamente perturbada – melhor estimativa da integridade biótica (próximo à condição natural); Condição menos perturbada – melhor estimativa das condições físicas, químicas e de habitat na paisagem; Melhor condição possível – melhor condição atingível entre o minimamente perturbado e menos perturbado. Desta forma, a melhor condição possível não será “melhor” que o minimamente perturbado e nem “pior” que o menos perturbado.

A definição da condição de referência está relacionada à expansão ou restrição na classificação do estado ecológico (nível de perturbação antropogênica), por isso a necessidade de esclarecimento do termo para que a escolha dos critérios seja consistente (Bailey et al., 2004; Stoddard et al., 2006). No caso dos reservatórios, objeto deste trabalho, o conceito de condição de referência não pode ser aplicado, por serem ecossistemas artificiais. Por isso, para fins comparativos, utiliza-se o conceito alternativo de “máximo potencial ecológico” (Comissão Europeia, 2000), que se refere ao melhor estado ecológico que às massas de águas artificiais podem atingir. No Brasil, o conceito de máximo potencial ecológico já foi utilizado por Molozzi et al. (2013), sendo aplicado a reservatórios em clima tropical.

Aqui, reunimos uma série de características para avaliação do estado ecológico de reservatórios. Essas características englobam os aspectos ambientais, avaliados através dos parâmetros físicos, químicos, biogeoquímicos (através do carbono orgânico dissolvido), do habitat e da paisagem e ainda os aspectos bióticos. Esses últimos foram avaliados aqui através dos padrões de flutuação dos componentes estruturais (riqueza, diversidade e abundância) e funcionais (atributos funcionais) da comunidade de macroinvertebrados. Selecionamos a comunidade de macroinvertebrados bentônicos principalmente por apresentar organismos ubíquos e serem representados por grupos taxonômicos diversificados (Moreno & Callisto, 2006; Roque et al., 2010); muitos taxa serem sedentários e com ciclo de vida longo, capazes de registrar efeitos acumulativos e alterações de habitat (Barbour et al., 1996), além de serem sensíveis a alterações físicas e químicas nos diferentes ecossistemas, e suas respostas, a estas alterações, serem detectáveis e mensuráveis (Bonada et al., 2007).

Consideramos que a análise da gama de características ecológicas em reservatórios e a variação desses aspectos em diferentes gradientes ambientais, subsidiam uma análise mais ampla do atual estado ecológico (ex. Boyer et al., 2009; Patrício et al., 2009; Fernandes et al., 2010; Sheaves et al., 2012; Sowińska-Świerkosz & Soszyński, 2014; Azevêdo et al., 2015a, b; Ramoelo et al., 2015; Renner et al., 2015; Zhang et al., 2018). Em particular, a análise dos padrões do carbono orgânico dissolvido e dos atributos funcionais das comunidades geram respostas que permitem inferir além das condições ambientais, as propriedades funcionais do ecossistema (ex. produtividade, metabolismo e transferências de energia entre níveis tróficos) (Zhang et al., 2009; Feio & Dolédec, 2012; Demars et al., 2012; Shang et al., 2018).

Características ambientais, estruturais e funcionais podem ser utilizadas para elaboração de ferramentas de classificação do estado ecológico. Essas ferramentas podem englobar algoritmos simples, índices bióticos, ferramentas multimétricas (conjunto de índices) ou multivariadas (modelos preditivos) (Johnson & Wiederholm, 1993). No presente trabalho de tese, utilizamos a abordagem multivariada, reunindo características preditivas que permitem o reconhecimento de padrões (Lücke & Johnson, 2009). A análise multivariada oferece vantagens sobre os métodos tradicionais, pois: i) possui indicativos específicos da comunidade; ii) indicam pressões antrópicas sutis (Ferreira, et al., 2004; Bonada et al., 2007) e iii) possuem características preditivas, baseado em um conjunto de elementos que categorizam ecossistemas aquáticos e relaciona o observado com o esperado (Lücke & Johnson, 2009; Chalar et al., 2010).

Muitas ferramentas multivariadas têm sido desenvolvidas para avaliação e classificação de ecossistemas aquáticos em diversas partes do mundo, a exemplo:

1) RIVPACS (River Invertebrate Prediction Classification System) - proposto em 1977 na Inglaterra, classifica a integridade ecológica em rios com base na comunidade de macroinvertebrados bentônicos. Ao longo das décadas, o modelo RIVPACS foi aperfeiçoado (atualmente com três versões), sobretudo, no que se refere aos protocolos de amostragens e o número de áreas de referência;
2) AUSRIVAS (Australian River Assessment Scheme) - proposto em 1992 pelo Programa Nacional da Austrália, para monitoramento de rios australianos. O modelo AUSRIVAS que, assim como RIVIPACS, utiliza a comunidade de macroinvertebrados bentônicos, mas difere deste, nos aspectos do protocolo de amostragem, análises estatísticas e nível taxonômico considerado (AUSRIVAS considera o nível de família e RIVIPACS considera nível específico). Atualmente o modelo AUSRIVAS, sofreu adaptações, sendo ampliada a utilização com outros elementos biológicos (ex. diatomáceas e peixes) (Smith et al., 1999; Clarke et al., 2003; Feio & Poquet, 2011);
3) BEAST (Benthic Assessment of Sediment) - proposto por Reynoldson et al. (1995), para avaliação da integridade biológica de sedimentos de lagos localizados na américa do Norte. Este modelo foi proposto com base na comunidade de invertebrados e sofreu adaptações, principalmente no que se refere ao número de locais de referência. O modelo BEAST, diferentemente do RIVIPACS, não produz probabilidade de captura de taxa;
4) DIATMOD (Diatom Predictive Model for Quality Assessment of Portuguese Running Waters) - modelo desenvolvido em Portugal (Almeida & Feio, 2012), baseado em diatomáceas e adaptado a partir dos modelos RIVIPACS e AUSRIVAS.

No Brasil, o emprego de abordagens multivariadas ainda assume uma posição inicial de investigação, com poucos trabalhos realizados em ecossistemas aquáticos (Moreno et al.; 2009; Molozzi et al., 2013).

Comumente, as ferramentas multivariadas são baseadas em análise de função discriminante e avalia a similaridade entre os sites e a existência de grupos de sites de referências (Gabriels et al., 2007, Feio & Poquet, 2011). No entanto, essas técnicas apresentam algumas desvantagens: requerem variáveis com distribuição normal, variáveis não co-lineares, e a criação de grupos de sites podem representar uma divisão artificial no conjunto de dados (Feio et al., 2014a, b; Strachan & Reynoldson, 2014). Outro aspecto é que estas ferramentas tradicionais são baseadas em condição de referência, o que reduz a acurácia de predição quando se utiliza dados sob algum nível de impacto (Linke et al., 2001). Por isso a modelagem de dados ecológicos é consideravelmente dificultosa, primeiro pelo fato de que áreas sem influências antropogênicas são cada vez mais raras e por envolver relações entre variáveis não bem conhecidas (Hoang, 2001).

Modelos baseados em aprendizado de máquinas (*machine learning techniques*) parecem ser uma solução para estas questões de limitação metodológica e vem sendo desenvolvidos e testados para biomonitoramento (Park et al., 2004; Linke et al., 2005; Lencioni et al., 2006; Gabriels et al., 2007; Feio et al., 2014a, b; Sarrazin-Delay et al., 2014). Estas técnicas exibem vantagens sobre as tradicionais, pois baseiam-se no aprendizado a partir do conjunto de dados, minimizando efeitos artificiais e com capacidade de capturar relações não colineares. Além disso, esses métodos são menos influenciadas por *outliers* e assim, não requer distribuição normal das variáveis integrantes (Gevrey et al., 2004, Rose et al., 2016). No presente trabalho, utilizamos a ferramenta HYDRA, desenvolvida por Feio et al. (2014a). Esta ferramenta emprega simultaneamente três técnicas de aprendizado de máquinas com alta acurácia, para a elaboração de modelo de predição: *Support Vector Machines* (SVM; Drake et al., 2006), *Multi-Layer Perceptron* (MLP, Rumelhart et al., 1986; Gallant, 1993, Gevrey et al., 2004) *K-Nearest Neighbor analysis (*KNN; Linke et al., 2005).

Para elaboração do modelo preditivo multivariado, utilizamos uma abordagem diferenciada dos modelos tradicionais baseados em sites de referência. Construímos o modelo com base no conjunto de variáveis de diferentes sites, sejam menos ou mais impactados, os chamados *dirty water models*, introduzidos por Norris et al. (2000) e ainda pouco testados (Hoang, 2001; Linke et al., 2001; Hoang et al., 2003). Esta abordagem permite considerar quaisquer variáveis que exercem alguma influência sobre a comunidade e prevê alterações a partir das mudanças nas variáveis, simulando cenários (Hoang et al., 2003).

Devido a potencialidade da ferramenta elaborada, também a empregamos na abordagem de reabilitação, simulando cenários de melhoria do estado ecológico. Esta abordagem representa mais um passo no contexto da ecologia aplicada, desenvolvendo um modelo de priorização que possa ser utilizado para proposição de ações foco sobre o quadro de melhoria da condição ecológica em ecossistemas sob alto nível de degradação (Hermoso et al., 2011; Maire et al., 2015). Neste contexto, acreditamos que o desenvolvimento dessa tese possibilitou a obtenção de dados inéditos e de alta qualidade que ampliam o conhecimento sobre a dinâmica em sistemas artificiais, tanto em relação aos componentes ambientais (parâmetros físicos, químicos, biogeoquímicos, habitat e paisagem) quanto às comunidades estudadas, considerando seus aspectos estruturais e funcionais. Além disso, a elaboração de um modelo preditivo representa uma ferramenta útil para a biomonitoramento, além do potencial suporte para medidas de reabilitação em ecossistemas aquáticos.

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2. OBJETIVOS DA TESE

O principal objetivo desta tese foi analisar características ecológicas de reservatórios no semiárido para classificar o estado ecológico e elaborar uma ferramenta multivariada de suporte à reabilitação.

Especificamente pretendeu-se:

I) Inventariar a biodiversidade em escala local (sites) e em escala mais ampla (bacia hidrográfica).

II) Avaliar a dinâmica de espécies de moluscos exóticos e determinar os principais fatores relacionados a sua densidade e distribuição.

III) Analisar a distribuição da diversidade inventariada em diferentes escalas espaciais (alfa, beta e gama) e determinar qual o principal mecanismo (substituição/*turnover* ou aninhamento) é responsável pela manutenção da beta-diversidade.

IV) Testar atributos funcionais da comunidade de macroinvertebrados bentônicos, como indicadores do estado ecológico em bacias hidrográficas em região semiárida, na intenção de obter indicadores da funcionalidade desses sistemas.

V) Testar a resposta do carbono orgânico dissolvido e carbono colorido ao longo de gradientes tróficos como indicador biogeoquímico de degradação ambiental de reservatórios e suas respectivas bacias hidrográficas.

VI) Elaborar um modelo preditivo baseado em *machine learning techniques* e testar o modelo construído na simulação de cenários de melhoria em reservatórios.

3. ESTRUTURA DA TESE

Para o melhor direcionamento desta tese, de forma a atingir os objetivos propostos, estruturamos o seu desenvolvimento em cinco capítulos. Cada um desses, resulta em um artigo científico submetido ou a ser submetido às revistas internacionais com classificação B1 superior, de acordo com a base estabelecida para área de Biodiversidade (CAPES 2015). Assim, as questões que nortearam cada capítulo foram:

Capítulo 1. *O inventário da biodiversidade em reservatórios no semiárido brasileiro indica dominância de molusco exótico, porque será?*

TESTING MULTIPLE FACTORS TO EXPLAIN THE DEGREE OF INVASION BY A NON-NATIVE MOLLUSK IN SEMI-ARID WATERSHEDS *(manuscrito a ser submetido à Aquatic Invasions)*

Hipóteses – Capítulo 1: i) o grau de invasão (alta abundância) de *Melanoides tuberculata* é explicada principalmente (maior contribuição correlativa) associada a fatores extrínsecos. ii) a abundância do gastrópoda invasor varia também em função da riqueza da comunidade nativa/residente; iii) condições de distúrbio, aqui representado pela diminuição no volume hídrico, apresenta menor importância correlativa para explicar o grau de invasão do gastrópoda invasor.

Predições – Capítulo 1: i) entre os fatores extrínsecos a temperatura é o fator com maior contribuição correlativa para o grau de invasão *Melanoides tuberculata*, pois este fator é intimamente associados a extensão do nicho (tolerância ecológica) e aspectos reprodutivos da espécie (ex. pressão de propágulo); ii) as maiores abundâncias/habitat do molusco não-nativo ocorre nos habitats mais pobres (baixa riqueza da comunidade nativa/residente), indicando um nível de resistência biótica (fator intrínseco) da comunidade residente em áreas mais ricas; iii) o grau de distúrbio representa o fator com menor importância para o grau de invasão de *Melanoides tuberculata*, considerando que a espécie apresenta diferentes estratégias fisiológicas (ex. capacidade de estivação) e comportamentais (ex. desenvolvimento infaunal das larvas) que confere alta capacidade de resistência e resiliência diante de condições adversas.

Capítulo 2. *Você sabia que mesmo durante um período de seca intensa a diversidade entre os locais é predominantemente mantida pela substituição de espécies e não por aninhamento?*

TURNOVER DRIVES Β-DIVERSITY OF MACROINVERTEBRATES IN WATERSHEDS DURING A SUPRA-SEASONAL DROUGHT IN SEMI-ARID REGION *(manuscrito submetido à Freshwater Science)*

Hipóteses – Capítulo 2: i) a diversidade beta é alterada ao longo de período de seca supra-sazonal em reservatórios no semiárido; ii) as dissimilaridades entre as diferentes escalas espaciais são mantidas pela substituição das espécies (*turnover*) ao longo do período de seca supra-sazonal.

Predições – Capítulo 2: i) a diversidade beta sofre aumento ao longo do período de contração contínua em reservatórios semiáridos (período de seca supra-sazonal) acompanhada da alta variação na condição ambiental e estrutura da comunidade (riqueza e diversidade) entre as bacias hidrográficas, reservatórios e sites; ii) a diversidade beta é principalmente sustenta por turnover, isto é, espécies melhor adaptadas as condições atuais substituem as dominâncias prévias.

Capítulo 3. *E o padrão funcional da comunidade? A perda de espécies durante a seca indica homogeneização biótica*

DIPTERAN ASSEMBLAGES AS FUNCTIONAL INDICATORS OF EXTREME DROUGHTS *(manuscrito submetido à Journal of Arid Environments)*

Hipóteses – Capítulo 3: i) os atributos funcionais de Diptera são alterados ao longo do período de seca supra-sazonal; ii) há alteração no padrão da diversidade funcional de Diptera, em resposta das alterações nos atributos funcionais.

Predições – Capítulo 3: i) Os atributos funcionais de Diptera mudam em resposta as alterações ambientais mudanças no ambiente ao longo do tempo; ii) com o aumento da degradação ambiental ao longo da seca, há aumento na uniformidade funcional e diminuição da riqueza funcional, como resultado da dominância de poucos atributos (categorias de atributos).

Capítulo 4. *O que determina a baixa qualidade ecológica dos reservatórios? Os pools de carbono orgânico podem indicar as respostas*

DISSOLVED ORGANIC MATTER POOLS IN SEMI-ARID RESERVOIRS: SUPPORT TO ASSESSMENT OF TROPHIC STATUS IN WATERSHEDS *(manuscrito a ser submetido à Aquatic Sciences)*

Hipóteses – Capítulo 4: i) o níveis carbono orgânico (COD) e suas propriedades ópticas mudam ao longo dos gradientes tróficos em reservatórios no semiárido.

Predições – Capítulo 4: i) sites com alto nível de trofia são aqueles com alto nível de carbono orgânico dissolvido (COD), especialmente COD autóctone, representado por moléculas de baixo peso molecular (maior M).

Capítulo 5. *E agora, o que fazemos diante da baixa qualidade ecológica dos reservatórios? Elaborando uma ferramenta preditiva para reabilitação do estado ecológico*

REHABILITATION SCENARIOS FOR RESERVOIRS: PREDICTING AQUATIC COMMUNITIES THROUGH MACHINE LEARNING *(manuscrito submetido à Ecological Indicators)*

Hipóteses – Capítulo 4: i) a melhoria na qualidade da água em reservatórios resulta no aumento da biodiversidade e na qualidade biótica dos ecossistemas; ii) no maior nível de melhoria da qualidade da água é onde ocorre a maior riqueza esperada; iii) a recuperação da biodiversidade é maior em sites severamente perturbados, do que em sites menos perturbados.

Capítulo 1. TESTING MULTIPLE FACTORS TO EXPLAIN THE DEGREE OF INVASION BY A NON-NATIVE MOLLUSK IN SEMI-ARID WATERSHEDS

Manuscrito submetido submetido à Aquatic Invasions

Reservatório Poções, Monteiro – Paraíba (PB) Brasil/ 2014. Foto: Luiz Carlos

Testing multiple factors to explain the degree of invasion by a non-native mollusk in semi-arid watersheds

Daniele Jovem-Azevêdo[1*], José Fernandes Bezerra-Neto[2], Maria João Feio[3], Rodrigo Fernandes[4] and Joseline Molozzi[5]

[1] Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais, Belo Horizonte, MG CEP 31270-901, Brazil. e-mail: daniele.jazevedo@gmail.com

[2] Departamento de Biologia Geral/ Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais, Belo Horizonte, MG CEP 31270-901, Brazil. e-mail: joseneto.ufmg@gmail.com

[3] MARE - Marine and Environmental Sciences Centre, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal. e-mail: feiomaria@gmail.com

[5] Programa Pós-Graduação em Ecologia e Conservação/ Departamento de Ciências Animais, Universidade Federal Rural do Semiárido, Mossoró, RN CEP 59625-900, Brazil. e-mail: rfernandes@ufersa.edu.br

[5] Departamento de Biologia/ Programa de Pós- Graduação em Ecologia e Conservação, Universidade Estadual da Paraíba, Campina Grande, PB CEP 58429-500, Brazil. e-mail: jmolozzi@gmail.com

*Corresponding author

Abstract

Invasive species change the dynamic of communities and ecological processes in ecosystems. Determining the mechanisms associated to invasion is thus essential to establish the effective management of undesirable species. Here we aimed to test the relative importance of intrinsic (biotic resistance) and extrinsic factors (abiotic factors and disturbance) to the degree of invasion (DI) of non-native mollusk species (Melanoides tuberculata) in freshwater ecosystems. With that purpose we sampled 478 sites distributed in six reservoirs of Piranhas-Assu river and Paraíba river watersheds, in Brazilian semi-arid in June, September, December (2014) and March (2015). A niche-based model (BRT's) explained 94% of the total variation in Melanoides turberculata abundance which included intrinsic and extrinsic factors. Temperature was the factor with the highest relative importance (15.8%) followed by the organic matter content (14%), which shows the importance of ecosystem productivity factors to explain the degree of the mollusk invasion. The invasibility (biotic resistance) and disturbance explained 5.9% and 5.8%, respectively. Our results indicate that intrinsic and extrinsic factors acts simultaneously to high abundance of *Melanoides tuberculata*, however extrinsic factors are more important to DI, especially factors associated to niche extent of the species. Biotic resistance also explained part of the degree of invasion, even though at a smaller scale. This shows that the preservation of native diverse communities from the earlier times of construction of a reservoir should be considered a potential important solution to the preservation of biodiversity of the semi-arid reservoirs, as they constitute natural controls to invasion

Keywords: niche-based models, intrinsic factors, extrinsic factors, invasibility, alien species, macroinbertebrates.

Introduction

Invasive species are cause impacts of several magnitudes on ecosystems and human societies (Holmes et al., 2009; Blackburn et al., 2014). In particular, the high abundance of invasive species may conduct to wide changes in composition, distribution and abundance of native species, promoting indirect effects on natural dynamic of ecological processes (Simberloff, 2011). Alterations in the ecosystems are one of the major threats to global biodiversity loss affecting the ecosystem services, which directly result in socio-economic impacts (Pimentel, 2002; Nghiem et al., 2013; Fenoglio et al., 2016). Other growing concern is the increasing impact of invaders on human health as many are vectors of dissimiation of parasites and pathogens (Cunningham et al., 2012; Conn et al., 2014; Hulme et al., 2014; Mazza et al., 2014; Schindler et al., 2015). Thus, it is fundamental to determine the drivers of arrival, establishment, and spread, as well as ecological impacts promoted by these species in the invaded ecosystems, to provide adequate management and conservation strategies (Lockwood et al., 2005; Havel et al., 2015; Colangelo et al., 2017).

The population growth in invaded habitat depends on intrinsic and extrinsic factors (Colautti et al., 2014). Intrinsic factors are related to *invasibility*, i.e. the vulnerability of the native community to invasion (Guo et al., 2015). This is associated with niche opportunities provided by native species, the availability of resources and the biotic resistance (Shea and Chesson, 2002; Hierro et al., 2005). The biotic resistance is represented by the competitive ability of species on the invaded community to prevent the establishment and spread of non-native species in similar niche (Byun and Lee, 2017). In contrast, the extrinsic factors represent the *degree of invasion*, i.e. the extent or level at which a community has been invaded (Guo and Symstad, 2007). The degree of invasion depends on the intrinsic property of the native community, but also on the propagule pressure, abiotic factors and disturbance (Davies et al., 2005; Clark et al., 2011). In particular, the propagule pressure is related to the number of non-native individuals produced on each reproductive event and the total number of reproductive events, associated to the life-history of the species (Lockwood et al., 2005) and to the environmental conditions, including the local resources (Catford et al., 2009). Thus, the interaction between intrinsic and extrinsic factors allows to infer the dominance level of invaders species on native species, and to know the mechanisms associated to invasion. However, to establish the relationships between the growth of non-native species populations and intrinsic and extrinsic factors it's not simple because these factors form a drivers network that can act individually or synergistically, and vary in space and time (Strayer and Heather, 2006; Pysek and Richardson, 2010). Furthermore, disturbance events, (e.g. drought event), act modifying species abundances, resource availability and habitat structure, which influences the invasion dynamic (Lake, 2000, 2003; Havel et al., 2005, 2015; Souza et al., 2017).

In this study, we analyzed simultaneously the relative importance of intrinsic and extrinsic factors in the densification of the non-native gastropoda *Melanoides tuberculata* (Müller, 1974) (Gastropoda: Thiaridae) in semi-arid reservoirs. *Melanoides tuberculata* is a mollusk from southeastern Asia. This species shows a larger global distribution, occurring in different ecosystems of world (e.g., Dugan et al., 2002; Peso et al., 2011; Rader et al., 2011; Cilia et al., 2013; Gashtarov and Georgiev, 2016). The first record of *M. tuberculata* in Brazil is from the southeastern, in São Paulo State (1967) and after that, the species was only registered again in 1984, in Distrito Federal (Vaz et al., 1986). Later it was expanded to other states: Bahia, Ceará, Goiás, Paraíba, Pernambuco, Minas Gerais, Rio de Janeiro, Rio Grande do Norte and Paraná (Silva et al., 1994; Paz et al., 1995; Mello and Cordeiro, 1999; Fernandez et al., 2003; Rocha-Miranda and Martins-Silva, 2006; Gregoric et al., 2007; Santos et al., 2007).

The introduction this non-native species may have occurred through the transport of ornamental fish and plant, ballast water or by migratory species (Silva et al., 1994; Santos and

Eskinazi-Sant'Anna, 2010). The species is viviparous, reproducing parthenogenetically, but originating differentiated clones. The sexual reproduction is rare (Facon et al., 2006). *M. tuberculata* show a high capacity of hybridization, which gives it numerous morphotypes (Facon et al., 2005). Probably this set of features favors the persistence of the species in several freshwater environments under different environmental conditions.

Various studies have documented the densification of *Melanoides tuberculata* and broad impacts on native macroinvertebrates (Santos and Eskinazi-Sant’Anna, 2010; Peso et al., 2011; Rader et al., 2011; Molozzi et al., 2013; Azevêdo et al., 2015; Azevêdo et al., 2017) and microphytobenthos communities (Vasconcelos et al., 2013; Raw et al., 2016a, 2016b). This species is also a host for several pathogens that affect human and animal health (Derraik, 2008; Pinto and Melo; 2010, 2012; Krailas et al., 2014; Najet et al., 2014). Most of these studies have correlated the high spread of *Melanoides tuberculata* with single abiotic factors but to our knowledge there isn’t any assessment of the influence of multiple factors on its densification. Knowing the dynamic and the factors that determine the high abundance of non-native species in the invaded ecosystems is highly relevant for the conservation of ecosystems as their large spread can change the ecosystem functioning and services (Ehrenfeld, 2003; McLaughlan et al., 2014; Jackson and Britton, 2014). Furthermore, to understand and model the fluctuation patterns of invasive species over time it essential and represent the first step to propose appropriate management, because non-native species are often well established in the new ecosystem, making it difficult to control propagation and densification (Schlaepfer et al., 2011).

Here, we used a correlative niche-based model to explore the relative importance of intrinsic (biotic resistance) and extrinsic factors (abiotic factors and disturbance) to the degree of invasion of reservoirs of the semi-arid by the non-native mollusk species (*Melanoides tuberculata*) in freshwater ecosystems, especially those under disturbance conditions (e.g.

supra-seasonal drought). Niche-based models are useful tools for resolve this issue, because they synthetize the information focusing on the population and integrating simultaneously multiples factors, which help us to identify the relative contribution of different drivers of population growth of invaders (Peters, 2004; Lindim, 2015; Corrales et al., 2017; Lohr et al., 2017). We expect the *Melanoides tuberculata* densification to be associated to water temperature and pH, as these factors are directly related to the niche extent (ecological tolerance) and reproductive aspects (e.g., propagule pressure) (Lockwood et al., 2005; Catford et al., 2009; Simberlof, 2009). We also believe that the highest abundance of non-native mollusk occurs in poorer areas, due to the biotic resistance of native/resident communities on taxonomic richest areas (Kennedy et al., 2002). Additionally, we hypothesized that the disturbance conditions, here represented by the reduction of water volume caused by a prolonged drought, have a similar importance than other drivers because it results in the loss of habitat and reduction in resources availability (Lake 2003,2011).

2. Material and Methods

2.1. Study area and sampling design

This study was conducted in six reservoirs located in Northeastern Brazilian: three reservoirs in Piranhas-Assu watershed (Rio Grande do Norte state) and three reservoirs in Paraíba watershed (Paraíba state) (Table S1 – Supplementary material, Figure 1). The climate of the region is BSh (dry semiarid) Köppen–Geiger with a mean rainfall of 400 mm/year for Paraíba River and 800 mm/year for Piranhas-Assu River. In both watersheds, the minimum air temperature varies between 18 and 22ºC (July and August) and the maximum temperature between 28 and 31ºC (November and December) (Alvares et al., 2013).

Sampling was performed along the littoral zone, in 66 sampling sites of Piranhas-Assu and 75 of Paraíba reservoirs (Figure 1). At all sites, sampling was conducted four times (June, September, December of 2014 and March 2015), to cover the normal climatic variability. However, during the study period, the reservoirs passed through a strong reduction in the hydrologic volume, according to the water agency “Agência Executiva de Gestão das Águas” (AESA), in the State of Paraíba and the environmental agency “Secretaria de Meio Ambiente e Recursos Hídricos” (SEMARH), in the State of Rio Grande do Norte. Thus, the sampling period covered an intense and prolonged drought, instead of well-marked dry (nine months per year) and wet seasons (February to April) (Alvares et al., 2013). Reservoirs characterization and hydric volume data were collected from of AESA and SEMARH agencies (Table S1)

2.2 Biotic data

The invertebrate samples were collected at each site with an Eckman-Birge dredge (area 225cm²). The samples were fixed *in situ* with formaldehyde. In the laboratory, the samples were washed with two sieves (0.5-1mm). The organisms were removed and identified under a stereomicroscope. Non-native mollusks and Chironomidae family were identified to species and genus, respectively (Peterson 1960; Boffi 1979; Péres 1988; Trivinho-Strixino and Strixino 1995; Merritt and Cummins 1996; Carvalho and Calil 2000; Epler 2001; Fernández and Domínguez 2001; Costa et al. 2006). We use the *Melanoides tuberculata* abundance as a proxy of the degree of invasion in the habitat.

2.3 Drivers variables

We used the richness of the native community as an invasibility proxy (biotic resistance). The abiotic variables assessed were: temperature (ºC), pH, conductivity (mS/cm) and dissolved oxygen (mg L^{-1}), measured *in situ* using a multiparameter probe (HORIBA U-50), and the depth (m) estimated with a sonar. One liter of water was collected from the sub-surface to determine the concentrations of total phosphorus (µg/L) and total nitrogen (µg/L), according to the "Standard Methods for the Examination of Water" (APHA 2005). The concentration of chlorophyll-*a* was estimated by extraction of the pigment in acetone 90% (Lorenzen 1967). Additionally, samples of sediment were collected from each sampling site using an Eckman-Birge dredge (area 225cm²) to measure their content in organic matter. Aliquots (content 3g) were incinerated in a muffle (550ºC; 4h), according to the gravimetric method. Phosphorus, nitrogen, chlorophyll-*a* concentrations and organic matter were used as variables related to ecosystems productivity.

We grouped the variables in two classes: i) intrinsic factors (native/resident community richness, proxy of invasibility) and ii) extrinsic factors (abiotic variables, productivity in ecosystem and disturbance). The ecological importance of drivers analyzed is shown in Table 1.

2.4 Data analysis

To assess the relative importance of intrinsic and extrinsic factors, we used Boosted Regression Trees techniques (BRTs). BRTs are robust statistical analysis that incorporates tree-based methods (decision trees) with machine learning techniques (boosting) (Dea'th 2007; Elith et al. 2008). While the decision trees work with the partitioning of explanatory variables for recursive binary splits (nodes), the boosting algorithms aim to improve the model accuracy by training data. Fitted models are produced by calibration of three major parameters: tree complexity (*tc*), learning rate (*lr*) and bag-fraction (*bf*). Tree complexity regulates the number of nodes into a tree (depth), the learning rate refers the contribution of each tree to the final model (shrinkage) and the bag-fraction is the proportion data taken randomly (stochasticity) (Elith et al. 2008). The best model, *i.e.* the simplest model with highest explicative performance, is selected by cross-validation (CV) using multiple iterations among these three parameters. The final model retained has the lowest CV deviance and Standard-Error (SE), i.e., the optimal settings (for more details about method see Elith et al. 2008).

Because our dataset is composed of many predictors with distinctive ecological influences on response variable, which may produce complex nonlinear relationships, we preferred BRT instead conventional methods (e.g. GLM, multiple regressions). The BRTs were used to quantify the relative importance of each explanatory variable on variation of abundance of *M. tuberculata*. We identified also interactive properties between pair of predictors that best explain the variability of the invasive gastropod. We built the BRT model based on Poisson distribution (count data) using the following values for training data: *tc* (1, 2, 3, 4 and 5), *lr* (0.0005, 0.0001, 0.005, 0.001, 0.05 and 0.01) and *bf* (0.5 and 0.75) (cf. Elith et al. 2008). Finally, the model performance was assessed by two metrics: 1) explained deviance (percentage of the null deviance) and 2) CV correlation. Both measures are considered goodness-of-fit of BRT models, where the first is analogous to the R^2 coefficient of the traditional regression analysis (Soykan et al. 2014). We performed all analytical procedures in R software (R Core Development Team 2017) using *dismo* package (Hijmans et al. 2017).

3 Results

3.1 Biotic pattern

During the study period, a total 61 macroinvertebrates taxa (7 Mollusca, 2 Annelida, 1 Nematoda, 1 Platyhelminthes and 50 Arthropoda) were identified from the 77,400 individuals counted. Among these, 30,266 individuals were *M. tuberculata*. The highest abundance/site was 591 individuals, and the average was 63.31±93.85 individuals/site. There were also sites without *M. tuberculata* and sites where we found only the invasive species (100% of contribution to community). In these last, the abundance varied between 3 and 511 individuals.

3.2 Predictors pattern

The water of the sampling sites had high temperatures during the entire period, varying between 26.37±1.36 and 27.58±1.05 ºC. The waters were predominantly alkaline, with average values ranged from 7.99±0.52 to 8.47±0.49 and with the highest levels of dissolved oxygen (7.31±3.09 mg L^{-1}). Regarding the productivity variables, the highest concentrations of total nitrogen occurred in habitats with high organic matter content (234.81±81.42 µg L^{-1}; 0.30±0.05 gDW, respectively) (Table 1). The water volume decreased strongly over time (see Table S2), due to long period of rainfall absence. Low richness was recorded among habitats, with average values of 6.4±2.0 taxa/site (Table 1).

3.2 Modelling intrinsic and extrinsic drivers

Fitted BRT models produced a reasonable performance (30.6% of explained deviance, 50.9 of correlation and 1200 trees) in explaining the variation of *M. tuberculata* abundance. The final model was attained with the highest tree complexity and lower learning rate (Table 3), indicating that predictors had partitioned contributions to the response variable. No predictor was removed by simplification method of the BRT, reinforcing that all contributed for *M. tuberculata* abundance.

The model built explained 94% of the total variation in *Melanoides turberculata* abundance. Among the intrinsic and extrinsic factors analyzed, the temperature had highest relative contribution to the abundance of the invasive species (15.8%). In general, the invasive gastropod was more abundant within dams with cooler water temperature (22-25ºC), lower depth (< 0.5 m) and low concentration of organic matter (0.0-0.2 mg.cm^{-3}) and electrical conductivity (0-5 mS.cm^{-1}) (Fig. 1). Another driver influencing positively the gastropod abundance was the concentration of total nitrogen > 230µg/L (7.4% of relative contribution), chlorophyll-a > 1µg/L (6.7%), alkaline pH (6.4%), and high dissolved oxygen (6.3%) and hydric volume (5.8%). In contrast, negative interactions with *M. tuberculata* abundance were found for total phosphorus (7.2%) and species richness > 3 species.m^{-2} (5.9%) (Figure 2).

A strong interaction (interaction size = 15.08) was recorded between water temperature and organic matter, with the *M. tuberculata* abundance sharply decreasing due warmer water temperature and enhanced organic matter (Figure 3).

4 Discussion

Determining the contribution of intrinsic and extrinsic factors to the degree of invasion provides information on invasion mechanisms and this may help in management strategies against invasive species (Guo and Symstad, 2007; Catford et al., 2012). Here, we showed that the niche-based model (BRT's) explained a high proportion in the variation of the *Melanoides tuberculata* abundance (94% of explained variation). The best model indicated that the degree of invasion of this species is conditioned by the interaction between intrinsic and extrinsic factors, but with a high relative contribution from extrinsic factors (abiotic variables).

We hypothesized that the high abundance rates of invaders would be related with the local water temperature, which indeed occurred. We found that coolest waters favored the invasive species, being the predictor variable with highest relative contribution to degree of invasion (15.8%). This was expectable as temperature has a direct influence on physiological aspects of mollusks species: it affects the larval development and recruitment rate in populations (Fortunato et al., 2015); and the consumption/digestion ratio, driving metabolic processes and the species role on local habitat (Raw et al., 2016a). In fact, the highest frequency of *Melanoides tuberculata* was been recorded in freshwater ecosystems with temperatures between 15 and 25 ºC (Kock1 and Wolmarans, 2009; Raw et al., 2016a), corroborating with our results.

The niche-based model built indicates that extrinsic factors (as temperature) were those with highest influence on *Melanoides tuberculata* degree of invasion. Extrinsic factors are frequently associated to the success of invasive species, as they limit the niche extent and reflect the tolerance degree of the species (Lockwood et al., 2005; Simberlof, 2009). Among these, abiotic characteristics showed high influence for abundance of invader mollusk. Abiotic factors are fundamental to organisms at local habitat, as they may determinate the ideal growing conditions and invaders reproductive success (propagule pressure) (Catford et al., 2009). Besides temperature other habitat characteristics (e.g., depth with 13.8%; and pH with 6.5%) were important to the high abundance of invaders.

The ecosystem productivity factors were also included as predictors of *Melanoides tuberculata* densification, with highlight to the organic matter content (14% of relative importance). However different from what we expected, the highest abundance of the invasive gastropoda occurred at sites with low organic matter concentrations. We have associated this fact to feeding preference of the species. *Melanoides tuberculata* show high plasticity and foraging strategies according resource availability (Vasconcelos et al., 2013 Raw at al., 2016a). Raw and collaborators (2016b) show through isotopic analyzes that the preferential resource of this invasive species are green algae and that alternative resources (e.g. detritus and particulate organic matter) may be additionated to feeding, according with the decreasing of the preferential resource. In fact, the sites with the highest organic matter content and total phosphorus concentrations (variables with negative influence) were those with lowest chlorophyll-*a* concentration (see Table 1), conditions that may indicate a greatest degradation level and low availability of preferential resource. The organic matter together with the temperature represents the variables (extrinsic factors) with highest relative importance to degree of invasion of these mollusks (29.8% of accumulated relative importance) (see Figure 3).

The extrinsic factors alone don't explained the high abundance of invasive gastropoda. The invasibility (intrinsic factor), represented by the biotic resistance, also showed influence in the degree of invasion, despite the low proportion (5.9% of relative importance). We have found that the high *Melanoides tuberculata* abundance occurs in sites with lowest native/resident community richness (< 3 taxa/site) and that richer areas (> 10 taxa/site) were those with low densification of invasive gastropoda. This could apparently indicate that native biodiversity has a resistance level to invasive species. Indeed, previous studies indicate that at richest areas native species show competition strategies, which make local community highly competitive avoiding the establishment of others species with a similar niche (Kennedy et al., 2002; Tilman, 2004).

The *Melanoides tuberculata* showed a contribution higher than 80% for community abundance in many sampling sites (>18%) and in some sites the invaders were even the unique taxa. The spread and long-term dominance of an invader occurs due the broad niche extent associated to high competitive ability, which favors larger population ratios and native species loss, resulting at poorer systems (lower native community richness) (Macdougall and Turkington 2005; Lima et al. 2013). Particularly, the *M. tuberculata* is reported as a species with high competitive ability and, in most cases, with its densities overcome the cover of the native species in the habitat (Santos and Skinazi-Sant'Anna, 2010;Azevêdo et al. 2015; Azevêdo et al. 2016; Raw et al., 2016b), which corroborates with our findings.

The decrease of hydric volume during the study period was a disturbance factor associated to degree of invasion from non-native species, however with a lower importance than other factors (5.8% of relative importance). Freshwater ecosystems at driest areas (as arid and semi-arid areas) are frequently subject to drought events, which changes the local conditions (Melo et al., 2017). Species that live on these seasonal conditions exhibit adaptations to such natural variations (Lytle and Poff 2004; Bogan et al., 2014). In particular, *Melanoides tuberculata* shows physiological strategies to survival time during drought periods especially when are humid protected microhabitats (Facon et al., 2004), which may explain the low influence of this factor in the degree of invasion. Furthermore, drought events act as disturbing agents on the availability resources and decreasing also the native community abundance open space to opportunist species, as invaders (Lake 2003; 2011; Raw et al., 2016a,b).

Reservoirs of the semi-arid region are probably more susceptible to invasion, due to the stability provided by the high water residence time in these ecosystems, variable between 3 and 5 years, due to prolonged drought events (Santos and Eskinazi-Sant'Anna 2010, Melo et al., 2017). In addition, the homogenous characteristics among habitats in the reservoirs tend to select more generalist species against specialist strategies (Devictor et al. 2008). The wide coverage of *Melanoides tuberculata* can also indicate its wide functional plasticity, considering that the studied watersheds have the strongest environmental degradation (e.g. phosphorus, nitrogen, chlorophyll-*a*).

Conclusions

The niche-based model showed be an useful tool to model the degree of invasion of *Melanoides tuberculata*, given the high proportion of explained variation in our dataset (94%), and provided important conclusions. First, our results reinforce the idea that intrinsic and extrinsic factors act simultaneously to results in a high abundance of *Melanoides tuberculata*, with the highest relative importance being attributed to the extrinsic factors. In particular, cooler waters and habitats with lowest organic matter content favor the greatest abundance/site, probably by representing factors associated to niche modelling of the species, which may determine the multiple invasion processes (introduction, establish and spread).

Second, biotic resistance also explained part of the degree of invasion, even though at a smaller scale. This shows that the preservation of native/resident diverse communities from the earlier times of construction of a reservoir should be considered a potential important solution to the preservation of biodiversity of the semi-arid reservoirs, as they constitute natural controls to invasion. In drier zones, reservoirs, in spite of being considered poor ecosystems in terms of aquatic diversity, represent a pool to conservation of local biodiversity, due absence of water in the rivers and streams during longs time and thus should be adequately managed. Thus, future management actions should aim to enhance the reservoirs water quality to promote the conservation of other species, as physically and chemically degraded habitats and poor in native species provides adequate conditions to harbor invasive and more tolerant species.

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Table 1. Drivers (mean and standard deviation) of invasion of non-native species mollusk (*Melanoides tuberculata*) in the reservoirs. Where: E = extrinsic factors; I = intrinsic factor.

Variables (units)	**Piranhas-Assu river**	**Paraíba river**	**Ecological importance**	**Predictor**
Abiotic variables			Variables related to niche extent, i.e environmental tolerance level of the species for success in introduction, establishment and spread (Lockwood et al., 2005; Catford et al., 2009; Simberlof, 2009).	E
Temperature (ºC)	27.58±1.05	26.37±1.36		
pH	7.99±0.52	8.47±0.49		
Conductivity (mS.cm^{-1})	1.34±0.29	2.15±0.44		
Dissolved oxygen (µg/L)	6.33±1.64	7.31±3.09		
Depth (m)	0.59±0.09	0.71±0.10		
Ecosystem Productivity				E
Total phosphorus (µg/L)	195.51±117.49	164.71±66.16		
Total nitrogen (µg/L)	153.48±51.76	234.81±81.42		
Chlorophyll-*a* (µg/L)	33.22±24.08	18.51±16.05		
Organic matter (gDW)	0.19±0.007	0.30±0.05		
Disturbance				
Hydric Volume (mm^3)	7.91±3.47	8.05±2.31	Factor conditioned to seasonal variation, modify the local conditions and limit the species spread (Lake 2003, 2011).	E
Biotic resistance				
Native community richness	6.42±2.03	2.93±0.61	Related to invasibility. Richest communities are more competitive, avoiding the establishment of species with similar niches to themselves (Kennedy et al., 2002; Tilman, 2004).	I

Table 2: Optimal setting (i.e. combination of parameters that produced the best model) and explicative performance of fitted BRT model aiming at explaining the variation of *M. tuberculata* abundance. tc – tree complexity, lr – learning rate, bf – bag-fraction, cv – cross-validation, nt – number of trees.

Optimal setting			Explicative performance			
Tc	*Lr*	*bf*	CV deviance ± SE	% explained deviance	CV correlation	nt
5	0.005	0.5	80.06 ± 5.21	30.6	50.9	1200

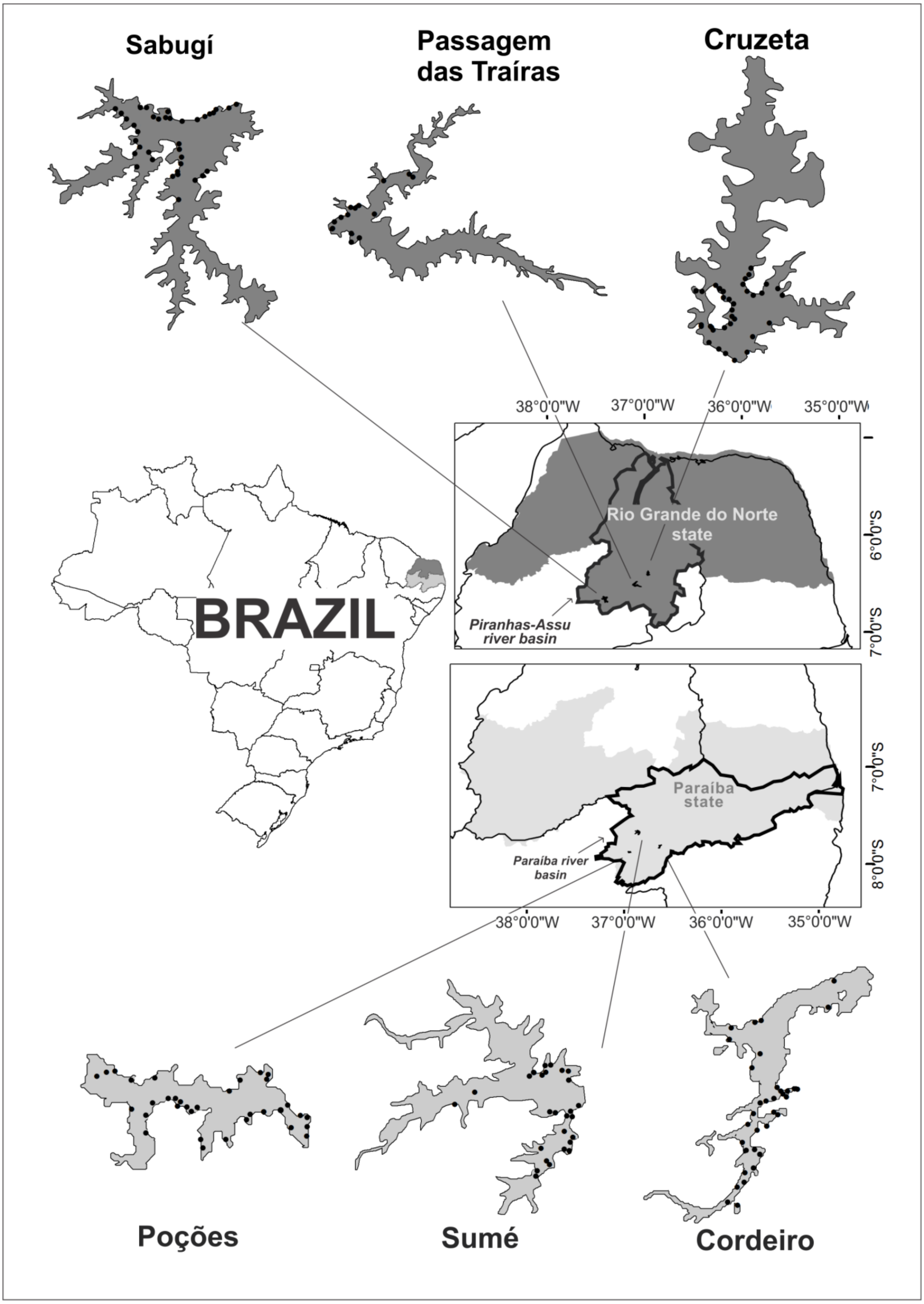

Figure 1. Distribution of the sampling sites (black points) in the reservoirs of Piranhas-Assu river (Rio Grande do Norte state) and Paraíba river (Paraíba state), Brazilian semi-arid region.

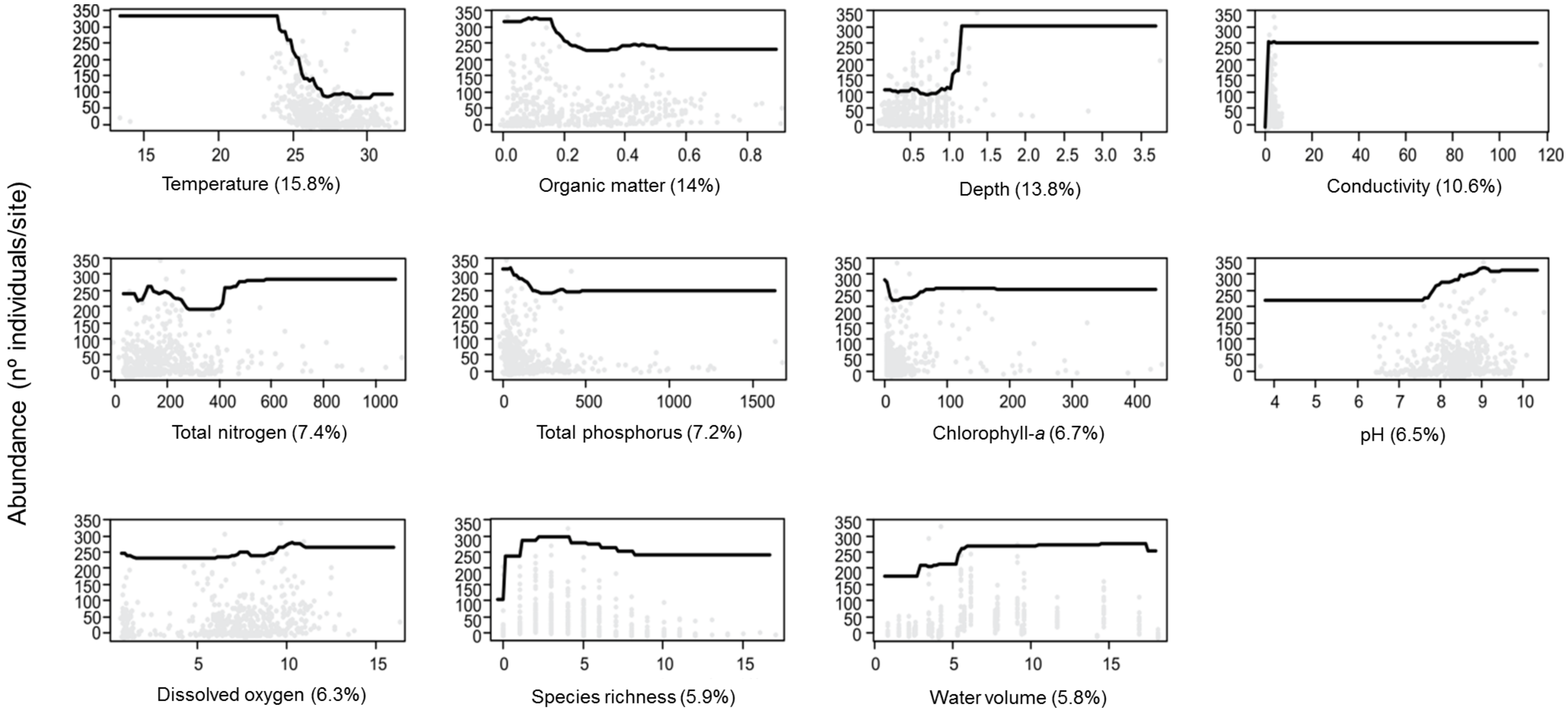

Figure 2: Plots of partial dependencies for the eleven predictors (summed 94% of total variance) discriminated, in order, by relative importance used into BRT model (niche-based) for explaining the degree of invasion of *Melanoides tuberculata* in semi-arid watersheds.

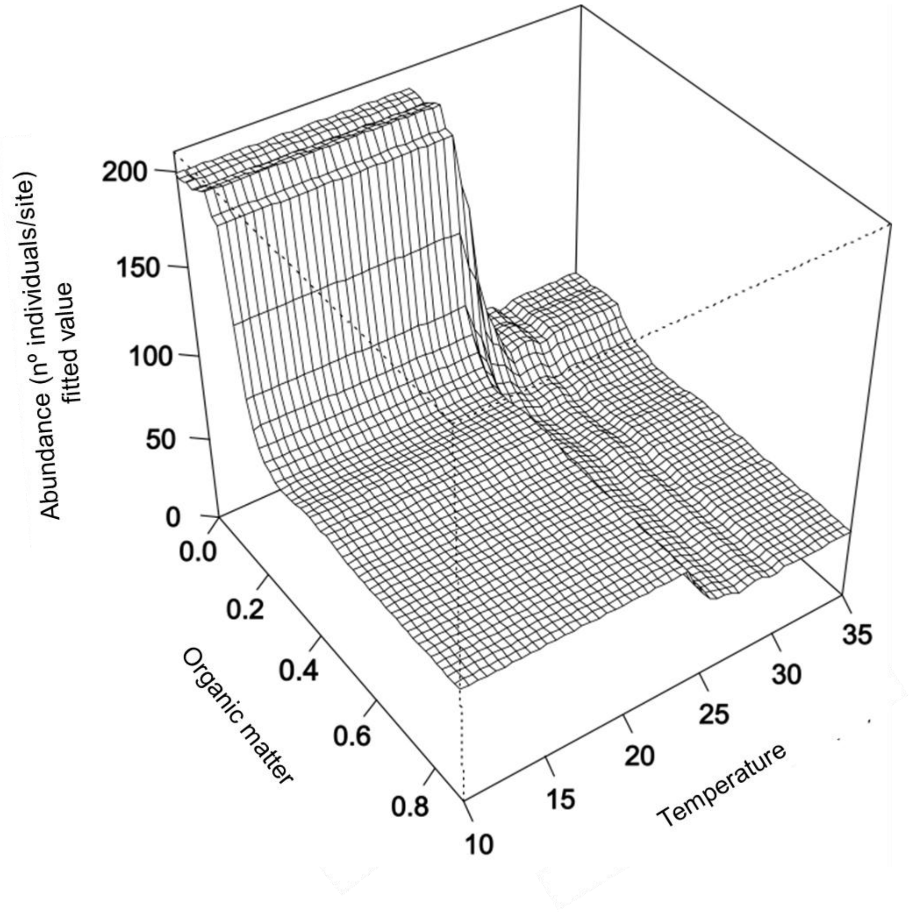

Figure 3: Strongest interactions of the fitted BRT model used for explaining the *M. tuberculata* abundance.

Supplementary material

Table 1. Characterization of reservoirs of Piranhas-Assu and Paraíba rivers watersheds. Data provided by Secretaria de Meio Ambiente e Recursos Hídricos State of Rio Grande do Norte (SEMARH) Agência Executiva de Gestão das Águas (AESA) State of Paraíba. * no record.

Features/Reservoirs	**Piranhas-Assu River**			**Paraíba River**		
	Sabugí	P. Traíras	Cruzeta	Cordeiro	Sumé	Poções
Geographic localization	06º43’06’’S 37º12’02’’W	06º27’16’’S 36º52’29’’W	06º24’42’’S 36º47’23’’W	7°47’38.00”S 36°40’14.04”W	7°29’8"S 37°12'20"W	7°53’38”S e 37°0’30”W
Altitude (m)	187	196	231	480	500	596
Approximate maximum capacity ($10^6 m^3$)	65	49	23	70	45	30
Construction year	1965	1994	1929	*	1953	1982
Main finality	Supply	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation
Time of retention water	3-5 years	3-5 years	3-5 years	3-5 years	3-5 years	3-5 years

Average volume ($10^6 m^3$)	June	September	December	March
Sabugí	18	14	9	7
Passagem das Traíras	3	2	1	0.648
Cruzeta	5	4	2	1
Cordeiro	9	8	5	4
Sumé	17	14	11	9
Poções	6	5	3	2

Capítulo 2. TURNOVER DRIVES Β-DIVERSITY OF MACROINVERTEBRATES IN WATERSHEDS DURING A SUPRA-SEASONAL DROUGHT IN SEMI-ARID REGION

Manuscrito submetido à Freshwater Science

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Turnover drives β-diversity

Turnover drives β-diversity of macroinvertebrates in watersheds during a supra-seasonal drought in semi-arid region

Daniele Jovem-Azevêdo[1,7], José Fernandes Bezerra Neto[2,8], Maria João Feio[5,11], and Joseline Molozzi [4,6,12]

[1]Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, sala 318, bloco L3. Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brazil.

[2]Departamento de Biologia Geral/ Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, sala 318, bloco L3. Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brazil.

[3] Programa de Pós-Graduação em Ecologia, Universidade Federal do Pará. Rua Augusto Corrêa, nº 01, Campus Guamá, CEP: 66075-110, Campina Grande - Paraíba, Brazil.

[4] Programa Pós-Graduação em Ciência Ambiental e Tecnologia, Universidade Estadual da Paraiba. Rua Juvêncio Arruda, S/N, Campus Universitário, CEP: 58429-600, Campina Grande - Paraíba, Brazil.

[5] MARE – Marine and Environmental Sciences Centre, University of Coimbra. Rua da Matemática nº 49, 3000-276 Coimbra, Portugal.

[6]Departamento de Biologia/ Programa de Pós- Graduação em Ecologia e Conservação, Universidade Estadual da Paraíba. Rua Baraúnas, nº. 351 - Complexo Três Marias, Prédio de Biologia, Térreo - sala 10. Universitário, CEP 58.429-500, Campina Grande - Paraíba, Brazil.

E-mail addresses: [7]daniele.jazevedo@gmail.com; [8] joseneto.ufmg@gmail.com; [9]carlindarailly@gmail.com; [10] wilmaizabelly@hotmail.com; [11] feiomaria@gmail.com; [12] jmolozzi@gmail.com

Abstract

Beta diversity provides information on the relationship between local and regional diversity and how the communities vary in space, according to geographic gradients, providing important insights for decision making on biodiversity conservation. Here, we analyzed the evolution of β-diversity of macroinvertebrate communities from 6 reservoirs of two watersheds located in the semi-arid region of Northeast Brazil, during a supra-seasonal drought. The sampling occurred four times between June and March along with an environmental characterization. A null model was employed to determine the deviation between the observed β-diversity and that expected at random. The turnover and nestedness in the β-diversity was analyzed by Sørensen dissimilarity. In addition, we performed a between-class analysis to assess if the environmental characteristics follow the same patterns of β-diversity. Environmental descriptors varied over time, at the site, reservoir and watershed scales ($p<0.001$), with a clear segregation of the watersheds between the first and last sampling periods. The highest communities' dissimilarity occurred between the first and the driest (last) sampling periods (73.8%). The greatest β-diversity (38%) occurred among watersheds (β3 scale). In agreement, the highest diversity variation was found at the largest scales (β2 and β3), with observed proportion larger than expected (Exp > Obs < 0.001). The lowest total diversity was obtained in the driest period (γ = 27). β-diversity was maintained mainly by species replacement (spatial turnover) in both watersheds, especially with replacement of Chironomidae genus. However, there was an increase in species nestedness, during the driest period. These results indicate that, under the future of climate change scenarios of more frequent and intense drought periods, the loss of species may become higher than species replacement, resulting in poor-communities and few tolerant dominant species. Thus the conservation of the aquatic diversity in dry regions where extreme droughts are expected under climate changes (e.g. arid and semi-arid regions), efforts should be directed to richer sites, as under extreme droughts the diversity among locals is maintained by arrival of species (nestedness), forming sub-sets of species from the regional pool.

Key words: dissimilarity; nestedness; species replacement; species diversity; conservation of species; reservoirs.

Introduction

In semi-arid areas seasonal droughts are natural events that act shaping the communities, and their spatial distribution (Stubbington et al., 2014; Rolls et al., 2016; Chadd et al., 2017) and the functional patterns (Chessman, 2015; Feio et al., 2015). These droughts promote the reduction of available habitat, refuge and resource availability influencing the biological communities (Lake 2000). Their predictable patterns shape the communities, leading to dominance of sets of species traits that confer them resistance and resilience (Lake et al., 2003, Bogan et al., 2014, Feio et al., 2015). However, the increase in global temperature has changed precipitation and evaporation rates (IPCC, 2014) leading to longer periods of reduced rainfall and to supra-seasonal droughts (Lake et al., 2003). Particularly in semi-arid regions, this alteration represents not only a risk to water availability but also to water quality resulting in potential species loss (Kim et al., 2013; Gunkel et al., 2015; Daneshvar et al., 2016; Melo et al., 2017). In addition, their unpredictable pattern can change communities traits selecting resistant organisms (Boulton, 2003, Wood and Armitage, 2004, Lake, 2011, Dézerald et al. 2015). Understanding the patterns of spatial organization of the diversity at multiple scales and the ecological mechanisms that maintain it during a climatic atypical event is important to predict communities responses against extreme events (Bogan and Lytle, 2011, Rolls et al., 2016). This information is essential to propose strategies for the management and conservation of biodiversity in aquatic ecosystems, especially when extreme events may be more frequent and intense (Bogan et al., 2014, Marengo and Bernasconi, 2015, Jewitt et al. 2016, Marengo et al., 2016).

β-diversity is a tools that has been used as a mean to analyse community spatial patterns in ecological studies. The term β-diversity, introduced by Whittaker (1960) refers to the degree of community differentiation in relation to a complex-gradient of environment and traduces the difference between gamma (regional) and alpha (local) diversities. Beta-diversity may reflect two phenomena: spatial turnover and nestedness, which act on differentiation of species identities among communities (Baselga et al. 2007, Baselga 2010, Anderson et al., 2011; Baselga and Leprieur 2015). The spatial turnover is associated with the replacement of species by others, due to stochastic environmental events (e.g. replacement of species over of the environmental gradient), spatial restriction (e.g. geographical barriers) or historical events. In the nestedness, the local pool of species represent a subset of richest areas. This local diversity results from selective colonization (immigration and migration), extinction and plasticity of species (Qian et al. 2005, Leprieur et al. 2011).

Accordingly, the processes that drive spatial turnover and nestedness and consequently the dissimilarity among places (β-diversity) are related to “ecological assembly rules” (Diamond 1975). These assembly rules refer to ecological and phylogenetic filters on composition and organization of communities, in consequence of random events (demographic process, dispersal patterns or ecological drift) or niche extent (Hubbell 2001, Chase and Leibold 2003, Götzenberger et al. 2012, Guichard 2017). Therefore, the distribution of species in different gradients is the result of two processes that can act in a non-exclusive form (Sommer et al. 2014, Boschilia et al. 2015).

In the last decades there was a wide debate on the best method to estimate β-diversity (Chao et al. 2012, Baselga 2013, Baselga and Leprieur 2015). Although the opinions differ, all authors agree that it provides useful information on species organization in space and the patterns of fluctuation among geographic gradients, including areas with large anthropogenic pressure (Kraft et al. 2011, Boschilia et al. 2015, Santana et al. 2017). Thus, β-diversity can be used to predict a scenario of species loss, to establish priority areas for conservation and to propose actions of preventions and management of invasive species (Gering et al. 2003, Wiersma and Urban 2005, Gabriel et al. 2006, Karp et al. 2012, Nobis et al. 2016, Santana et al. 2017). Additionally, information on turnover and nestedness mechanisms is also useful to optimize the decision-making, focusing on the conservation at multiple scales or only on richer areas (Socolar at al., 2015).

In dry and arid regions of the world the reservoirs are, nowadays, frequent elements in the landscape, and important aquatic ecosystems as they maintain a large amount of water and are often the only perennial systems where many of the rivers and streams dry during a large period of the year. Therefore, these ecosystems have a very relevant role in the maintenance of aquatic biodiversity. In addition they provide important services to human populations constitution an important source of freshwaters for human consumption or agriculture, food or providing humidity and contributing to lower air temperature (Boschilia et al., 2015; Souza-Barroso et al., 2017). However, these ecosystems are also frequently subject intensive and frequent disturbances, as result of such as fishing, tourism, boats traffic, sewage discharges, water abstraction, flood pulses or low water volumes that lead to changes in their ecological quality, putting in risk their biodiversity and compromising ecosystem services at the local and regional scales (e.g. watershed) (Sugunan 2000, Chellappa et al. 2009, Barbosa et al. 2012).

However, very few studies analysed the diversity patterns in reservoirs and none was done in the semi-arid region neither considering the influence of extreme droughts (Molozzi et al. 2013, Boschilia et al. 2015, Wojciechowski et al. 2017b). And yet, due to its extreme conditions, the semi-arid region, can provide important insights on the effect of climate changes in biodiversity. This information can contribute to the preservation of the most arid regions of the planet, which are often affected by social and economical problems making water one of the most valuable goods. We intended to overcome that lack of information by analysing over time the effect of an increasingly stronger supra-seasonal drought in the β-diversity of six reservoirs located in the semi-arid region of Brazil. More specifically, our hypotheses were: 1) β-diversity increases over the continuous water contraction period in semi-arid reservoirs (supra-seasonal drought), accompanying the variation in environmental conditions and communities' structure (species richness and diversity) among watersheds, reservoirs and sites; and 2) β-diversity is mainly sustained by turnover, i.e., species better adapted to new conditions substitute the previous in their dominance (Figure 1). We focused our analyses on the benthic macroinvertebrates communities because they are abundant and diversified communities of reservoirs, include ubiquitous species and are sensitive to environmental variations (Shao et al. 2008, Beghelli et al. 2012, Molozzi et al. 2013, Azevêdo et al. 2015).

METHODS

Study area and sample design

The sampling design was structured in four hierarchical levels of diversity, following the spatial hierarchy pattern described by Paivone et al. (2016) and applied to rivers: α - sampling site, β1 - dissimilarity among sampling sites, β2 - dissimilarity among reservoirs and β3 – dissimilarity among watersheds (Figure 2). Thus, the study was conducted in six reservoirs distributed in two watersheds located in the semiarid region of Brazil: Sabugí, Passagem das Traíras and Cruzeta reservoirs (Piranhas-Assu river, Rio Grande do Norte state) and Cordeiro, Sumé and Poções reservoirs (Paraíba river, Paraíba state) (Figure 3). All the reservoirs are used for water supply and irrigation (Table S1 - supplementary material).

According to Köppen–Geiger classification, BSh (dry semiarid) is the dominant climate in the region. In Paraíba watershed the mean rainfall is ca. 400 mm/year, while in Piranhas-Assu watershed is ca. 800 mm/year. The minimum air temperature varies between 18 and 22ºC (July and August) and the maximum between 28 and 31ºC (November and December). In both regions, the wet period lasts about 3 months (usually from February to April) (Alvares et al. 2013).

Sampling occurred in 60 sites (10/reservoir) in June, September and December of 2014 and March of 2015. This period was classified as a supra-seasonal drought during which the reservoirs had a strong reduction of their hydric volumes (Table S1) (Agência Executiva de Gestão das Águas - Paraíba State; and Secretaria de Meio Ambiente e Recursos Hídricos - Rio Grande do Norte State). In fact, since 2012 the Brazilian semi-arid is suffering a prolonged drought, which is considered the most severe of the last decades (Marengo et al. 2016).

Sampling and processing of benthic macroinvertebrates

The individuals were sampled with an Eckman-Birge dredge (225 cm²) and fixed in situ with formaldehyde 10%. In the laboratory, the samples were washed under a sieve with 0.5mm mesh size and the individuals were removed from sediment. Invertebrates were identified to family level, except for Chironomidae that were identified to genus level (Peterson 1960, Boffi 1979, Péres 1988, Trivinho-Strixino and Strixino 1995, Merritt and Cummins 1996, Carvalho and Calil 2000, Epler 2001, Fernández and Domínguez 2001, Costa et al. 2006).

Environmental descriptors

I) Physical and chemical parameters

At each site, temperature, turbidity, dissolved oxygen and total dissolved solids were measured using a multiparameter probe (HORIBA U-50). The water transparency was assessed with a Secchi disk and the depth with sonar. Water (1L) was sampled in the sub-surface of each site and analysed in laboratory to determine the concentration of total phosphorus, soluble reactive phosphorus, total nitrogen, nitrate and nitrite (for NOx-N) and ammonium ion, according to APHA (2005). The concentrations of chlorophyll-*a* were estimated by extraction of the pigment in acetone 90% (Lorenzen 1967).

II) Habitat characterization

Sediment aliquots were sampled with an Eckman-Birge dredge (0.225 cm^2) to assess the granulometric composition and organic matter content. Granulometric composition was analysed according to the method proposed by Suguio (1973), modified by Callisto and Esteves (1996), considering the following classes: gravel (>1 mm), coarse sand (500-1000 µm), middle sand (250-500 µm), fine sand (63-250 µm) and silt/mud (< 63µm). For organic matter, we incinerated sediment (3 g) in muffle (550°C for 4h) and calculated the differences between first and last weight, considering the standard deviation < 0.004 among replicates.

Data analyses

To test our first hypothesis, we evaluated the changes in the composition of benthic macroinvertebrates' communities (square root transformation, Bray-Curtis similarity coefficient, 9999 permutations) and environmental descriptors (log x+1 transformation, Euclidian distance, 9999 permutations; $p<0.005$) through multivariate "Permutational Multivariate Analysis of Variance" (PERMANOVA; Anderson 2001a, b; Anderson and Braak 2003, Anderson et al. 2008). All above analyses addressed three random factors: sites (sixty levels), reservoirs (six levels), watersheds (two levels) and the sampling period (four levels). In addition, univariate PERMANOVA (Euclidean distance; $p<0.005$) was employed to analyse the differences among sampling periods in taxonomic richness/α-diversity (S) and diversity (H', given by the Shannon-Wiener index) and in environmental conditions, for each variable measured. Previously, highly correlated (Spearman r >0.75) environmental variables were excluded (e.g. total phosphorus, soluble reactive phosphorus, chlorophyll-*a*, transparency, gravel, coarse sand, middle sand and silt/mud). Posteriorly, all the environmental variables were normalized. The most representative taxa and environmental variables were determined for each sampling period through the "similarity percentages analysis" (SIMPER). Biological data was previously transformed by square root and Bray-Curtis similarity measure was used; environmental data was transformed by log (x+1) and Euclidian distance was used as similarity measure. All the analyses were performed in PRIMER + PERMANOVA 6 software (Clarke and Gorley, 2006).

To analyse the distribution of sites based on community composition in function of the environmental variation over time, we employed "Between-class coinertia analysis" (bca.coinertia) (Franquet et al. 1995). This analysis results from the combination of "Principal component analysis"

(PCA) for biological and environmental matrix and co-inertia analysis for calculation of maximal covariance among samples (environmental and biological data) (Dolédec and Chessel 1994, Dray et al. 2003). Furthermore, the bca.coinertia allows the insertion of spatial and temporal data as categories, enabling to analyze the segregation among classes. For analysis of bca.coinertia only the significant environmental variables were considered (see Table 2). These analyses were performed in R statistical environment (R Development Core Team 2016), using “ade4” (Dray 2017).

Additionally, we calculated the β-diversity through additive partitioning, which has been considered the most operational approach (Veench et al. 2002, Cris et al. 2003, Chiarucci et al. 2010, Baselga 2012). Total diversity is obtained by the sum of each hierarchical levels of the sampling design, thus in our study: $\gamma = \alpha + \beta 1 + \beta 2 + \beta 3$. The α diversity represents the mean number of species in the sites and β is the average diversity that is absent from a given sample, considering the hierarchical levels (Crist and Veech 2006). A null model was employed to determine the deviation of the observed (Obs) from the expected partition (Exp) by random distribution in space (Crist et al. 2003). The significance level was calculated by the difference among Exp and Obs, considering 9999 permutations. High proportions ($\text{Prop}_{\text{exp>obs}}>0.975$) indicate that Obs was significantly lower that Exp, while low proportions ($\text{Prop}_{\text{exp>obs}}<0.025$) indicate that Obs was significantly higher that Exp. These analysis were conducted in Partition software 3.0 (Veech and Crist 2009).

Finally, to answer to the second hypothesis, the spatial β-diversity was determined by measuring dissimilarities among multiple sites based on species presence/absence (Baselga 2010). To analyse the influence of the turnover and nestedness in β-diversity among sampling periods, the Sørensen dissimilarity (β_{SOR}) was divided in two components: spatial turnover (β_{SIM}) and nestedness (β_{NES}) (Baselga 2010). Analyses were performed in R statistical environment (R Development Core Team 2016), using “vegan” (Oksanen et al. 2017) and “betapart” packages (Baselga 2012).

RESULTS

Pattern of α-diversity

In our samples, 47 taxa were identified from the 31469 individuals (Tables S2 and S3). In the reservoirs of Paraíba river (15884 individuals) *Melanoides tuberculata* was the dominant taxa (63.5 contributive % - SIMPER). In the reservoirs of Piranhas-Assu, the invasive mollusc also was identified, but Oligochaeta was the most representative taxa (15585 individuals, 36.7% - SIMPER). The greater total abundance occurred during the highest hydric volume in June, in both watersheds (4971 individuals in Piranhas river and 4159 in Paraíba river), and decreased gradually over the drought period (Tables S2 and S3).

The highest community dissimilarity occurred between the first sampling period (June) and the driest period (March) (73.8%). *M. tuberculata* (29.6% contribution) and Oligochaeta (27.6% contribution) were the most representative taxa of this dissimilarity. Chironomidae genus, such as *Goeldichironomus* and *Aedokritus* (9.0% and 4.4%, respectively), also contributed for differences in the community over time. The differences in communities were significant among sites (PERMANOVA: Pseudo-$F_{58.205}$ = 1.95; p = 0.0001), reservoirs (Pseudo-$F_{5.205}$ = 13.65; p = 0.0001), watersheds (Pseudo-$F_{1.205}$ = 30.02; p = 0.0001) and sampling periods (Pseudo$F_{3.205}$ = 3.87; p = 0.0001).

In accordance, the highest average richness (S) and diversity (H') was obtained in Piranhas-Assu reservoirs (S = 8.5±3; H' = 0.8±0.05) and the lowest values in Paraíba reservoirs (S = 2.6±0.2; H' = 0.3±0.1). A gradual reduction in richness and diversity was observed with the intensification of the drought (Table 1). There was a significant difference in species richness among sites (Pseudo-$F_{59.239}$ = 3.22; p = 0.0001), reservoirs (Pseudo-$F_{5.239}$ = 27.97; p = 0.0001), watersheds (Pseudo-$F_{1.239}$ = 31.97; p = 0.0001) and sampling periods (Pseudo-$F_{3.239}$ = 11.80; p = 0.0001). The Shannon-Wiener diversity was also significantly different among sites (Pseudo-$F_{59.239}$ = 2.22; p = 0.0001), reservoirs (Pseudo-$F_{5.239}$ = 13.88; p = 0.0001), watersheds (Pseudo-F1.239 = 19.91; p = 0.0003) and sampling periods (Pseudo$F_{3.239}$ = 6.18; p = 0.0007).

Environmental drivers of community patterns

The watersheds and their respective reservoirs vary in their trophic status. Overall, values of environmental variables varied over time with intensification of drought (see table 2). In Piranhas-Assu and Paraíba river all water chemical and physical parameters varied significantly during the study period (p< 0.005, Table 2). Considering the habitat descriptors only gravel varied in Piranhas-Assu river (p = 0.0003, see table 3) while in Paraíba river, organic matter (p = 0.003) and fine sand (p = 0.003) changed significantly over time. Thus, as for community patterns, the greatest average squared distance among environmental descriptors was found between the first sampling period (June) and the driest period (March) (26.2), mainly due to dissolved oxygen, turbidity and temperature (15%, 14.3% and 13.9% contribution to average dissimilarity, respectively). Increasing with drought intensity, these environmental variables reached their highest values the driest period along with total dissolved solids and trophic index status, indicating a greater environmental degradation.

In the beginning of the study period, the communities were negatively correlated with turbidity (-0.89 - axis 1 - total inertia 59.7%), especially in Piranhas-Assu sites (Figure 4A). Over of the study period, other environmental descriptors, such as total dissolved solids and temperature, became the best explanatory variables for both watersheds (total inertia 53.1 at September and 31.4 % at December) (Figure 4B and 5A). Differently than observed in previous months, during driest period (total inertia 22.7%), the NOx-N was mainly correlated with sites of Paraíba river (Figure 5B). In the beginning of the study (June) the sites were segregated by watershed, but over time (September and December) the communities became more similar, until the last sampling period when the initial pattern of segregation was re-established.

Diversity partitioning, Turnover and Nestedness

The greatest diversity variation occurred among watersheds (β3 scale) (Figure 6) in the period of June, December and March, with proportions of 38.3, 37.6, and 38.0%, respectively. In September, the highest dissimilarity occurred among sites (β1 scale = 30.6%). According to the null model, the observed values of richness were lower than expected at random (Exp > Obs > 0,999) in the smaller spatial scale (α and β1 scales), except in driest period (March), where the observed values were bigger than expected at β1 scale (Figure 6). For β2 and β3 scales, the observed values were higher than expected (Exp > Obs < 0,001) in all months (Figure 6). The lowest total diversity was found in the driest period (March, γ = 27) (Figure 6).

In Piranhas-Assu watershed, the greatest global β-diversity was observed in the beginning of the study (June) and in the driest period (March) (Figure 7A). In this watershed, the decomposition of β-diversity showed that dissimilarities were predominantly maintained by turnover, except in last period (March) when greater nestedness occurred (Figure 7A). At Paraíba river, β-diversity was also higher in last period (March) and similar in June and December (Figure 7B) and the community was driven mainly by species turnover over.

DISCUSSION

Beta-diversity varies with environmental dissimilarity, randomly or by historical factors. Among these, the environmental factors create gradients that segregate species according to their niches extent (Chase and Leibold 2003, Leprieur et al. 2009, Boschilia et al. 2015). In agreement, we found an increase in β-diversity over of a drought period, accompanying the increasing dissimilarity between watersheds and reservoirs. In deed, the highest β-diversity occurred on driest period (March), when the smallest total diversity was recorded. Ours results support the hypothesis that the increase of environmental dissimilarity, considering the variation in the abiotic components (see Heino et al., 2015), provides different niche conditions, selecting species more adapted to local environmental conditions or with greater extent niche, which increases the variation of communities among sites (Leibold et al., 2004, Cottenie, 2005).

The pattern of spatial shaping of community by environmental dissimilarity among scales is predictable (Gröroons et al., 2013, Bini et al., 2014, Heino et al., 2015). However, here the environmental dissimilarity may have been accentuated by distance. The drought events decrease connectivity and increase the distance among sites (in this case, between reservoirs or watersheds, due to the intermittency of rivers), which limits the dispersal of species and, consequently, increases β-diversity (Soininen et al., 2007; Ligeiro et al. 2010, Angeler and Johnson 2012, Gray and Arnott 2012). Here the intensive drought represents thus a disturbance of an even larger magnitude leading to changes in the spatial arrangement of communities over of the time (Chase, 2003, Trexler et al., 2005, Garner et al., 2015).

In line with our predictions, we found that the β-diversity was maintained mainly by turnover in both watersheds. This means that, communities composed of taxa resistant to hydric stress mainly compose the diversity during the drought period. But, under extreme seasonal the community may be conducted to a pattern out of its physiological limit, resulting in impoverished communities and high rotation rates of species among sites (Boersma et al., 2013; Aspin et al., 2018).Yet, resistant species (wide plasticity and high competitive ability) frequently show smaller dispersion abilities, as a trade-off evidence (Tilman, 1994; Pyšek and Richardson, 2010; Darrigran and Damborenea 2011). In fact, we have found high rates of *Melanoides tuberculata* and Oligochaeta along the sampling period, which are taxa with high tolerance and resistance level, but limited dispersion mechanisms (Giovanelli et al., 2003; Guimarães et al., 2001; Santos and Eskinazi-Sant'Anna, 2010, Azevêdo et al., 2015; Azevêdo et al., 2016). Furthermore, our dataset shows that, despite *M. tuberculata* and Oligochaeta dominance, over of drought period there was a change in the dominant Chironomidae genus, as *Goeldichironomus* and *Chironomus* were substituted by *Tanytarsus* and *Polypedilum* (see table S2 and S3), reinforcing the replacement pattern.

The turnover is a process frequently associated to historical factors (niche conservatism), stochastic, geographic barriers and dispersal limitation (Qian et al. 2005, Wang et al. 2017). In deed, in our study, the absence of connectivity among reservoirs (in consequence of the supra-seasonal drought) creates barriers for dispersion, limiting local diversity and making the random processes stronger in communities' assembly (Chase et al. 2003). Previous studies showed a similar pattern, where the environmental gradients were considered the strongest factor for the fast replacement of species (Boschilia et al. 2015, Medeiros et al. 2016).

On the other hand, against our predictions, we also found that in Piranhas-Assu watershed, during driest period, the local communities were the most nested, indicating that, rather than species replacement, there was species loss. In addition, the increasing of the species nestedness during driest period indicates that rare species were restricted to few richer sites, revealing a risk for conservation at the watersheds level (Larsen and Ormerod, 2010). This increase of nestedness was probably caused by the higher level of environmental degradation (e.g. total dissolved solids and nitrogen concentration) observed during the driest period on the watershed. In fact, nestedness can be related to environmental pressure on sensitive species, favouring the arrival, colonization or densification of tolerant species, common in all communities in the regional pool (Qian et al. 2005, Heino, 2011, Leprieur et al. 2011, Gutiérrez-Cánovas et al. 2013). The nested pattern can be common in aquatic ecosystems across-regions, where colonization dynamics modulate the arrival of species from richer areas (Taylor and Warren 2001, Heino 2005, Heino et al. 2009, Ramos-Jiliberto et al. 2009, Florencio et al. 2011, Mitsuo et al. 2011, Soininen and Köngäs 2012).

The increase of species nestedness during the driest period was represented by a species subset found in previous periods, but with a lower abundance of common species and without rare taxa. Drought events of high intensity may act selecting rare species through variation of environmental conditions or common species by dispersion limitation (Bogan and Lytle, 2011, Petsch et al., 2015). Furthermore, under the intense disturbance, promoted by extreme drought, the local extinction process assumes an important role in community shaping, increasing the nestedness pattern (Trexler et al., 2005, Lake 2011, Shukla and Bhat 2018). Our study corroborated this pattern showing that it also occurs in artificial systems as the studied reservoirs and not only for rivers, as previously found.

Knowing the patterns of diversity and their drivers in the ecosystems is important to propose strategies for conservation and management (Jankowski et al., 2009; Socolar et al., 2015). We showed that this is also true for ecosystems of reservoirs, in watersheds where they have a relevant presence and are often the largest aquatic ecosystems during long dry periods, as those of semi-arid regions. Guerin et al. (2013) modelling the pattern of diversity change in ecotones (mesic-arid environments), emphasized that environments dominated by turnover can be more susceptible to climate changes with greater probability of species loss. Here, we showed that the turnover is the dominant phenomena for diversity of reservoirs during prolonged drought in semi-arid watersheds. In addition, nestedness also occurs favouring tolerant species, with low dispersal abilities. This means that communities of already more poor systems, as the reservoirs, may become even poorer under climate changes, leading to modifications in the dynamic and functioning on the ecosystem. Under these conditions, the best strategy for conservation will be the preservation of a network of sites distributed throughout the watersheds, aiming to increase the probability of conservation regional diversity (Florencio et al. 2011, Loiseau et al. 2016).

Finally, it could be argued that the different taxonomic levels used for Diptera (genus) and for the remaining taxa (family) could have been influenced our results. Yet, and considering the impossibility of identifying all taxa to genus level, as these families are mostly constituted by a single genus and Chironomidae are in deed the majority of the community, we performed a preliminary test, comparing the analyses of the entire community (identified to family and genus level) with those considering only Diptera (identified to genus level). Since our results showed a similar pattern in both cases with predominance of the turnover, we opted to use the entire community to broaden our results.

CONCLUSIONS

The analyses of beta-diversity of invertebrate communities of reservoirs of the semi-arid during a supra-seasonal drought enabled important conclusions. First, we showed that the prolonged drought act as a trigger in the changes of the environmental conditions, through the strong decrease of hydric volume, leading to the loss of connectivity among reservoirs and decrease in water quality. These changes promoted changes in spatial arrangement of diversity, resulting in high dissimilarities between communities (β-diversity) and among sites in watersheds.

In addition, community dissimilarities (β-diversity) were predominantly maintained by the random replacement of species (turnover), indicating that tolerant species are determinant to the colonization of reservoirs in the dry period. However, with the increase of disturbance, the diversity is mainly determined by a species subset from regional pool (nestedness). This suggests that under climate changes, with the increase of drought events, the loss of species may overcome their replacement, resulting in poorer communities dominated by generalists.

Finally, conservation actions of areas where the intensity and frequency of drought events is expected to increase (e.g. arid and semi-arid regions), should be undertaken at the may be directed to richer sites, as under extreme droughts the dissimilarity is maintained by arrival of species from the regional pool (nestedness).

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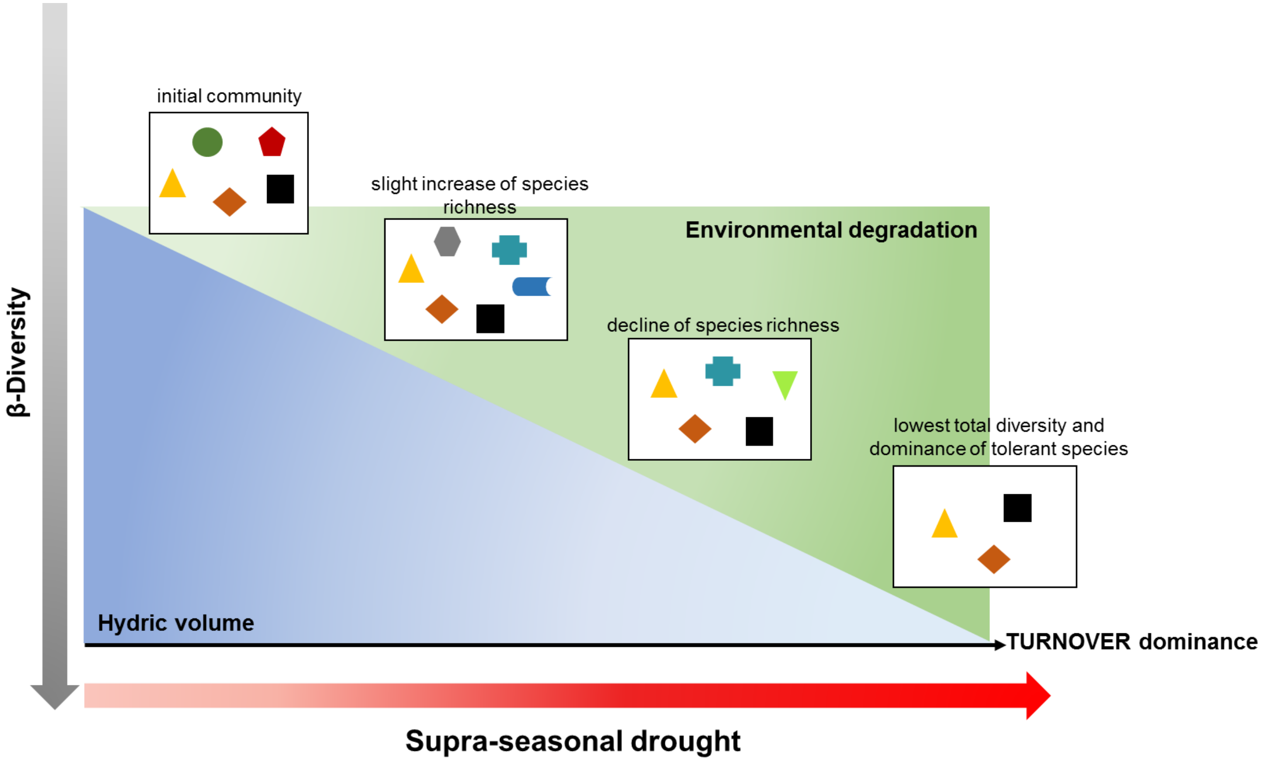

Figure 1. Conceptual model of the evolution of β-diversity of macroinvertebrate communities over of a supra-seasonal drought period, according to continuous water contraction and increase in environmental degradation. Species in a community are represented by symbols inside the squares

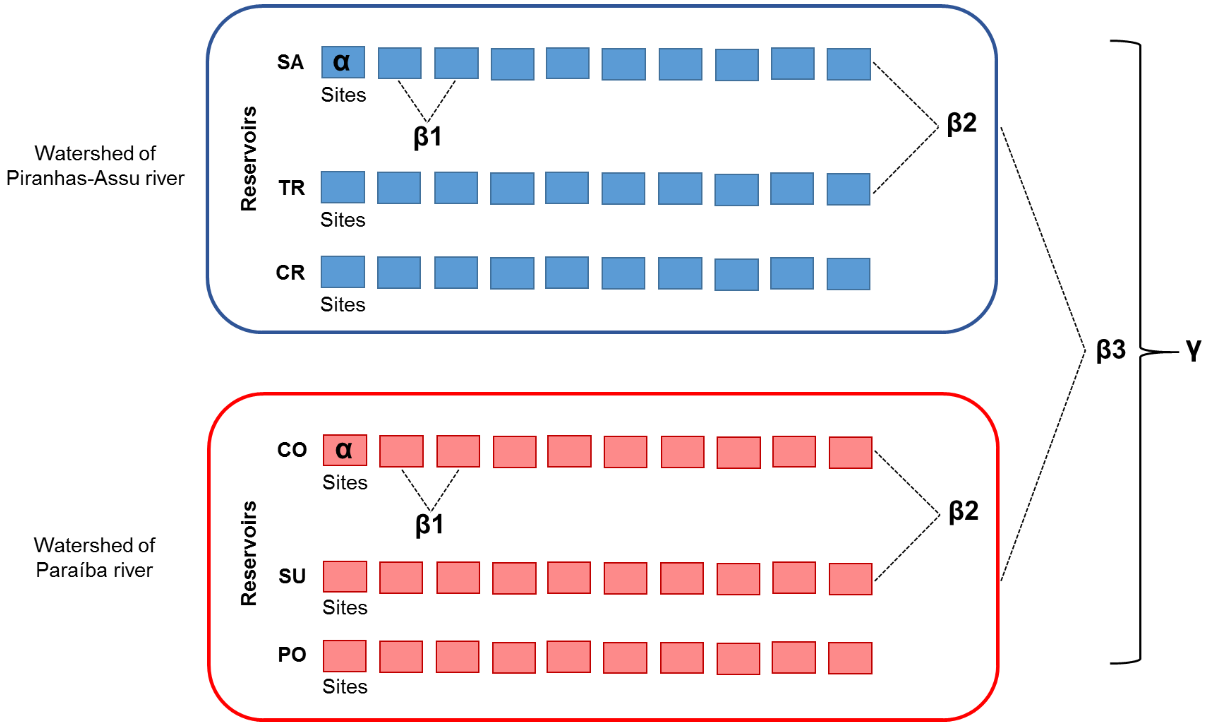

Figure 2. Representative scheme of the hierarchical design made for diversity partitioning in semi-arid watersheds. Where: SA = Sabugí, TR = Passagem das Traíras, CR = Cruzeta, CO = Cordeiro, SU = Sumé and PO = Poções.

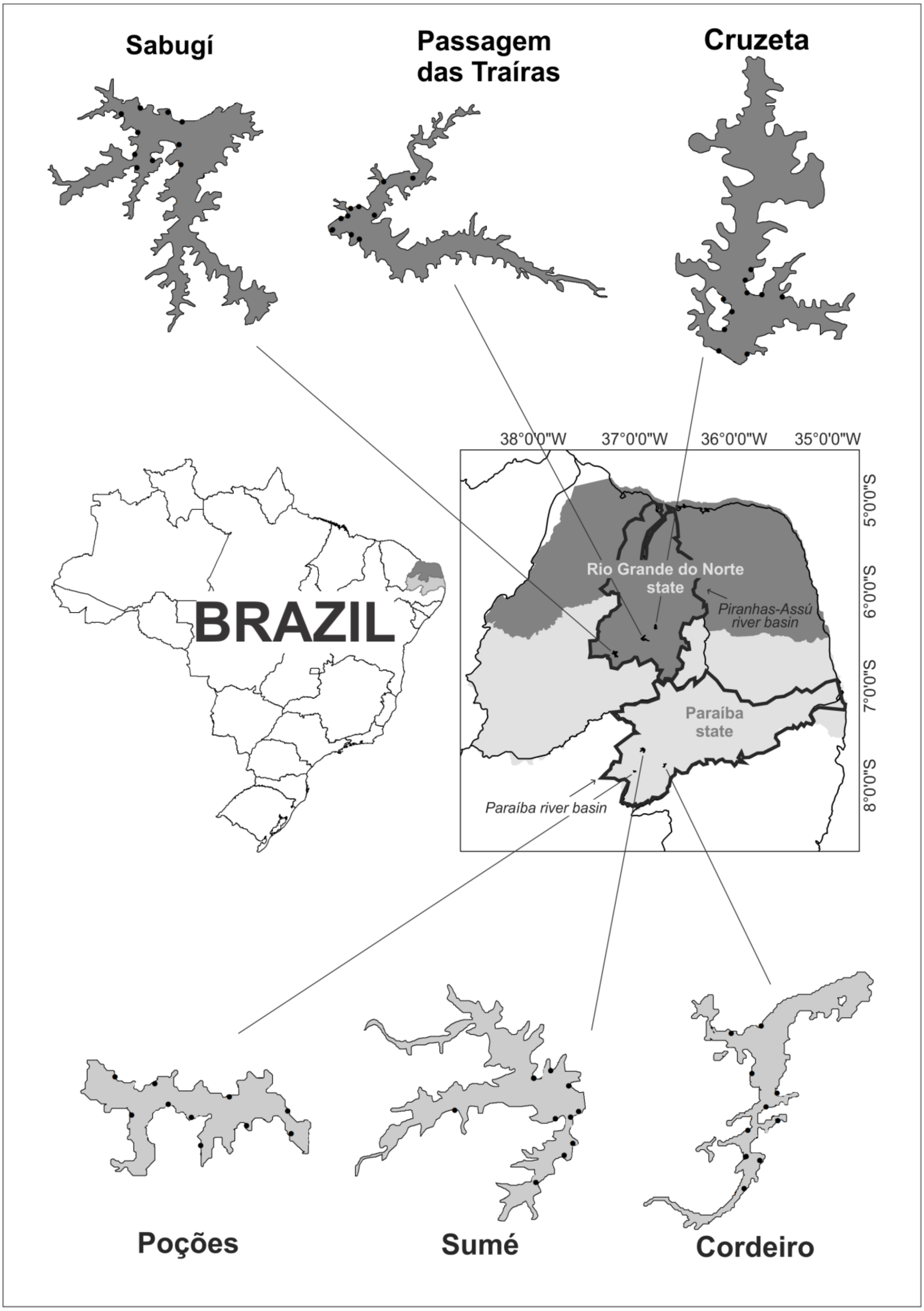

Figure 3. Distribution of study sites (black dots) in the reservoirs of Piranhas-Assu river (Rio Grande do Norte state) and Paraíba river (Paraíba state), Brazilian semi-arid region.

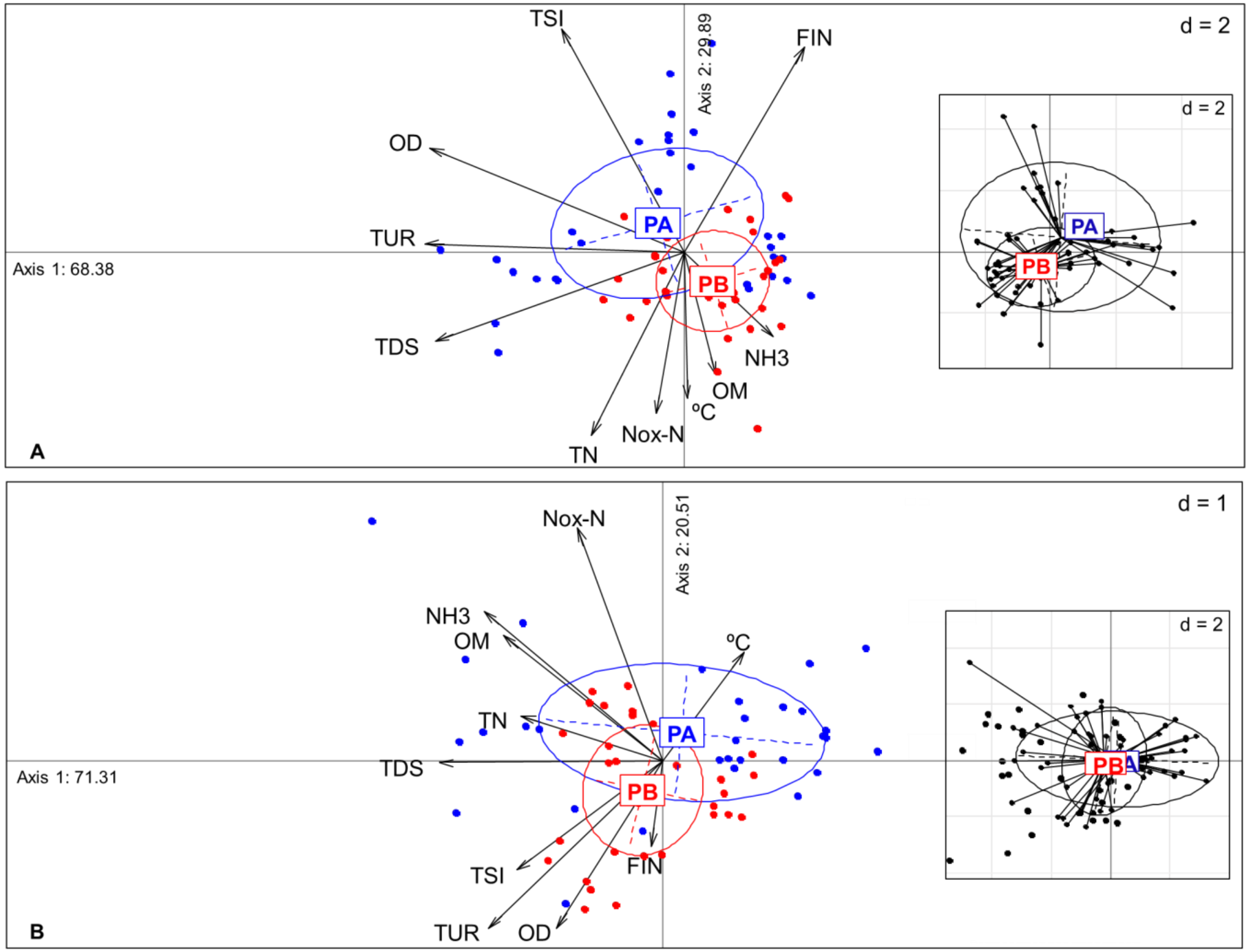

Figure 4. Results of "bca.coinertia" analysis between environmental variables and community composition. In blue and red, circles indicate the distribution of the community composition in Piranhas-Assu river and Paraíba river, respectively. On the right, "within-class analysis" (part of the bac.coinertia) indicate common centre of distribution of the dataset among classes. Where: A = June; B = September.

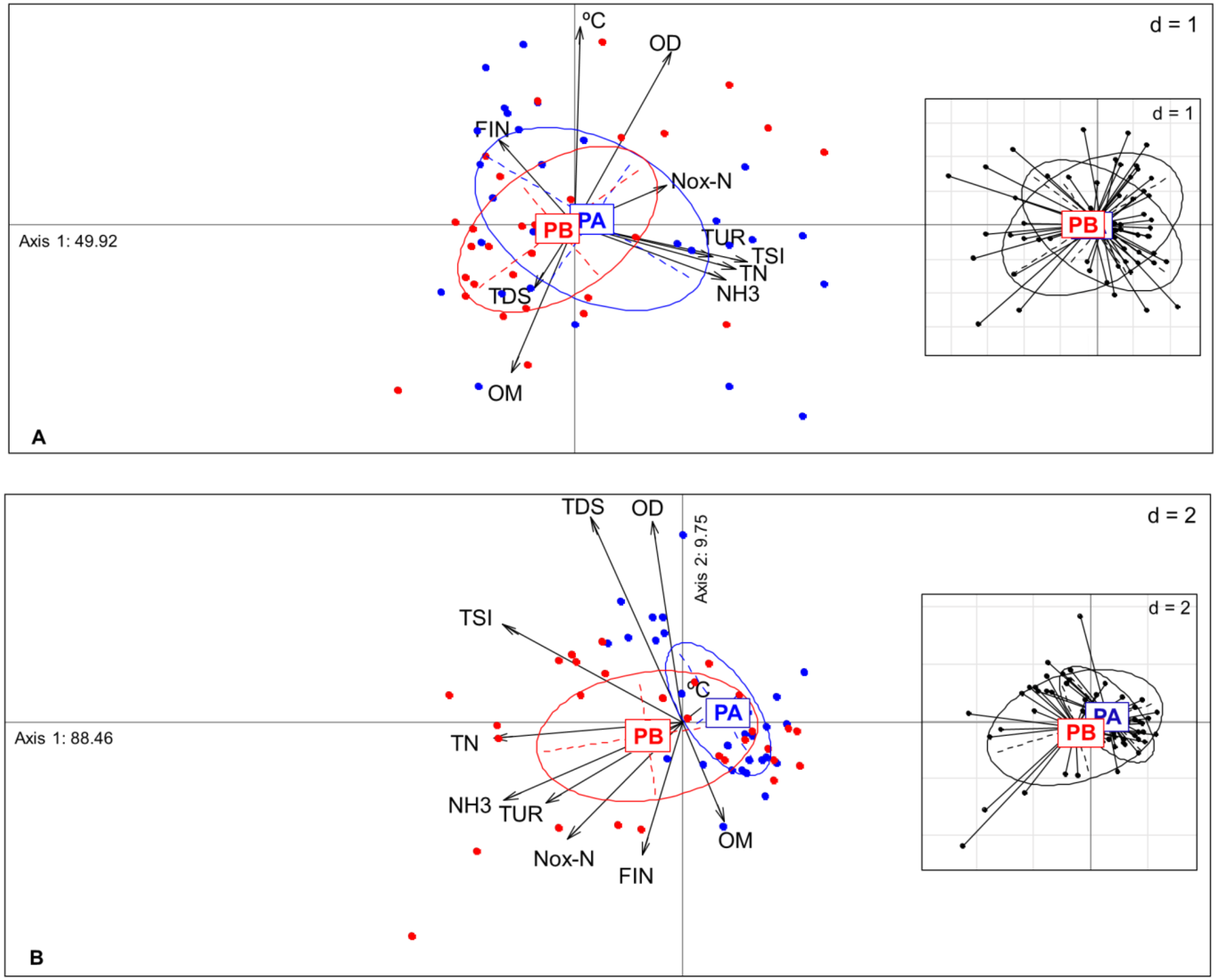

Figure 5. Results of the "bca.coinertia" analysis between environmental variables and community composition. In blue and red, circles indicate the distribution of the community composition on sites of Piranhas-Assu river and Paraíba river, respectively. On the right, "within-class analysis" (part of the bac.coinertia) indicate common centre of distribution of the dataset among classes. Where: A = December; B = March.

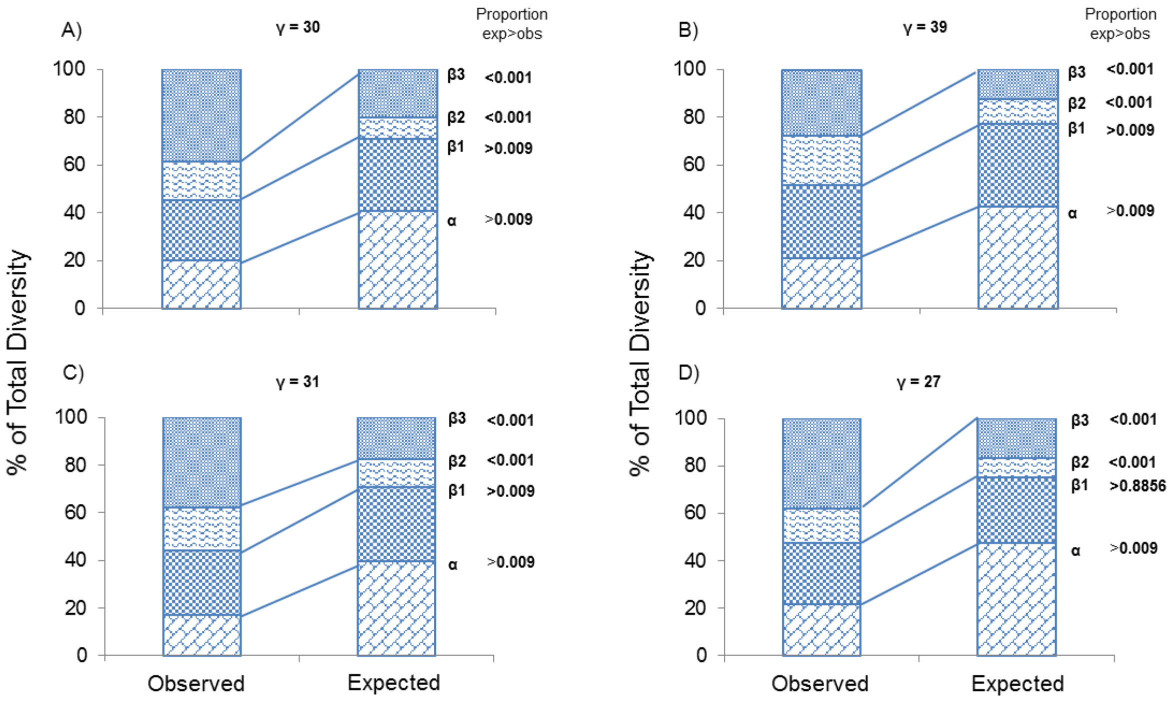

Figure 6. Observed and expected diversity partitioned in alpha and beta components during June (A), September (B), December (C) 2014 and March (D) 2015 in semi-arid watersheds. Bold numbers indicate the proportion of the observed and expected partition randomly. Where: α = mean species richness in the sites; β1 = dissimilarity among sites; β2 = dissimilarity among reservoirs (Sabugí, Passagem das Traíras, Cruzeta, Cordeiro, Sumé and Poções); β3 = dissimilarity among watersheds (Piranhas-Assu river and Paraíba river); and γ = total diversity during sampling period. (A) = June; (B) = September; (C) December and (D) = March.

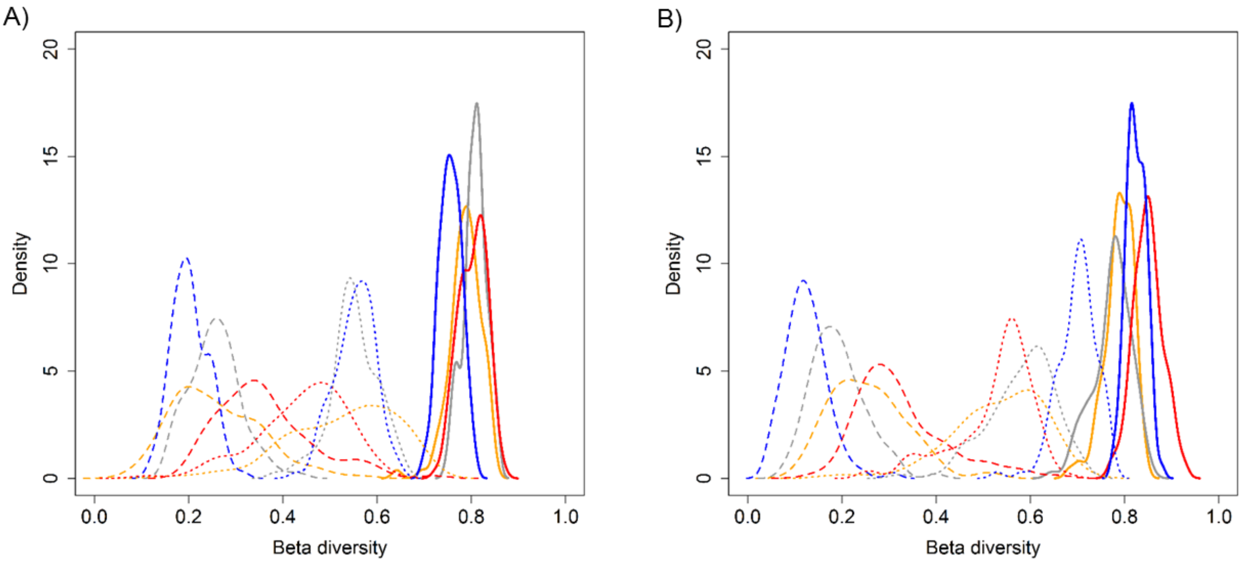

Figure 7. Beta diversity and contribution of turnover and nestedness mechanisms in the Piranhas-Assu river (A) and Paraíba river (B). Where: continuous lines = indicate general beta diversity, dotted lines = indicate turnover and dashed lines = indicate nestedness. The colors of the lines represent the different sampling periods, where: June = grey, September = blue, December = orange and March = red.

Table 1. Mean and standard deviation (SD) of the species richness and Shannon-Wiener diversity of macroinvertebrate communities of sites from the six reservoirs in Piranhas-Assu river and Paraíba river watersheds, during sampling period: June, September and December (2014) and March (2015).

	Richness (mean ±SD)				**Shannon-Wiener** (mean ±SD)			
Piranhas-Assu river	**JUN**	**SEP**	**DEC**	**MAR**	**JUN**	**SEP**	**DEC**	**MAR**
Sabugí	8.7 ±2.4	11.3±4.2	7.6±2.4	6.7±3.3	0.8 ±0.1	0.8±0.06	0.8±0.07	0.7±0.2
P. Traíras	1.7±1.2	5.3±1.5	2.5±1.2	1.8±0.9	0.3±0.2	0.7±0.08	0.4±0.3	0.4±0.3
Cruzeta	5.1±3	9±4.2	7±3.5	4±3.3	0.6±0.3	0.7±0.3	0.7±0.2	0.5±0.3
Paraíba river								
Cordeiro	4.2±2.3	5±3	1.6±1.9	1.5±1.9	0.5±0.2	0.6±0.2	0.3±03	0.2±0.3
Sumé	3.3±1.7	5.4±2.6	4.7±1.7	5.2±1.4	0.5±0.2	0.6±0.2	0.6±0.2	0.6±0.1
Poções	3.1±1	4.7±2.4	1.7±1.1	1.2±1.5	0.5±0.2	0.5±0.2	0.3±0.3	0.2±0.3

Table 2. Environmental variables (mean and standard deviation) from of all sampling sites in the watersheds of the Piranhas-Assu River and Paraíba River, during June, September, December (2014) and March (2015). Trophic typology based on Trophic State Index (Carlson 1977, modified Toledo et al., 1983). In bold variables contributing significantly to the differences between periods. Pseudo-F* 3.119.

Variables/ Abreviations	**Piranhas-Assu River**													
	JUNE			SEPTEMBER			DECEMBER			MARCH			**P**	**Pseudo-F***
	Sabugí	P.Traíras	Cruzeta	Sabugí	P.Traíras	Cruzeta	Sabugí	P.Traíras	Cruzeta	Sabugí	P.Traíras	Cruzeta		
Physical and chemical parameters														
Temperature (ºC) / ºC	27±0.5	26±0.81	24±5	28±1	27±1	27±1	29±1	26±0.7	26±1	29±1	28±0.9	29±2	**0.0001**	10.4
Turbidity (NTU) / TUR	3±6	124±50	37±20	9±10	86±34	26±39	23±11	167±104	51±22	35±11	165±54	70±10	**0.0002**	3.1
Dissolved oxygen (mg/L) / OD	5±0.4	8±1	7±1	7±2	8±2	7±2	8±1	8±2	6±1	7±0.6	8±3	7±1	**0.0001**	9.0
Total dissolved solids (mg/L) / TDS	0.1±0.01	2±0.4	0.5±0.1	0.3±0	2±0.5	0.4±0.01	0.4±0.01	3±0.1	0.6±0.01	0.4±0	3±0.9	0.9±0.4	0.2	1.3
Total nitrogen (µg/L) / TN	86±9	118±57	56±9	140±34	274±144	301±44	125±47	277±122	99±21	165±22	246±72	166±47	**0.0001**	26.0
NOx-N (µg/L) / NOx	25±28	33±23	12±16	61±27	80±39	47±16	23±7	31±11	16±6	6±7	7±5	9±14	**0.0001**	30.0
Ammonium ion (µg/L) / NH_4^-	6±6	6±2	4±0.9	31±21	189±128	34±20	65±18	179±96	48±17	22±45	39±46	34±52	**0.0001**	35.1
Trophic Status Index / TSI	52±4	64±4	75±2	44±5	65±6	58±6	62±5	85±6	72±9	49±4	70±7	58±5	**0.0001**	15.7
Habitats descriptors														
Organic matter (gDW) / OM	0.09±0.09	0.3±0.2	0.1±0.09	0.05±0.03	0.3±0.2	0.2±0.1	0.2±0.1	0.2±0.2	0.3±0.2	0.3±0.2	0.3±0.1	0.3±0.09	0.008	4.0
Gravel (%) / GRA	26±15	9±11	50±44	29±23	6±14	31±35	20±20	0.9±1	5±11	8±10	0.3±0.5	2±2	**0.0003**	7.0
Coarse sand (%) / COA	22±10.1	11±10	5±4	21±9	16±25	6±5	22±8	5±4	9±9	20±21	2±5	20±16	0.4	1.0
Middle sand (%) / MID	18±5	12±4	9±10	19±10	23±19	10±11	16±6	13±16	13±12	18±7	10±12	15±14	0.07	2.3
Fine sand (%) / FIN	22±12	35±16	10±11	20±11	17±12	22±14	23±10	46±15	29±20	28±17	18±19	14±10	0.02	3.2
Silt (%) / SIL	9±7	25±11	18±19	8±4	17±16	19±15	13±10	28±10	31±18	17±8	15±17	14±11	0.02	3.3
Mud (%) / MUD	2±2	8±5	7±8	2±1	11±9	10±8	7±5	7±4	13±8	8±6	4±5	5±4	0.2	1.6

Variables/ Abreviations	**Paraíba River**													
	Cordeiro	Sumé	Poções	Cordeiro	Sumé	Poções	Cordeiro	Sumé	Poções	Cordeiro	Sumé	Poções	**P**	**Pseudo-F***
Physical and chemical parameters														
Temperature (ºC) / ºC	26±1	26±0.6	26±0.6	25±2	26±0.7	26±1	26±1	27±1	27±2	28±0.7	28±0.6	28±2	**0.0001**	46.3
Turbidity (NTU) / TUR	11±22	8±11	34±28	35±20	29±6	83±29	124±115	58±59	132±49	124±104	39±36	328±347	**0.0001**	44.9
Dissolved oxygen (mg/L) / OD	2±2	1±0.2	6±0.7	10±1	9±1	10±2	7±0.7	7±1	10±2	7±2	9±2	9±3	**0.0001**	83.3
Total dissolved solids (mg/L) / TDS	0.9±0.2	0.3±0.02	0.8±0.04	1±0.05	0.5±0.02	1±0.07	21±61	0.6±0	2±0.02	2±0.3	0.9±0.4	2±0.7	**0.0001**	7.3
Total nitrogen (µg/L) / TN	121±46	67±37	168±39	299±67	199±58	324±131	241±56	142±36	302±95	268±221	134±197	514±229	**0.0001**	13.7
NOx-N (µg/L) / NOx	54±52	25±15	52±48	80±24	35±6	28±5	56±34	38±25	101±167	94±100	21±20	44±37	**0.001**	10.6
Ammonium ion (µg/L) / NH_4^-	8±5	7±5	1±0	98±16	83±14	61±23	35±44	33±27	47±31	131±151	49±29	278±155	**0.0001**	19.0
Trophic Status Index / TSI	52±9	62±3.5	62±4.3	48±8	49±6	62±4	58±8	58±5	72±5	55±9	50±15	78±3	**0.004**	4.6
Habitats descriptors														
Organic matter (gDW) / OM	0.5±0.1	0.3±0.2	0.2±0.1	0.3±0.09	0.2±0.1	0.2±0.09	0.5±0.1	0.4±0.2	0.2±0.1	0.3±0.1	0.3±0.1	0.2±0.1	**0.003**	4.7
Gravel (%) / GRA	3±4	29±35	12±17	0.8±2	37±17	14±19	0.8±1	23±23	12±16	5±9	13±12	7±11	0.01	3.4
Coarse sand (%) / COA	7±5	18±7	30±11	9±13	25±8	23±13	11±15	16±11	21±16	29±15	19±13	23±19	0.09	2.1
Middle sand (%) / MID	29±10	19±12	22±9	31±17	13±5	20±10	27±13	16±8	10±5	25±10	13±7	12±11	0.02	3.0
Fine sand (%) / FIN	26±3	17±10	21±9	20±4	14±7	20±9	30±14	24±15	22±7	25±12	22±15	15±11	**0.003**	4.7
Silt (%) / SIL	23±6	10±6	10±7	20±9	6±4	13±7	25±25	14±10	23±15	13±7	9±6	15±14	0.01	3.5
Mud (%) / MUD	12±5	6±6	5±7	19±10	4±4	10±7	5±5	6±5	12±11	4±3	4±4	8±7	0.07	2.3

Supplementary material

Table S1. Characterization of Piranhas-Assu and Paraíba river reservoirs. Data provided by Secretaria de Meio Ambiente e Recursos Hídricos Rio Grande do Norte (SEMARH) and Agência Executiva de Gestão das Águas (AESA), Paraíba. 1* = no record; () = hydric volume (%).

Features/Reservoirs	Piranhas-Assu river			Paraíba river		
	Sabugí	**P. Traíras**	**Cruzeta**	**Cordeiro**	**Sumé**	**Poções**
Geographic localization	06º43’06’’S/ 37º12’02’’W	06º27’16’’S/ 36º52’29’’W	06º24’42’’S/ 36º47’23’’W	7°47’38.00”S/ 36°40’14.04”W	7°29’8"S/ 37°12'20"W	7°53’38”S/ 37°0’30”W
Altitude (m)	187	196	231	480	500	596
Approximate maximum capacity ($10^6 m^3$)	65	49	23	70	45	30
Construction year	1965	1994	1929	^{1*}	1953	1982
Main finality	Supply	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation
Approximate water volume ($10^6 m^3$) by period^{2*} (2014-2015)						
June	18 (27.7)	3 (8)	5 (20.8)	9 (12.8)	17 (37.7)	6 (20)
September	14 (21.5)	2 (4)	4 (16.6)	8 (11.4)	15 (33.3)	5 (16.6)
December	9 (13.8)	1 (2)	2 (8.3)	6 (8.5)	12 (26.6)	3 (10)
March	8 (12.3)	0.649 (1.2)	1 (4.16)	4 (5.7)	9 (20)	2 (6.6)

Table S2. Composition of macroinvertebrate communities (number of individuals/taxa) of sites from the three reservoirs in Piranhas-Assu river, during sampling period: June, September and December (2014) and March (2015). * no record.

Taxa	**Piranhas-Assu River**											
	JUNE			**SEPTEMBER**			**DECEMBER**			**MARCH**		
	Sabugí	**P.Traíras**	**Cruzeta**	**Sabugí**	**P.Traíras**	**Cruzeta**	**Sabugí**	**P.Traíras**	**Cruzeta**	**Sabugí**	**P.Traíras**	**Cruzeta**
ANNELIDA												
Hirudinea	*	*	*	*	*	1	*	1	4	*	*	*
Oligochaeta	1731	338	416	884	426	249	307	116	503	464	143	148
ARACNÍDEO												
Acarina	*	*	*	*	*	*	*	*	*	*	*	*
Oxidae	*	*	*	*	*	*	*	*	*	*	*	*
CRUSTÁCEO												
Decapoda	*	*	*	*	*	*	*	4	*	*	2	*
Ostracoda	*	*	1	26	*	*	*	*	*	*	*	*
INSECTA												
Chironomidae												
Chironominae -												
Aedokritus (Roback 1958)	7	*	*	85	160	12	183	27	142	153	9	135
Asheum (Sublette 1964)	397	*	*	139	3	45	5	*	21	39	*	*
Chironomus (Meigen 1803)	81	*	25	20	20	1	*	*	6	*	*	*
Dicrotendipes (Kieffer 1913)	15	*	3	24	*	16	12	*	3	5	*	*
Fissimentum (Cranston and Nolte 1996)	1	*	*	15	*	1	149	*	*	21	*	*
Goeldichironomus (Fittkau 1965)	798	1	297	191	15	228	3	*	45	64	*	7
Parachironomus (Lenz 1921)	3	1	5	2	*	*	*	*	*	*	*	*
Pelomus (Reis 1989)	36	*	3	52	2	9	1	*	*	*	*	*
Polypedilum (Kieffer 1912)	104	*	1	286	6	260	6	1	89	13	1	23
Tanytarsus (Van der Wulp 1874)	182	1	30	610	28	355	1007	*	165	122	*	6
Tanypodinae -												
Ablabesmyia (Johannsen 1905)	*	*	*	3	*	1	1	*	*	*	*	*
Brundiniella (Roback 1978)	*	*	*	*	*	*	*	*	*	3	*	*
Coelotanypus (Kieffer 1913)	4	*	1	2	2	26	40	*	58	53	*	18
Djalmabatista (Fittkau 1968)	*	*	*	17	*	104	1	*	1	*	*	*
Larsia (Fittkau 1962)	5	*	*	*	*	1	*	*	*	*	*	*
Procladius (Skuse 1889)	*	*	*	*	*	*	*	*	3	1	*	*
Tanypus (Meigen 1803)	*	*	*	11	*	*	*	*	*	*	*	*
Coleoptera												
Gyrinidae	*	*	8	3	*	11	*	*	*	*	*	*
Diptera												
Chaoboridae -												
Chaoborus (Lichtenstein 1980)	14	17	7	1	22	*	*	*	*	*	*	*

Ceratopogonidae	8	*	5	15	2	10	7	*	7	17	*	3
Ephemeroptera												
Baetidae	*	*	*	*	*	*	*	*	*	*	*	*
Caenidae	*	*	*	*	*	*	*	*	*	*	*	*
Polymitarcydae	*	*	*	*	*	*	*	*	*	*	*	*
Hemiptera												
Belostomatidae	*	*	*	*	*	*	*	*	*	*	*	*
Corixidae	6	*	1	1	*	3	*	*	*	*	*	*
Odonada												
Coenogrionidae	*	*	*	*	*	*	2	*	3	*	*	*
Gomphidae	*	*	*	*	*	*	2	*	*	2	*	2
Phyllocycla	*	*	*	1	*	*	*	*	*	*	*	*
Libellulidae	*	*	*	1	*	*	*	*	*	*	*	*
Trichoptera												
Leptoceridae	*	*	*	*	*	*	2	*	*	*	*	*
Polycentropodidade	*	*	*	1	*	*	1	*	*	*	*	*
MOLLUSCA												
Gastropoda												
Ancylidae	*	*	*	9	*	*	2	*	*	*	*	*
Bulimidae	*	*	*	*	*	*	*	*	*	*	*	12
Planorbidae	1	*	2	1	*	*	*	*	1	*	*	*
Ampullariidae - *Pomacea*	1	*	2	4	*	*	4	*	*	2	*	*
Thiaridae - *Melanoides tuberculatus* (Müller 1774)	*	389	24	13	170	28	154	516	213	364	19	258
Lymnaeidae	*	*	*	*	*	*	*	*	*	*	*	*
Bivalve												
Corbiculidae - *Corbicula largillierti* (Philippi 1844)	*	*	*	*	*	*	*	*	*	*	*	*
Sphaeriidae	*	*	*	*	*	*	*	*	*	*	*	*
NEMATODA	*	*	*	23	*	*	*	*	*	*	*	*
PLATYHELMINTHES	*	*	*	13	*	*	*	*	*	*	*	*

Table S3. Composition of macroinvertebrate communities (nº of individuals/taxa) of sites from the three reservoirs in Paraíba river, during sampling period: June, September and December (2014) and March (2015). * (taxon no recorded).

Taxa	**Paraíba River**											
	JUNE			**SEPTEMBER**			**DECEMBER**			**MARCH**		
	Cordeiro	**Sumé**	**Poções**	**Cordeiro**	**Sumé**	**Poções**	**Cordeiro**	**Sumé**	**Poções**	**Cordeiro**	**Sumé**	**Poções**
ANNELIDA												
Hirudinea	11	11	2	3	3	*	1	15	*	9	3	*
Oligochaeta	114	512	194	108	121	826	206	1063	816	200	427	203
ARACNÍDEO												
Acarina	*	*	*	2	*	*	*	*	*	*	*	*
Oxidae	1	*	*	1	*	*	*	*	*	*	*	*
CRUSTÁCEO												
Decapoda	*	*	*	9	*	*	1	*	3	2	*	4
Ostracoda	*	*	*	9	2	*	*	*	*	*	2	*
INSECTA												
Chironomidae												
Chironominae -												
Aedokritus (Roback 1958)	*	*	6	*	*	21	*	1	4	7	*	23
Asheum (Sublette 1964)	*	*	*	*	3	3	*	*	*	*	*	*
Chironomus (Meigen 1803)	1	*	13	*	*	5	*	*	*	*	*	*
Dicrotendipes (Kieffer 1913)	*	*	*	*	*							
Fissimentum (Cranston and Nolte 1996)	*	*	*	*	2	*	*	4	*	*	5	*
Goeldichironomus (Fittkau 1965)	2	3	44	*	6	12	*	13	*	*	1	*
Parachironomus (Lenz 1921)	3	*	*	1	*	*	*	*	*	*	1	*
Pelomus (Reis 1989)	*	4	*	*	*	5	*	*	*	*	*	*
Polypedilum (Kieffer 1912)	*	24	*	*	17	88	*	2	*	1	2	*
Tanytarsus (Van der Wulp 1874)	*	9	*	*	*	4	*	*	*	*	*	*
Tanypodinae												
Ablabesmyia (Johannsen 1905)	*	*	*	*	2	*	*	1	*	*	13	*
Brundiniella (Roback 1978)	*	*	*	*	*	*	*	*	*	*	*	*
Coelotanypus (Kieffer 1913)	16	1	10	68	3	*	9	6	1	7	1	*
Djalmabatista (Fittkau 1968)	1	*	*	2	1	*	*	*	*	*	*	*
Larsia (Fittkau 1962)	*	*	*	*	2	*	*	*	*	*	*	*
Procladius (Skuse 1889)	*	*	*	*	*	*	*	*	*	*	*	*
Tanypus (Meigen 1803)	*	*	*	*	*	1	*	*	*	*	*	*
Coleoptera												
Gyrinidae	*	*	*	1	*	*	*	*	*	*	*	*
Diptera												
Chaoboridae -												
Chaoborus (Lichtenstein 1980)	*	*	*	*	*	*	*	*	*	*	*	*

Ceratopogonidae	2	*	*	*	1	1	1	4	*	*	2	*
Ephemeroptera												
Baetidae	1	*	*	*	*	*	*	*	*	*	*	*
Caenidae	*	*	*	*	1	*	*	*	*	*	9	*
Polymitarcydae	2	*	1	5	*	*	17	*	*	*	*	*
Hemiptera												
Belostomatidae	*	1	*	*	*	*	*	*	*	*	*	*
Corixidae	*	1	*	*	*	*	*	1	*	*	*	*
Odonada												
Coenogrionidae	1	*	*	2	1	1	*	1	*	*	*	*
Gomphidae	*	*	*	*	*	*	*	1	*	*	1	*
Phyllocycla	*	*	*	*	1	1	*	*	*	*	*	*
Libellulidae	5	*	*	4	*	*	*	*	*	2	*	*
Trichoptera											*	*
Leptoceridae	*	*	*	*	*	*	*	*	*	*	*	*
Polycentropodidade	*	*	*	*	*	*	*	*	*	*	*	*
MOLLUSCA												
Gastropoda												
Ancylidae	*	*	*	*	*	*	*	*	*	*	*	*
Bulimidae	*	*	*	*	*	*	*	*	*	*	*	*
Planorbidae	20	2	*	44	2	*	*	5	*	*	1	*
Ampullariidae -												
Pomacea	*	*	*	1	1	*	*	*	*	*	1	*
Thiaridae -												
Melanoides tuberculatus (Müller 1774)	1169	866	1104	1050	1200	735	349	820	787	928	927	157
Lymnaeidae	*	*	*	*	*	*	*	*	*	*	1	*
Bivalve												
Corbiculidae -												
Corbicula largillierti (Philippi 1844)	*	*	*	*	15	*	*	155	*	*	55	*
Sphaeriidae	*	*	*	*	1	*	*	*	*	*	*	*
NEMATODA	*	*	*	*	*	*	*	*	*	*	*	*
PLATYHELMINTHES	*	2	*	11	11	*	*	6	*	10	6	*

Capítulo 3. DIPTERAN ASSEMBLAGES AS FUNCTIONAL INDICATORS OF EXTREME DROUGHTS

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Dipteran assemblages as functional indicators of extreme droughts

JOVEM-AZEVÊDO, D.[1]; BEZERRA-NETO, J. F[2]; AZEVÊDO, E. L.[3]; GOMES, W. I. A.[4]; MOLOZZI, J.[5];
FEIO, M. J. [6]

[1]Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, sala 318, bloco L3. Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brazil.

[2]Departamento de Biologia Geral/ Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, sala 318, bloco L3. Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brazil.

[3]Departamento de Biologia Geral, Instituto Federal de Educação, Ciência e Tecnologia do Ceará. Alameda José Quintino, S/N, Prado, CEP 63.400-000, Cedro - Ceará, Brazil.

[4] Programa Pós-Graduação em Ciência Ambiental e Tecnologia, Universidade Estadual da Paraiba. Rua Juvêncio Arruda, S/N, Campus Universitário, CEP: 58429-600, Campina Grande - Paraíba, Brazil.

[5]Departamento de Biologia/ Programa de Pós- Graduação em Ecologia e Conservação, Universidade Estadual da Paraíba. Rua Baraúnas, nº. 351 - Complexo Três Marias, Prédio de Biologia, Térreo - sala 10. Universitário, CEP 58.429-500, Campina Grande - Paraíba, Brazil

[6] MARE – Marine and Environmental Sciences Centre, University of Coimbra. Rua da Matemática nº 49, 3000-276 Coimbra, Portugal

E-mail of the corresponding author: daniele.jazevedo@gmail.com / Telephone number: +55 (83) 998350274

Abstract

In the present study we assessed the indicator potential of Dipteran as functional indicators of extreme droughts. Dipteran assemblages were sampled four times between 2014/2015 in 141 sites, from six reservoirs located in two semi-arid watersheds. Ours results show that the Dipteran functional traits changed over the extreme drought period ($p<0.0001$) and were associated to different environmental variables. The body size, feeding group, feeding strategy, preferential habitat and tubules positions changed their dominance over time. Initially, small engulfers (G1 and G2 groups) were dominant and significantly related to variables of water quality, as chlorophyll-a, turbidity and total dissolved solids. In the driest months, negative correlations were found between smaller organisms, presence of haemoglobin, short pseudopods and sprawler habits with water and habitat parameters (e.g organic matter). We found that during the study period occurs a reduction in functional richness and increase of the functional evenness in communities, indicating functional loss and a greater biotic homogenization. Therefore, future management plans of reservoirs and others freshwater ecosystems, especially in areas with frequent drought events (as arid and semi-arid areas), should consider the importance of maintaining a certain level of water quantity to guarantee the ecosystem functioning during the dried season.

Keywords: semi-arid; reservoirs; macroinvertebrates; functional richness; functional evenness; environmental degradation.

Introduction

Climate projections shows the intensification of drought in arid and semi-arid regions, due the reduction in precipitation level and increase in the temperature (up to 4.8 ºC until 2100 in South America), which may represent a risk for quantity and quality of available water (IPCC 2014; Marengo et al., 2016). Aquatic ecosystems are particularly sensible to extreme changes such as, floods and droughts (Melo et al., 2017). Drought events act as natural disturbance forces on biota, however, when these events are of high intensity and duration, as supra-seasonal droughts, the effects over the aquatic communities may be severe (Lake, 2003). Under such scenarios, understanding the patterns of change in aquatic communities and ecosystem processes is more challenging (Bogan et al., 2014), but simultaneously an essential step to a more suitable management of aquatic resources.

Species traits are considered potential tools for ecological assessment as well as to evaluate the effect of natural disturbance on functional structure of the communities (e.g. Bonada et al., 2007; Serra et al., 2017a; Vinagre et al. 2017). Traits that are related to fitness performance and a specific function of organisms in the ecosystems are called functional traits (Cianciaruso et al., 2009) and can also be used to measure the functional diversity and dynamic of niches in ecosystems (Mason et al. 2005).

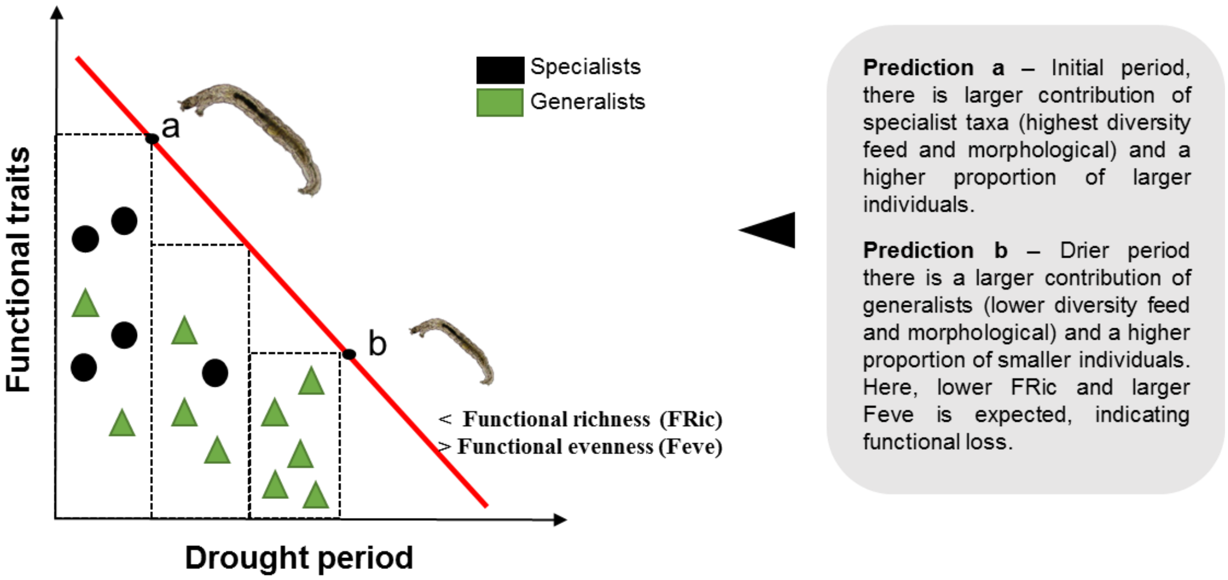
The functional diversity evaluates the differences between the functional traits in the communities, inferring over the complementary or functional overlap of the species (Díaz and Cabido, 2001). The analysis of communities' traits and their functional diversity has been used in the assessment of aquatic ecosystems and can be viewed as a surrogate of ecosystems functions, reflecting the environmental changes over time caused by human disturbance or even climate change (e.g., Dolédec and Statzer 2010; Cotter et al. 2017). The climate changes result in shifts of species distribution patterns and in the fluctuation of populations in the globe (e.g. extinction, emigration and immigration) (Pearson and Dawson 2003). When there is species loss, changes in ecological functions and local ecosystem services and the functional homogenization of communities are expected (Clavel et al. 2011; Feio et al. 2015).

The measurement of functional diversity (including the homogenization) provides information on how species organize themselves in the functional space and how changes in the local community affect the ecosystem processes (Ricotta and Moretti, 2011). Particularly, the homogenization represents the increase of the similarity between communities and in their functional role (Villéger et al., 2008). In this study, we evaluated the potential differences in the functional traits and diversity of Diptera over of extreme drought period. Among Diptera, Chironomidae is the most abundant and frequent and diversified family of aquatic insects

(Nicacio and Juen, 2015). Recent studies have demonstrated that this family is highly sensitive to environmental changes and has a potential as bioindicator of global changes in aquatic ecosystems, including indirect effects of the drought (Cañedo-Argüelles et al., 2016; Serra 2017a,b).

Different traits can provide information on functional changes in the space/time in response to environmental variation The traits body size and feeding groups are fundamental traits that have been related to the fitness of a species (responses) and variations of environmental conditions (effect) (Beauchard et al. 2017). Body size is associated with the life history of a species, varying according with the dynamic of the local ecological processes (Xu et al. 2016). The feeding groups of Diptera are related to the availability of resources, providing information about the mechanisms that drive the ecosystems diversity (Serra et al. 2017a). In addition, behavioural and physiological specializations (e.g. pseudopods, Lauterborn organs, hemoglobin) allow them to colonize different environments and perform different functions (Serra et al. 2017b).

In the present study we assessed the indicator potential of Dipteran as functional indicators of extreme droughts. Understanding their variation can contribute to a better management of water quantity and quality of dry areas including reservoirs where they constitute the most abundant group. Our main hypotheses are: a) Dipteran functional traits change over the extreme drought period, in response to changes in the environment; b) functional evenness increases and functional richness decreases as result of the dominance of fewer traits (trait categories) over the drought period (Figure 1).



Prediction a – Initial period, there is larger contribution of specialist taxa (highest diversity feed and morphological) and a higher proportion of larger individuals.

Prediction b – Drier period there is a larger contribution of generalists (lower diversity feed and morphological) and a higher proportion of smaller individuals. Here, lower FRic and larger Feve is expected, indicating functional loss.

Figure 1: Predictions made for the relationship between traits of Diptera (INSECTA) larvae during the prolonged drought in reservoirs of the semi-arid region.

Material and methods

Study area and sampling design

The present study was conducted in two watersheds located in the semiarid region of Brazil: 1) Piranhas-Assu river (PA; state of Rio Grande do Norte) and Paraíba river (P; state of Paraíba). According Köppen–Geiger classification, the climate of the region is BSh (dry semiarid). The mean rainfall is 400 mm/year in Paraíba and 800 mm/year in Piranhas-Assu river. The minimum air temperature varies between 18 and 22ºC (July and August) and the maximum temperature between 28 and 31ºC (November and December). In both regions the period has about 9-10 months (Alvares et al., 2013).

In the study area, 66 sites were selected in Piranhas-Assu river and 75 sites in Paraíba river (Figure 2). Sampling was performed in the months of June, September, December of

2014 and March 2015. In each watershed, three reservoirs were selected: Sabugí, Cruzeta and Passagem das Traíras (Piranhas-Assu river), Cordeiro, Sumé and Poções (Paraíba river) (Table S1 - Supplementary material).

During the study period the reservoirs had a strong reduction of the hydric volume (Table S1), indicating a year of intense and prolonged drought, according to the Agência Excutiva de Gestão das Águas (AESA – Paraíba State) and Secretaria de Meio Ambiente e Recursos Hídricos (SEMARH - Rio Grande do Norte State).

Dipteran sampling

The larvae were sampled with an Eckman-Birge dredge (225 cm²), in the littoral zone at each sampling site, to 0.6 meters of maximum depth. The samples were fixed *in situ* with formaldehyde 10%. In the laboratory, the samples were washed and the individuals sorted and identified to the family level, except for Chironomidae, which were identified to the genus level (Trivinho-Strixino 2011).

Dipteran traits

Eight traits and the respective 28 categories related to functional role of the organisms were used: body size, feeding trophic groups, feeding strategy, preferential habitat, hemoglobin presence, pseudopods length, Lauterborn organ presence and tubules position on body (Table 1). These traits are associated to species fitness and life strategies that provide ability of colonization, perception, exploration, as well as the performance of functions in ecosystems with varied environmental conditions (Armitage et al., 1995; Trivinho-Strixino, 2011; Beauchard et al. 2017).

The body size was obtained by direct measurement of individuals, from the cephalic capsule to the last segment of the body, excluding the cephalic and terminal appendices.

Based on this measure, the individuals were grouped into body size classes, as proposed by Serra et al. (2015): G1: < 2.5mm, G2: 2.5-5.0mm; G3: 5.0-10mm; G4: 10-20mm; and G5: 20-40mm. Regarding the feeding groups, the larvae were divided into five classes according to their food preferences, as proposed by Cummins et al. (2005): collectors (fine particulate organic matter), filterer-collectors (filter fine particulate organic matter from water column), gatherer-collectors (fine particulate organic matter from sediment), predators (prey) and shedders (coarse particulate organic matter). The feeding strategy categories were analyzed based on size and type of ingested organic particles, according Buttaka et al. (2016) and Saulino et al. (2016): fine particles (filter), middle particles (gatherer), coarse particles (herbivore), fragments of animals and algae (engulfer). The habitat is analyzed according to the preferential habit: free-life (sprawler); inside own tubes (silk tube); attached to rocks, macrophytes or shells (climber); and plant structures (miner). The categories above were attributed based on a search in studies developed in Neotropical ecosystems. The morphological traits were analyzed for each identified individual and classified according to specialized literature (Armitage et al., 1995; Trivinho-Strixino, 2011) (see Table 1). Trait categories were assigned to Chironomidae genus and families of the remaining Diptera and were quantified according to their proportion in each sample.

Environmental descriptors

Physical and chemical parameters

At each site, temperature (ºC), pH, turbidity (NTU), dissolved oxygen (mg/L) and total dissolved solids (g/L) were measured with a multiparameter probe (Horiba U-50). The transparency of the water was estimated using a Secchi disk. In addition, water (1L) was sampled in the sub-surface (0.6 meters of maximum depth) to determine the concentration of total phosphorus (TP - µg/L), reactive soluble phosphate (SRP - µg/L) and total nitrogen (TN - µg/L), according to APHA (2005). Chlorophyll-a was determined by extraction in acetone 90% (Lorenzen 1967). The trophic classification of each site was calculated with the application of the trophic state index (TSI) proposed by Carlson (1977), and modified by Toledo et al. (1983).

Habitat descriptors

Aliquots of sediment were collected with an Eckman-Birge dredge (225cm²), in the same sites of Diptera sampling, to estimate the content of organic matter and granulometric composition. The organic matter was determined through the gravimetric method: samples were oven-dried for 72h to 60ºC and, posteriorly, aliquots of 3g were burned in a muffle furnace at 450ºC for 4h, to estimate the ash-free dry weight. The granulometric composition followed Suguio (1973) protocol, modified by Callisto and Esteves (1996). The particles were classified in the following categories: gravel (>1mm), coarse sand (500-1000µm), middle size sand (250-500µm), fine sand (125-250µm), silt (63-125µm) and mud (<63µm).

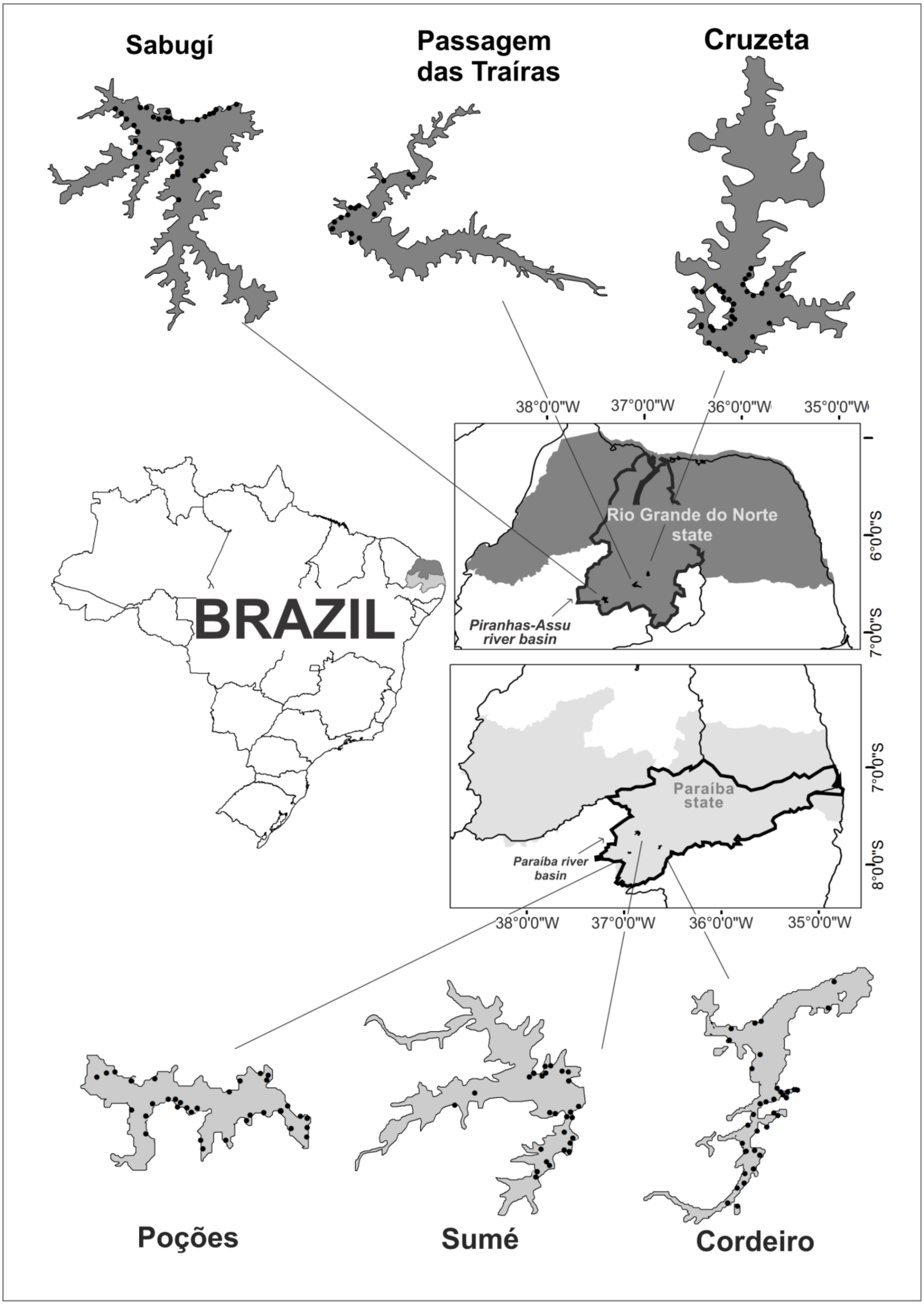


Figure 2. Distribution of the sampling sites (black points) in the reservoirs of Piranhas-Assu river (Rio Grande do Norte state) and Paraíba river (Paraíba state), Brazilian semi-arid region.

Table 1: Traits and respective categories and code, used in data analyses.

Trait	Categories	Code	References
Body size	< 2.5mm 2.5-5.0mm 5.0-10mm 10-20mm 20-40mm	G1 G2 G3 G4 G5	Serra et al. (2015)
Feeding trophic groups	Filterer-collectors Gatherer-collectors Predators	FT-FC FT-GC FT-PR	Henrique-Oliveira et al., (2003); Sanseverino and Nessimian (2008); Zili et al. (2008); Silva et al. (2009); Butakka et al. (2014); Santos and Rodrigues (2015); Saulino et al. (2016)
Feeding strategy	Filters Gatherer Herbivore Engulfer	FS-FI FS-GA FS-HE FS-EN	Silva et al. (2008); Butakka et al. (2014, 2016); Saulino et al. (2016)
Habitat	Sprawler Silk tube Climber Miner	HA-SP HA-ST HA-CL HA-MI	Perticarrari et al. (2004); Borkent and Spinelli (2007); Saulino et al. (2016)
Hemoglobin	Present Absent	HE-PR HE-AB	Armitage et al. (1995); Trivinho-Strixino (2011)
Pseudopods	Elongated Short Absent	PS-EL PS-SH PS-AB	Trivinho-Strixino (2011)
Sensorial organ	Present Absent	SO-PR SO-AB	Trivinho-Strixino (2011)
Tubules	Abdominal Anal Abdominal and Anal Absent	TU-ABD TU-AN TU-AB/AN TU-AB	Trivinho-Strixino (2011)

Data Analysis

To test our first hypothesis, we analysed the variation of functional traits over the study period through a "Permutational Multivariate Analysis of Variance" (PERMANOVA, Bray-Curtis similarity coefficient, 9999 permutations; Anderson et al., 2008), based on the traits by site matrix (square root transformation; Bray-Curtis coefficient). Post-hoc tests were conducted to evaluate the differences to pairs between sampling periods. We also evaluated the changes in taxonomic composition of Diptera assemblages (square root transformation) comparing sampling periods (June, September, December and March) through PERMANOVA. Additionally, we calculated the Shannon-Wiener diversity index (Shannon and Weaver, 1963) and analysed its variation (univariate PERMANOVA, Euclidean distance) to understand if differences in the traits were related to changes in diversity.

The dataset environmental descriptors was previously explored analyzing the correlation between variables (physical and chemical parameters and habitat) by a series of bivariate Spearman correlations (Draftsman's Plot) and one of the highly correlated variables (>0.75) was excluded. The environmental data with skewed distribution was transformed by log (x +1) while the habitat data was transformed by arc-sine. All environmental data were posteriorly normalized. A PERMANOVA with post-hoc test (based on the Euclidean Distance) was performed to assess the differences between sampling periods. To analyse the variability in the hydric volume between the sampling months, we performed an univariate PERMANOVA, considering Euclidian Distance as similarity matrix. All these analyses were made in PRIMER + PERMANOVA 6 software.

Additionally, to analyse the association of functional traits with environmental descriptors (among physical and chemical parameters and habitat descriptors) during the sampling period, a RLQ analysis was performed. This analysis generates ordinations that combine the environmental variables matrix (R), abundance of community (L) and traits of community (Q) (Dolédec et al. 1996), inferring how the community distribution and their traits vary over the environmental gradients. Complementarily, the fourth-corner method (9999 permutations) (Dray et al., 2014) and false discovery rate method (FDR - Benjamini and Hochberg, 1995) for correction of *p* values were performed to test if the correlations between the environmental variables and traits were significant. Here, we selected the model 6, which combines two permutations methods (model 2 and model 4) to analyze the significance among traits and environmental variables (Dray et al., 2014). RLQ analyze and FDR methods were performed in R statistical environment (The R Development Core Team, 2016), using vegan (Oksanen et al., 2016) and FD (Laliberté et al., 2014) packages.

In respond to our second hypothesis, the functional diversity of the communities was analysed through the "Distance-Based Functional Diversity Indices" analysis on species composition and traits abundance matrixes (Villéger et al. 2008). This analysis computes the functional diversity indices from traits (type and number) and species abundance matrices. The functional evenness index (FEve) corresponds to the sum of the points distributed (in expansion tree) in T dimensional space, by relative abundance (Villéger et al., 2008). Functional richness (FRic) represents the amount of niche filled by the community in the dimensional space (Villéger et al., 2008). Weighted means of trait values (CWM) reflecting the dominance of the traits on community, from traits (continuous or binary) and relative abundances of the species (Lavorel et al., 2008). We selected these indexes, because they provide information on the evenness in the distribution of the abundance in the functional space (FEve), the value of functional space occupied by community (FRic) and there are dominant traits (CWM) (Villéger et al., 2008; Ricotta and Moretti, 2011). These can indicate the levels of homogenization and functional loss in communities over time. Finally, we used the indices values to test the functional variability over prolonged drought period through

PERMANOVA (Bray-Curtis similarity). The indexes (FRic and FEve) variability was assessed through univariate PERMANOVAs (Euclidean distance).

Results

Environmental descriptors

The hydric volume of the reservoirs was significantly different between periods (ANOVA: Pseudo-$F_{3.23}$: 21.98, p = 0.0001, 9958 permutations), which is also reflected in the differences in other environmental descriptors: physical and chemical parameters (PERMANOVA: Pseudo-$F_{3.352}$: 42.86, p = 0.0001, 9896 permutations) and habitat (PERMANOVA: Pseudo-$F_{3.352}$: 4.98 p = 0.0001, 9928 permutations). For all sampling periods, significant differences occurred in physical and chemical parameters (p = 0.0001). There were no significant differences in the habitat descriptors between June x September and December x March (p> 0.05) (Table 2). During the driest period (March) occurred the highest concentrations of nutrients related to degradation (turbidity, ammonium, total phosphorus and soluble reactive phosphate), and the fine sediment was predominant (Table S2).

Dipteran assemblages

In our samples, 17809 individuals belonging to three families were found: Ceratopogonidae, Chaoboridae and Chironomidae (19 genus), being *Tanytarsus* and *Goeldichironomus* the most abundant taxa (5.809 and 4.446 individuals, respectively) (Table S3). The lowest global abundance occurred during the driest period (1951 individuals) and a similar pattern was found for community diversity (0.25±0.25) (Table S3). The Dipteran assemblages (PERMANOVA: Pseudo-F3.352: 10.49, p = 0.0001, 9888 permutations) and diversity (ANOVA: Pseudo-F3.352: 4.22, p = 0.005, 9956 permutations) differed significantly among the sampling periods. Differences in community structure were however higher and significant between all combinations of months, according post-hoc tests (Table 2).

Variation of traits and functional diversity during prolonged drought

The body size, feeding group, feeding strategy, preferential habitat and tubules position changed significantly over the sampling period ($p = 0.0001$ for all). In addition, the post-hoc tests showed that there were significant differences ($p< 0.04$) among almost all pairs of months (Table 2).

The differences between the functional traits were also shown by the dominance some categories identified through community-weighted means (CWM). During the first sampling period (June) when the hydric volume in all reservoirs was the highest, the communities were dominated by small and intermediate individuals for body size (G2 and G3), while on the others periods, the smallest organisms dominated the community (G1 and G2). In addition, larger individuals (G4) were considerably reduced and G5 was excluded in September (Figure 3A).

Table 2: Results of the post-hoc tests from PERMANOVA's and ANOVA's for structural components of Dipteran assemblages, categories of functional traits, functional diversity and descriptors environmental.

	Trait														
	Body size			***Feeding trophic groups***			***Feeding strategy***			***Habitat***			***Hemoglobin***		
Post-hoc tests	T	P-perm	perms	t	P-perm	perms	t	P-perm	perms	t	P-perm	perms	t	P-perm	perm
Jun X Sep	4.83	**0.0001**	9951	1.34	0.16	914	1.63	**0.09**	252	4.99	**0.0001**	769	0.66	0.61	23
Jun X Dec	3.92	**0.0001**	9939	3.08	**0.0003**	738	5.74	**0.0001**	449	5.75	**0.0001**	440	0.51	0.71	23
Jun X Mar	5.36	**0.0001**	9944	2.05	**0.03**	936	6.83	**0.0001**	640	5.84	**0.0001**	442	1.62	0.14	22
Sep X Dec	2.68	**0.0009**	9948	1.81	**0.04**	476	3.96	**0.0001**	476	2.18	**0.01**	498	0.11	1	21
Sep X Mar	5.71	**0.0001**	9958	1.30	0.18	799	5.08	**0.0001**	809	3.99	**0.0001**	786	1.04	0.39	23
Dec X Mar	5.58	**0.0001**	9945	2.05	**0.01**	690	2.59	**0.001**	576	2.13	**0.01**	419	1.08	0.34	22

	Trait									**Community structure**					
	Pseudopods			***Sensorial organ***			***Tubules***			***Abundance***			***Shannon-Wiener diversity***		
Post-hoc tests	T	P-perm	perms	t	P-perm	perms	t	P-perm	perms	t	P-perm	perms	t	P-perm	perms
Jun X Sep	0.85	0.42	448	1.03	0.35	25	6.73	**0.0001**	68	3.29	**0.0001**	9926	3.30	**0.001**	9829
Jun X Dec	7.90	1	219	0.20	0.85	22	7.90	**0.0001**	65	4.24	**0.0001**	9950	1.51	0.13	9835
Jun X Mar	1.74	**0.06**	399	1.75	0.11	22	7.98	**0.0001**	122	3.73	**0.0001**	9932	0.33	0.73	9822
Sep X Dec	0.70	0.54	695	0.78	0.50	22	1.44	0.17	16	2.93	**0.0001**	9934	1.62	0.10	9827
Sep X Mar	1.03	0.31	932	0.83	0.40	21	2.05	**0.04**	29	2.87	**0.0001**	9938	2.51	**0.01**	9821
Dec X Mar	1.58	0.10	671	1.50	0.15	20	0.80	0.50	20	1.47	**0.03**	9955	1.0	0.32	9831

	Functional diversity									**Environmental descriptors**					
	FRic			*FEve*			*CWM*			***Physical and chemical parameters***			***Habitat***		
Post-hoc tests	T	P-perm	perm	t	P-perm	perm	t	P-perm	perm	t	P-perm	perm	t	P-perm	perm
Jun X Sep	3.16	**0.001**	9830	2.53	**0.01**	9828	4.48	**0.0001**	9947	7.53	**0.0001**	9938	1.13	0.25	9956
Jun X Dec	1.64	0.10	9838	1.94	**0.05**	9829	3.79	**0.0001**	9957	7.26	**0.0001**	9932	2.61	**0.0002**	9953
Jun X Mar	0.82	0.40	9807	0.56	0.56	9821	5.29	**0.0001**	9954	5.70	**0.0001**	9937	2.73	**0.0001**	9940
Sep X Dec	1.59	0.10	9824	0.38	0.70	9824	2.49	**0.002**	9944	5.25	**0.0001**	9950	2.48	**0.0004**	9944
Sep X Mar	3.55	**0.0009**	9836	1.61	0.10	9833	5.34	**0.0001**	9947	6.21	**0.0001**	9948	2.67	**0.0003**	9944
Dec X Mar	2.39	**0.01**	9830	1.15	0.24	9844	5.21	**0.0001**	9943	5.83	**0.0001**	9942	1.11	0.27	9937

Attending to the feeding group, the community was almost fully dominated by gathering-collectors during the entire study, which achieved the highest proportion during the highest hydric volume (June). The filterer-collectors increased their importance continuously to December, but in drier period (March) their contribution was the lowest. During this last period (March), predators had their highest contribution (Figure 3B). A similar pattern was found for feeding strategies. Gatherers dominated during the highest hydric volume (June and September), being their dominance replaced by engulfers during the driest period, when the abundance of predators increased and others organisms have the algae as preferential feed (Tables 3 and S4; Figure 3C).

The tube builders and those with abdominal/anal tubules were more representative of the community in the first periods (June to September), however, over the drought the community were substituted by sprawlers and animals with anal tubules (Figure 3D-H). The traits hemoglobin presence, presence of Lauterborn organ and pseudopods length weren't variable over the sampling period ($p > 0.05$). The first two and short pseudopods were dominant during the entire study period (Figure 3E-G).

The functional diversity indices confirm the variation in the traits' categories. The highest mean functional richness (FRic) was found in September (30.6), period where the largest taxonomic richness was obtained. However, a gradual loss in the FRic occurred up to the driest period (March) (19.9). In contrast, FEve index showed that, over time, the community has become more functionally even, with the high value recorded in drier period (0.64) (Table S3). FRic and FEve differed significantly among months (ANOVA: Pseudo-$F_{3.52} = 6.27$, $p = 0.0006$, 9956 permutations; Pseudo-$F_{3.52} = 2.49$, $p = 0.05$, 9944 permutations, respectively) and the post-hoc tests confirm that this variability occurs between the all combinations of months (Table 2).

Table 3: Results from of RLQ analysis between the sampling periods.

	June	September	December	March
Inertia % Axis 1	78.61	71.37	78.66	53.77
Correlation	0.53	0.39	0.43	0.43
p Model 2 (RxL)	0.0001	0.0001	0.02	0.40
p Model 4 (LXQ)	0.03	0.3	0.09	0.15

Response of the functional traits to environmental descriptors

Functional traits were related to different environmental descriptors over of study period, according RLQ analyses (Table 3). When the hydric volume was higher (June), predators, engulfers and organism with elongated pseudopods were positively related to total dissolved solids and organic matter (r=0.44 to 0.47). In contrast, the presence of haemoglobin and individuals with short pseudopods were negatively related to these variables (r=0.44 to 0.45) (Figure 4A). During this period, the most abundant taxa organisms were predators, with elongated pseudopods and with no haemoglobin as the *Coelotanypus* (129 individuals) (Tables S3 and S4).

In the other periods, the traits' categories began to show a higher relationship with variables associated to water quality. In September, high correlations were found for the engulfer category with total dissolved solids (r = 0.47), high rate of organic matter (r=0.36) and turbidity (0.34) and chlorophyll-*a* (0.32) (Figure 4B). The engulfer taxa, with haemoglobin and short pseudopods, common in this period, were *Aedokritus*, *Cladopelma* and *Zavreliela*, being *Aedokritus* the most representative (518 individuals) (Tables S3 and S4).

During the driest months (December and March), negative correlations were found between smaller organisms (G1 and G2), presence of hemoglobin, short pseudopods and sprawler habits with water and habitat parameters (ph, total dissolved solids, total phosphorus, dissolved oxygen, soluble reactive phosphate and organic matter) (r=-0.26 to -0.41) (Figure 5A-B, respectively). The most common taxa was *Aedokritus* (699 individuals).

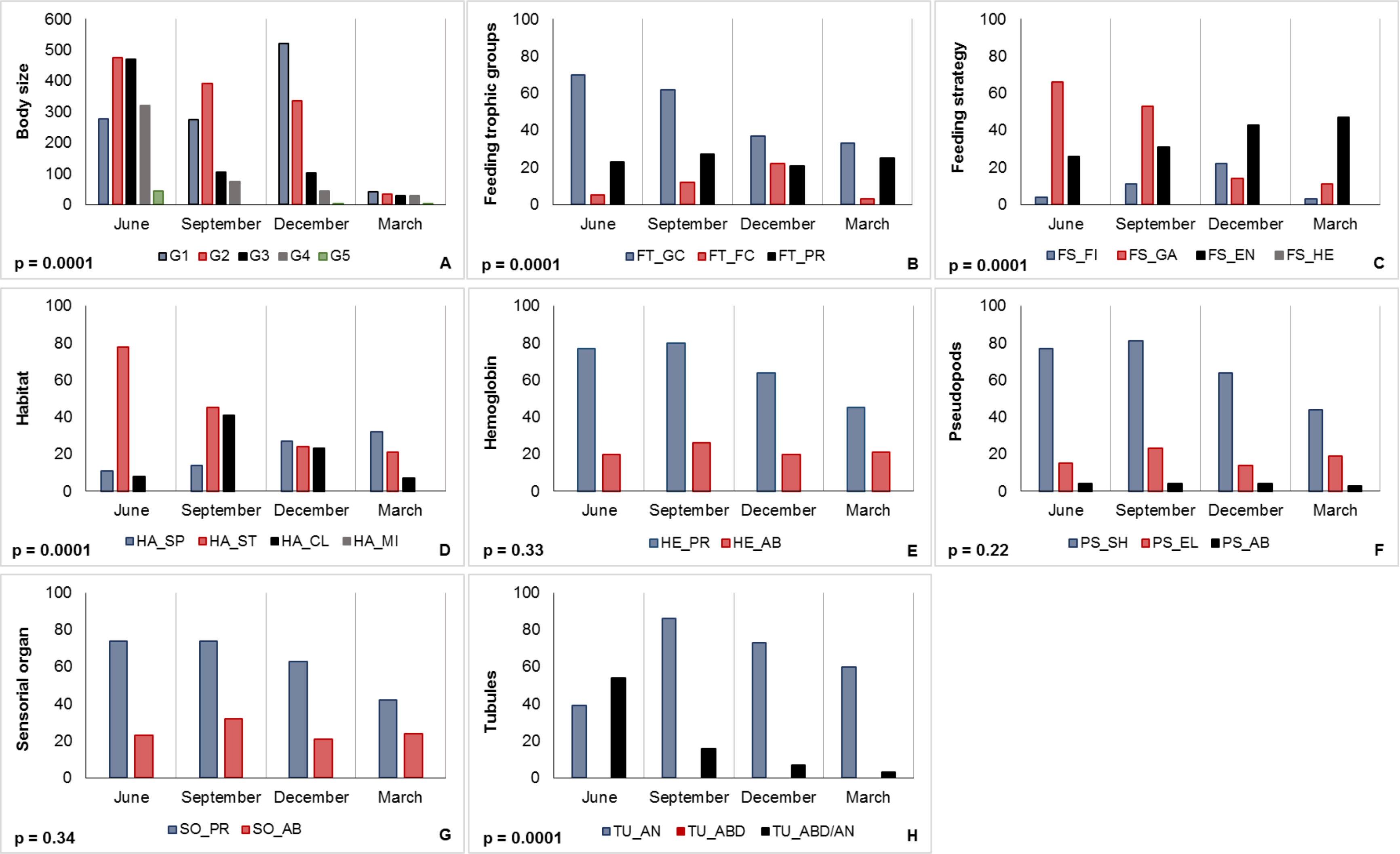
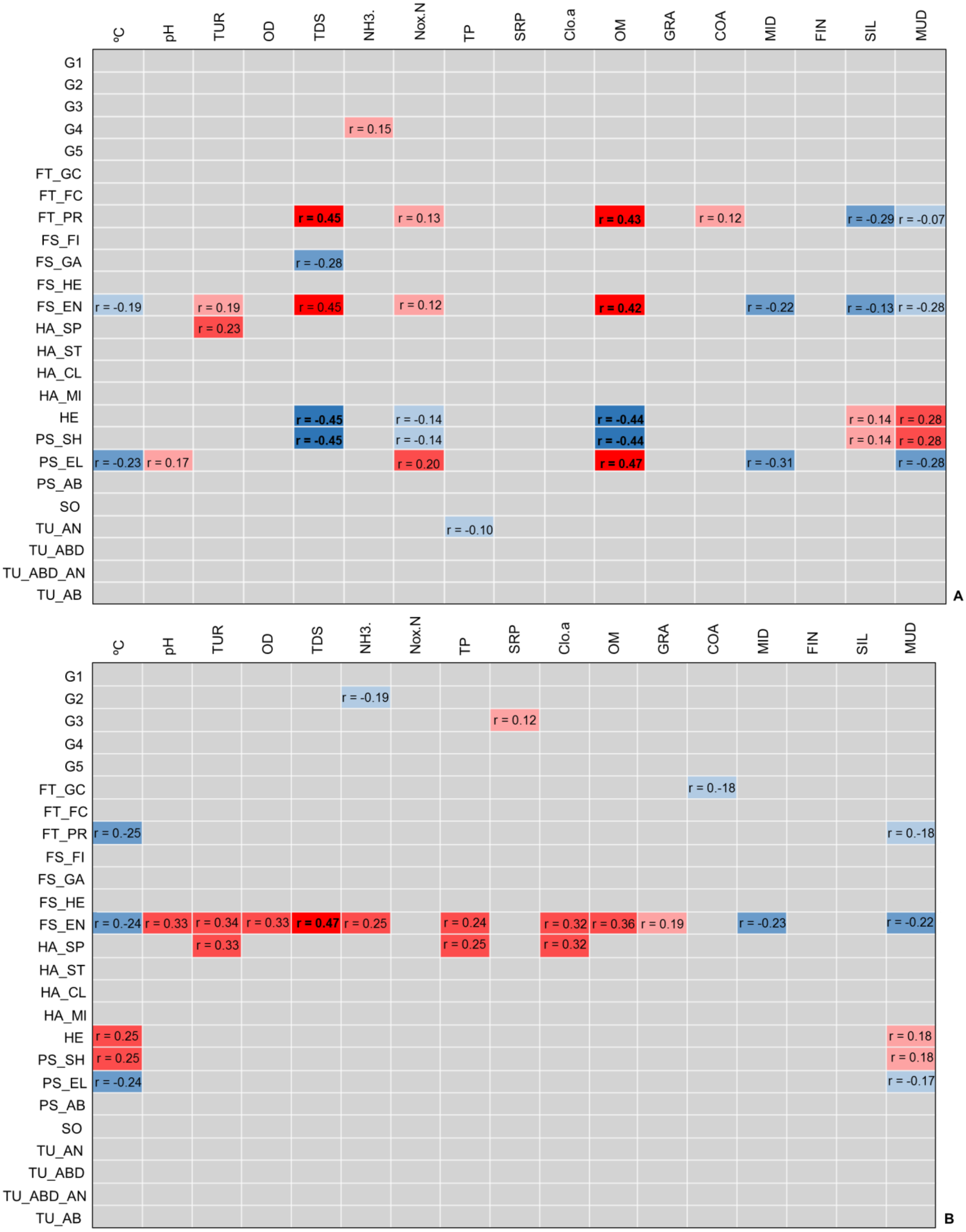


Figure 3: Dominant traits categories by abundance in the Diptera communities between sampling periods, according CWM index for reservoirs in two semi-arid watersheds (Piranhas-Assu river and Paraíba river), Northeast Brazil. See Table 1 for code of the traits categories.



A

	°C	pH	TUR	OD	TDS	NH3.	Nox.N	TP	SRP	Clo.a	OM	GRA	COA	MID	FIN	SIL	MUD
G1																	
G2																	
G3																	
G4						r = 0.15											
G5																	
FT_GC																	
FT_FC																	
FT_PR					**r = 0.45**		r = 0.13				**r = 0.43**		r = 0.12			r = -0.29	r = -0.07
FS_FI																	
FS_GA					r = -0.28												
FS_HE																	
FS_EN	r = -0.19		r = 0.19		r = 0.45		r = 0.12				**r = 0.42**			r = -0.22		r = -0.13	r = -0.28
HA_SP			r = 0.23														
HA_ST																	
HA_CL																	
HA_MI																	
HE					**r = -0.45**		r = -0.14				**r = -0.44**					r = 0.14	r = 0.28
PS_SH					**r = -0.45**		r = -0.14				**r = -0.44**					r = 0.14	r = 0.28
PS_EL	r = -0.23	r = 0.17					r = 0.20				**r = 0.47**			r = -0.31			r = -0.28
PS_AB																	
SO																	
TU_AN								r = -0.10									
TU_ABD																	
TU_ABD_AN																	
TU_AB																	

B

	°C	pH	TUR	OD	TDS	NH3.	Nox.N	TP	SRP	Clo.a	OM	GRA	COA	MID	FIN	SIL	MUD
G1																	
G2						r = -0.19											
G3									r = 0.12								
G4																	
G5																	
FT_GC													r = 0.-18				
FT_FC																	
FT_PR	r = 0.-25																r = 0.-18
FS_FI																	
FS_GA																	
FS_HE																	
FS_EN	r = 0.-24	r = 0.33	r = 0.34	r = 0.33	**r = 0.47**	r = 0.25		r = 0.24		r = 0.32	r = 0.36	r = 0.19		r = -0.23			r = -0.22
HA_SP			r = 0.33					r = 0.25		r = 0.32							
HA_ST																	
HA_CL																	
HA_MI																	
HE	r = 0.25																r = 0.18
PS_SH	r = 0.25																r = 0.18
PS_EL	r = -0.24																r = -0.17
PS_AB																	
SO																	
TU_AN																	
TU_ABD																	
TU_ABD_AN																	
TU_AB																	

Figure 4: Results of fourth-corner tests for the relationship between functional traits and physical and chemical parameters and habitat descriptors, during June and September (A and B, respectively). Grey boxes represent non-significant correlations (p > 0.05); red boxes, positive and significant co-relations (p < 0.05); blue boxes, negative significant co-relations. In bold, the highest values of correlation (r>0.4). Trait categories codes in Table 1; environmental variables codes in Table S3.

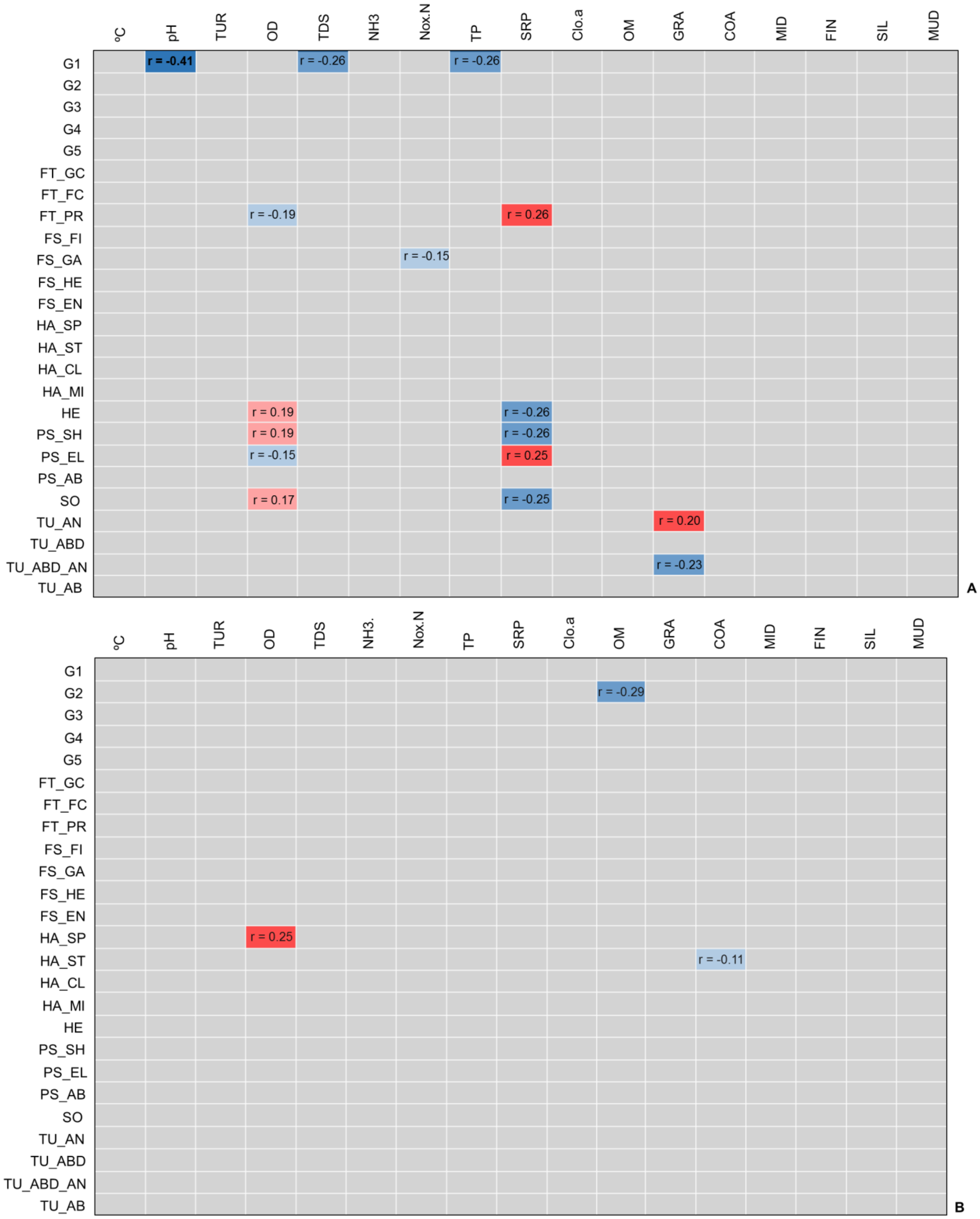


Figure 5: Results of fourth-corner tests for the relationship between functional traits and physical and chemical parameters and habitat descriptors, during December and March (A and B, respectively). Grey boxes represent non-significant correlations ($p > 0.05$); red boxes, positive and significant co-relations ($p < 0.05$); blue boxes, negative significant co-relations. In bold, the highest correlations ($r>0.4$). Trait categories codes in Table 1; environmental variables codes in Table S3.

Discussion

The assessment of communities' functional traits provides direct information on the relationships between biological responses and environmental conditions (Poff et al., 2010). Semi-arid freshwater ecosystems are frequently subject to seasonal variations, which changes the environmental conditions due to the increase in the nutrients level by decrease of the hydric volume during the drought (Melo et al., 2017). Therefore, as predicted, in our study the Dipteran functional traits changed over the extreme drought period and were associated to different environmental variables.

However, we not all traits were variable over time, contradicting our expectations. The body size, feeding group, feeding strategy, preferential habitat and tubules positions changed their dominance over time. Yet, the presence of haemoglobin, and Lauterbourn organ and pseudopods length were constant and dominant over the sampling period. The haemoglobin respiratory pigment is a physiological adaptation of Chironmidae larvae, which allows for tolerance to anoxia conditions on sediments, increasing their capacity of colonization of different habitat (Armitage at al., 1995). The dominance of organism with short pseudopods and the sensorial organ is associated with a type of locomotion (rapid undulations) and habitat exploration common in ecosystems subjected to environmental instability (Trivinho-Strixino 2011; Serra et al., 2017b). Species that live in habitats subject to strong hydric variation, as ecosystems of arid and semi-arid zones, already exhibit adaptations that confer them resistance and resilience to these natural fluctuations (Lake 2003; Lytle and Poff 2004; Bogan et al., 2014). However, extreme events can overcome the tolerance threshold of communities, decreasing their capacity of natural recuperation, promoting local extinctions, facilitating the invasion by non-native species and leading to changes in ecosystem patterns (Chessman, 2014). Ultimately, the present situation of extreme drought can lead to such permanent alterations in the studied aquatic ecosystems.

The smallest individual (G1 and G2) were dominant during the entire period and individuals with larger sizes were even excluded during a period of time. This is in accordance with a previous study by Feio et al. (2015), where smaller size organisms increased during periods with extremely high temperatures and low precipitation, in a Mediterranean stream, where communities are also adapted to a dry period (summer). Others showed that the decrease of larger body size organisms could be an indirect effect of temperature increase with a lower water volume and increase on degradation level (Melo et al., 2017), which may favour smallest individuals (Daufresne et al. 2009). Smallest individuals commonly show rapid growth and reproduction taxa, indicating a selection of r-strategists species with a higher capacity of renovating the community under perturbation conditions (Bonada et al., 2007; Clavel et al., 2011; Giam et al., 2017).

The main feeding group was the gatherer-collecters, despite the increase in the contribution of predators in the driest period (March), differing from our expectations. However, the increase of predators was mainly due to one genus, the *Coelotanypus*. Although this genus is classified as predator (ingesting mainly other Chironomidae), it has a wide plasticity assuming a generalist behaviour and feeding on CPOM, FPOM, microalgae and inorganic micro-particles, depending on the availability of resources (Lemes-Silva et al. 2013). Such organisms, called versatile generalists, have thus the capability to change the expected phenotype in response of the environmental condition, as an adaptive strategy (Clavel et al. 2011). This was confirmed by the RLQ and Fourth-corner methods, which showed that predators are significantly correlated with dissolved total solids and organic matter, during highest water volume. In accordance to this, filter-feeders increased during the drought period (December), probably favoured by the higher concentration of fine particles with the reduced water quantity. However, they could not resist the harsher conditions, decreasing in proportion in March.

Initially, the community had as main feeding strategy the gatherer category (June and September). However, during drier period the dominance of gathers was substituted for engulfer organisms. In this same period, the engulfers were related to variables of water quality (e.g. turbidity, total phosphorus and chlorophyll-a), when there was also an increase in the abundance of *Aedokritus*. The engulfer genus may feed predominantly on algae, which is common in the Chironominae subfamily (Henriques-Oliveira et al., 2003; Butakka et al., 2016). And in fact, during the drier period the phytoplankton biomass may have increased (as indicated by the higher chlorophyll-a, turbidity and total dissolved solids values) favouring these engulfer organisms (Costa et al., 2016).

The low diversity of feeding groups and strategies may be related to the difficulty that most species from rivers have to adapt to reservoirs conditions, which consequently become a filter for the colonization of the sensible species, promoting the homogenization of the local biota (Havel et al. 2005). Moreover, in spite of the progressing drought conditions observed in the study period, the reservoirs were already in a water reduction situation when we started the study, and therefore to an initial degradation level and consequently a relatively low diversity. The disturbance of high intensity and frequency, as drought extreme, acts as drivers limiting to species diversity (Lake, 2003; Bogan and Lytle 2011), which indicates be an alert to biodiversity loss, once the extreme events will be even more common, especially in drier regions (arid and semi-arid) (Marengo et al., 2016)

We also expected an increase in functional evenness and decrease in the functional richness of the communities. Despite of the dominance of different traits (or trait categories) over the drought period the analysis of functional diversity reinforces the idea of a functional loss over time. We found a significant decrease in the FRci and increase on the FEve. The functional richness represents the amount of species that occupy the functional space and, in a complementary manner, the functional evenness indicates the distribution of the abundance in these occupied niches (Mason et al., 2005). In our study, the driest period had a lower functional richness and higher functional evenness, indicating loss of function and a greater homogenization level of the community, as we expected. In fact, the higher level of environmental degradation during the driest period limits the feeding resources, which select the set of traits resistant to disturbance, as recorded in previously studies (Bogan et al., 2014; Feio et al., 2015). Furthermore, the decrease in functional richness and increase of functional evenness indicate that, many individuals occupy few niches in the functional space (Karadimou et al., 2016). Thus, in the driest period the communities are functionally more similar, representing an increasing on biotic homogenization process, and functional loss (McGoff et al., 2013).

This study showed that a prolonged drought, especially when associated to poor water quality, is able to cause changes in functional traits, favouring resistant traits. The changes in functional traits resulted in a decrease of functional richness and increase of the functional homogenization in reservoirs. This is in accordance with recent studies that show that extreme drought select species with resistance traits, but also that this pattern occurs even in systems naturally adapted to strong water reduction periods, as those of the semi-arid region. In accordance, some traits categories were not able to cope with very dry conditions. Future management plans of reservoirs and others freshwater ecosystems, especially those localized in vulnerable areas to frequent droughts, should consider the importance of maintaining a certain level of water quantity to guarantee the preservation of water quality, integrity biodiversity, and ecosystem functioning during the dried season and avoid definitive biodiversity and functional traits losses and preserve ecosystems services.

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Supplementary material

Table S1. Characterization of Piranhas-Assu and Paraíba river reservoirs. Data provided by Secretaria de Meio Ambiente e Recursos Hídricos Rio Grande do Norte (SEMARH) and Agência Executiva de Gestão das Águas (AESA), Paraíba. 1* no record; 2* data in parentheses: hydric volume (%).

Features/Reservoirs	**Piranhas-Assu river**			**Paraíba river**		
	Sabugí	**P. Traíras**	**Cruzeta**	**Cordeiro**	**Sumé**	**Poções**
Geographic localization	06º43’06’’S/ 37º12’02’’W	06º27’16’’S/ 36º52’29’’W	06º24’42’’S/ 36º47’23’’W	7°47’38.00”S/ 36°40’14.04”W	7°29’8"S/ 37°12'20"W	7°53’38”S/ 37°0’30”W
Altitude (m)	187	196	231	480	500	596
Approximate maximum capacity ($10^6 m^3$)	65	49	23	70	45	30
Construction year	1965	1994	1929	^{1}*	1953	1982
Main finality	Supply	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation
Approximate water volume ($10^6 m^3$) by period^{2}* (2014-2015)						
June	18 (27.7)	3 (8)	5 (20.8)	9 (12.8)	17 (37.7)	6 (20)
September	14 (21.5)	2 (4)	4 (16.6)	8 (11.4)	15 (33.3)	5 (16.6)
December	9 (13.8)	1 (2)	2 (8.3)	6 (8.5)	12 (26.6)	3 (10)
March	8 (12.3)	0.649 (1.2)	1 (4.16)	4 (5.7)	9 (20)	2 (6.6)

Table S2. Mean and standard deviation of the environmental variables (physical and chemical parameters, habitat and landscape) measured during the sampling period (June-December, 2014 and March, 2015) in the watersheds of the Piranhas-Assu (Sabugí, P. Traíras and Cruzeta reservoirs) and Paraíba river (Codeiro, Sumé and Poções reservoirs).

Environmental variables	**Sampling Period**				**Variables code**
	June	**September**	**December**	**March**	
Physical and chemical parameters					
Temperature (°C)	26.2±1.6	26.4±1.7	27.2±1.6	28.3±1.3	°C
pH	8.5±0.7	7.7±0.6	7.7±0.6	8.7±0.4	pH
Turbidity (NTU)	21.0±31.2	38.0±36.0	75.9±120.7	86.4±165.7	TUR
Dissolved oxygen (mg/L)	3.1±2.5	8.8±1.9	7.4±1.8	7.5±1.7	OD
Total dissolved solids (mg/L)	0.6±0.4	0.9±0.7	1.0±0.8	0.9±0.7	TDS
NOx-N (µg/L)	36.42±40.4	58.01±30.2	36.12±55.6	17.8±31.1	NOx-N
Ammonium ion (µg/L)	6.6±11.6	71.2±70.9	57.4±45.7	84.8±136.7	NH_3^-
Total phosphorus (µg/L)	113.88±92.4	115.22±164.2	154.4±234.1	282.23±268.7	TP
Soluble reactive phosphate (µg/L)	48.34±73.1	16.61±17.6	45.6±136.1	142.96±179.6	SRP
Chlorophyll-*a* (µg/L)	36.15±52.4	9.06±14.1	18.8±38.2	23.3±34.6	Chlo-*a*
Habitat descriptors					
Organic matter (gAFDW)	0.2±0.1	0.2±0.1	0.3±0.2	0.3±0.2	OM
Gravel (%)	19.6±14.1	19.0±11.9	9.3±9.4	7.4±5.9	GRA
Coarse sand (%)	16.1±2.7	17.3±7.3	14.2±4.7	21.1±4.6	COA
Middle sand (%)	19.1±2.4	19.1±5.3	17.4±3.8	19.4±1.9	MID
Fine sand (%)	22.8±4.7	20.8±4.2	30.0±2.3	25.5±3.5	FIN
Silt (%)	15.7±4.0	14.4±4.4	22.2±6.0	19.2±2.4	SIL
Mud (%)	6.7±1.2	9.4±2.5	8.1±3.8	7.4±1.8	MUD

Table S3. List of Diptera taxa. Abundance value, mean of Shannon-Wiener diversity and functional diversity indexes (FRic and FEve) in the different sampling periods, from sites of Piranhas-Assu river and Paraíba river watershds. In bold are the most abundant taxa in each period. * (taxon no recorded).

TAXA	Sampling period			
	JUN (n=126)	**SEP (n=121)**	**DEC (n=117)**	**MARCH (n=114)**
CERATOPOGONIDAE	31	81	74	65
CHAOBORIDAE	57	24	2	24
CHIRONOMIDAE				
Chironominae				
Aedokritus (Roback, 1958)	23	518	764	**699**
Asheum (Sublette, 1964)	1,026	202	57	86
Chironomus (Meigen, 1803)	248	85	15	1
Cladopelma (Kieffer, 1921)	*	5	2	2
Dicrotendipes (Kieffer, 1913)	20	116	65	13
Fissimentum (Cranston and Nolte, 1996)	2	53	260	57
Goeldichironomus (Fittkau, 1965)	**3,035**	940	268	203
Parachironomus (Lenz, 1921)	24	10	*	1
Pelomus (Reis, 1989)	66	148	3	*
Polypedilum (Kieffer, 1912)	187	1,450	164	60
Tanytarsus (Van der Wulp, 1874)	323	**2,138**	**2,826**	522
Zavreliella (Kieffer, 1920)	*	6	*	*
Tanypodinae				
Ablabesmyia (Johannsen, 1905)	*	21	5	24
Clinotanypus (Kieffer, 1913)	*	3	5	*
Coelotanypus (Kieffer, 1913)	129	184	317	283
Djalmabatista (Fittkau, 1968)	*	162	5	*
Larsia (Fittkau, 1962)	8	12	1	*
Procladius (Skuse, 1889)	*	*	75	*
Tanypus (Meigen, 1803)	*	46	*	*
Shannon-Wiener diversity	0.26±0.22	0.36±0.26	0.30±0.24	0.25±0.25
FRic	24.13±0.5	30.62±0.04	26.10±0.6	19.9±0.43
FEve	0.59±0.11	0.60±0.09	0.63±0.18	0.64±0.16

Table S4. Diptera functional traits from reservoirs in two semi-arid watersheds (Piranhas-Assu river and Paraíba river), Northeast Brazil.

	Trait								
Taxa	**Body size/mm (mean±SD)**	**Body size/mm (min-max)**	**Feeding trophic**	**Feeding strategy**	**Habitat**	**Hemoglobin**	**Pseudopods**	**Sensorial organ**	**Tubules**
CERATOPOGONIDAE	7.5±5.4	1.3-30.0	FT-PR	FS-EN	HA-SP	HE-AB	PS-AB	SO-AB	TU-AB
CHAOBORIDAE	4.4±1.9	1.5-10.0	FT-PR	FS-EN	HA-SP	HE-AB	PS-AB	SO-AB	TU-AB
CHIRONOMIDAE									
Chironominae									
Aedokritus (Roback, 1958)	9.3±5.3	1.2-31.2	FT-GC	FS-EN	HA-SP	HE-PR	PS-SH	SO-PR	TU-AN
Asheum (Sublette, 1964)	4.5±2.2	0.5-19.1	FT-GC	FS-GA	HA-ST	HE-PR	PS-SH	SO-AB	TU-AN
Chironomus (Meigen, 1803)	12.7±10.1	0.5-57.6	FT-GC	FS-GA	HA-ST	HE-PR	PS-SH	SO-PR	TU-ABD/AN
Cladopelma (Kieffer, 1921)	1.8±0.6	0.6-2.5	FT-GC	FS-EN	HA-SP	HE-PR	PS-SH	SO-AB	TU-AN
Dicrotendipes (Kieffer, 1913)	4.1±3.1	0.2-12.0	FT-GC	FS-GA	HA-SP	HE-PR	PS-SH	SO-PR	TU-ABD/AN
Fissimentum (Cranston and Nolte, 1996)	5.3±3.3	0.7-21.8	FT-GC	FS-GA	HA-SP	HE-PR	PS-SH	SO-PR	TU-AN
Goeldichironomus (Fittkau, 1965)	7.2±5.0	0.3-36.3	FT-GC	FS-GA	HA-ST	HE-PR	PS-SH	SO-PR	TU-ABD/AN
Parachironomus (Lenz, 1921)	3.7±2.5	0.99-16.1	FT-GC	FS-GA	HA-SP	HE-PR	PS-SH	SO-PR	TU-AN
Pelomus (Reis, 1989)	2.1±0.9	0.4-7.3	FT-GC	FS-GA	HA-ST	HE-PR	PS-SH	SO-AB	TU-AN
Polypedilum (Kieffer, 1912)	3.1±1.1	0.6-6.0	FT-GC	FS-GA	HA-CL	HE-PR	PS-SH	SO-PR	TU-AN
Tanytarsus (Van der Wulp, 1874)	2.4±1.2	0.3-28.3	FT-GC	FS-FI	HA-CL	HE-PR	PS-SH	SO-PR	TU-AN
Zavreliella (Kieffer, 1920)	1.3±0.5	0.7-1.8	FT-PR	FS-EN	HA-ST	HE-PR	PS-SH	SO-PR	TU-AN
Tanypodinae									
Ablabesmyia (Johannsen, 1905)	5.1±3.8	0.6-15.4	FT-PR	FS-EN	HA-SP	HE-AB	PS-EL	SO-AB	TU-AN
Clinotanypus (Kieffer, 1913)	9.1±7.1	4.0-14.2	FT-PR	FS-EN	HA-ST	HE-AB	PS-EL	SO-AB	TU-AN
Coelotanypus (Kieffer, 1913)	5.0±4.0	0.2-36.5	FT-PR	FS-EN	HA-ST	HE-AB	PS-EL	SO-AB	TU-AN
Djalmabatista (Fittkau, 1968)	3.2±2.6	0.3-16.2	FT-PR	FS-EN	HA-SP	HE-AB	PS-EL	SO-AB	TU-AN
Larsia (Fittkau, 1962)	4.2±2.8	0.8-10.5	FT-PR	FS-EN	HA-SP	HE-AB	PS-EL	SO-AB	TU-AN
Procladius (Skuse, 1889)	2.2±1.5	0.3-5.1	FT-GC	FS-GA	HA-SP	HE-AB	PS-EL	SO-AB	TU-AN
Tanypus (Meigen, 1803)	3.0±3.7	0.5-28.6	FT-PR	FS-EN	HA-SP	HE-AB	PS-EL	SO-AB	TU-AN

Capítulo 4. DISSOLVED ORGANIC MATTER POOLS IN SEMI-ARID RESERVOIRS: SUPPORT TO ASSESSMENT OF TROPHIC STATUS IN WATERSHEDS

Manuscrito a ser submetido à Aquatic Sciences

Reservatório Sabugí, São João do Sabugí – Rio Grande do Norte (RN), Brasil/ 2014. Foto: Luiz Carlos

Dissolved organic matter pools in semi-arid reservoirs: support to assessment of trophic status in watersheds

Daniele Jovem-Azevêdo[1]; Ludmila Brighenti[2]; Maria João Feio[3]; Joseline Molozzi[4]; Jose Fernandes Bezerra-Neto[2]

[1]Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brasil.

[2]Laboratório de Limnologia, Ecotoxicologia e Ecologia Aquática - Limnea, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brasil.

[3]MARE – Marine and Environmental Sciences Centre, Faculty of Sciences and Technology, Department of Life Sciences, University of Coimbra. Rua da Matemática nº 49, 3000-276 Coimbra, Portugal

[4] Departamento de Biologia/ Programa de Pós-Graduação em Ecologia e Conservação, Universidade Estadual da Paraíba. Rua Baraúnas, nº. 351 - Complexo Três Marias, Prédio de Biologia, Térreo - sala 10. Universitário, CEP 58.429-500, Campina Grande - Paraíba, Brasil.

E-mail of the corresponding author: daniele.jazevedo@gmail.com / Telephone number: +55 (83) 998350274

Abstract

The dissolved organic matter (DOM) participates directly in the carbon cycle and plays an important role on physical, chemical and biological functions in aquatic ecosystems. Here, we main aim was assess the applicability DOM pattern (quantitative and quality aspects) as a tool for monitoring of environmental quality in freshwater ecosystems, complementarily to traditional methods (trophic state index, TSI). Our dataset was obtained on 120 sites from six semi-arid reservoirs of Brazilian Northeast. We analyzed TSI correlated to dissolved organic carbon (DOC) and Chromophoric dissolved organic matter (CDOM). DOC values showed high carbon level for semi-arid reservoirs. However, the DOC levels decreasing along time, in contrast to increasing of TSI. The autochthonous sources showed highest contribution to organic carbon pools, given the low molecular weight fractions and high chlorophyll-a concentrations. Our data demonstrated that the degradation of DOC was caused both photodegradation and microbial activities. Between both, the photodegradation seem be the more influence factor, same in driest period and high eutrophic conditions. In our study, CDOM indexes are useful indicators to monitoring of trophic status when compared with DOC values alone, as they were able to reflect particular conditions (as highest photodegradation of organic material) with implications for trophic status.

Keywords: dissolved organic carbon, CDOM, autochthonous carbon, trophic state index.

Introduction

Climate changes associated to extensive development of human activities has promote disturbance in various scales on natural ecosystems (Duffy et al., 2007; Kim et al., 2013), which boost ecologists to study monitoring tools and mitigation measures of impacts (Moreno et al., 2009; Tixier et al., 2011). Several metrics are employed for this purpose and help us to assess health status of ecosystems (Jørgensen, 2005). Particularly in aquatic ecosystems, the health assessment is made through abiotic and biotic metrics or its simultaneous use, as by multimetric approach (Kearns et al., 2005; Demars et al., 2012).

Frequently, the ecological health assessment is focused on traditional metrics (e.g., phosphorus, nitrogen and chlorophyll-a) (Carlson, 1977; McMurtry et al., 1989). However, metabolism estimates and organic matter processing should be incorporated in aquatic ecosystems monitoring (Williamson et al., 2009). In fact, dissolved organic matter (DOM) measured through content, and quality of carbon is important for processes and functions in aquatic ecosystems (Graeber et al., 2017; Zhuang and Yang, 2017).

The DOM is formed by a complex mixture of organic components and represents the main source of organic carbon in aquatic ecosystems (Fellman et al., 2010). DOM is originated from the decomposition of dead organic matter (e.g., animal, plants and microorganisms), and could be derived from two main sources: allochthonous and autochthonous. The first is coming from terrestrial sources carried from the drainage basin, being more susceptible to photodegradation, due to the high capacity of absorbing ultraviolet radiation (Brandão et al., 2018). The Allochthonous source represents an essential linkage between terrestrial and aquatic ecosystems so that changes in riparian structure directly affects its dynamic on aquatic habitats (Messetta et al., 2017). The second, the autochthonous one, is produced from phytoplankton, macrophytes and microbial activity, which results at others organic components (e.g., carbohydrates and lipids) (Zhang et al., 2011; Mao et al., 2016).

Recent studies have demonstrated that DOM concentrations may vary according to land use, especially in agricultural areas (Stanley et al., 2011; Graeber et al., 2012; Bodmer et al., 2016).

The DOM participates directly in the carbon cycle and plays an important role on physical, chemical and biological functions in aquatic ecosystems. The chromophoric fraction (chromophoric dissolved organic matter/CDOM) of DOM is optically active and participates in the attenuation of ultraviolet (UV) and visible radiation on water column, affecting primary productivity and consequently secondary productivity (Shang et al., 2018). Beyond radiation attenuation, the photodegradation and microbial degradation of DOC/CDOM change the nutrient concentration in the water column, acting on the trophic level of freshwater bodies (Fukushima et al., 1996; Zhang et al., 2011).

In rivers and streams, the DOM/CDOM allochthonous sources may be dominant, based at Riverine Continuum Concept (Vannote et al., 1980) and Flood Pulse Concept (Junk et al., 1989). However, those models seem applicable to perennial watersheds, differing in intermittent watersheds, which are common in arid and semi-arid zones. Freshwater bodies in the semi-arid region, represents especially by reservoirs, are frequently subject to drought events, which change not just the water availability, but the quality of the remaining water (Melo et al., 2017). Prolonged drought periods act as an important disturbance factor to structure habitat, including the dynamic of the DOC due to high water residence times in these ecosystems (Lake, 2003;2011; Song et al., 2013).

In the context of climate changes, the drought events will be even more intense and frequent, which represent a stronger influence to DOC natural dynamic and a risk factor to water quality (Marengo et al., 2016; Song et al., 2018). Several previous studies focused in DOC/CDOM dynamics in rivers and lakes, with few studies for reservoirs (e.g., Brandão et al., 2016; Coelho et al., 2017; Shang et al., 2018; Song et al., 2018). However, the construction of reservoirs and large dams is growing in all world, especially after the 70's (Havel, 2005). It is estimated that about 700 dams are constructed per year, which represents a key-factor in changes of natural dynamic on watersheds and consequently of organic carbon (Gunkel et al., 2015; Shang et al., 2018).

Particularly, the semi-arid reservoirs, objects of this study, may be important models to this issue, as it's frequently subjects to drought disturbance and representing important elements for intermittent watersheds dynamic (Maltchik, 1999; Gunkel et al., 2015). Furthermore, the high nutrients levels, hydric volume, light and DOM are fundamental to metabolism in these ecosystems, which may be a relevant factor to global dynamic of carbon, including the high greenhouse gases emission (Barros et al., 2011; Deemer et al., 2016; Mendonça-Júnior et al., 2018). Thus, the DOC/CDOM variation pattern may be an essential metric to reservoirs monitoring, as differences in DOC pools and their quality (CDOM measures) represent biogeochemical transformations, providing information about environmental conditions, including disturbance conditions (Toming et al., 2016).

We aim to assess the applicability of dissolved organic matter variation pattern (quantitative and qualitative aspects) as a tool for monitoring of environmental quality in freshwater ecosystems, compared to traditional methods (trophic state index, TSI). Here, we measure the CDOM through radiation absorption coefficients: absorption 254nm (a_{254}) and specific UV absorbance (SUVA_{254}) as aromaticity indicators; ratio between 250-365nm spectra (E_2:E_3 or M) for relative molecular size; spectral slope ($S_{275\text{-}295}$ and $S_{350\text{-}400}$) as indicator of photodegradation or biodegradation; and slope ratio (SR) as indicator of M and photodegradation (Weishaar et al., 2003; Helms et al., 2008; Coelho et al., 2017).

We firstly tested if there was an increase in the DOC concentration and change in DOC quality during prolonged drought. Secondly, we tested if sites with high TSI level are those with high DOC levels, especially autochthonous DOC, which are composed from low molecular weight molecules (highest *M*) (Brandão et al., 2016; Zhang et al., 2009, 2018). Finally, we tested the relationship between DOC/CDOM absorption and the TSI, as complementary tool to monitoring of reservoirs.

Material and methods

Study area and sampling design

The present study was conducted on six reservoirs located in two Brazilian watersheds at the semi-arid region: Sabugí, Passagem das Traíras, Cruzeta (Piranhas-Assu river, Rio Grande do Norte state), Cordeiro, Sumé and Poções (Paraíba river, Paraíba state) (Table S1 – Supplementary material). The climate in the region is BSh (hot semi-arid), with mean accumulated rainfall of 400 mm/year in Paraíba river and 800 mm/year in Piranhas-Assu river. The dry period varies from 9 to 10 months, with the maximum temperature between 28 and 31ºC in the driest months (Alvares et al., 2013).

In each reservoir, five sites were selected in the limnetic zone, distributed between the dam (highest depth) and input of main tributary (lowest depth) (Figure 1). The sampling was performed in four times: June, September, and December (2014) and March (2015). The study period was classified as of prolonged drought, according with environmental agencies in Rio Grande do Norte state and Paraíba state (Secretaria de Meio Ambiente e Recursos Hídricos/ SEMARH and Agência Excutiva de Gestão das Águas/ AESA, respectively) being March the driest period, characterized by a sharp reduction in hydric volume on reservoirs (Table S1).

Traditional parameters to water quality assessment

At each site, we measured temperature (ºC), pH, turbidity (NTU), total dissolved solids (g/L) and dissolved oxygen (mg/L) through a multiparameter probe (HORIBA U-50).

The depth was assessed with sonar, and the water transparency was analyzed by the disappearance of Secchi disk. In addition, we sampled one liter of water at 0.5 meter to estimate the concentrations of total phosphorus (µg/L), reactive soluble phosphate (µg/L) and total nitrogen (µg/L), according (APHA, 2005). The chlorophyll-a was estimated through pigment extraction by acetone (concentration 90%) (Lorenzen, 1967). The values of total phosphorus, reactive soluble phosphate, chlorophyll-*a* and water transparency were used to calculate the trophic level of reservoirs, trough trophic state index (TSI) proposed by Carlson (1977), and modified by Toledo et al. (1983).

Dissolved organic carbon (DOC)

To DOC analyze, we collected at each sampling site 500ml of water at 0.5 meter, the samples were cooled until filtration process. Posteriorly, samples were filtered (100ml) in glass-microfiber filters with pore 0.22µm. Additionally, the DOC content determination was obtained by catalytic oxidation method of high temperature using the Total Organic Carbon Analyzer (TOC - Shimadzu 5000A). To estimate the carbon quality, we analyzed the absorption spectra of chromophoric dissolved organic matter (CDOM) through a spectrophotometer (UV–vis Shimadzu). The spectra considered varied between 250 and 700nm, being used to spectral reading a quartz cuvette with 5cm. Readings were performed in replicate and Milli-Q water was used as reference. Specific UV absorbance ($SUVA_{254}$, mg C L^{-1} m^{-1}) was estimated from ratio between a_{254} (m^{-1}) by the concentration of DOC (mg L^{-1}) (Weishaar et al., 2003). The relative molecular size (M) was calculated through of the absorbance coefficient a_{250} divided by a_{365}, where the low ratio (low M value) indicate higher molecular weight (Peuravuori and Pihlaja, 1997; Zhang et al., 2011). The spectral slopes $S_{275-295}$ and $S_{350-400}$ were calculated using a nonlinear regression of the log-transformed absorbance coefficients for the interval from 275 and 295 nm ($S_{275-295}$) and 350 and 400 nm ($S_{350-400}$) (Helms et al., 2008).

Data analyses

To test our first hypothesis, we analyzed the variation of DOC (quantitative and qualitative aspects) and traditional environmental parameters through "Permutational Multivariate Analysis of Variance", based in matrix of DOC concentration, absorption coefficients and environmental matrix (PERMANOVA - Euclidian distance, 9999 permutations; Anderson 2001a, b; Anderson and Braak 2003; Anderson et al., 2008). A "Permutational Univariate Analysis of Variance" was employed to analyze the variation in trophic level (ANOVA, based Euclidian distance). Three fixed factors were considered: reservoirs (six levels: Sabugí, Passagem das Traíras, Cruzeta, Cordeiro, Sumé and Poções), watersheds (two levels: Piranhas-Assu river and Paraíba river) and sampling periods (four levels: June, September, December, and March).

The environmental data were previously analyzed through a bivariate series of correlation (Draftsman's Plot), and highly correlated variables (r>0.75) were excluded. Variables with skewed distribution were transformed by log (x +1) (conductivity, dissolved oxygen, total phosphorus, reactive soluble phosphate, total nitrogen and chlorophyll-a) and posteriorly normalized.

Additionally, to our second hypothesis we performed a "Principal component analysis" (PCA) based on DOC/CDOM matrix with superposition of the TSI matrix (Bubble plot) to view the segregation of sites among scores of DOC/CDOM and along of trophic gradients (Anderson et al., 2006). All these analysis were performed in PRIMER6 & PERMANOVA+ software (Clarke and Gorley, 2006).

Finally, to test if the relationship between DOC/CDOM and TSI, we employed a series of Spearman bivariate correlations and, posteriorly, we build a correlations graphic with significance test. For this, we used Corrr and Corrplot packages in R software (Jackson, 2016; Wei, 2017).

Results

Environmental pattern

During the study period, the water of the reservoirs showed high temperatures (> 25ºC), with the highest value recorded in the drier period (28.67±1.06 ºC, Piranhas-Assu river). The alkaline aspect was predominant over the period (7.7-9.0±0.45-0.26). The content of suspended solids in water column increased gradually (0.67-2.3 ±0.26-4.2 g/L), accompanied by water more turve (11.8-150.5±15.2-249.5 NTU) and shallow along drought. Under this same condition, we found high oxygen level (9.5/L±2.1 mg). Concordantly, we recorded high concentrations of total phosphorus, reactive soluble phosphate, total nitrogen and chlorophyll-*a*, during the most intense drought. However, contradicting the pattern found, in sites of Paraíba river there was a decrease in the chlorophyll-a rate in function of drought (see Table 1 and Figure 2). The differences observed were, in fact, significant among reservoirs (p = 0.0001), watersheds (0.0007) and sampling periods (p = 0.0001) (Table 2).

DOC/CDOM and TSI pattern

The DOC values showed high carbon rate for semi-arid watersheds along time, with variation between (20.9-80.7mg/L±11.8-52.6). Unlike of what we found for the abiotic pattern, the DOC concentration decreased during the sampling period, being the lowest concentrations recorded in the driest period (20.9 mg/L in March, Paraíba river). Analyzing the CDOM components, we observed that a_{254} exhibited values ranges 48.7±15.6 (driest period, Paraíba river watershed) to 66.8±28.8 (Piranhas-Assu river watershed). We found an increasing in SUVA along time, with high values recorded during the most intense drought (2.7±1.1 in March). Complementarily, the relative molecular size (M) ranged from 6.6±1.6 (Piranhas-Assu river watershed in September) to 10.4±3.1 (Paraíba river watershed in September), indicating that CDOM with low-weight molecules was most representative to sites of Paraíba river watershed. Additionally, the different sources of organic carbon in these environments are those commonly photodegraded. This relationship is indicated by the highest values of the spectral slope $S_{275\text{-}295}$ ($0.025\text{nm}^{-1}\pm0.003$) and SR values (1.7±0.4), in contrast with lowest values of $S_{350\text{-}400}$ ($0.015\text{nm}^{-1}\pm0.003$). The variation from quantity and quality of DOC was significant among analyzed coefficients ($p<0.05$), except to spectral slope ($S_{275\text{-}295}$ and $S_{350\text{-}400}$) and to absorption coefficient a_{254} among watersheds and sampling periods ($p>0.05$), respectively (see Tables 1 and 2; Figure 3).

The trophic classification showed that during all sampling periods the reservoirs varied between mesotrophic and eutrophic, with highest degradation level recorded in the driest period (62.4-76.1±8.6-9.1), when we found low DOC level. Unlike of the CDOM coefficients, the trophic classification significantly ranged among reservoirs, watersheds and sampling periods ($p<0.05$) (Table 2).

Complementarity between DOC/CDOM and TSI

The Principal components analysis (PCA) showed that the variation of organic carbon (DOC) and chromophoric carbon (CDOM) results in segregation among the distribution pattern of sites, with 67.5% of explained variation on the dataset (Table 3). Analyzing this variation along trophic gradients (superimposed analysis), we found that sites with highest degradation level (high TSI values) are those where occurs high spectral slope values ($S_{275\text{-}295}$)

and molecular low-weight components (high M values) (axis 1: 0.49 and 0.52, respectively). These same sites were negatively associated to high scores of SUVA (axis 1: -0.40). In contrast, sites with high DOC and slope spectral ($S_{350-400}$) tend to be negatively related to middle TSI level (mesotrophic sites) (axis 2: -0.42 and -0.30, respectively) (Table 3, Figure 4).

In additional manner, we analyzed the correlation of the DOC quantity and quality with trophic level on the sites. We observed that TSI values were significantly correlated with all DOC/CDOM parameters, except with spectral slope $S_{275-295}$ (Figure 5). The eutrophic condition (high TSI values) was positively correlated with high a_{254} and SUVA values ($r = 0.44$ and 0.30, respectively), indicators of aromaticity. In contrast, the high TSI values occurred when there was low $S_{350-400}$ values, indicated by significant negative correlation ($r = -0.31$).

Analyzing the associations among components of DOC/CDOM, we found the high DOC values were negatively correlated with SUVA ($r = -0.73$) and unlike, positively related with spectral slope $S_{350-400}$ ($r = 0.40$). The relative molecular size (M) show stronger positive relationship with $S_{275-295}$ and $S_{350-400}$ ($r = 0.92$ and 0.66, respectively) (Table 4, Figure 5).

Discussion

The dissolved organic matter dynamics and its components represent an important factor to understanding of processes and functioning in freshwater (Coelho et al., 2017). Therefore, we believe that the dissolved organic matter variation in quantity and quality represents a useful tool for monitoring of reservoirs, including those under intense disturbance condition (natural or anthropic). We first tested the hypothesis that DOC level and its quality indexes differ significantly during a period of prolonged drought. Our dataset showed that indeed there is a shift on dissolved organic carbon during a prolonged drought period, confirming our expectations.

Although the extended period without allochthonous organic carbon inputs, due to the absence of rainfall, the variation of the organic carbon pools in semi-arid reservoirs seem to be influenced by intrinsic factors of these ecosystems. Since, the accentuated decrease in hydric volume and high evaporation rates (hydro-climate factors) may accelerate the photodegradation and microbial metabolism, changing DOC content (Braga et al., 2015). Furthermore, the synergic action hydro-climate factors frequently shift the nutrients concentration (high values), becoming these ecosystems most vulnerable to degradation process (Gunkel et al., 2015; Wiegand et al., 2016).

The reservoirs varied between mesotrophic and eutrophic, indicating a condition of initial degradation. Under these trophic conditions, also were observed high pools of dissolved organic carbon (see Table 2), which to our knowledge represents unusual values compared to those found in previous studies in reservoirs (e.g., Herzsprung et al., 2012; Bittar et al., 2015; Brandão et al., 2016; Shang et al., 2018). However, Curtis and Adams (1995) analyzing the DOM pattern in a Canadian lakes complex recorded values highest than ours, and they attributed this condition to low water level occurred during seasonal drought, reinforcing our idea on the high influence of hydro-climate factors to the DOM dynamics. Regions where the evaporation overcomes the rainfall rates, there is accentuated decrease from dilution of organic components, increasing their concentrations water column (Gergel et al., 1999).

Further these factors, we attributed the high DOC values finding to long time of water residence in semi-arid reservoirs, which associated to common hydro-climate factors favors the nutrients concentration intensifying the degradation status (Barbosa et al., 2012). We found in previous studies that the residence time, in semi-arid ecosystems, varies between 95 days and 14 years from water retention (Santos and Eskinazi-Sant'Anna, 2010; Silva and Costa 2015). Time considerably long without water renovation, becoming the poorer quality status common, especially during atypical drought (Bouvy et al., 2003). Particularly, the studded reservoirs shows residence time between 3 to 5 years, according environmental Agencies (SEMARH and AESA).

The high DOC content finding in semi-arid reservoirs is worrisome, as reservoirs under this conditions were reported as ecosystems with high potential on the greenhouse gases emission (e.g., CO_2 and CH_4) (Tadonléké et al., 2005; Deermer et al., 2016). This occur, as the CO_2 and CH_4 emission is strongly influenced by reservoirs age, chlorophyll-*a* concentrations, precipitation rate, dissolved oxygen, wind speed, depths, but especially by dissolved organic carbon (Barros et al., 2011; Yang et al., 2014). Furthermore, reservoirs in arid and semi-arid regions rely on an additional factor: high residence time associated to warm temperatures, which accelerate the decomposition process of the organic matter on conditions anoxic, increasing gases emissions (Therrien et al., 2005). If we consider eutrophic ecosystems, the gases emission data are further alarming (Deermer et al., 2016).

Although of the high DOC levels during all time, we have finding that the DOC levels decreased, contradicting our expectations. We previously believed that high nutrients concentrations providing a favorable environment to the growth of tolerant organisms to high degradation level and low transparency (e.g., cyanobacteria and mixotrophic individuals), resulting in high DOC level coming from autochthonous sources predominantly (Brandão et al., 2018; Costa et al., 2016, 2018). The decreasing of DOC over time may indicate that part of the initial organic pool was removed.

Simultaneous manner, we have observed that of there was an increasing in specific UV absorption (SUVA) and similar values of a_{254}mn during over time, indicating that the DOC initial pool, predominantly autochthonous (strong association between DOC and $S_{350\text{-}400}$, see Table 4 and Figure 5) was degraded with the increasing of drought, remaining only more aromatic components. Materials with high aromaticity level are derived from humic components, as result of the plant decomposition (Wagner et al., 2015). Aromatic components are compounds by hydrocarbons arrangement able to absorb shorter wavelengths and with more energy, as indicated by a_{254} and SUVA values (Weishaar et al., 2003; Jaffé et al., 2008). We have confirmed this pattern, as the aromatic components seem occurs in sites with high TSI level, given stronger correlation of this parameter with a_{254} and SUVA (see Table 4 and Figure 5), indicating remaining material from degradation DOC.

Here, the degradation of DOC was caused both photodegradation and microbial activities ($S_{275\text{-}295} > S_{350\text{-}400}$). Between both, the photodegradation seem be the more influence factor and, same in driest period with high eutrophic conditions, the autochthonous sources showed highest contribution to organic carbon pools, given the low molecular weight fractions (M and SR values) and high chlorophyll-a concentrations (especially to Paraíba river) (Helms et al., 2008). Indeed, the photodegradation and biodegradation are complementary mechanisms to organic carbon mineralization in aquatic ecosystems (Amado et al., 2007). In first case, a series of photochemical reactions may degrade the DOC, originating components with environmental and biological importance to intrinsic processes in these ecosystems (e.g., carbohydrates, proteins, lipids) (Hanamachi et al., 2008). Although, the photodegradation reactions of DOC origins toxic components and with the lowest aromaticity and therefore can represent a stronger factor to decline DOC (Hudson et al., 2003; Waiser and Robarts, 2004; Zhang et al., 2014; Bittar et al., 2015).

Additionally, the DOC degradation may also be performed through microbial mineralization process, producing low molecular weight compounds (stronger correlation between DOC and $S_{275\text{-}295}$). The autochthonous organic material constitutes an important carbon source to heterotrophic organisms, stimulating the growing and higher activities of these organisms (Hanamachi et al., 2008; Tessarolli et al., 2018). Previous studies corroborates with ours results and demonstrates the high contribution of the autochthonous DOC (especially from phytoplankton) to changes on CDOM in aquatic ecosystems (Zhang et al., 2009, 2013; Hiriart-Baer et al., 2017). Regardless of the DOC degradation processes, various changes in the water chemical properties may be observed, including ionic potential (pH) (Zhang et al., 2014). Our results also show that the decline of DOC rates accompanied an increase in pH values. Alkaline environments make the fulvic acids most soluble, which, associated with increasing temperature, high evaporation rates and shallow waters (due to intensity drought), favors the photodegradation processes over the microbial metabolism of DOC in water column (Curtis and Adam, 1995; Kritzberg et al., 2006; Sobek and Tranvik, 2007).

Ours analyzes evidence a negative and weak relationship between DOC values and TSI level, and this may be related to abrupt decreasing of DOC along drought. Nevertheless, CDOM indexes showed strong relationship with the trophic gradients finding, indicating the changes occurred on dissolved organic matter during studied period. Thus, CDOM indexes was more importantly to characterization reservoir trophic level, demonstrating be an useful metric to monitoring of the water quality. Others have demonstrated the association between CDOM indexes and TSI characterization, corroborating the use idea to assessment of trophic gradients (Niu et al., 2014; Brandão et al., 2016; Maizel et al., 2017; Zhang et al., 2018).

Thus, the analysis of organic carbon pool in semi-arid reservoirs provides important insights into monitoring aspects:

(1) High DOC pools in semi-arid reservoirs were found, and despite the gradual reduction, their rates seem unusual. This increase the alert to future analysis of the contribution of these systems to greenhouse gases emissions.

(2) The degradation autochthonous DOC during the drought prolonged occurs mainly through photodegradation ($S_{275-295}$ > $S_{350-400}$, and SR), resulting in sites with highest trophic level and high aromatic CDOM content remaining (a_{254} and SUVA values).

(3) Future studies may consider analyze the variation pattern of the DOC quantity and quality from increasing of the water column, by input of water rainfall, relating with the fluctuation local trophic condition. Furthermore, studies to assessment of greenhouse gases emission by reservoirs on extreme condition are encouraged, as results would provide evidences from potential contribution these ecosystems to gases emission in atmosphere, especially CO_2 and CH_4.

(4) Finally, the CDOM indexes are useful indicators to monitoring of trophic status when compared with DOC values alone, as they were able to reflect particular conditions (as highest photodegradation label organic material) which can influence trophic status. Although, the DOC pools are important as it represents the carbon dynamic along time.

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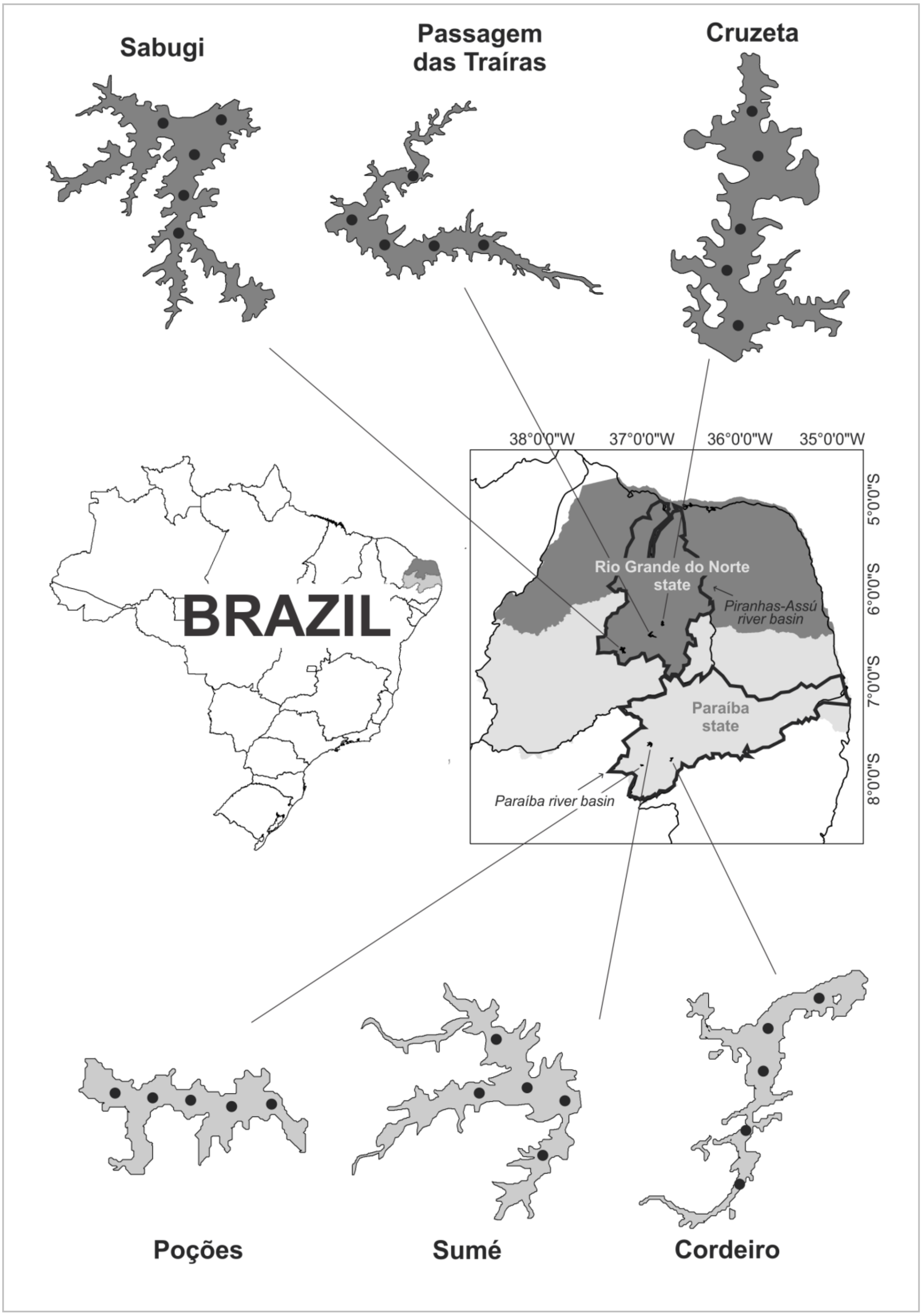

Figure 1: Sampling sites (black points) distributed on Sabugí, Passagem das Traíras, Cruzeta (Piranhas-Assu river, Rio Grande do Norte state), Cordeiro, Sumé and Poções reservoirs (Paraíba river, Paraíba state).

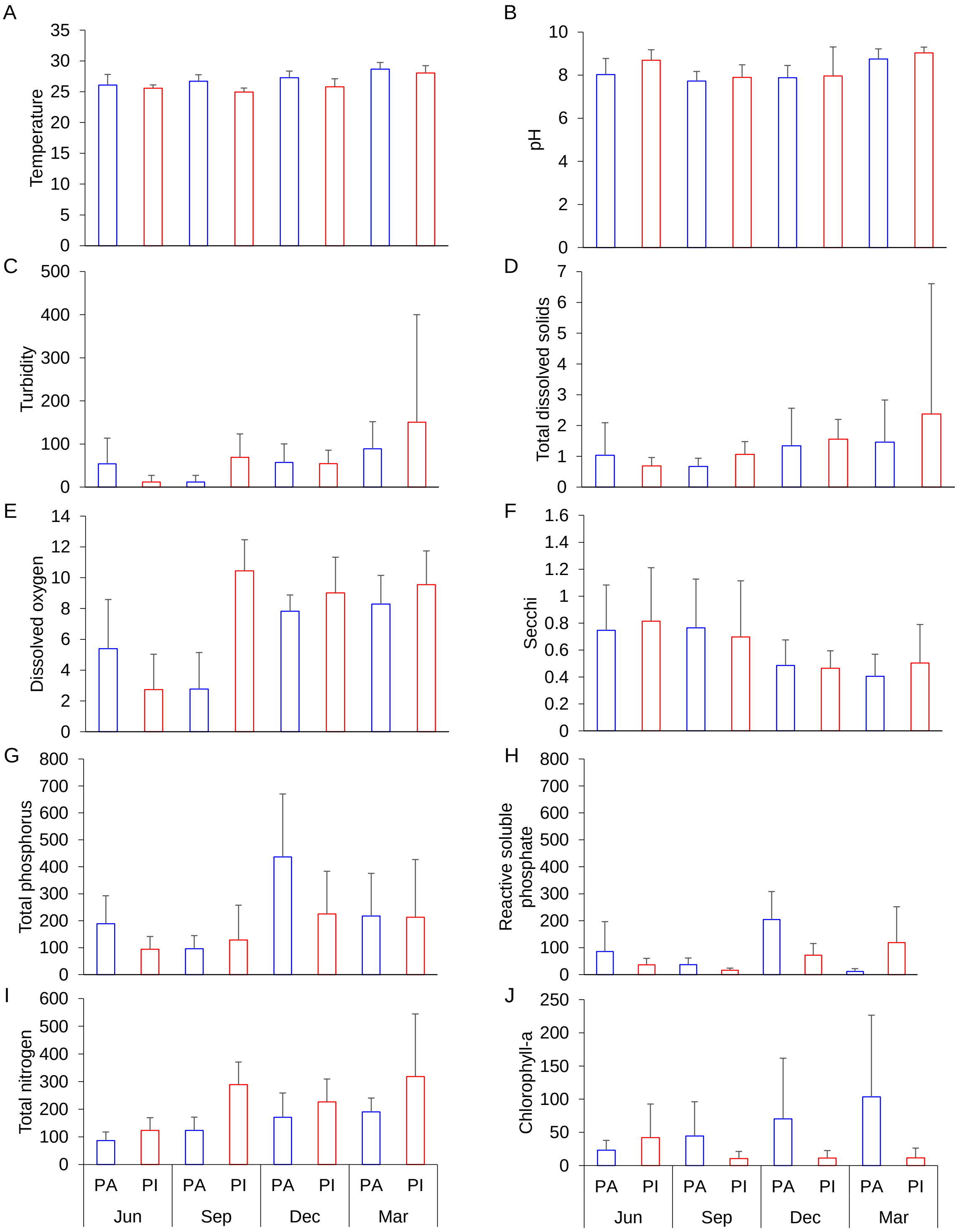

Figure 2: Variation of environmental parameters among the watersheds and sampling periods. Where: PA = Piranhas-Assu river and PI = Paraíba river. Dataset from 120 sites belonging to six reservoirs in semi-arid region in Brazilian Northeast.

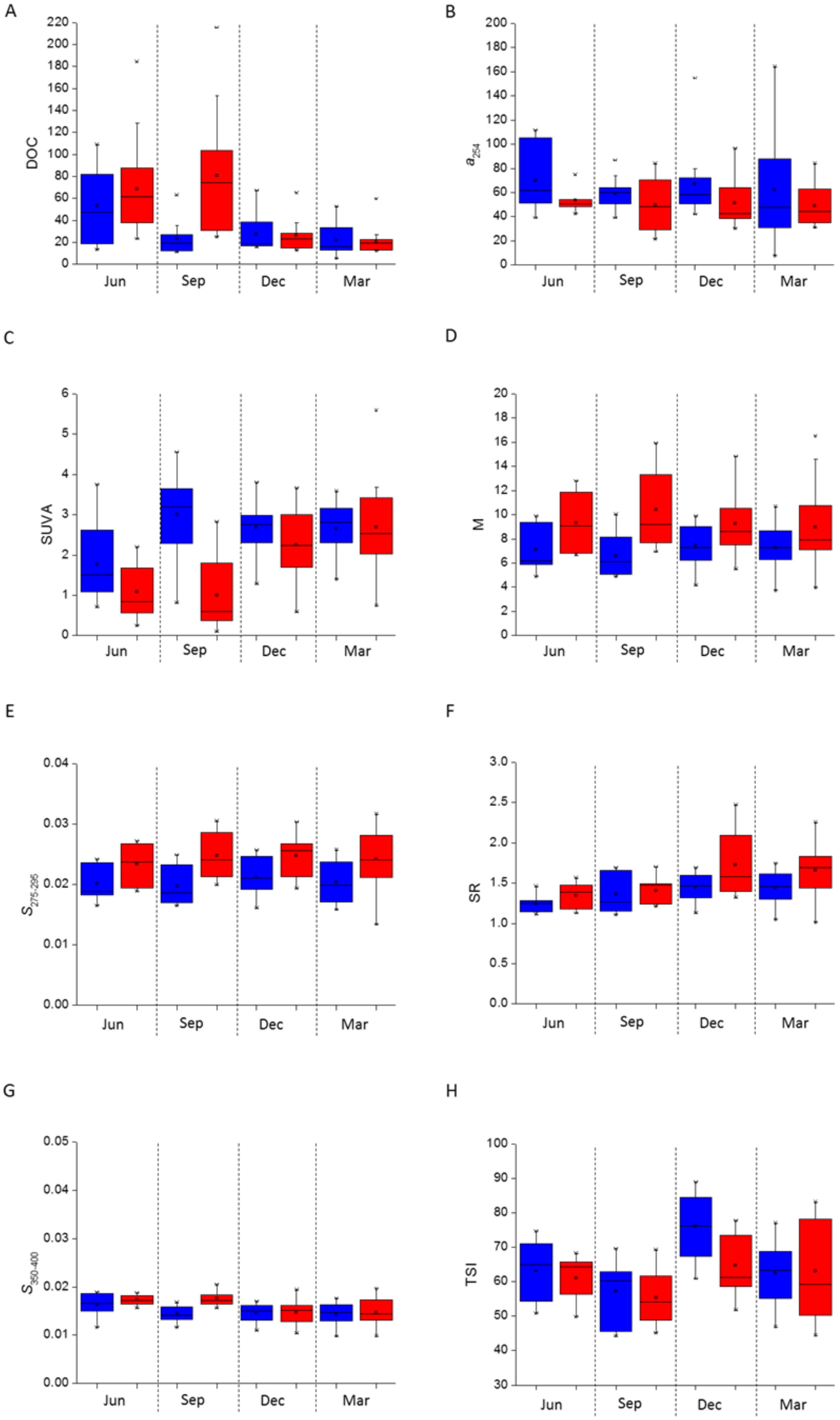

Figure 3: Variation of dissolved organic carbon (DOC) and chromophoric dissolved organic matter - CDOM (through a_{254}, SUVA, *SR*, spectral slopes $S_{275\text{-}295}$ and $S_{350\text{-}400}$ and *M*) and trophic status index (TSI) among watersheds (in blue Piranhas-Assu river; and red Paraíba river) and sampling periods. The horizontal lines on boxes represent the average value; central point is median value; vertical lines indicate the pattern error and * indicate the maximum and minimum values.

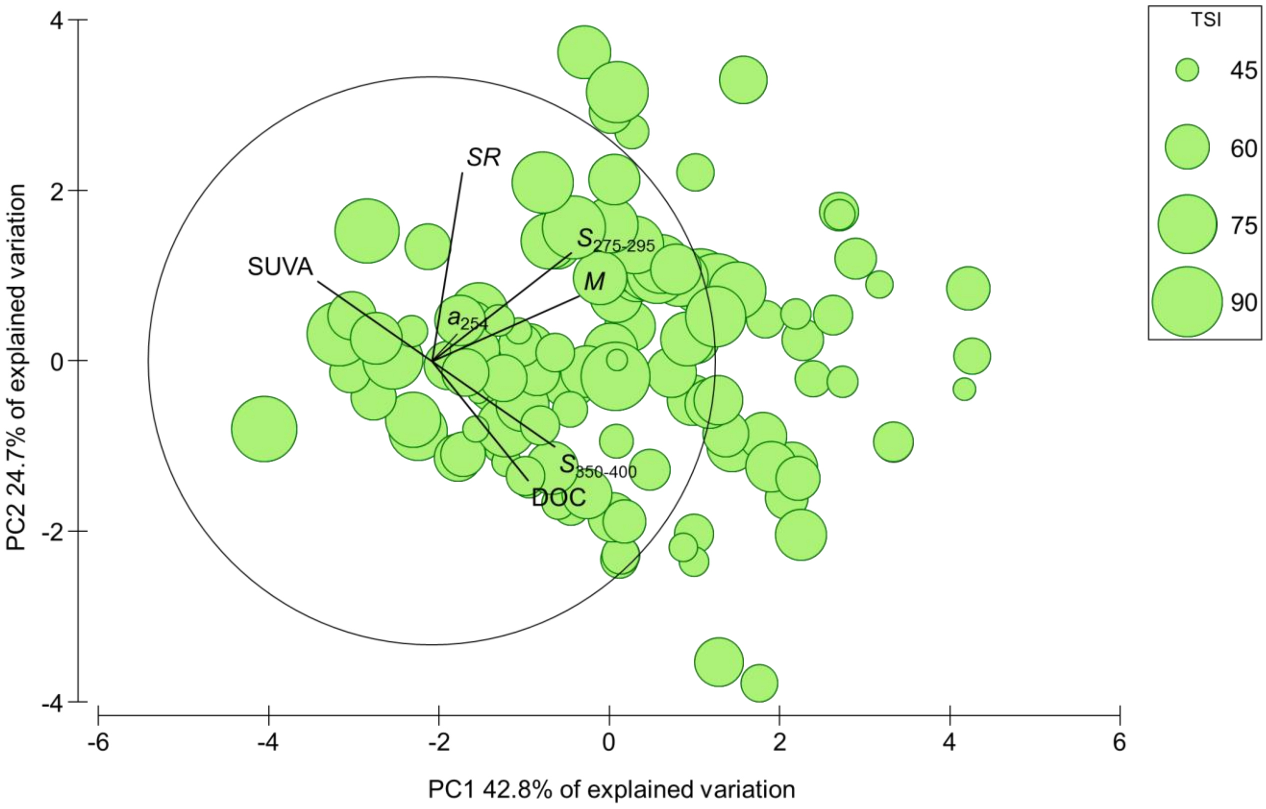

Figure 4: Results from Principal components analysis (PCA) to spatial variation of DOC and CDOM (through SUVA, S275-295 and S350-400) along of trophic gradients (TSI) - superimposed PCA.

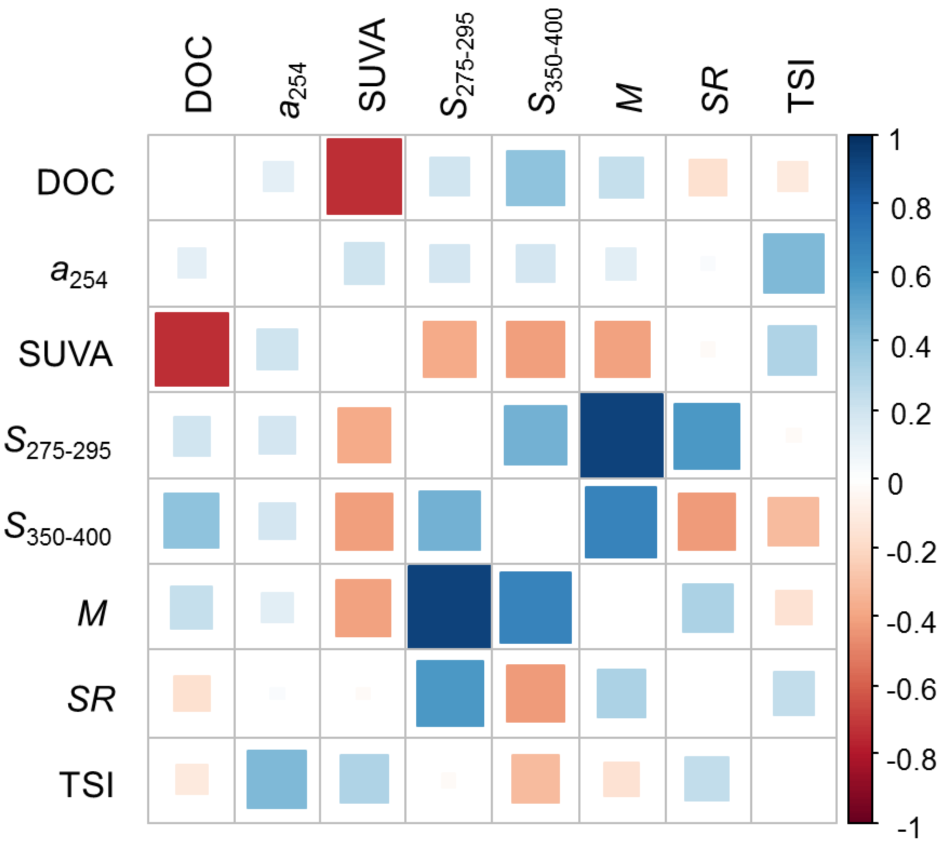

Figure 5: Correlation among DOC/CDOM parameters and TSI. Where: Blue square represents significant positive correlations and red square represents significant negative correlations ($p<0.05$). Square without color represents no-significant correlations ($p>0.05$). The colors intensity and size are related with the correlation force (see variation bar to right)

Table 1: Results of the tests from PERMANOVA's and ANOVA's for, abiotic parameters, dissolved organic carbon/colored (DOC/CDOM, respectively) and trophic status index (TSI) from 120 sites distributed in Piranhas-Assu river and Paraíba river. In bold significant p (<0.05).

Variables	**Factors**											
	Reservoirs				Watersheds				Periods			
	df	Pseudo-F	P-perm	perms	df	Pseudo-F	P-perm	perms	df	Pseudo-F	P-perm	perms
Abiotic parameters	5	25.63	**0.0001**	9878	1	4.96	**0.0007**	9950	3	26.99	**0.0001**	9908
DOC		3.63	**0.008**	9957		1.14	0.29	9812		23.81	**0.0001**	9945
a_{254}		46.41	**0.0001**	9966		10.06	**0.0001**	9684		1.89	0.13	9947
$SUVA_{254}$		8.96	**0.0001**	9961		3.65	**0.05**	9843		31.59	**0.0001**	9949
M		65.68	**0.0001**	9956		30.16	**0.0001**	9848		0.50	0.68	9948
$S_{275-295}$		15.73	**0.0001**	9946		2.58	0.86	9841		53.73	**0.0001**	9952
$S_{350-400}$		6.42	**0.0003**	9955		1.26	0.25	9852		17.38	**0.0001**	9953
SR		25.60	**0.0001**	9946		12.08	**0.0006**	9827		23.18	**0.0001**	9959
TSI		93.19	**0.0001**	9951		3.58	**0.05**	9825		63.33	**0.0001**	9957
Total	119				119				119			

Table 2. Mean and standard deviation of the environmental variables (physical and chemical parameters) measured from 120 sites distributed in the watersheds of the Piranhas-Assu (Sabugí, P. Traíras and Cruzeta reservoirs) and Paraíba rivers (Codeiro, Sumé and Poções reservoirs).

Environmental variables	**Piranhas-Assu river**				**Paraíba river**			
	June	**September**	**December**	**March**	**June**	**September**	**December**	**March**
Physical and chemical parameters								
Depth (m)	4.2±1.3	3.2±1.3	2.4±1.1	1.8±0.9	5.5±2.1	4.5±2.1	3.2±2.2	2.7±1.6
Temperature (ºC)	26.1±1.8	26.0±1.0	27.3±1.1	28.7±1.1	25.5±0.5	25.0±0.6	25.8±1.3	28.0±1.2
pH	8.0±0.7	7.7±0.4	7.9±0.6	8.7±0.5	8.7±0.5	7.9±0.6	8.0±1.3	9.0±0.3
Turbidity (NTU)	54.0±59.5	29.0±32.4	57.4±42.6	88.8±63.0	11.9±15.2	69.0±54.1	54.3±31.3	150.6±249.6
Total dissolved solids (mg/L)	1.0±1.0	1.12±0.8	1.3±1.2	1.5±1.4	0.7±0.3	1.1±0.4	1.5±0.6	2.4±4.2
Dissolved oxygen (mg/L)	5.4±3.2	7.1±1.2	7.8±1.0	8.2±1.8	2.7±2.3	10.4±2.0	9.0±2.3	9.5±2.2
Secchi	0.74±0.33	0.64±0.33	0.48±0.2	0.40±0.16	0.81±0.3	0.69±0.4	0.46±0.1	0.5±0.3
Total phosphorus (µg/L)	188.8±104.0	130.9±72.7	436.6±233.8	217.3±158.3	94.3±47.3	128.7±128.9	225.2±158.2	212.7±214.0
Soluble reactive phosphate (µg/L)	85.8±110.8	26.6±19.6	204.3±103.6	11.4±10.5	36.3±24.0	16.0±8.0	72.2±43.0	118.7±133.0
Total nitrogen	86.4±31.5	235.3±4.1	171.0±87.4	190.4±49.6	123.2±46.4	288.9±81.7	226.7±82.8	318.4±226.0
Chlorophyll-*a* (µg/L)	23.0±14.8	14.1±19.2	71.0±91.4	103.5±123.0	42.01±50.6	10.4±10.8	11.1±11.5	11.6±14.6
Organic matter								
Dissolved organic carbon (mg/L)	53.0±33.2	23.3±13.2	27.4±16.0	22.1±16.0	68.6±43.8	80.8±52.6	26.2±14.5	20.9±11.8
a_{254}	65.8±26.0	58.8±12.1	66.8±28.8	62.5±42.3	53.7±9.6	49.5±19.5	51.5±20.1	48.7±15.6
SUVA (mg C L–1 m–1)	1.8±0.9	3.0±1.0	2.7±0.7	2.6±0.7	1.1±0.6	1.1±0.8	2.3±0.9	2.7±1.1
Relative molecular size (*M*)	7.1±1.9	6.6±1.6	7.4±1.6	7.3±1.9	9.3±2.3	10.4±3.1	9.2±2.3	8.9±3.6
$S_{275-295}$ (nm^{-1})	0.020±0.003	0.020±0.003	0.021±0.003	0.020±0.003	0.023±0.003	0.025±0.04	0.025±0.03	0.024±0.005
$S_{350-400}$ (nm^{-1})	0.016±0.002	0.015±0.002	0.015±0.002	0.014±0.002	0.017±0.001	0.018±0.002	0.015±0.003	0.015±0.003
SR	1.2±0.1	1.4±0.2	1.4±0.2	1.4±0.2	1.3±0.1	1.4±0.1	1.7±0.4	1.6±0.3
Trophic status index								
TSI	63.1±8.6	57.1±9.3	76.1±9.1	62.4±8.7	61.1±5.7	55.3±7.1	64.7±8.0	63.2±13.9

Table 3: Results from Principal components analysis (PCA) to DOC/CDOM dataset superimposed with TSI pattern from 120 sites distributed between Piranhas-Assu river and Paraíba rivers, in semi-arid region of Brazilian Northeast**.**

Variables	**PC1**	**PC2**
DOC	0.33	-0.42
a_{254}	0.08	0.09
SUVA	-0.40	0.28
$S_{275\text{-}295}$	0.49	0.38
$S_{350\text{-}400}$	0.43	-0.30
M	0.52	0.22
SR	0.10	0.66

Table 4: Correlation results among DOC quantity and quality parameters and TSI from 120 sites in reservoirs of the Piranhas-Assu river and Paraíba river watersheds, Brazilian semi-arid.

	DOC	a_{254}	**SUVA**	$S_{275-295}$	$S_{350-400}$	M	SR	**TSI**
DOC		0.11	-0.73	0.19	0.40	0.23	-0.16	-0.11
a_{254}			0.20	0.18	0.18	0.12	0.02	0.44
SUVA				-0.37	-0.41	-0.40	-0.02	0.30
$S_{275-295}$					0.47	0.92	0.57	-0.02
$S_{350-400}$						0.66	-0.42	-0.31
M							0.31	-0.15
SR								0.24

Supplementary material

Table S1. Characterization of reservoirs of Piranhas-Assu and Paraíba rivers watersheds. Data provided by Secretaria de Meio Ambiente e Recursos Hídricos State of Rio Grande do Norte (SEMARH) Agência Executiva de Gestão das Águas (AESA) State of Paraíba. * no record.

Features/Reservoirs	**Piranhas-Assu River**			**Paraíba River**		
	Sabugí	P. Traíras	Cruzeta	Cordeiro	Sumé	Poções
Geographic localization	06º43’06’’S 37º12’02’’W	06º27’16’’S 36º52’29’’W	06º24’42’’S 36º47’23’’W	7°47’38.00”S 36°40’14.04”W	7°29’8"S 37°12'20"W	7°53’38”S e 37°0’30”W
Altitude (m)	187	196	231	480	500	596
Approximate maximum capacity (10^6m^3)	65	49	23	70	45	30
Construction year	1965	1994	1929	*	1953	1982
Main finality	Supply	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation
Time of retention water	3-5 years	3-5 years	3-5 years	3-5 years	3-5 years	3-5 years

Average volume (10^6m^3)	June	September	December	March
Sabugí	18	14	9	7
Passagem das Traíras	3	2	1	0.648
Cruzeta	5	4	2	1
Cordeiro	9	8	5	4
Sumé	17	14	11	9
Poções	6	5	3	2

Capítulo 5. REHABILITATION SCENARIOS FOR RESERVOIRS: PREDICTING AQUATIC COMMUNITIES THROUGH MACHINE LEARNING

Manuscrito submetido à Ecological Indicators

Reservatório Sabugí, São João do Sabugí – Rio Grande do Norte (RN), Brasil/ 2014. Foto: Luiz Carlos

Rehabilitation scenarios for reservoirs: predicting aquatic communities through machine learning

AZEVÊDO, D. J. S.[1]; BEZERRA-NETO, J. F [2]; MOLOZZI, J.[3]; FEIO, M. J [4]

[1]Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, sala 318, bloco L3. Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brasil.

[2] Departamento de Biologia Geral/ Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais. Avenida Presidente Antônio Carlos nº 6627, sala 318, bloco L3. Pampulha, CEP 31270-910, Belo Horizonte – Minas Gerais, Brasil.

[3]Departamento de Biologia/ Programa de Pós- Graduação em Ecologia e Conservação, Universidade Estadual da Paraíba. Rua Baraúnas, nº. 351 - Complexo Três Marias, Prédio de Biologia, Térreo - sala 10. Universitário, CEP 58.429-500, Campina Grande - Paraíba, Brasil.

[4] MARE – Marine and Environmental Sciences Centre, Faculty of Sciences and Technology, Department of Life Sciences, University of Coimbra. Rua da Matemática nº 49, 3000-276 Coimbra, Portugal

E-mail of the corresponding author: daniele.jazevedo@gmail.com / Telephone number: +55 (83) 998350274

Abstract

In present study, we tested the applicability of a machine-learning tool (HYDRA) to simulate rehabilitation scenarios of reservoirs. The HYDRA was used to build a “dirty-water” predictive model based on 129 training sites from semi-arid reservoirs of SW Brazil, covering a gradient of disturbance, and including predictive variables translating anthropogenic disturbance (contrary to predictive models based on the Reference Condition Approach). The model was used to simulate the effects of the rehabilitation measures (treatment of the urban effluents discharging into the reservoirs or associated rivers) in the macroinvertebrate communities, through the manipulation of predictive variables related to eutrophication (phosphorus, soluble reactive phosphates, transparency and chlorophyll-a, dissolved oxygen, total dissolved solids and turbidity). Two different rehabilitation scenarios were built (D1 = lower improvement, 25% of change; D2 = higher improvement, 75% of change); and three initial levels of disturbance were considered (Severely disturbed-SD, Intermediately disturbed-ID, Least Disturbed-LD), based on PCA analyses. The effects were analyzed in terms of changes in Expected taxa (E) against Observed (O) richness (OE ratios), communities' composition, biotic and trophic status indices and spatial distribution of sensitive taxa. The "dirty-water" model had a high accuracy in taxa prediction (mean > 0.73; sd 0.15-0.19) and precision (O/E = 0.78; SDOE = 0.17; OE linear regression R2 = 0.89, slope = 0.86, intersection = -0.75). All simulations resulted in the significant improvements (PERMANOVAs: $p<0.001$) for all disturbance levels and indicators (trophic status index, biotic index for invertebrates and total richness). Sensitive taxa from Trichoptera, Ephemeroptera and Diptera expanded their distribution across the reservoirs. The greatest differences in richness were found for the SD sites but richness reached higher levels in LD sites under D2 scenario. The simulations showed also that rehabilitation measures, even in artificial systems such as the reservoirs, could result in a higher biodiversity, biological quality and water quality. In addition we showed that there is still room for improvement in communities even at the least disturbed sites. The HYDRA tool and the "dirty-water" models showed a high potential for management and conservation purposes, enabling the determination of the most cost-effective rehabilitation measures and their follow-up.

Keywords: Semi-arid; HYDRA; predictive models; restoration; freshwater ecosystem; macroinvertebrates

Introduction

Predictive models are considered useful tools for the bioassessment of aquatic ecosystems and elaboration of management strategies and have been used all over world (e.g., Clarke et al., 2003; Feio et al., 2007; Strachan and Reynoldson, 2014; Ashofteh et al., 2017). These models evaluate the environmental quality of a site through the deviation of the observed biological communities to those expected under reference conditions (Reynoldson et al., 1997; Stoddard et al., 2006; Feio et al., 2009; Hawkins et al., 2010). The predictive and exploratory ability of these models provides useful information about present and future dynamic of ecosystems (Kwang-Seuk et al., 2005).

Predictive models based on machine learning techniques have been developed and tested for bioassessment of rivers (e.g., Linke et al., 2005; Gabriels et al., 2007; Feio et al., 2014; Sarrazin-Delay et al., 2014). These approaches have the ability to model and predict the species distribution in the dimensional space, with the advantages of: not requiring an *a priori* reference sites grouping that can be viewed as artificial; capturing non-linear relationships not requiring normal distribution of variables; and for being less influenced by outliers (Gevrey et al., 2004, Rose et al., 2016). In this study we used the HYDRA machine learning tool (Feio et al. 2014a,b), which enables the prediction of communities through the simultaneous use of three different techniques, allowing for greater accuracy in taxa predictions: Support Vector Machines (SVM;), Multi-Layer Perceptron (MLP) and K-Nearest Neighbor analysis (KNN). This tool was already widely tested with large datasets from Europe, North America and Oceania rivers and showed a good precision and accuracy in identifying alterations in aquatic invertebrate communities (Feio et al. 2014a,b).

In spite of the great evolution in bioassessment techniques and the large implementation of large-scale assessment programs over the world, the recovery of aquatic systems is still unsatisfactory (e.g., Langhans et al. 2014; Hwang et al., 2014; Muhar et al., 2016; Feio et al. 2017). Among the main reasons are the lack of measureable ecosystem health objectives and association between measures-effects in restoration projects. In that context simulation tools are one of the keys for a successful restoration because they can incorporate multiple information on the indicators of degradation and test different combinations of rehabilitation scenarios (Hermoso et al., 2011; Brudvig et al., 2017). Yet, these tools have been used seldom and their outcomes are mostly restricted to specific species or diversity metrics (Joy and Death, 2004; Mérigoux et al., 2015; Rose et al., 2016; Laughlin et al., 2017).

Here, differently from what is normally aimed with predictive models for bioassessment, we tested the applicability of HYDRA to predict the effect of rehabilitation measures in the aquatic communities of reservoirs instead of assessing their level of degradation. Thus, the models are built over environmental and biological data from sites covering a disturbance gradient, and environmental predictors that are sensitive to disturbance, such as nitrate or phosphate concentration, opposing to conventional predictive models, where only reference data and non-pressure environmental variables (as altitude or geology) are used to build a model. These models are called “dirty-water models” following a concept proposed by Norris et al. (2000) which was however only rarely tested (but see Linke et al., 2001; Hoang et al. 2001, 2003).

The HYDRA “dirty-water” modeling approach, was applied to 129 sites from 6 reservoirs located in two watersheds of the semi-arid region of Brazil (NE). These systems vary in their degree of disturbance regarding the land use in the surroundings and contamination by nutrients derived from urban sewage. Yet, in spite of being artificial systems, the maintenance of a good ecological quality (or good ecological potential, according to the Water Framework Directive, WFD; European Comission 2000) is crucial to preserve the biodiversity in the catchment, avoid the proliferation of exotic species and maintain ecosystem services (e.g., food production, sources of clean water for irrigation or human consumption, nutrients cycling) (e.g, Havel et al., 2005; Chellappa et al., 2009; Vidal-Abarca et al. 2014, Gunkel et al., 2015). We expect to show that the HYDRA can be a useful tool for the management and rehabilitation planning of reservoirs, by providing clear indications on the most efficient measures to enhance biodiversity and improve the ecological quality the reservoirs and consequently of their watersheds.

Material and methods

Data base

The data used to build and test the HYDRA models and rehabilitation scenarios was obtained from 129 sites distributed through six reservoirs localized in two semi-arid watersheds: Piranhas-Assu (Rio Grande do Norte state) and Paraíba rivers (Paraíba state) (Table S1 – Supplementary material; Figure 1). Sampling occurred four times, in June, September and December 2014 and March 2015. The climate in the region is BSh type (dry semiarid), following Köppen–Geiger classification. In both regions, the dry period has about 9-10 months (Alvares et al., 2013) and the mean rainfall is 400 mm/year in Paraíba and 800 mm/year in Piranhas-Assu rivers, respectively. The minimum air temperature varies between 18 and 22ºC (July and August) and the maximum temperature between 28 and 31ºC (November and December).

The benthic macroinvertebrate community and environmental descriptors (e.g. locality, precipitation, geology, physical and chemical parameters, habitat and landscape) were characterized for each site (Table 1). The macroinvertebrate community was sampled in each site using an Eckman-Birge dredge (225cm²). All individuals were identified to family level and the Chironomidae larvae to genus level (Peterson, 1960; Boffi, 1979; Péres, 1988;

Trivinho-Strixino and Strixino, 1995; Merritt and Cummins, 1996; Carvalho and Calil, 2000; Epler, 2001; Fernández and Domínguez, 2001; Costa et al., 2006).

The characterization data (location, precipitation and geology) were obtained from the official environmental agencies of the studied areas ("Secretaria de Meio Ambiente e Recursos Hídricos" in Rio Grande do Norte state and "Agência Executiva de Gestão das Águas" in Paraíba state). Physical and chemical parameters were measured *in situ* with multiparameter probe (Horiba U-50): water temperature (ºC), pH, turbidity (NTU), dissolved oxygen (mg/L) and total dissolved solids (g/L). Water samples were collected to determine the concentration of total phosphorus (TP - µg/L), reactive soluble phosphate (SRP - µg/L), ammonium (NH_3^- - µg/L), and nitrogenous compounds (NOx-N - µg/L) (APHA, 2005). The concentration of chlorophyll-*a* was estimated by methods proposed by Lorenzen (1967). Sediment samples were collected using an Eckman-Birge dredge ($225cm^2$) to determine the organic matter content and granulometric composition. The landscape was characterized through the River Habitat Survey protocol, modified by Rowan et al. (2006) for lentic freshwater ecosystems. Fifty-meters of the margins were assessed in each sampling site considering: 1) urban development (e.g. residence, transmission lines and fence); and 2) agricultural areas (e.g. pastures and agricultural plantations). The coverage values vary between 0-1, where values near 1 represent those sites with highest anthropogenic occupation.

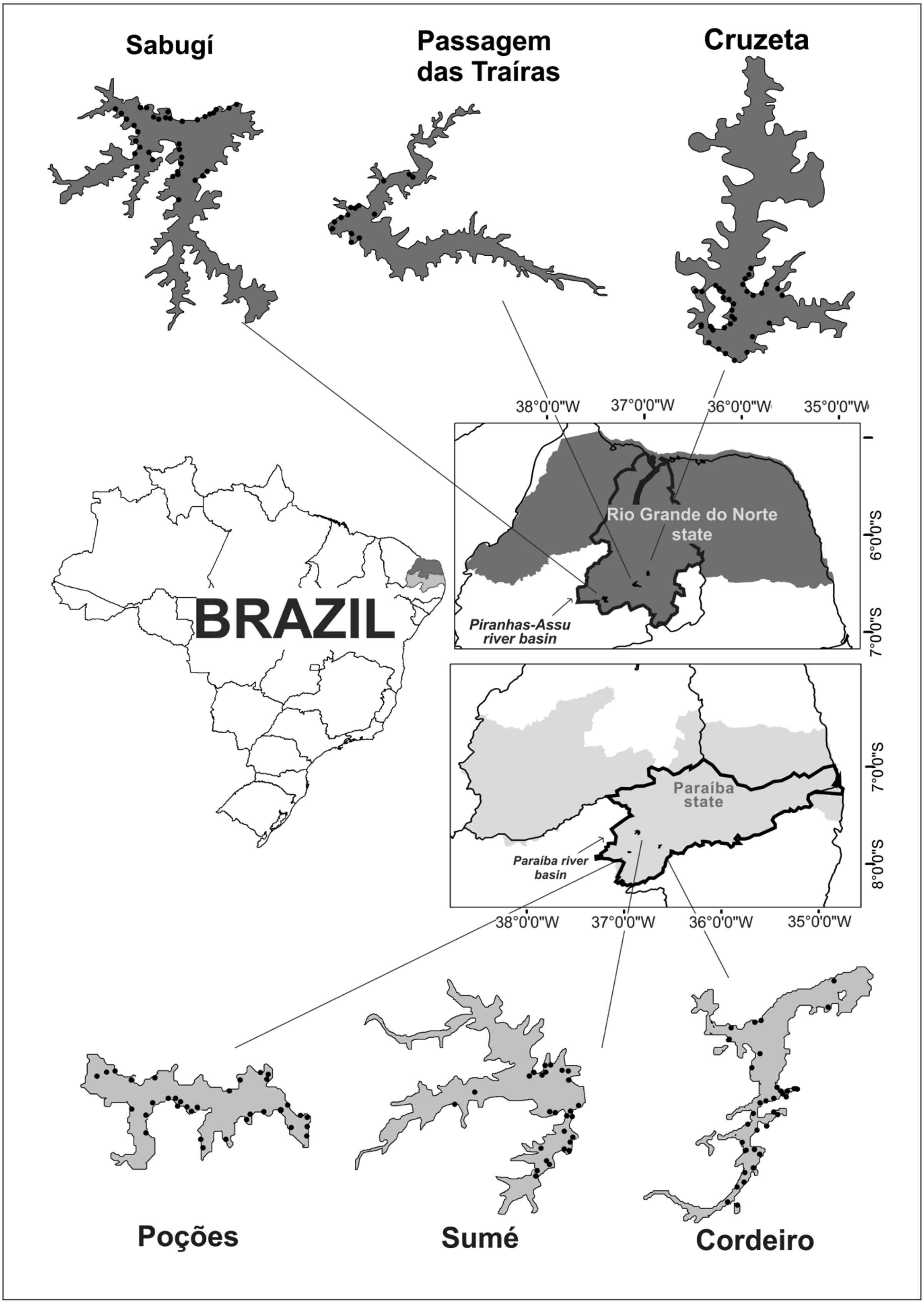

Figure 1. Distribution of sampling sites (black dots) in the reservoirs of Piranhas-Assu river (Rio Grande do Norte state) and Paraíba river (Paraíba state), Brazilian semi-arid region.

Table 1: Mean and standard deviation (±) for environmental variables measured in the 129 sites of the six studied reservoirs LD = Least disturb; ID = Intermediate disturb; SD = severe disturb; Min/Max = minimum, maximum values for LD, ID, and SD sites (1, 2 and 3, respectively). TSI D1 = Trophic Status Index for lower environmental improvement level (25% of change); TSI D2 = Trophic Status Index for higher environmental improvement level (75% of change).

Variables	LD	Min/Max 1	ID	Min/Max 2	SD	Min/Max 3
Characterization						
Latitude	-37.2±0.2	-36.7 / -37.2	-36.9±0.17	-36.7 / -37.2	-36.9±0.09	-36.8 / -37.0
Longitude	-6.7±0.4	-6.4 / -7.9	-7.6±0.4	-6.7 / -7.9	-6.98±0.7	-6.4 / -7.9
Precipitation	33.7±7.5	5.7 / 36.5	12.5±12.2	4.3 / 36.5	31.3±3.9	26.7 / 36.5
Altitude	223.9±97.8	187.0 / 599.0	473.1±114.6	187.0 / 599.0	360.3±185.0	207.0 / 599.0
Lithology	1.33±0.6	1.0 / 3.0	3.2±0.9	1.0 / 4.0	2.4±0.5	2.0 / 3.0
Depth	0.6±0.1	0.4 / 0.8	0.7±0.1	0.3 /1.1	0.6±0.2	0.2 / 1.3
Physical and chemical parameters						
Secchi	0.5±0.1	0.3 / 0.8	0.5±0.1	0.2 / 0.7	0.3±0.1	0.2 / 0.6
Turbidity	19.9±10.9	4.6 / 47.2	49.0±45.8	7.2 / 248.4	104.6±68.9	29.6 / 347.2
Oxygen dissolved	5.8±1.0	4.0 / 8.7	6.7±1.1	4.2 / 9.0	7.8±1.6	4.5 / 10.6
Total dissolved solids	0.43±0.2	0.3 / 1.1	0.9±0.5	0.3 / 1.8	1.5±0.9	0.6 / 3.0
Nitrogen oxides	31.3±20.0	4.8 / 125.7	45.8±26.9	11.54 / 129.6	37.8±28.8	9.8 / 162.1
Ammonium ion	32.7±16.0	1.1 / 64.8	53.8±29.1	1.1 / 167.9	79.8±48.6	13.1 / 229.1
Total phosphorus	106.2±62.4	43.2 / 276.3	123.8±77.8	43.2 / 407.3	313.5±107.9	106.5 / 557.8
Soluble reactive phosphate	39.9±48.7	4.0 / 187.6	43.1±33.2	4.0 / 198.8	111.7±65.9	8.2 / 321.4
Chlorophyll-*a*	12.8±11.0	2.2 / 66.2	16.3±25.8	2.3 / 122.0	58.0±51.3	11.0 / 188.1
Habitat						
Organic matter	0.2±0.1	0.04 / 0.4	0.3±0.1	0.04 / 0.55	0.2±0.1	0.1 / 0.5
Gravel	1.3±0.2	1.0 / 1.6	1.4±0.1	1.0 / 1.6	1.5±0.1	0.8 / 1.6
Coarse sand	1.4±0.09	1.2 / 1.6	1.4±0.09	1.1 / 1.6	1.4±0.09	1.2 / 1.5
Middle sand	1.39±0.08	1.1 / 1.5	1.3±0.07	1.1 / 1.5	1.4±0.08	1.2 / 1.5
Fine sand	1.33±0.07	1.2 / 1.5	1.3±0.07	1.1 / 1.5	1.3±0.08	1.1 / 1.4
Silt	1.44±0.05	1.3 / 1.5	1.4±0.07	1.2 / 1.5	1.3±0.07	1.2 / 1.5
Mud	1.51±0.04	1.4 / 1.5	1.5±0.04	1.4 / 1.6	1.5±0.04	1.4 / 1.5
Landscape						
Residence	0.2±0.2	0.0 / 0.7	0.2±0.2	0.0 / 0.7	0.5±0.36	0.0 / 1.0
Pasture	0.3±0.2	0.0 / 1.0	0.2±0.2	0.0 / 0.7	0.4±0.3	0.0 / 1.0
Agricultural	0.1±0.2	0.0 / 0.5	0.2±0.2	0.0 / 0.7	0.4±0.3	0.0 / 1.0
Eutrophication level						
Observed TSI	59.7±6.1	47.0/72.1	59.6±6.1	51.0/74.0	73.2±5.4	58.0/83.0
TSI D1	56.2±6.4	43.0/68.5	55.7±6.1	47.4/70.0	69.5±5.4	54.0/79.0
TSI D2	43.2±6.1	34.4/55.6	42.9±6.0	35.0/57.0	56.6±5.4	41.0/66.0

Data analyses

Development of the HYDRA dirty-water model

The HYDRA allows the construction and application of predictive models based on a combination of three supervised machine learning techniques (SVM, MLP and KNN; Feio et al. 2014a,b). Similarly to RIVPACS models (e.g. Wright et al., 2000; Hart et al., 2001, Feio et al. 2009), the HYDRA produces a list of Expected taxa (E), defined as those taxa with a probability of occurrence above 0.5; and an Observed/Expected ratio (OE) that translates the deviation between the observed taxa and that expected by the models. Yet, for each taxa in the training dataset (data used in the learning process to construct the models), models based on HYDRA three machine learning techniques are constructed through a supervised learning process. The best model is used to define the final probabilities of finding a taxa at a site, based on all environmental variables available. The minimum taxa accuracy to accept a taxa prediction is 0.5. The best models are those with an Observed/Expected ratio taxa (OE)≈1; a low standard deviation OE ratios (SDOE) and a good OE regression: $R^2 \geq 0.5$; $p<0.05$; intersection close to origin (a range of -1.5 to 1.5 is acceptable) and slope ≈ 1 (acceptable range 0.85 and 1.15) (Linke et al., 2005; Feio et al., 2014a, b). The HYDRA tool, described in detail in Feio et al. 2014a,b, is implemented in the AQUAWEB platform (aquaweb.uc.pt) developed in the R language using the *Rminer* package (Cortez 2010). Here we built a model based on a training biological and environmental dataset from 129 sites. Rare taxa (present in less than 5% of the sites) were previously excluded.

Rehabilitation scenarios and simulations

In a second phase the HYDRA model developed with training sites was applied to simulated data, used as test sites, to determine the expected taxa after rehabilitation measures.

Thus here, the OE ratios produced by the HYDRA model for the simulated data indicate the deviation in richness under rehabilitation measures and are expected to become smaller with the improvement of reservoirs (higher Expected than Observed values), contrary to normal predictive models used in bioassessment (e.g. Clarke et al., 2003; Hose et al., 2004; Hargett et al., 2007; Nichols et al., 2014).

The rehabilitation scenarios tested here considered the improvement in water quality associated to a potential treatment of urban waste-water effluents which are discharging into the rivers or the reservoirs with none or insufficient treatment (WHO, 2015; UNESCO, 2017). Thus, values of all variables associated to eutrophication (phosphorus, soluble reactive phosphates, transparency and chlorophyll-*a*) were changed in the simulations. Moreover, we changed the values of dissolved oxygen, total dissolved solids and turbidity, as they are variables intimately associated to variation in the transparency and chlorophyll-*a*. All values were reduced, except for dissolved oxygen that was reduced only for concentrations > 7 mg/L. Sites with low oxygen concentrations were not changed, as freshwater ecosystems with oxygen inferior to 4 mg/L are considered anoxic (CONAMA 357/05). The values of transparency were also increased for lower eutrophication scenarios, which frequently correspond to clearer waters (see Figure 2 for steps). Furthermore, we simulated two levels of change in water quality: D1 = lower improvement (25% of change) and D2 = higher improvement (75% of change).

To enable a finer analysis of the effect of rehabilitation measures namely by attempting on the initial disturbance level, we did an initial division of training sites based on their distribution in a PCA (Principal Components Analysis) using disturbance variables only. The variables with non-normal skewed distribution (e.g. OD, TDS, NOx-N, NH3- and OM) were transformed by log (x +1) and habitat data was transformed by arc-sine. All environmental data were posteriorly normalized. When variables were highly correlated

(Spearman correlations; >0.75) only one was used in further analyses. From their position in the PCA axes, we selected least disturbed sites (LD), sites with intermediate disturbance (ID) and severely disturbed sites (SD). Significantly differences between these groups were checked with a Permutational Multivariate Analysis of Variance (PERMANOVA, Distance Euclidian coefficient, 9999 permutations; Anderson 2001a, b; Anderson and Braak 2003; Anderson et al., 2008). Differences in the invertebrate communities were also confirmed through a multivariate PERMANOVA (Bray Curtis similarity, 9999 permutations).

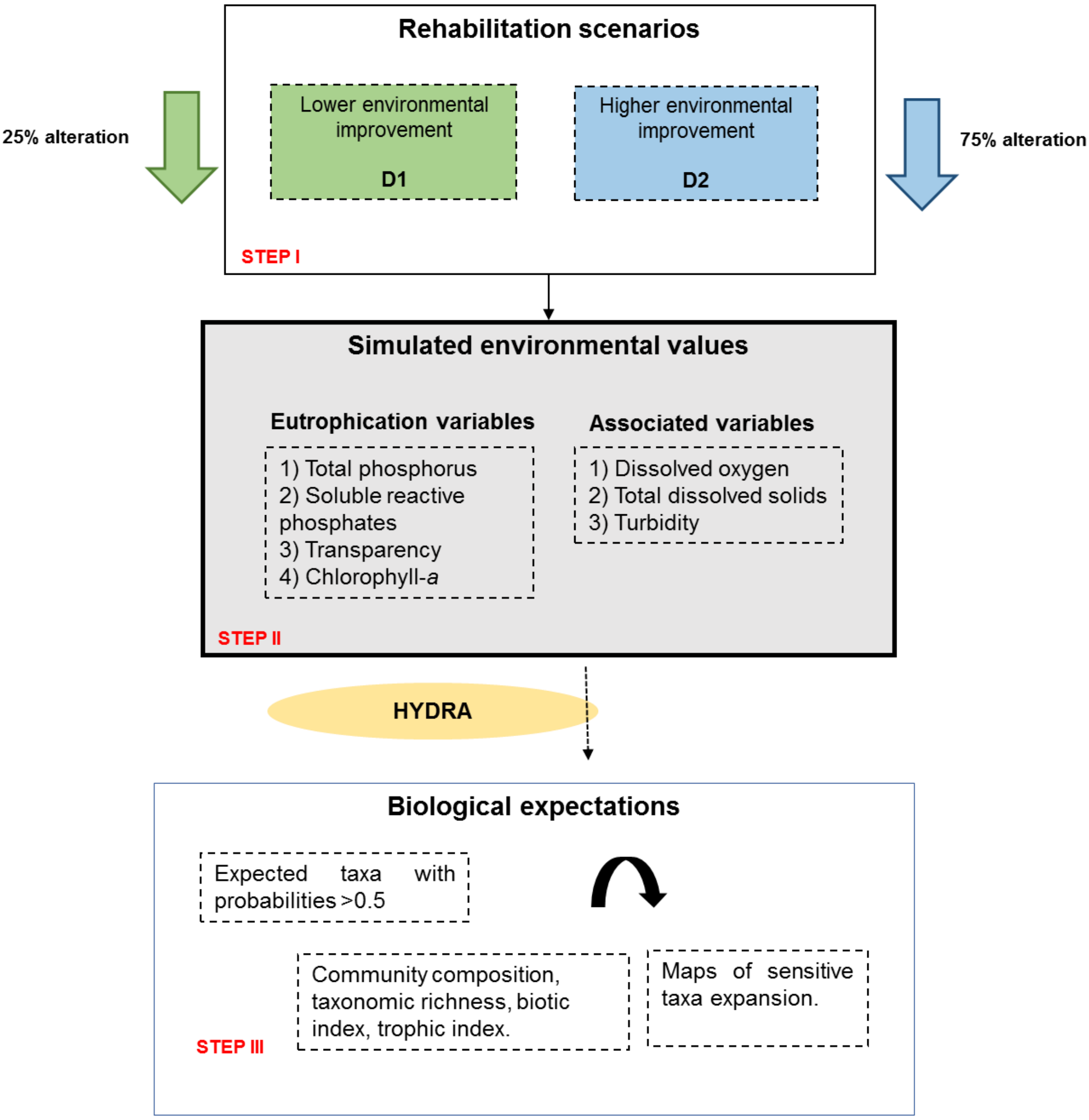

Figure 2: Schematic representation of the step-by-step process followed to analyze the rehabilitation scenarios and their effects in the benthic invertebrate communities of the studied reservoirs.

Based on the Expected data (presence/absence of taxa in simulated sites), we calculated the taxonomic richness and the biotic index IBMWP (Iberian Biological Monitoring Working Party; Alba-Tercedor et al., 2002; Jáimez-Cuéllar et al., 2002) for both Observed and Expected sites to analyze the biotic improvement. The IBMWP index weights the sensitivity of each invertebrate family present at a site to disturbance (mostly organic contamination and nutrients) and considers five quality status according to its final scores: good (101-150), acceptable (61-100), deficient (36-60), bad (16-35) and very bad (<15). Here we used this scale for comparative purposes only, as the index was developed for rivers and temperate systems where the diversity is naturally higher. In addition we calculated the Trophic Status Index (TSI), proposed by Carlson (1977) and modified by Toledo et al. (1983), commonly used to assess reservoirs in Brazil (Molozzi et al., 2013; Azevêdo et al., 2015) for both Observed and Expected lists. Finally, we selected three sensitive taxa of different orders to analyze the potential changes in their spatial distribution resulting from rehabilitation measures: the Ephemeroptera Caenidae, the Trichoptera Polycentropodidade and the Diptera Chironomidae *Zavreliela.*

The PERMANOVA was again used to assess for significant differences between Observed and Expected communities (Bray-Curtis similarity, 9999 permutations) and differences in IBMWP and TSI values (Euclidian Distance coefficient, 9999 permutations). All statistical analyses were performed in PRIMER + PERMANOVA 6 software.

Results

HYDRA model

All techniques performed well and the mean taxa prediction accuracies were: 0.84 (SVM), 0.73 (MLP) and 0.82 (KNN) (sd 0.15-0.19). A total of 17 rare taxa were excluded from the biological dataset. The remaining taxa (44 taxa) were predicted with an accuracy >0.5 by at least one of the techniques. The selected model had a mean OE=0.78 (SDOE = 0.17) and the OE regression was within acceptable values (R^2 = 0.89; slope = 0.86; intersection = -0.75; Figure 3).

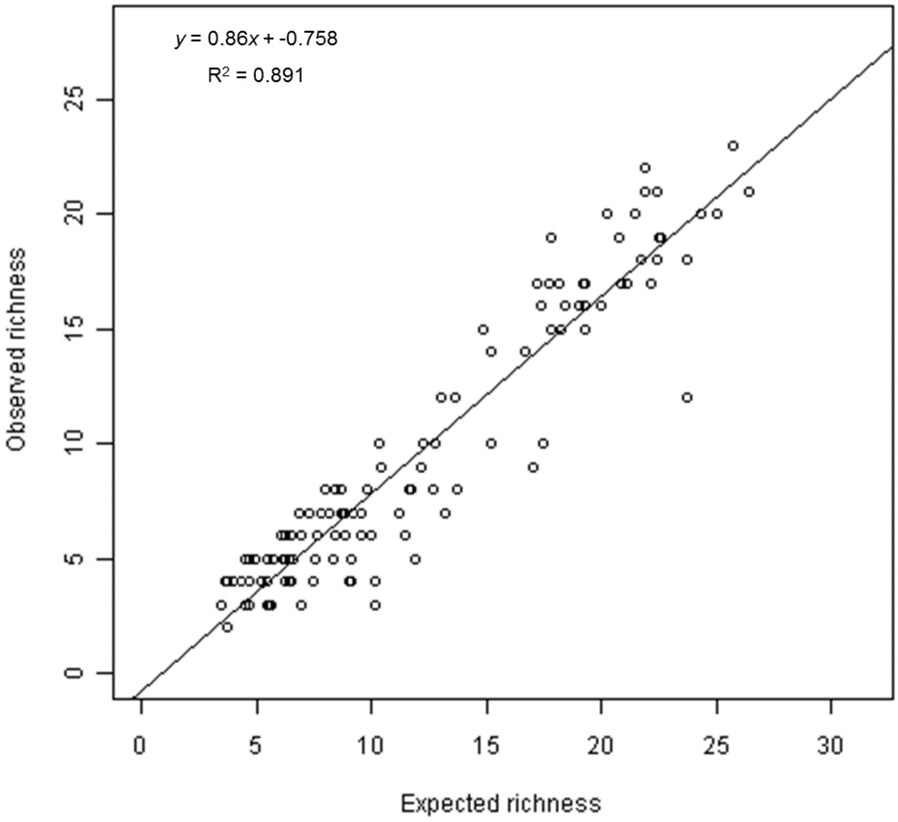

Figure 3: Observed/Expected taxa regression for the final HYDRA dirty-water model built with biological and environmental data from 129 sites (training sites) located in Sabugí, Cruzeta, Passagem of the Traíras reservoirs (Piranhas-Assu watershed) and Cordeiro, Sumé and Poções reservoirs (Paraíba watershed).

Training sites characterization and disturbance categories

The first three axes of PCA based on environmental data explained 54.0% of data variability. PC1 alone explained 22.5% and was better correlated with characterization variables (e.g. localization, altitude, lithology and precipitation: 0.31-0.33 coefficient at linear combinations); PC2 (19.5%) was correlated to pressure variables (chlorophyll-*a*, total phosphorus) and habitat (silt) (0.29-0.33 coefficient of linear combinations) (Table 2). Based on sites distribution in the PCA plot, sites were grouped into three disturbance levels (Figure 4): the first group included 30 least disturbed sites (LD); the second included 54 sites with intermediate disturbance (ID); and the remaining 45 sites were considered severely disturbed group (SD) (Table 1). These groups were significantly different considering: their environmental characteristics (PERMANOVA: Pseudo-$F_{2.128}$ = 25.7; p = 0.0001); and Trophic Status Index (PERMANOVA: Pseudo-$F_{2.128}$ = 78.4, p = 0.0001), with the highest eutrophication level obtained for SD group, with a maximum value of 83.0.

Total richness was similar in LD, ID and SD groups (46, 43 and 43 identified taxa, respectively), from a total of 61 taxa identified. However, the average richness differed between groups (PERMANOVA: Pseudo-$F_{2.128}$ = 19.6; p = 0.0001) and was lower (4.7±1.9) in SD sites, intermediate in ID sites (5.8±2.0) and highest in LD sites (6.7±2.0) (Table S2 – Supplementary material). The biotic index showed a similar pattern (PERMANOVA: Pseudo-$F_{2.128}$ = 13.8, p = 0.0001), with the lowest IBMWP scores in the SD (12.2±8.2), intermediate at ID (16.6±7.6) and the highest in LD sites (22.6±9.8) (Table S2).

Table 2: Results of the Principal Components Analysis (PCA). Coefficients of linear combinations for axes 1 and 2 (PCs) of the PCA based on environmental data.

Variables	Code	PC 1	PC 2
Latitude	Lat	-0.31	0.003
Longitude	Lon	0.32	-0.19
Precipitation	Prec	0.31	-0.25
Altitude	Alt	-0.32	0.14
Lithology	Lit	-0.33	0.14
Depth	Deph	-0.17	0.09
Secchi	Sec	-0.03	0.22
Turbidity	Tur	-0.23	-0.25
Oxygen dissolved	Od	-0.17	-0.16
Total dissolved solids	Tds	-0.25	-0.18
Nitrogen oxides	NOx-N	-0.19	0.02
Ammonium ion	NH_3^-	-0.18	-0.19
Total phosphorus	TP	-0.01	-0.31
Soluble reactive phosphate	Srp	-0.03	-0.20
Chlorophyll-*a*	Clo-*a*	0.008	-0.33
Organic matter	OM	-0.29	0.01
Gravel	Gra	-0.22	-0.15
Coarse sand	Coa	-0.04	-0.14
Middle size sand	Mid	0.21	-0.09
Fine sand	Fin	0.01	0.21
Silt	Sil	0.11	0.29
Mud	Mud	0.19	0.16
Residence	Res	0.03	-0.28
Pasture	Pas	0.04	-0.23
Agriculture	Agr	-0.08	-0.23

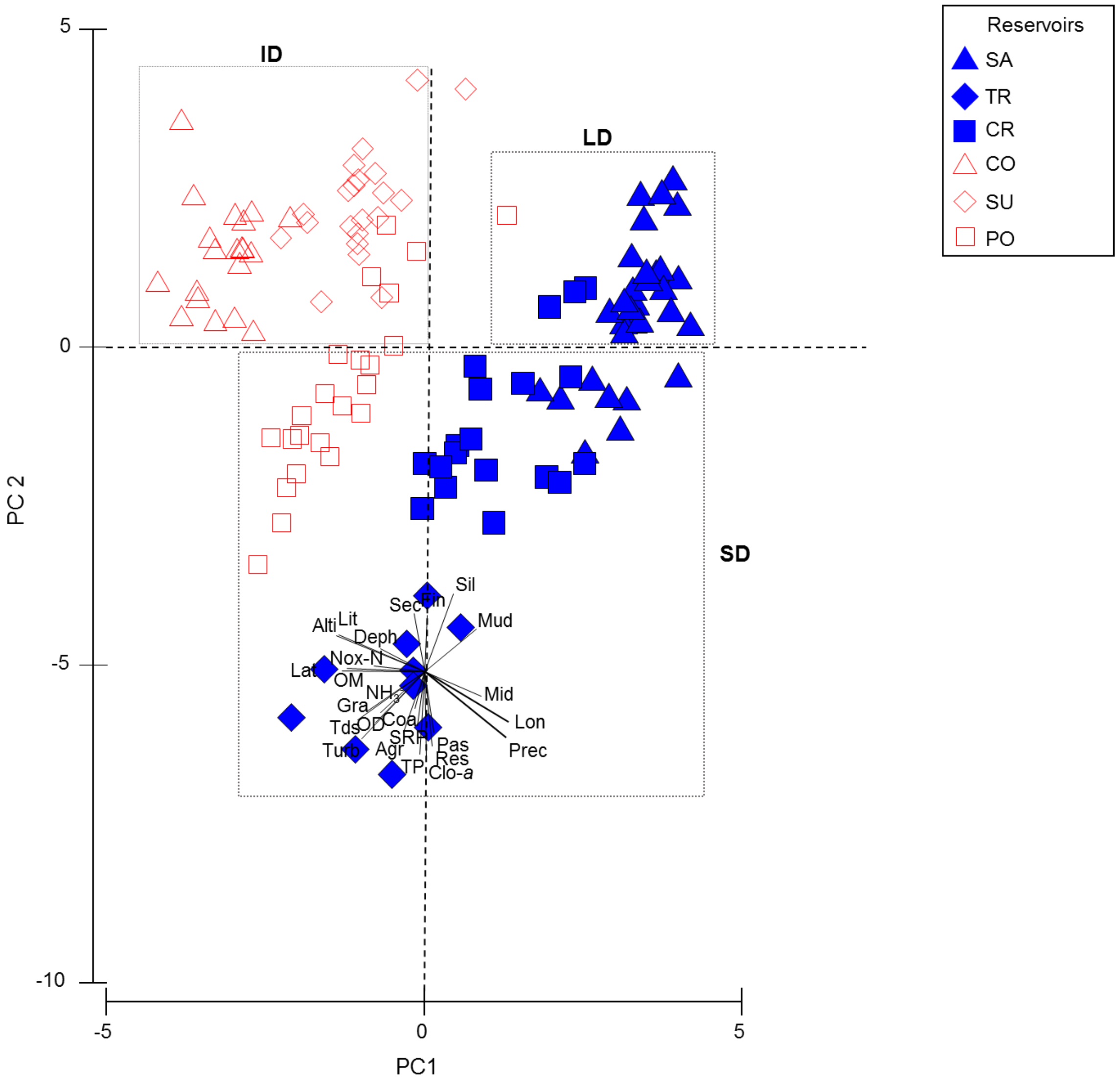

Figure 4: Principal Component Analysis (PCA) based on environmental data (e.g. locality, precipitation, geology, physical and chemical parameters, habitat and landscape) of 129 sites from Sabugí, Passagem das Traíras and Cruzeta reservoirs (Piranhas-Assu watershed), Cordeiro, Sumé and Poções reservoirs (Paraíba watershed). Where LD are Least disturbed sites; ID are Intermediated disturbed sites and SD are Severely Disturbed sites. Blue dots correspond to Piranhas-Assu and red dots to Paraíba watershed. For variables abbreviation see Table 2.

Rehabilitation scenarios

In general, our rehabilitation scenarios resulted in the improvement of ecosystems for all levels of disturbance, and the improvements were always stronger for the D2 scenario, considering all indicators: total richness and trophic status and biotic index (Table S2 and Figure 5). In addition, the decrease of OE values with the degree of impairment (from LD to SD), especially in D2 scenario (Table 3) show that the simulations resulted in communities' enhancement, which was more evident for sites with an initially poorer quality. The expected communities under D1 and D2 scenarios were significantly different (PERMANOVA: Pseudo-$F_{2.386}$ = 127.9, p = 0.0001).

There was a general increase in sites richness with the arrival of new species to sites were they weren't present before (Tables S2), especially in D2 scenario. The greatest differences were verified for the most impaired sites (SD) but the highest mean richness is expected in the D2 scenario for LD sites (10.6±1.5) Richness was significantly different between the D1 and D2 scenarios (PERMANOVA: Pseudo-$F_{1.257}$ = 14.9, p = 0.0001).

The IBMWP scores were also significantly different between D1 and D2 scenarios (PERMANOVA: Pseudo-$F_{2.386}$ = 85.7, p = 0.0001) and the highest expected IBMWP scores were obtained with D2 scenario for LD sites (44.0±8.2) (Table S2 – Supplementary material). The total number of sites with severe of biotic degradation decreased (Figure 6).

These changes in the biotic index values are not only due to a higher richness, but also to the appearance of more sensitive taxa in the reservoirs balancing the observed predominance of Diptera. For example, the Ephemeroptera Caenidae increased considerably their distribution in the studied reservoirs for LD (from being present at any site to a distribution through 15 sites), ID (from 5 to 22 sites) and SD (from 1 to 8 sites) groups. An even greater expansion was observed for the Trichoptera Polycentropodidae in LD (from 5 to 24 sites), ID (from 0 to 3 sites) and SD (from 1 to 7 sites) (Figures 7 and 8). The Diptera

Chironomidae, *Zavreliela* increased also its distribution but only in Least Disturbed and Severely Disturbed Sites (LD: from 2 to 7 sites; SD: from 2 to12 sites) (Table S2, Figure 9).

Table 3: Mean OE (Observed/Expected ratio) obtained by simulations of rehabilitation measures in two scenarios (D1= 25% improvement and D2= 75% improvement) for the 129 study sites with three levels of impairment. LD = Least Disturbed; ID = Intermediately Disturbed and SD = Severely Disturbed.

Groups	D1 Scenario
LD	0.917±0.11
ID	0.826±0.15
SD	0.884±0.15
	D2 Scenario
LD	0.729±0.12
ID	0.582±0.16
SD	0.588±0.17

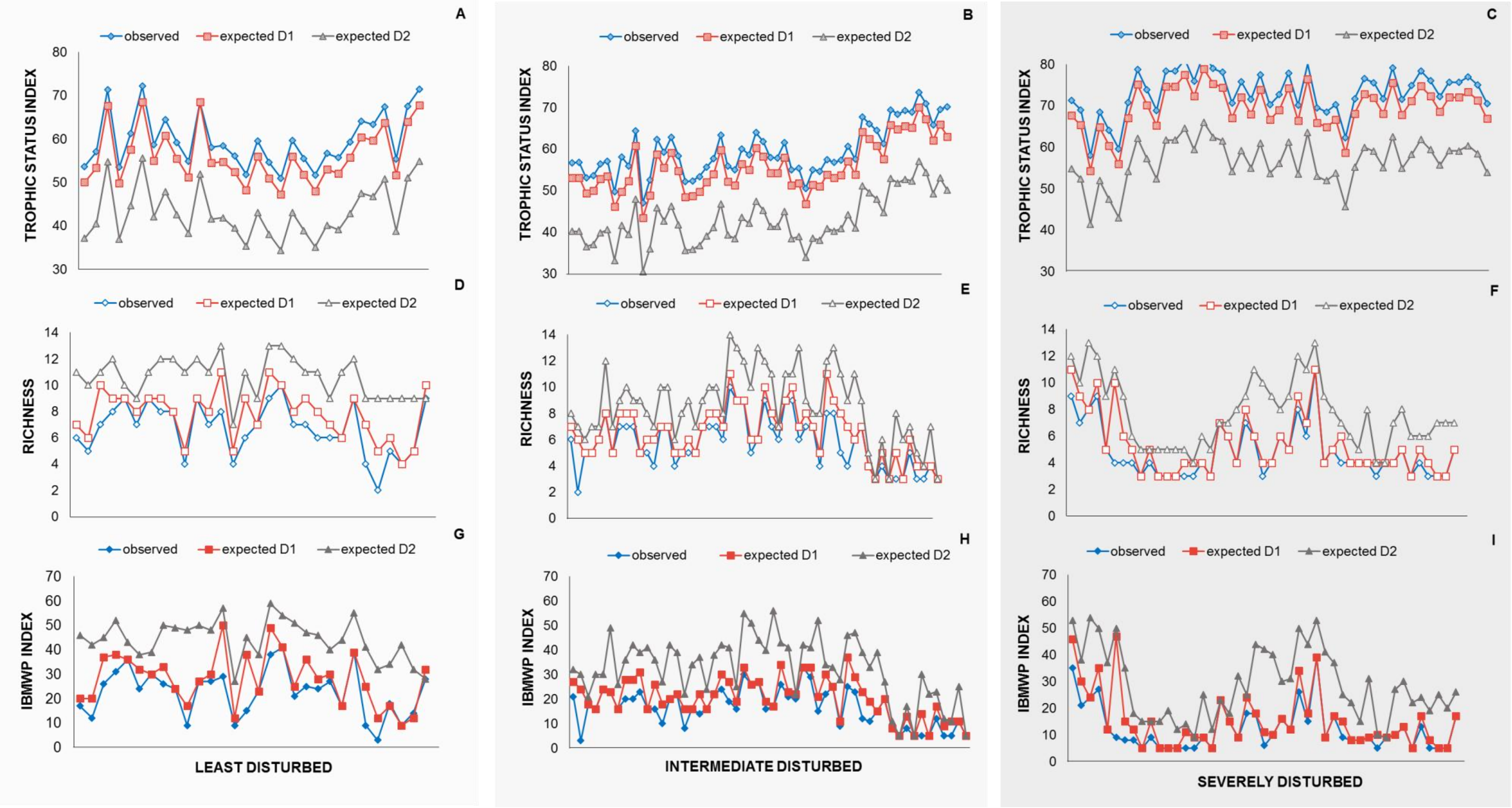

Figure 5: Comparison of the observed TSI (A-C), richness (D-F) and IBMWP index (G- I) and expected values from scenarios D1 (25% improvement) and D2 (75% improvement) for 129 sites with three levels of disturbance: Least disturbed, Intermediate disturbed and Severely disturbed.

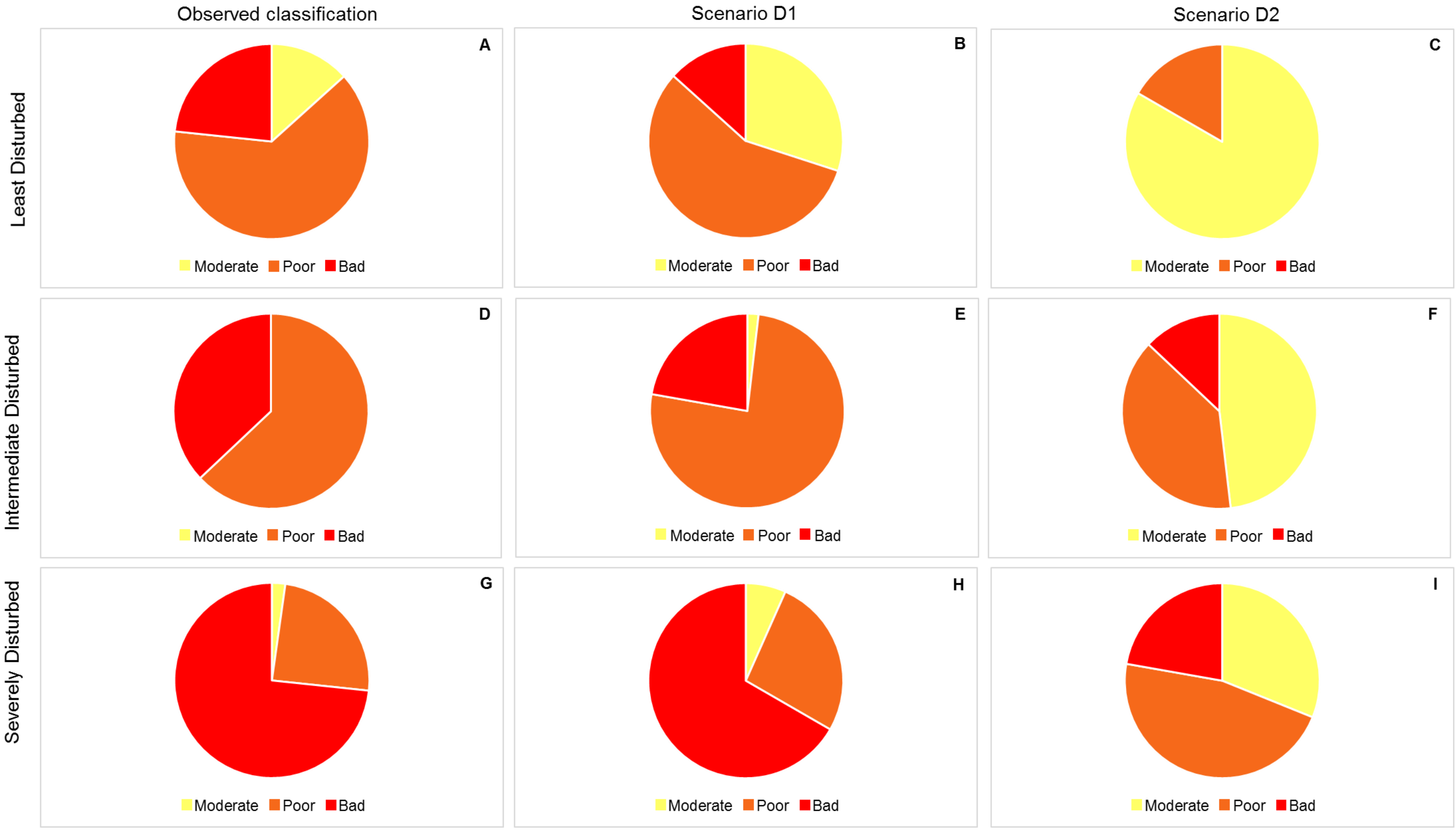

Figure 6: Comparison between observed (A, D, G) IBMWP scores and expected from D1 (B, E,H) and D2 (C,F,I) scenarios for the 129 study sites divided by their disturbance level (Least Disturbed, Intermediately Disturbed and Severely Disturbed). Where the IBMWP scores: Moderate = 36-60; Poor = 16-35 and Bad = <15.

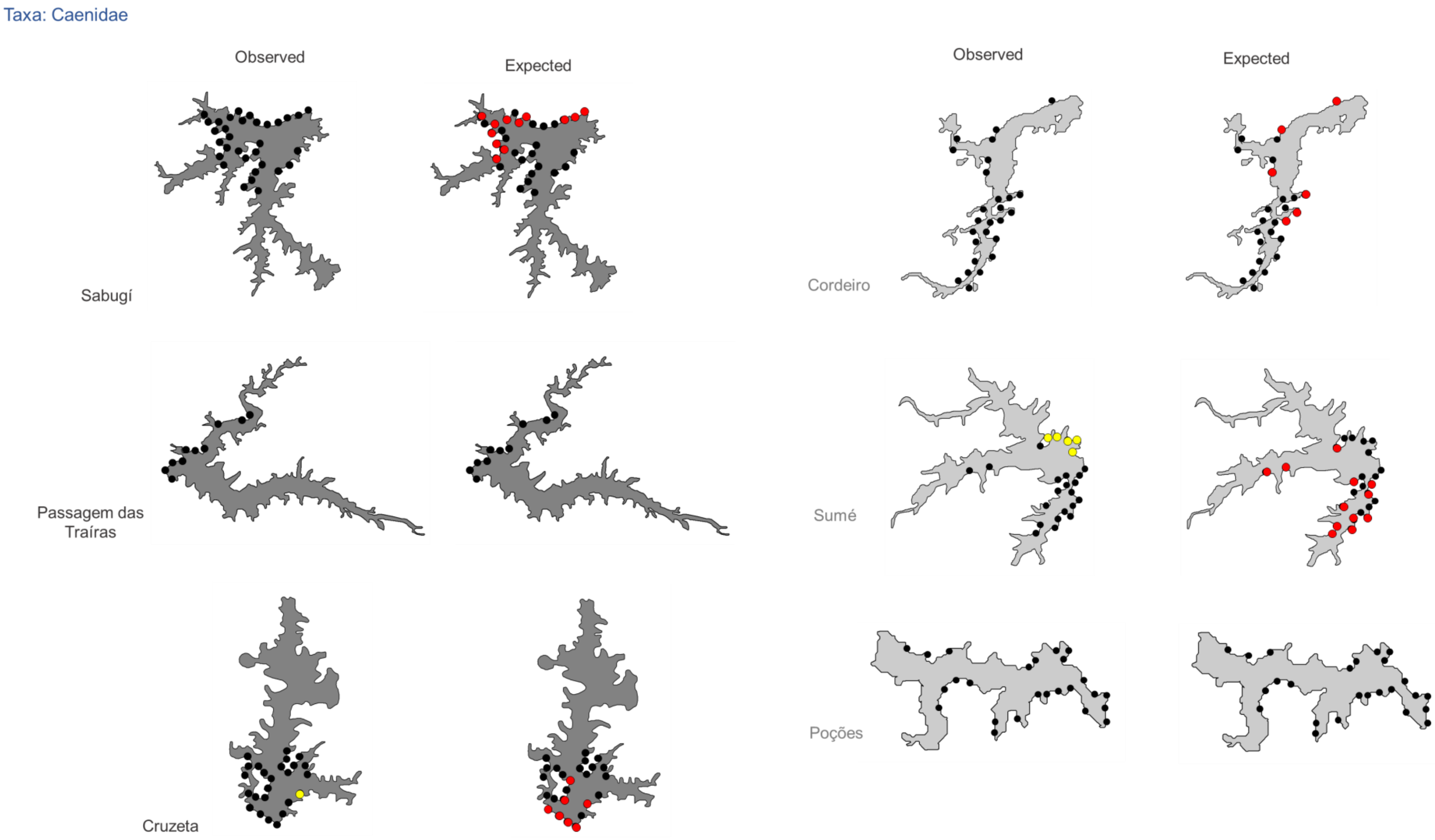

Figure 7: Expected spatial distribution of Caenidae taxa based on D2 scenario in Sabugí, Passagem das Traíras and Cruzeta reservoirs (Piranhas-Assu watershed), Cordeiro, Sumé and Poções reservoirs (Paraíba watershed). Yellow circles represents the observed presence and red circles the expected presence of the taxa. Black circles are the corresponding to sampling sites.

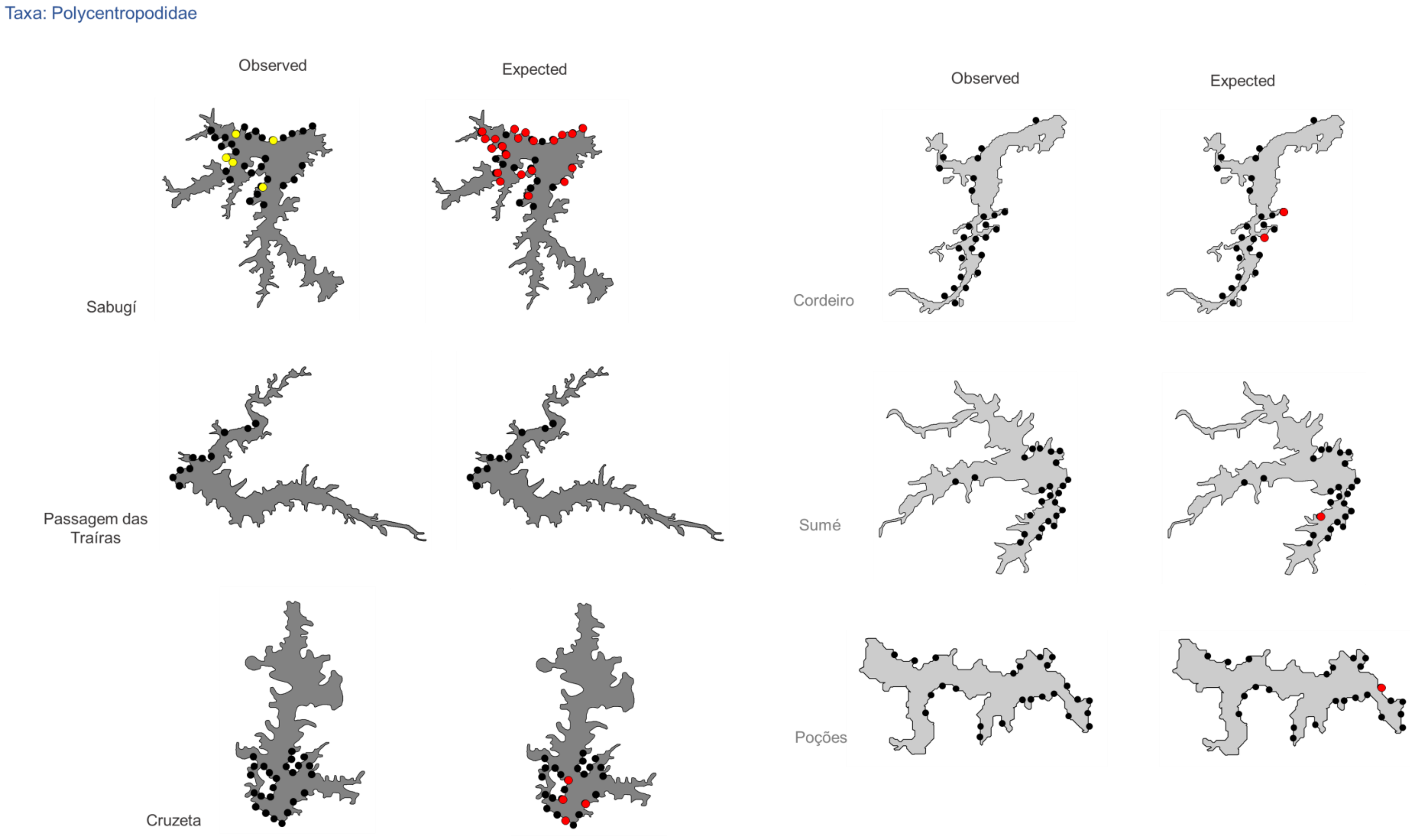

Figure 8: Expected spatial distribution of Polycentropodidae taxa based on D2 scenario in Sabugí, Passagem das Traíras and Cruzeta reservoirs (Piranhas-Assu watershed), Cordeiro, Sumé and Poções reservoirs (Paraíba watershed). Yellow circles represents the observed presence and red circles the expected presence of the taxa. Black circles are the corresponding to sampling sites.

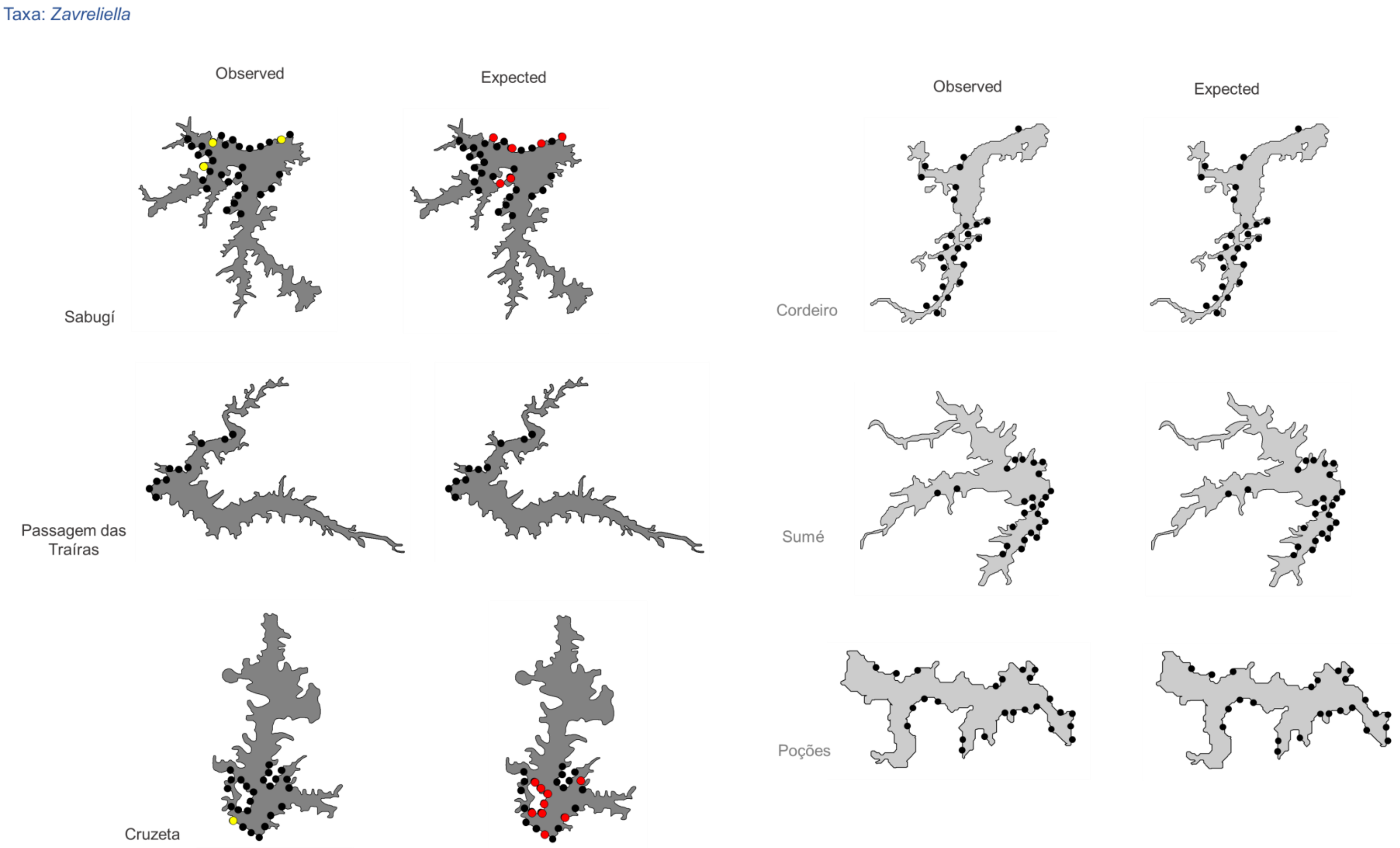

Figure 9: Expected spatial distribution of *Zavreliella* taxa based on D2 scenario in Sabugí, Passagem das Traíras and Cruzeta reservoirs (Piranhas-Assu watershed), Cordeiro, Sumé and Poções reservoirs (Paraíba watershed). Blue circles represents the observed presence and red circles the expected presence of the taxa. Black circles are the corresponding to sampling sites.

Discussion

Here, we showed that a predictive model based on a combination of machine learning techniques, is a useful tool to determine the effect of rehabilitation measures in reservoirs. It is the first time that a combined machine learning approach and the specifically the HYDRA were used to develop “dirty-water” models, showing to be effective in predicting the increase in richness and biological quality of benthic invertebrate communities from scenarios of improvement of reservoirs’ water quality. The success of HYDRA was probably due to the high accuracies obtained for each taxa (>0.7 with several >0.9), as result of the selection of the most accurate of the three techniques available for each taxa, differently from what occurs when a single modeling approach is used, as most more often done (Feio et al. 2014a).

In detail, our simulations of rehabilitation measures in reservoirs showed that the improvement in the wastewater treatment could result in an alteration in the trophic status, from eutrophic to oligotrophic, especially in LD and ID groups. This had implications in richness, as the highest expected richness site occurred in the scenario of greater eutrophication improvement (D2) for all groups, and more interestingly in the recovery of territory by sensitive taxa, which were previously spatially constrained. These results suggest that the reduction in eutrophication provides new favorable conditions for the colonization of species with lower niche amplitude and specific requirements (Büchi and Vuilleumier, 2014; Parmar et al., 2016).

From the management point of view, the HYDRA enabled several important conclusions. First, it is worthwhile to improve the wastewater treatment and consequently decrease nutrients enrichment and organic contamination as those measures result in a higher biodiversity (more taxa), higher biological quality (more sensitive taxa) and higher water quality (less eutrophic waters) in the reservoirs. These changes will consequently enhance the ecosystems services that are presently highly compromised, such as providing leisure areas, food (fishing) and water for irrigation and human consumption while requiring less treatment (Gunkel et al., 2015; Keitel et al., 2015; Melo et al., 2017). In addition, our study highlighted the importance in investing in the improvement of least disturbed sites, and not only the worse ones, as measures applied to them could result in a significant improvement in aquatic communities and biodiversity and a reduction in the eutrophication level (Langford et al., 2008; Jähnig et al., 2010; Kail et al., 2012). A second major conclusion is that sites under high level of degradation, as some found in P. Traíras and Poções reservoirs, require other rehabilitation actions (e.g. recovery of the forested areas in the watershed and riparian areas, urban planning) beyond of wastewater treatment to effectively enhance local biodiversity, in agreement with literature (Quinn et al., 2009; Palmer et al. 2014; Muhar et al., 2016). Third, the simultaneous predictions of many taxa for a large number of sites allowed by the HYDRA, enables the elaboration of maps with the distribution of species (as the examples given here); the follow-up of the implementation of measures over time; analyzing changes in the spatial distribution of taxa relevant for conservation in a given region (e.g., endangered, sensitive taxa).

Our predictions in spite of showing a clear improvement of reservoirs quality can have underestimated the potential changes. In spite of the large number of sites, we used only a restricted list of taxa for predictions, corresponding to those actually found in reservoirs, while species from rivers and streams in the watershed, that presently do not find adequate conditions for survival in the reservoirs were not used (Havel et al. 2005). Yet, if the improvement of water quality becomes a reality, and in spite of great differences in habitats and other conditions (depth, sediment), some other sensitive taxa that can cope with lentic conditions, may be able to colonize these reservoirs drifting from upstream river sites. Future developments of this model will test the inclusion of running water sites in the training dataset.

In conclusion, the HYDRA "dirty-water" models can be a useful tool for restoration planning of water bodies and their future management, allowing an easy follow-up of the effect of measures or their adjustments over time in biodiversity, community composition or quality index.

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Supplementary material

Table S1. Characterization of Piranhas-Assu and Paraíba river reservoirs. Data provided by Secretaria de Meio Ambiente e Recursos Hídricos Rio Grande do Norte (SEMARH) and Agência Executiva de Gestão das Águas (AESA), Paraíba. 1* no record; 2* data in parentheses: hydric volume (%).

Features/Reservoirs	**Piranhas-Assu river**			**Paraíba river**		
	Sabugí	**P. Traíras**	**Cruzeta**	**Cordeiro**	**Sumé**	**Poções**
Geographic localization	06º43’06’’S/ 37º12’02’’W	06º27’16’’S/ 36º52’29’’W	06º24’42’’S/ 36º47’23’’W	7°47’38.00”S/ 36°40’14.04”W	7°29’8"S/ 37°12'20"W	7°53’38”S/ 37°0’30”W
Altitude (m)	187	196	231	480	500	596
Approximate maximum capacity ($10^6 m^3$)	65	49	23	70	45	30
Construction year	1965	1994	1929	^{1*}	1953	1982
Main finality	Supply	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation	Supply and irrigation
Approximate water volume ($10^6 m^3$) by period^{2*} (2014-2015)						
June	18 (27.7)	3 (8)	5 (20.8)	9 (12.8)	17 (37.7)	6 (20)
September	14 (21.5)	2 (4)	4 (16.6)	8 (11.4)	15 (33.3)	5 (16.6)
December	9 (13.8)	1 (2)	2 (8.3)	6 (8.5)	12 (26.6)	3 (10)
March	8 (12.3)	0.649 (1.2)	1 (4.16)	4 (5.7)	9 (20)	2 (6.6)

Table S2. Number of Least disturbed, Intermediately Disturbed and Severely Disturbed **sites** where each taxa was observed (O) and number of sites where it was predicted under the two rehabilitation scenarios (D1 and D2) improvement. Below are the mean richness/site and mean IBMWP scores. O = observed richness, D1= 25% improvement and D2= 75% improvement. (-) no observed or expected. In bold, taxa with predicted expansion.

Taxa	Least Disturbed			Intermediately Disturbed			Severely Disturbed		
	O	D1	D2	O	D1	D2	O	D1	D2
ANNELIDA									
Hirudinea	-	-	-	25	**26**	25	10	**12**	10
Oligochaeta	30	30	30	54	54	54	45	45	45
ARACNÍDEO									
Acarina	-	-	-	1	1	1	-	-	-
Oxidae	-	-	-	2	2	2	-	-	-
CRUSTÁCEO									
Decapoda	-	-	-	11	**19**	**19**	5	**6**	5
Ostracoda	18	**21**	**23**	2	**3**	**5**	7	**10**	**12**
INSECTA									
Chironomidae									
Chironominae									
Aedokritus (Roback, 1958)	25	**30**	**29**	15	**17**	15	36	**41**	**40**
Asheum (Sublette, 1964)	29	**30**	**30**	6	6	6	23	**26**	**35**
Chironomus (Meigen, 1803)	26	**29**	**29**	8	8	8	25	**30**	**28**
Cladopelma (Kieffer, 1921)	3	3	**8**	-	-	**4**	2	2	**8**
Dicrotendipes (Kieffer, 1913)	21	**27**	**26**	-	-	-	16	**19**	**17**
Fissimentum (Cranston and Nolte, 1996)	23	**25**	**25**	6	6	6	6	**8**	**8**
Goeldichironomus (Fittkau, 1965)	29	**30**	**29**	25	**32**	**30**	35	**45**	**45**
Parachironomus (Lenz, 1921)	6	**7**	**7**	8	**17**	**18**	6	**9**	**17**
Pelomus (Reis, 1989)	23	**28**	**28**	5	5	5	11	**16**	**16**
Polypedilum (Kieffer, 1912)	30	30	30	17	**18**	**22**	31	31	**34**
Saetheria (Jackson, 1977)	1	1	1	-	-	-	-	-	-
Tanytarsus (Van der Wulp, 1874)	30	30	30	13	13	13	28	**33**	**33**
Zavreliella (Kieffer, 1920)	2	**5**	**7**	-	-	-	2	**7**	**12**
Tanypodinae									
Ablabesmyia (Johannsen, 1905)	10	**22**	**15**	7	**9**	7	4	**5**	**11**
Brundiniella (Roback, 1978)	2	2	2	-	-	-	-	-	-
Clinotanypus (Kieffer, 1913)	0	0	0	2	2	2	1	1	1
Coelotanypus (Kieffer, 1913)	28	**30**	**30**	37	**46**	**50**	24	**25**	**44**
Denopelopia (Roback and Rutter, 1988)	-	-	-	1	1	1	0	0	0
Djalmabatista (Fittkau, 1968)	14	**18**	**23**	3	3	3	12	**13**	**21**
Larsia (Fittkau, 1962)	6	**7**	**19**	4	**6**	**9**	4	**7**	**15**
Monopelopia (Fittkau, 1962)	0	0	0	1	1	1	-	-	-
Parapentaneura (Fittkau and Serrano, 2006)	0	0	0	1	1	1	-	-	-
Procladius (Skuse, 1889)	3	**6**	**22**	-	**1**	**4**	4	**10**	**12**
Tanypus (Meigen, 1803)	9	**11**	**13**	-	**4**	**2**	2	**6**	**7**
Coleoptera									
Disticidae	1	1	1	-	-	-	-	-	-
Elmidae	-	-	-	-	-	-	1	1	1
Gyrinidae	7	**12**	**27**	1	**4**	**19**	8	**10**	**17**
Diptera									
Chaoboridae	5	5	5	1	1	1	8	8	**9**
Chaoborus (Lichtenstein, 1980)	4	4	4	3	3	3	5	**7**	**8**
Ceratopogonidae	28	**30**	**29**	16	16	16	25	**29**	**33**
Ephemeroptera									
Baetidae	1	**2**	1	2	2	2	1	1	1

Caenidae	-	-	**15**	5	**6**	**22**	1	1	8
Polymitarcydae	-	-	-	10	**11**	10	1	1	**2**
Hemiptera									
Belostomatidae	-	-	-	1	1	1	-	-	-
Corixidae	5	5	5	2	2	2	5	5	**7**
Heteroptera	1	1	1	-	-	-	-	-	-
Odonada									
Coenogrionidae	5	5	**19**	7	**14**	**43**	3	**4**	**29**
Dicteriadidade	1	1	1	-	-	-	-	-	-
Gomphidae	10	**11**	**24**	5	**9**	**21**	4	**6**	**28**
Phyllocycla	3	**8**	**20**	2	2	**23**	1	**2**	**4**
Progomphus	1	1	1	0	0	0	0	0	0
Libellulidae	2	**10**	**19**	9	**16**	**30**	2	**5**	**15**
Trichoptera									
Leptoceridae	1	1	1	-	-	-	-	-	-
Polycentropodidade	5	**10**	**24**	-	-	3	1	**2**	**8**
MOLLUSCA									
Gastropoda									
Ancylidae	7	**9**	**10**	-	-	-	1	**6**	**6**
Bulimidae	1	1	1	-	-	-	6	6	6
Planorbidae	12	**14**	**16**	25	**29**	**40**	9	9	**25**
Ampullariidae - *Pomacea*	17	17	**20**	8	**11**	**30**	7	**11**	**15**
Thiaridae - ***Melanoides tuberculatus*** **(Müller, 1774)**	29	29	29	54	54	54	42	42	**43**
Lymnaeidae	0	0	0	1	1	1	-	-	-
Bivalve									
Corbiculidae - *Corbicula largillierti* (Philippi, 1844)	-	-	-	19	**22**	**23**	-	-	-
Sphaeriidae	-	-	-	5	5	5	-	**1**	-
NEMATODA	1	1	1	-	-	-	-	-	-
PLATYHELMINTHES									
Planariidae	10	**11**	**13**	19	**22**	**23**	2	**4**	**8**
Mean richness/site									
Observed	6.7±2.0			5.8±2.0			4.7±1.9		
D1	7.8±1.9			6.5±2.0			5.3±2.2		
D2	10.6±1.5			8.6±2.7			7.7±2.5		
Mean IBMWP/site									
Observed	22.6±9.8			16.6±7.6			12.2±8.2		
D1	28.0±10.8			20.4±8.1			15.2±10.8		
D2	44.0±8.2			32.6±12.7			28.6±13.3		

4. CONSIDERAÇÕES FINAIS

O conjunto de dados obtidos a partir da execução desse trabalho permitiu a inferir acerca dos aspectos estruturais e funcionais das comunidades de macroinvertebrados bentônicos e a aplicação desses nos contexto da avaliação ecológica, manejo e reabilitação de corpos de água doce. Aqui, reunimos informações sobre a flutuação dos padrões estruturais e funcionais das comunidades sob uma condição de perturbação intensa, como durante o período de seca prolongada na região semiárida, o que pode direcionar nosso olhar para o contexto futuro das mudanças climáticas.

Analisando o padrão de flutuação da espécie de molusco não-nativo (*Melanoides tuberculata*) foi condicionado por múltiplos fatores (intrínsecos e extrínsecos), os quais agem simultaneamente para o grau de invasão da espécie. Entre esses a temperatura deve ser destacada, uma vez que tem maior importância correlativa entre os fatores extrínsecos (capítulo 1). Ações de manejo da qualidade desses corpos aquáticos são encorajados, objetivando a conservação de outras espécies e prevenindo a invasão em outras áreas das bacias hidrográficas.

A seca de fato agiu como um gatilho nas mudanças das condições ambientais e perda da qualidade da água (capítulo 2). E assim como para o padrão de dissimilaridade ambiental, o aumento na intensidade da seca resultou em altas dissimilaridades nas comunidades entre os reservatórios e bacias hidrográficas (β-diversidade). As dissimilaridades das comunidades entre as escalas espaciais foram predominantemente mantidas pela substituição aleatória das espécies (*tunorver* espacial), mas no período mais seco, subconjuntos de espécies a partir do pool regional contribuem significativamente para a dissimilaridade (aninhamento) entre os locais. Assim, ao longo de um período de seca prolongada, a perda de espécies torna-se maior, o que resulta em comunidades empobrecidas e com dominância de espécies generalistas.

Nosso conjunto de dados demonstrou que a redução na diversidade de macroinvertebrados ao longo do tempo, resulta em alterações nos atributos funcionais, com alternância na dominância das categorias (capítulo 3). Além disso, o padrão de flutuação dos atributos funcionais demonstra que durante o período de seca prolongada há aumento na uniformidade funcional e redução na riqueza funcional, indicando perda funcional e aumento na homogeneização biótica.

O alto nível de degradação dos corpos aquáticos estudados e o pobre estado ecológico é uma realidade e o emprego de simulações para cenários de melhoria mostrou-se eficaz para o manejo e reabilitação do estado de degradação. A redução no nível de nutrientes associados a eutrofização resulta efetivamente no aumento da biodiversidade, qualidade biótica e da água, mas sobretudo na expansão da cobertura de taxa sensíveis (ex. Ephemeroptera e Trichoptera). A reabilitação promove maiores níveis de melhorias em sites mais impactados, mas as melhorias ambientais e bióticas também ocorrem em sites sob um menor nível de degradação, indicando que as estratégias de restauração podem garantir melhorias ao longo dos gradientes tróficos. Destaca-se que a abordagem *"dirty-water models"* é eficiente a planos de restauração, representando uma ferramenta mais realista para o quadro de ausência de sites de referência.

A situação de degradação dos corpos aquáticos, em muitos casos, é reconhecida por parte do gestores e assim, o grande desafio é propor medidas eficientes para reversão do estado de degradação, garantindo o funcionamento ecológico, mas associando, de forma estratégica e planejada, o uso adequado dos recursos. Este trabalho trouxe novas contribuições para abordagem da reabilitação, mas ainda temos outros desafios, e entre esses destacamos os que nos parece mais próximos: i) estender a aplicabilidade da ferramenta a sistemas naturais; ii) considerar outros níveis de reabilitação isolada e simultaneamente (inclusão da paisagem, habitat, metais) e por fim, iii) considerar as possíveis ameaças para ecossistemas e simular seus impactos potenciais para a perda da qualidade ecológica.