



Universidade Federal de Minas Gerais  
Instituto de Ciências Biológicas  
Programa de Pós-graduação em Ecologia, Conservação  
e Manejo da Vida Silvestre.



**Atributos Físicos, Químicos e Biológicos do Solo como Indicadores de  
Recuperação de Áreas de Mata Ciliar na Ecoregião de Cerrado em  
Minas Gerais**

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**Atributos Físicos, Químicos e Biológicos do Solo como Indicadores de  
Recuperação de Áreas de Mata Ciliar na Ecoregião de Cerrado em  
Minas Gerais**

Tese apresentada à Universidade Federal de Minas Gerais, como pré-requisito do Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, para a obtenção do título de Doutor em Ecologia, Conservação e Manejo da Vida Silvestre.

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Dedico:

“À minha família pelo apoio e suporte nesta longa  
jornada.”

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## Resumo

Matas ciliares correspondem à formação florestal situada às margens de cursos d'água naturais, conectando ecossistemas aquáticos e terrestres. Estes amortecedores ripários trabalham na redução da perda de nutrientes, na ancoragem do solo e na estabilização e prevenção da erosão através da ação de conexão do sistema radicular e formação de matéria orgânica húmica no solo. Sua localização estratégica sujeita-as a eventos de inundação periódica, que embora seja um processo-chave para estes ecossistemas, pode ocasionar mudanças significativas na química, física e biologia do solo. Neste estudo duas áreas de Mata Ciliares inundáveis do Bioma Cerrado localizadas uma na Bacia do rio das Velhas e outra na Bacia do rio Uberaba no estado de Minas Gerais foram submetidas a processos de recuperação. A primeira foi recuperada usando espécies nativas e na última foi feito um monocultivo de *Syzygium cumini* (jambolão). Nosso objetivo foi avaliar a recuperação destas duas áreas utilizando como indicadores de recuperação as características físico-químicas e bióticas do solo envolvidas em importantes processos biogeoquímicos. Cada área de estudo foi composta por uma área experimental (em recuperação ou ES), e duas outras áreas de mata ciliar, uma área de referência que é preservada (PS) e outra degradada (DS) utilizada como controle negativo e todas delimitadas no mesmo fluxo d'água. As avaliações na bacia do rio das Velhas, aos 6 após plantio, revelaram solos com pH elevado e alto conteúdo de fósforo. Parâmetros como capacidade de troca catiônica (CEC), soma de bases (SB) e saturação de bases (BS) indicaram uma recuperação da fertilidade do solo em ES. Outros parâmetros como nitrogênio total (NT), nitrato ( $\text{NO}_3^-$ ), ácidos húmicos (HA) e fúlvicos (FA) colocaram ES em processo intermediário de recuperação entre DS e PS. Este fato foi atribuído ao elevado conteúdo de  $\text{NH}_4^+$  e deficiência na produção de nitrato observado no solo de ES e DS, sendo  $\text{DS} > \text{ES}$ . Tal acúmulo de amônio não foi registrado em PS. A comunidade microbiana estimada pelo PLFA e TGGE mostrou que tanto a mineralização como a desnitrificação foram inibidas em ES e especialmente DS ( $\text{DS} > \text{ES}$ ). Na área preservada o ciclo do N foi completo e a desnitrificação impediu acúmulo de amônio. Assim este acúmulo de amônio em DS e ES ( $\text{DS} > \text{ES}$ ) foi atribuído a descarga de esgoto na área de estudo. O aumento da desnitrificação em ES sugere que esta área está evoluindo em direção à PS com indícios de maior resiliência para superar o impacto. Nossos resultados na bacia do rio Uberaba mostraram que a área recuperada (ES) com espécie exótica após 15 anos do plantio, está em processo intermediário de recuperação. Parâmetros como bases trocáveis e fertilidade (CEC, SB e BS) colocam ES em posição intermediária entre PS e DS. Similaridades com uma área preservada foram observadas quanto à estabilidade de agregados e porosidade do solo. Entretanto, quimicamente a matéria orgânica produzida em ES foi distinta de PS. Com ES apresentando mais FA e compostos alifáticos que PS. Estes resultados sugerem que embora um plantio de espécie exótica tenha proporcionado à recuperação de serviços ecossistêmicos relacionados à agregação e porosidade do solo, diferenças qualitativas entre a área preservada e a área experimental referente à matéria orgânica formada indicam que a introdução de um plantio monoespecífico em uma área de mata ciliar degradada é insuficiente para restaurar todas as propriedades do solo.

Palavras-chave: matas ciliares, indicadores de recuperação, microrganismos do solo, química do solo, estabilidade do solo.

## Abstract

Riparian Forests refer to forest patches located at the margins of water bodies, connecting terrestrial and aquatic ecosystems. The roles of such riparian buffers are related to reduction of nutrient losses as well as the anchoring and soil stabilizing effect promoted by plant root system and soil organic matter, resulting in erosion prevention. Riparian forest are commonly subjected to flooding events which, although being a key-process for terrestrial-aquatic ecosystems interface, may result in significant changes in soil physical, chemical and biological properties. This study evaluated two riparian forests under restoration located within the Cerrado biome and subjected to periodic flooding conditions: the first located at Velhas River basin and the second at Uberaba river basin. The former was restored with native species belonging to the Cerrado biome while the latter was cultivated by local landowners with *Syzygium cumini* (jambolão) under monoculture. The aim of this study was to assess the restoration of both riparian sites by using soil physical, chemical and biological properties, particularly those involved in C and N biogeochemical cycles, as restoration indicators. Each study area was composed of an experimental site (ES) under restoration which was compared with a preserved site (PS) and a disturbed site (DS) located within the same water body. In the Velhas River basin experimental site, 6 year after restoration, it was found a high soil pH and P content. The evaluation of parameters as soil cation exchange capacity (CEC), sum of bases (SB) and base saturation (BS) indicates the recovery of soil fertility in ES. However, other parameters as total nitrogen (TN), nitrate ( $\text{NO}_3^-$ ), humic and fulvic acids (HA, FAs) revealed that ES is in an intermediate stage of restoration as compared to the reference sites. It was particularly influenced by the high soil  $\text{NH}_4^+$  content and deficient nitrate production observed in both ES and DS, following this order:  $\text{DS} > \text{ES}$ . Such ammonium accumulation was not registered in PS. The soil microbial communities as assessed by the Phospholipid derived fatty acids (PLFA) and Temperature Gradient Gel Electrophoresis (TGGE) techniques showed that both the mineralization and denitrification were inhibited in ES and especially in DS ( $\text{DS} > \text{ES}$ ). In contrast, in the PS it was observed a complete N biogeochemical cycle that prevented ammonium accumulation. The soil ammonium accumulation in ES and DS ( $\text{DS} > \text{ES}$ ) was attributed to the swage discharge which the river is subjected and the effect of flooding. Thus, the increase of denitrification in ES suggests that this site is evolving towards the condition of PS, showing evidence of increase of resilience to the impact. In relation to the 15 years old site under restoration with a single exotic species in the Uberaba river basin, parameters as exchangeable bases and soil fertility (CEC, SB e BS) put it in an intermediate stage of restoration. It was found similarities between ES and PS in relation to soil aggregation and porosity but the chemical composition of the soil organic matter (SOM) was different. While ES showed a high FA and aliphatic compounds, the PS produced a more aromatic humic acid. These results suggest that, although some ecosystems properties related to soil aggregation and porosity have been restored under a single plantation with an exotic species, the qualitative differences in SOM do not allow to conclude that the soil properties of this plantation is fully restored. Overall, these results show that soil physical as well as C and N biogeochemical parameters can be considered suitable indicators of riparian forest restoration in light of different environmental impacts and conditions they are subjected.

Key-words: riparian forests, recovery indicators, soil microorganisms, soil chemistry, soil stability.



## Lista de Figuras

### Capítulo 1

Figure 1. Mechanisms of nitrate retention/ removal in riparian forest 28

### Capítulo 2

Figure 1. Location of Stream Sabará and Velhas River basin in Minas Gerais state (SEMAM 2004) and studies sites (ES: experimental site; PS: preserved site, and DS: disturbed site). 45

Figure 2. Dendrograms based on TGGE profiles, using UPGMA (Dice coefficient of similarity) of microbial community of soils sampled at a disturbed, experimental and preserved sites. 53

Figure 3. Component principal analysis (PCA) biplot of microbial community's structure fitted predictor variables. Studied areas are abbreviated: Disturbed Site (DS), Experimental Site (ES), and Preserved Site (PS). 57

### Capítulo 3

Figure 1. A: Location of Grande River basin and Uberaba River basin in Minas Gerais state (SEMAM 2004); B: Experimental design in study sites. 67

Figure 2. Comparison of soil variables (a: aggregate stability; b: total porosity; c: macro-porosity; d: micro-porosity) among the sites: DS (disturbed site), PS (preserved site) and ES (experimental site). 69

Figure 3. Comparison of soil variables (a: glomalin content; b: C-organic content; c: humic acid content; d: fulvic acid content) among the sites: DS (disturbed site), PS (preserved site) and ES (experimental site). 70

Figure 4. Comparisons of the contribution of aromatic (a), aliphatic (b) and carbohydrate (c) compounds in the humic sample solutions based on H-NMR spectra. ES: experimental site, PS: preserved site, DS: disturbed site. 71

Figure 5. Principal component analysis based on soil properties measured on disturbed (DS), preserved (PS) and experimental sites (ES) 72

## Lista de Tabelas

### Capítulo 1

Table 1. Comparison of effect of riparian forest on the nitrate-nitrogen ( $\text{NO}_3^-$ - N) content retention	34
---	----

### Capítulo 2

Table 1. Chemical properties of soils sampled at a disturbed, experimental and preserved sites.	49
Table 2. Carbon, nitrogen and glomalin concentrations of soils sampled at a disturbed, experimental and preserved sites.	51
Table 3. Shannon-Weaver index ( $H'$ ) and band richness ( $R$ ) of microbial community of soils sampled at a disturbed, experimental and preserved sites. The data were derived from the TGGE profiles.	51
Table 4. PLFA concentrations ( $\mu\text{g/g}$ ) of functional groups of microbial community at soils sampled at a disturbed, experimental and preserved sites.	55

### Capítulo 3

Table 1. Chemical and physical properties of soils sampled at a disturbed, experimental [i.e., a monospecific plantation of <i>Syzygium cumini</i> (L) Skeels] and preserved sites.	69
---	----

## Abreviações

Actino – actinomycetes;	$\text{NO}_2^-$ - nitrite;
Ali C - aliphatic carbon;	$\text{NO}_3^-$ - nitrate;
AMF – arbuscular micorrhizal fungi;	NT – nitrogen total;
AOA - ammonia-oxidizing archaea;	OM – Organic matter;
AOB – ammonia-oxidizing bacteria;	OrgC – organic carbon;
Ar C - aromatic carbon;	P – Fósforo;
AS - aggregate stability;	PCR - Polymerase chain reaction;
$\text{Ca}^{2+}$ - Calcium;	PLFA – Phospholipid Fatty Acid;
Carb – carbohydrates;	PS – Preserved site;
CEC - Cation Exchange Capacity;	SOC - Soil organic carbon;
Cmic – microbial carbon;	SB - exchangeable bases;
DGGE – Denaturing Gradient Gel Electrophoresis;	TGGE – Temperature Gradient Gel Electrophoresis;
DNA - deoxyribonucleic acid;	TP - total porosity;
DS – Disturbed site;	V% - base saturation.
ES – Experimental site;	
FA – Fulvic Acid;	
Glom – glomalin;	
Gram <sup>+</sup> - gram-positive bacteria;	
Gram <sup>-</sup> - gram-negative bacteria;	
H+Al - potential acidity;	
HA – Humic Acid;	
HSs – humic substances;	
Hum – humin;	
K <sup>+</sup> - potassium;	
Mg <sup>2+</sup> - magnesium;	
SOM – Organic matter soil;	
N – nitrogen;	
N <sub>2</sub> - nitrogen gas;	
NH <sub>4</sub> <sup>+</sup> - ammonium;	
NH <sub>3</sub> – ammonia;	

## Sumário

Resumo	vi
Abstract	vii
Lista de Figuras	viii
Lista de Tabelas	ix
Abreviações	x
Introdução Geral	13
Objetivos	18
Áreas de Estudos	19
Referências	21
<b>Chapter 1 - Nitrogen transformations in flooding soils of riparian forest</b>	<b>24</b>
Resumo	25
Abstract	25
Introduction	26
Influence of riparian forest on N dynamic	27
Input by biological nitrogen fixing	29
Nitrogen mineralization and immobilization process	31
Nitrification process	32
Denitrification process	33
Concluding remarks	35
Literature cited	36
<b>Chapter 2 - Implications of ammonia accumulation for the riparian forest restoration</b>	<b>41</b>
Abstract	42
Introduction	43
Materials and Methods	44
Description of the area	44
Experimental design and soil sampling	45
Physicochemical analysis	46
DNA extraction and PCR-TGGE	46
PLFA analysis	47

Data Analysis	48
Results and Discussion	48
Physicochemical properties of soils	48
Characterization of soil microbial community	51
TGGE	51
PLFA	54
Relation between microbial community composition and soil properties	56
Conclusions	58
Literature cited	59
<b>Chapter 3 - Riparian reforestation with a single exotic species restores soil aggregation and porosity but not humic substances</b>	<b>64</b>
Abstract	65
Introduction	65
Materials and Methods	66
Study site	66
Experimental design	67
Textural and soil fertility analyses	67
The determination of soil aggregation and porosity	67
Fractionation of soil organic matter (SOM) and glomalin-related proteins (PRSG-T)	68
<sup>1</sup> H-Nuclear magnetic resonance (NMR)	68
Statistical analyses	68
Results and Discussion	68
Soil physical and chemical properties	68
Humic substances	70
Principal component analysis	72
Conclusions	73
References	73
Conclusões Gerais	75

## ***1. Introdução Geral***

Em uma paisagem florestal, os tampões ribeirinhos ou mata ciliares são frequentemente referidos como zonas não manejadas ao longo de córregos e riachos, cuja localização estratégica na interface entre os ecossistemas aquáticos e terrestres confere-lhe uma gama de gradientes ambientais, processos ecológicos e diversidade de plantas (Gregory et al. 1991; Krupek & Felski 2006; Naiman & Decamps 1997; Schultz et al. 2004; Svejcar 1997). Funcionalmente, matas ciliares são definidas como um sistema de três zonas responsáveis pela interação entre os ecossistemas (Gregory et al. 1991). A primeira zona comporta espécies de planta com enraizamento profundo que atuam na ancoragem do solo conferindo-lhe reforço mecânico e estabilização. Imediatamente adjacente temos a segunda zona composta principalmente por espécies de plantas produtoras de biomassa e formadoras de matéria orgânica humificada e que contribui para a fertilidade, o tamponamento químico e a estabilização do solo. A terceira e última zona trabalha reduzindo a perda de sedimento e nutrientes pelo controle da erosão laminar e redução do escoamento superficial (Gregory et al. 1991; Schultz et al. 2004; Welsch 1991). Tais características conferem ao ecossistema o status de fundamentalmente importante para a manutenção dos corpos hídricos onde estão inseridas (Gregory et al. 1991; Krupek & Felski 2006; Naiman & Decamps 1997; Schultz et al. 2004; Svejcar 1997).

A decomposição da liteira pela atividade microbiana é a principal via de formação da matéria orgânica do solo na mata ciliar (Craswell & Lefroy 2001; Gregory et al. 1991; Six et al. 2002; Zech et al. 1997). Além da quantidade de liteira vegetal, sua composição e suas propriedades são fatores essenciais para a formação de matéria orgânica e processos de humificação em solos (Kögel-Knabner 2002). A decomposição dos resíduos vegetais é mediada pela ação dos microrganismos do solo através da produção/ação de enzimas extracelulares que gradualmente transformam a liteira em matéria orgânica húmica (Sinsabaugh et al. 1991; Tian et al. 2000). De acordo com suas características físico-químicas e diferentes solubilidades as substâncias húmicas (SHs) podem ser operacionalmente definidas em três categorias: ácidos húmicos, ácidos fúlvicos e humina (DiDonato et al. 2016; Piccolo 2001; Simpson et al. 2013; Stevenson 1994). Entretanto estudos recentes derivados da espectrometria de massa de alta resolução têm permitido classificá-las segundo seus grupos moleculares baseado no

diagrama de van Krevelen que considera a taxa molar de H/C versus a taxa molar de O/C (DiDonato et al. 2016; van Krevelen 1950).

Substâncias húmicas participam diretamente do processo de estruturação do solo promovendo sua estabilidade estrutural através da formação dos agregados e da porosidade do solo. Os agregados formados resultam da união de partículas mediada pela matéria orgânica do solo (Bronick & Lal 2005; Oades 1993). Inicialmente, partículas primárias como argilas e cátions livres são unidas ao redor da matéria orgânica formando microagregados estáveis, que por sua vez, são ligados entre si formando macroagregados. Este último processo é mediado principalmente pela ação dos microrganismos do solo, agentes de ligação temporários (hifas fungicas e raízes de plantas) e transientes (polissacarídeos microbianos e derivados de plantas) (Craswell & Lefroy 2001; Six et al. 2004; Zech et al. 1997). Raízes e hifas fungicas mantêm as partículas juntas por ação mecânica ou produção de exsudatos e compostos extracelulares, respectivamente (Borie et al. 2008). Um exemplo particular da atuação fungica neste processo é a produção de uma glicoproteína (glomalina) pelos fungos micorrízicos arbusculares (AMF sigla em inglês) (Rilling 2004; Rilling et al. 2006; Wright et al. 1996).

A porosidade do solo consiste no volume de espaços vazios formados entre as partículas sólidas que formam os agregados. Os espaços dos poros, seu tamanho e quantidade podem influenciar o estoque e trocas de carbono orgânico no solo; por outro lado, o carbono orgânico do solo e a textura podem influenciar a porosidade (Thomsen et al. 1999). A porosidade do solo é também característica essencial para difusão de ar e capacidade de retenção e percolação de água através dos solos (Bronick & Lal 2005).

Hidrologicamente, matas ciliares estão sujeitas a distúrbios ocasionados por inundações frequentes (Gregory et al. 1991; Naimam & Decamps 1997) que alteram a dinâmica da comunidade ciliar periodicamente. Embora eventos de inundações periódicas sejam um processo-chave nas áreas ribeirinhas (Tockner & Stanford 2002) estes podem aumentar o input de insumos orgânicos fornecidos (principalmente carbono, nitrogênio e fósforo) e a lixiviação de nutrientes para os corpos d'água, alterar a comunidade microbiana e os processos biogeoquímicos associados a eles, reduzir a disponibilidade de luz, além de estabelecer estresse veiculado pela alternância da oxigenação do solo (Baldwin & Mitchel 2000; Broadmedoaw & Nisbet 2004; Sabater et al. 2000).

A capacidade destes tampões ripários em controlar os fluxos de nutrientes descarregados no sistema aquático é particularmente importante para manutenção do nível de nitrogênio nos solos (Baker et al. 2006; Dosskey et al. 2010; Gift et al. 2010; Okazawa et al. 2010), uma vez que as transformações de nitrogênio em solos alagados são marcadamente diferentes daquelas em solos drenados e arejados devido a alternância na oxigenação do solo e modificações na composição da comunidade microbiana (Patrick 1982). Além disso, em solos úmidos e/ou saturados, o que comumente ocorre em zonas ribeirinhas, decomposição da matéria orgânica prossegue mais lentamente, isto acontece porque quando o solo é inundado a água cria regiões de anaerobiose devido à depleção do oxigênio e aumento do CO<sub>2</sub> produzido pela respiração microbiana. O consumo do O<sub>2</sub> então limita os processos biológicos pela baixa disponibilidade de energia (Hill 2000).

A remoção/retenção de nitrogênio é influenciada pelas condições redox do solo, pela disponibilidade de nitrogênio dissolvido para as raízes das plantas e pela estrutura/composição da comunidade microbiana (Naimam & Decamps 1997). Outras propriedades do solo, como textura, teor de argila, pH e temperatura, também podem afetar a troca de nitrogênio nos solos, influenciando os processos de ciclagem de nutrientes microbianos e, portanto, regulando a disponibilidade de nutrientes e a produção primária líquida nos ecossistemas (Chapman et al. 2006; Schweitzer et al. 2004). Além disso, o nitrogênio em contraste com outros nutrientes tem uma via alternativa de grande importância na maioria das florestas ciliares a desnitrificação, considerada a principal via de remoção de nitrogênio nestas matas (Dosskey et al. 2010; Gift et al. 2010; Lowrance et al. 1984; Okazawa et al. 2010). Sob este ponto de vista, Vidon e Hill (2004) concluíram que a eficiência na retenção e remoção do nitrogênio pelas matas ciliares é diretamente proporcional ao estado de conservação do ecossistema.

Por serem os microrganismos indicadores sensíveis na avaliação da saúde do solo e conseqüentemente de um ecossistema, atualmente várias abordagens biológicas moleculares estão sendo utilizadas para obter uma melhor compreensão da ecologia das comunidades microbianas do solo (Nakatsu 2004). Dentre os variados métodos utilizados para caracterização microbiana dois deles se destacam pelo amplo uso em trabalhos que abordam a avaliação da comunidade microbiana no solo. A análise de ácidos graxos fosfolipídios (PLFA sigla em inglês) um método independente de cultura usado para avaliar a estrutura da comunidade microbiana do solo e determinar as



mudanças de crescimento que acompanham os distúrbios. Uma consideração importante no uso destas moléculas para descrever comunidades microbianas é que a presença de ácidos graxos particulares é indicativa de grupos específicos de microrganismos (Hill 2000). E a eletroforese em gel com gradiente de desnaturação ou de temperatura (DGGE/TGGE sigla em inglês) separa dois fragmentos de DNA de fita dupla produtos da amplificação por reação em cadeia de polimerase (PCR sigla em inglês) através de diferentes gradientes de desnaturação (Muyzer et al. 1993; Muyzer & Smalla 1998). Seu poder de separação baseia-se então no comportamento de fusão da molécula de DNA. Uma vez que o comportamento de fusão é ditado pela sequência de nucleotídeos, a separação resultará em bandas individuais, cada uma correspondendo a uma única sequência (Hill 2000) dando origem a bandas diferentes que são de mesmo comprimento, mas diferem em sequência (Muyzer et al. 1993; Muyzer & Smalla 1998).

O conhecimento das variações na comunidade microbiana relacionadas às mudanças nas propriedades físico-químicas do solo são um importante e sensível indicador de ambas as mudanças a longo e curto prazo na saúde do solo (Hill 2000). Assim a conservação, restauração e manejo adequados das zonas ribeirinhas através da recuperação das propriedades físico-químicas e biológicas do solo desempenham um papel crítico na manutenção e melhoria dos serviços ambientais fornecidos por este ecossistema (Sweeney & Czapka 2004).

Esta tese está dividida em três capítulos na forma de artigo de revisão e artigos de pesquisa. Todas as questões abordadas estão intimamente ligadas à recuperação e conservação de ecossistemas ciliares. O **primeiro capítulo** traz uma descrição detalhada do ciclo do nitrogênio em ecossistema ciliar, apresentando a influência da mata ciliar na dinâmica do nitrogênio do solo e suas vias de transformações (input pela fixação biológica, mineralização e imobilização, nitrificação e desnitrificação) e o reflexo que a disponibilidade deste nutriente tem nas comunidades microbianas associadas a elas. No **segundo capítulo** detalhamos a avaliação do processo de recuperação de mata ciliar situada às margens do Rio das Velhas, seis anos após o plantio de espécies nativas em sistema de zoneamento, processo realizado pela equipe do Grupo de Estudos em Recuperação Ambiental (GERA), usando como indicador de restauração o nitrogênio do solo e a estrutura da comunidade microbiana. No **terceiro capítulo** detalhamos a avaliação do processo de recuperação realizado de área de mata ciliar, efetuado pelo proprietário da área na Bacia do rio Uberaba, usando um plantio de monocultura de espécie exótica. Os indicadores de recuperação foram aqueles

associados à estrutura (por exemplo, estabilidade de agregados e porosidade) e composição da matéria orgânica humificada do solo. Capítulo aceito para publicação no periódico *Soil Use and Management*.

## **2. Objetivos**

### *Objetivo Geral*

O objetivo geral do presente estudo foi avaliar a recuperação de duas áreas de mata ciliar inundáveis utilizando como indicadores de recuperação as características físico-químicas do solo (tais como fertilidade, matéria orgânica e substâncias húmicas do solo) e bióticas (comunidade microbiana do solo) envolvidas em importantes processos biogeoquímicos.

### *Objetivos específicos*

- Revisar as vias de entrada e transformações do nitrogênio em solos de mata ciliar inundável e a importância da comunidade microbiana associada a cada etapa do processo;
- Avaliar a recuperação da mata ciliar urbana com espécies nativas através da análise dos atributos físico-químicos e biológicos de qualidade do solo relacionados ao ciclo biogeoquímico do nitrogênio como indicador de recuperação;
- Avaliar a utilização de plantio de monocultura de espécie exótica na recuperação dos serviços ecossistêmicos da mata ciliar através de indicadores físico-químicos e bióticos do solo.

### 3. *Áreas de Estudos*

#### *Área experimental na bacia do rio das Velhas*

O estudo foi conduzido no Ribeirão Sabará, tributário do Rio das Velhas, Minas Gerais, sudeste do Brasil (19° 53' 11" S e 43° 48' 24" W). O clima regional é tropical (Aw), com verões úmidos e precipitação média anual de 1500 mm. Sua flora é típica de Cerrado brasileiro. O Ribeirão Sabará recebe descargas de esgoto domésticos oriundos da cidade de Sabará e do tributário Gaia. A área experimental consiste em área de mata ciliar anteriormente convertida a pasto e que passou por processo de recuperação há cerca de 6 anos. Diferentes espécies de plantas nativas foram utilizadas nesta recuperação, destacando-se a leguminosa *Mimosa bimucronata* (DC.) Kuntze inoculada com os esporos de fungos micorrízicos arbusculares (AMF) da coleção do Laboratório de Interação Microrganismos-Plantas da Universidade Federal de Minas Gerais, Brasil. Amostras de solo foram coletadas na floresta reabilitada e em outras duas áreas utilizadas como referência, anteriormente nomeadas: Área Experimental (ES) - consistindo de área ciliar recuperada há cerca de 6 anos; Área Preservada (PS) e Área Perturbada (DS) como local de referência e controle negativo, respectivamente.

#### *Área experimental na Bacia do rio Uberaba*

O estudo foi conduzido em área de mata ciliar do Bioma Cerrado, localizada na Bacia do rio Uberaba município de Uberaba, Minas Gerais, sudeste do Brasil (19° 43' 23.5" S e 47° 57' 6.7" W). O clima regional é típico de savana com verões húmidos e invernos secos, e precipitação média anual de 1400 mm. Sua flora é típica do Cerrado. A área experimental consiste em área de mata ciliar inserida em propriedade partícula que é principalmente utilizada para criação intensiva de gado, esta área passou por processo de recuperação há cerca de 15 anos realizada pelo proprietário da terra usando plantio da espécie vegetal *Syzygium cumini* (L.) Skeels popularmente chamada “jambolão” em sistema de monocultura. Duas outras áreas foram delimitadas nos mesmo fluxo d’água, uma área de referência consistindo de mata ciliar preservada e um controle negativo consistindo de área de mata ciliar perturbada usada para atividade de pastagem e que tem sido colonizada por espécies de gramíneas invasoras. As amostras de solo foram coletadas da floresta reabilitada e áreas utilizadas como referência,

anteriormente denominadas: sítio experimental (ES) - floresta recuperada a cerca de 15 anos; Área Preservada (PS) e Área Perturbada (DS) usadas como local de referência e controle, respectivamente.

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## **Chapter 1**

### **Nitrogen transformations in flooding soils of riparian forest**

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Article review

## **Nitrogen transformations in flooding soils of riparian forest**

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### **Resumo**

As áreas ribeirinhas têm grande importância ecológica devido aos serviços ecossistêmicos prestados. Mais especificamente, os tampões ripários desempenham um papel importante na manutenção dos níveis de nitrogênio, atuando principalmente na interceptação do excesso de nitrogênio derivado das terras agrícolas ou adicionadas por aporte direto durante eventos de inundação. Os passos da transformação do nitrogênio na mata ciliar são regidos por um grande número de fatores, entre os quais a participação de microrganismos com ação específica em diferentes etapas até a qualidade e quantidade de matéria orgânica produzida e disponível. Este trabalho teve como objetivo fazer uma revisão de literatura sobre as vias de transformação e retenção do nitrogênio em ecossistemas ciliares, destacando a sua influência e o reflexo destas transformações sobre a disponibilidade de nutrientes e na comunidade microbiana do solo. A influência da floresta ripária na dinâmica do nitrogênio, a entrada deste nutriente pela fixação, o processo de mineralização e imobilização, a nitrificação e desnitrificação são temas abordados neste estudo. Em solos ripários a concentração de nitrogênio está quase exclusivamente sob a forma de compostos inorgânicos, tais como amônia e nitrato. Sendo os principais mecanismos responsáveis pela retenção de nitrogênio correspondem à absorção por plantas e imobilização microbiana, mineralização e amonificação, respectivamente. Entretanto, como resultado das condições de inundação a que é submetido, o processo de desnitrificação em ambientes ciliares torna-se o principal responsável pela remoção definitiva do excesso de nitrogênio do sistema. Seu papel na atenuação do nitrato faz de florestas ripárias de grande importância para a saúde dos ecossistemas aquáticos e terrestres, dando maior significado à sua preservação e manutenção.

**Palavras-chave:** remoção de nitrogênio, tampões ripários, oxidantes de amônia, mineralização, nitrificação, fixação biológica de nitrogênio, desnitrificação.

### **Abstract**

Riparian areas have a greater ecological importance because of their nutrient filtering capacity. More specifically, riparian buffers play an important role in maintaining nitrogen (N) levels, acting in the interception of N excess derived from agricultural land or added by direct input during flood events. The steps of the transformation of N in the riparian forest are governed by a greater number of factors, among which the participation of microorganisms with a specific action in different steps up to the quality and quantity of organic matter (OM) produced and available. The objective of this work was to review the literature on nitrogen retention transformation pathways in riparian

ecosystems, highlighting their influence and the reflection of these transformations on the availability of nutrients and on the soil microbial community. The influence of riparian forest on the N dynamics, the entry of this nutrient by the fixation, the process of mineralization and immobilization, nitrification and denitrification are topics addressed in this study. In riparian soils the N concentration is almost exclusively under the form of inorganic compounds, such as ammonium and nitrate. The main mechanisms responsible for N retention correspond to plant absorption and microbial immobilization, mineralization, and ammonification, respectively. However, as a result of flood conditions to which it is submitted, denitrification process becomes the main responsible for definitive excess N removal from the system. Its role in nitrate attenuation make of riparian forests of great importance to the health of the aquatic and terrestrial ecosystems, also gives greater meaning to their preservation and maintenance.

**Key-words:** nitrogen removal, riparian buffers, ammonia oxidizers, mineralization, nitrification, biological nitrogen fixation, denitrification.

## *1. Introduction*

Riparian forests are comprised of mosaics of landforms, communities, and landscape. In a forest landscape riparian buffers are often referred to as forest types along water bodies (Gundersen et al. 2010; Schultz et al. 2004) which exhibit a high degree of structural and compositional diversity, and play an important role in controlling nutrient fluxes from terrestrial to aquatic ecosystems (Gregory et al. 1991). Furthermore, plant cover reduces erosion and stabilizes stream banks (Gundersen et al. 2010). Protected and managed they also can provide the natural functions of filtering and processing of suburban pollutants associated with runoff from roads, lawns, and construction sites, act with excellent nutrient sinks and buffer the nutrient loss from surrounding agroecosystems to streams (Lowrance et al. 1984; Schultz et al. 2004).

Hydrological connectivity of vegetation areas with water flow is a key process and responsible for the energy transfer mediated by water, matter, and organisms within or between elements these areas (Tockner & Stanford 2002). Nitrogen (N) is limiting element for growing in ecosystems, thus, vegetation demand is large for this nutrient (Dosskey et al. 2010). In riparian soils duration of floods the vegetation played an important role in the N dynamics (Poiani et al. 2000). Apart from their ability to control nutrients fluxes from entering the stream ecosystem (Baker et al. 2006); riparian forest especially plays an important role in maintaining levels of N discharged by agricultural

fields flows into river water via base flow runoff by uptake by plant and microorganisms and denitrification (Okazawa et al. 2010).

In riparian soils, the vegetation is a major source of organic matter (OM) for ecosystem through the plant debris which controls the quantity and type of terrestrially derived OM (Dosskey et al. 2010; Gregory et al. 1991). In addition, OM produced plays an important role in the nitrogen soil dynamic by regulating N-cycle microbial growth (Gift et al. 2010).

The decomposition or mineralization processes are reduced when the OM input is poor and the limiting N is found immobilized into microbial cells (Pinay 2002). The gains, losses, and transformations of this nutrient within the soil/plant system affect the availability of N to plants and the transfer of N into the wider environment (Cameron et al. 2013). Litter decomposition can be also compromised by impacts as flooding which affects the redox conditions becoming the decomposition process slow, compromising the N available. In spite of this, under flooding conditions, the N-inorganic forms are more subject to leaching (Glazebrook & Robertson 1999).

Biogeochemical processes, especially those involved N, are sensitive to the oxidation-reduction state of the soil. For example, ammonification of organic nitrogen can be performed both under aerobic and anaerobic conditions, but the nitrification process that requires oxygen can occur only in aerated soil or sediment. Therefore, under anaerobic conditions the  $\text{NH}_4^+$  ion will accumulate. On the other hand, the denitrification process that occurs under anaerobiosis can reduced both nitrate and  $\text{NH}_4^+$  accumulation (Pinay et al. 2002).

The objective of this work was to review the literature on nitrogen retention transformation pathways in riparian ecosystems, highlighting their influence and the reflection of these transformations on the availability of nutrients and on the soil microbial community. The influence of riparian forest on the N dynamics, the entry of this nutrient by the fixation, the process of mineralization and immobilization, nitrification and denitrification are topics addressed in this study.

## **2. *Influence of riparian forest on N dynamic***

Soils commonly contain between 0.1% and 0.6% nitrogen in the top 15 cm. This usually represents between 2000 and 12.000 kg N ha<sup>-1</sup> depending on the soil type (Cameron et al. 2013). Soil nitrogen is present in four major forms: (a) soil organic

matter (SOM) as plant debris or in decomposition and humic substances; (b) soil organisms and microorganisms; (c) ammonium ions ( $\text{NH}_4^+$ ) held by clay minerals and OM and (d) mineral N forms in soil solution, including  $\text{NH}_4^+$ , nitrate ( $\text{NO}_3^-$ ) and low concentrations of nitrite ( $\text{NO}_2^-$ ) (Cameron et al. 2013).

Due to their unique, low-lying position in the landscape, riparian zones are ideally placed to receive and process  $\text{NO}_3^-$  transported via groundwater from agricultural areas towards rivers and streams (Lowrance et al. 1984). Several biogeochemical processes are responsible for the transformation and retention of N in riparian zones, such as related to hydrology, geomorphology, soil type, and biological process; this results in a large range of efficiencies (Pinay et al. 1995; Lowrance et al. 1997). A riparian zone may have high nitrate removal efficiency but fluxes may be very small or seasonally absent. Conversely, some riparian zones may display a lower efficiency but receive high  $\text{NO}_3^-$  loading throughout the year and therefore be larger  $\text{NO}_3^-$  sinks at the watershed scale (Vidon & Hill 2004).

The major mechanisms of N retention in riparian zones are uptake by plants and microbial immobilization and depend upon the age of the forest stand. Many studies have summarized the principal mechanisms of this nitrogen retention process in riparian forest and these are showed in Figure 1. Plant uptake only temporarily retains N, which returns to the available pool when the plant, or part of the plant, dies and is mineralized. Denitrification is a permanent loss of N to the atmosphere as  $\text{N}_2$ , which is unavailable for use by most organisms (Schade et al. 2002).

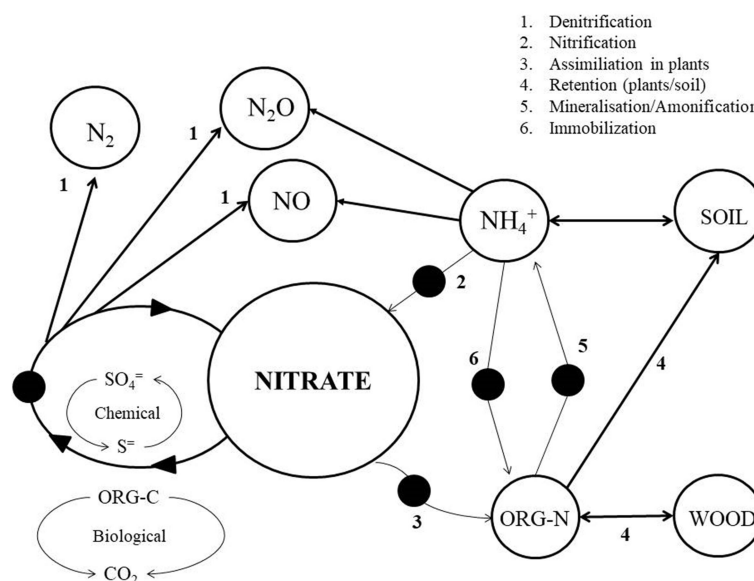


Figure 1. Mechanisms of nitrate retention/removal in riparian forest. Adapted from Correl (1996).

Noteworthy, the biogeochemical processes involved in the soil N cycle depend primarily on moisture conditions (Hefting et al. 2004). Therefore, the soil moisture regime and water table elevation controls to a great extent the end products of the soil N cycling in riparian zones, and ultimately, their buffer capacity (Pinay et al. 2007). Others soils properties, such as texture, clay content, pH, and temperature also can affect N turnover in soils in several ways by influence microbial nutrient cycling processes and thus regulation of nutrient availability and net primary production in terrestrial ecosystems (Champman et al. 2006; Schweitzer et al. 2004). Nitrogen transformations in submerged soils are markedly different from those in drained, aerated soils. These differences affect the prevalent soil microorganisms and microbial activities and the turnover, availability, and losses of N (Patrick 1982). There are an enormous biodiversity and metabolic capability of N conversions hidden in the microbial world (Jetten 2008).

### **2.1 Input by biological nitrogen fixing**

The ability to reduce inert atmospheric N to  $\text{NH}_3$  is restricted to prokaryotes that are able to break the strong triple bond within the  $\text{N}_2$  molecule, with the key participation of the nitrogenase enzyme complex that catalyse  $\text{N}_2$  reduction to produce ammonia ( $\text{NH}_3$ ) and hydrogenous ( $\text{H}_2$ ) (Christiansen et al. 2001; Olivares et al. 2013).

Atmospheric nitrogen ( $\text{N}_2$ ) can be converted back to  $\text{NO}_3^-$  by nitrogen-fixing microorganisms associated with the roots of some plants (e.g., Leguminosae) (Groffman et al. 1991; Lowrance, 1992), with the classic example of the bacterium *Rhizobium* infecting the roots of leguminous plants. Although the majority of N fixation is carried out by bacteria that live in association with plants, free living diazotrophs also contribute to N input (Cleveland et al. 1999).

Biological N fixation reflects the activity of a phylogenetically diverse list of prokaryotes (Vitousek et al. 2013) that harbor the *nifH* gene, one of the genes codings for the structural part of nitrogenase (Henneck 1981). *NifH* gene is widely used biomarkers and assumed to be highly conserved among diverse microorganisms, and therefore it has been extensively applied to assess the diversity of nitrogen-fixing bacteria in aquatic and terrestrial environments (Jetten 2008). For example, Sosa et al. (2018) used *nifH* gene with biomarker to assess N cycling gene abundance across an extensively sheep grazed riparian area. Moseman et al. (2009) assess the diversity and

functional responses of nitrogen-fixing microbes to three wetland invasions. However, studies using the *nifH* gene as a biomarker of the nitrogen-fixing community targeting the riparian ecosystems are still scarce.

The capacity of biological fixers to convert  $N_2$  to organic N is substantial, more than enough to maintain N pools in the ecosystem and replenish N losses (Butterbach-Bahl & Gudersen 2011). Biological N fixation still can be natural (e.g.  $N_2$  fixing trees that are present in forest ecosystems) or anthropogenic (e.g.  $N_2$  fixation by leguminous agricultural crops) (Reed et al. 2011). Interactions of microorganisms with various groups of plants are the most common symbiotic association for N assimilation (Kneip et al. 2007). In many ecosystems including riparian forests, the contribution of others N fixing organisms, such as symbiotic, cyanobacteria or free living heterotrophic bacterial is assumed to be significant and needs to be accounted for (Butterbach-Bahl & Gudersen 2011).

Nitrogen fixation is a highly energy-demanding process and, therefore, free-living fixers have limited significance compared than plant-associated fixers, which can obtain their energy requirements from plant exudates (Olivares et al. 2013). The enzyme complex is highly  $O_2$  sensitive (Paerl & Carlton 1988), and requires specific conditions, particularly related to the redox conditions of its environment. Other favourable factors are low mineral N contents (Hansen et al. 1992; Colnaghi et al. 1997), but good availability of other nutrients especially phosphorous (P), iron (Fe) and molybdenum (Mo) (Giller & Wilson 1991).

Flooded soil systems, as such as riparian forest, tend to be more favorable sites for nonsymbiotic nitrogen fixation than well-drained soils. However, the alternations in redox status in periodic flooding sites can favor the symbiotic  $N_2$  fixation. Many of the characteristic features of flooded soils indicate the suitability of these systems for nitrogen fixation. The near neutral pH and low redox potential found in most flooded soils also are apparently favorable for free living nitrogen fixation (Buresh et al. 1980).

To maintain balance in the global N budget,  $N_2$  fixation must be matched by denitrification, the return of fixed N to  $N_2$ . Currently, the fixation of  $N_2$  by industrial and biological pathways far exceeds denitrification rates; causing a rapid increase in biologically available N forms (Galloway et al. 2003).

## 2.2 *Nitrogen mineralization and immobilization process*

It is through OM decomposition by the saprophytic microorganisms that N is released in the mineral form depending on its recalcitrance and resistance to microbial attack (Philippot & Germon 2005). Definition of N mineralization takes into account two steps: (1) the depolymerization of organic macromolecules to bioavailable DON (dissolved organic nitrogen) and (2) mineralization/ammonification of bioavailable DON to  $\text{NH}_4^+$  (Butterbach-Bahl & Gundersen 2011; Schimel & Bennett 2004). Once  $\text{NH}_4^+$  is produced, it can be (1) fixed by clays or by SOM, (2) volatilized as  $\text{NH}_3$ , (3) assimilated by plants and microorganisms or finally (4) converted to  $\text{NO}_3^-$  by highly specialized bacteria during a secondary process: nitrification (Philippot & Germon 2005).

Nitrogen mineralization is regulated by chemical environmental factors such as soil temperature, pH, moisture and OM quality and quantity (e.g. lignin content) (Burt et al. 2010). This process is the rate limiting step of N transformations (Schimel & Bennett 2004). In non-fertilized areas, it can be a major controlling factor of the availability of N for plants and microorganisms (Haynes 1986). Furthermore, the forest age could be reflecting net mineralization rate. Studying restored and natural forests Amazonas et al. (2011) found mineralization rate was higher in the natural forest and the nitrogen amount was less concentrated in the soil of restored forests than in the natural forest.

Mineralization rates are generally also slow where disturbance has reduced soil OM content. Because N mineralization is influenced by soil OM chemistry, the proportion of total N mineralized (relative N mineralization) can be used as an indirect index of substrate quality (Zak & Grigal, 1991). The enzyme activities involved in mineralization could be negatively affected by nutrient availability because of the cost-efficiency strategy of microbes, known as the “microbial nitrogen mining” hypothesis (Craine et al. 2007; Shi 2011).

The main factor that affects the N transformations in riparian forest is the soil moisture due to shifts in the redox conditions of the soil determines the nutrient removal capacity of the riparian zones (Hefting et al. 2004). In well-drained soils, there is a faster N transformation which leads to greater mineral N contents than in poorly drained soils. Associate to soil moisture Ullah and Moore (2009) found an increase in litter N input accompanied by an increase in N mineralization and nitrification rates in forests



soils on southern Quebec, suggesting that quantity and quality of litter produced regulates the mineralization soil process too.

### 2.3 *Nitrification process*

Nitrification requires well drained aerobic sediments for oxygen ( $O_2$ ) respiration with a supply of OM and  $NH_3$  from the decomposition of plant and animal detritus (Burt et al. 2010). In the soils of many ecosystems worldwide, the ammonium is produced by the ammonifying bacteria, but also by fungi and animals, and is used as an energy source by bacteria autotrophic for carbon: the nitrifiers communities (Abbadie & Lata 2006).

The nitrification reaction refers to the oxidation ammonium ( $NH_4^+$ ) or ammonia ( $NH_3$ ) to  $NO_3^-$  via the intermediary step of nitrite ( $NO_2^-$ ). These nitrogen transformations lead to the production of several intermediaries which can be lost from the ecosystem, depending on its age and development. Nitrous oxide gas ( $N_2O$ ) produced during both the ammonia oxidation and nitrite oxidation steps is either emitted into the atmosphere, or utilized by  $N_2O$  reducing bacteria in the soil (Wrage et al. 2001).

Ammonia oxidation is rate-limiting step in the nitrification process and is performed by aerobic lithotrophic nitrifiers including bacterial and archaeal ammonia oxidizers (AOB and AOA, respectively) (Xia et al. 2015). A key functional enzyme in both bacterial and archaeal ammonia oxidizers is ammonia monooxygenase (AMO), which oxidizes ammonia to hydroxylamine (in AOB) (Gubry-Rangin et al. 2010). Until recently, ammonia oxidation was thought to be performed almost exclusively by bacteria. However, with the advancement of metagenomic studies, followed by laboratory isolation, was possible demonstrate the potential for significant ammonia oxidation by archaea, whose ecosystem function was previously unknown (Prosser & Nicol 2008; Wang et al. 2014). Studies performed by Leininger et al. (2006) found evidence that AOA can represent the most abundant ammonia-oxidizing organisms in soil ecosystems on Earth. They observed high numbers of AOA in various ecosystems and at greater soil depths indicate that these organisms are adapted to a broad range of growth conditions and might, therefore, have a more versatile metabolism than AOB, perhaps being able to grow mixotrophically. Evaluating the abundance of AOA and AOB in the riparian and agricultural environment, Wang et al. (2014) found that AOA and AOB might have a different contribution to nitrification in soils with the dominance

of AOA over AOB found in the slightly alkaline riparian soils. Studying riparian zones of Baiyangdian Lake (China), Zheng et al. (2017) observed that number of AOA genes was highest near land-water interface, suggesting a dominant role the AOA in environments with low dissolved oxygen content.

The importance of nitrifiers to ecosystem function is substantial: although some nitrate enters ecosystems in acid rain or as fertilizer, in most ecosystems nitrate are formed in situ via nitrification. Because nitrate is an anion, it shows more solubility than ammonium, the ionized source of  $\text{NH}_3$  in soil water (Robertson & Groffman 2015). According to Abbadie and Lata (2006), the rate of nitrification is generally high and most of the soils are richer in nitrate than in ammonium. In soils nitrification process is performed by two separate microorganisms and happens in a two-step process, carried out by separate groups of bacteria: the ammonia oxidizers or primary nitrifiers and nitrite oxidizers or secondary nitrifiers, respectively. These two groups together are called Nitrobacteriaceae (Wrage et al. 2001). Autotrophic nitrifiers are obligate aerobes (Robertson & Groffman 2015; Wrage et al. 2001) using  $\text{NH}_4^+$  as an energy source for the fixation of carbon dioxide ( $\text{CO}_2$ ) (Wrage et al. 2001).

The nitrifying organism in a flooded, wetland soil is likely to be concentrated closest to the surface of the soil due to the obligate  $\text{O}_2$  requirement (White & Reddy 2003) and its activity can be modified by flooding periodicity.

Plant species composition may also affect AOB populations indirectly by differentially affecting mineralization patterns, i.e., via production of variable litter quality and quantity (Kowalchuk & Stephen 2001). However, the most important factor regulating nitrification in the majority of soils is ammonium supply, but the oxygen is another important regulator. Where decomposition and thus N mineralization is low or where  $\text{NH}_4^+$  uptake and thus N immobilization by heterotrophs or plants is high, nitrification rates will be low. Conversely, any ecosystem disturbance that increases soil  $\text{NH}_4^+$  availability will usually accelerate nitrification unless some other factor is limiting (Robertson & Groffman 2015).

## ***2.4 Denitrification process***

The denitrification process is mediated by a particular group of bacteria called denitrifying and consists of the production of  $\text{NH}_3$  and eventually  $\text{N}_2$  (Robertson & Groffman 2007). Several studies have quantified the nitrate retention potential for the

riparian ecosystem, highlighting the potential of riparian vegetation in stock for excess nitrate (Table 1). Denitrification can be a major pathway of N removal from N-enriched groundwater in riparian zones, but it is apparently concentrated in the upper soil layers (Dosskey et al. 2010; Hill 1996; Lowrance 1992).

Table 1 Comparison of effect of riparian forest on the nitrate-nitrogen ( $\text{NO}_3^-$  - N) content retention

Location	Input ( $\text{mg L}^{-1}$ )	Retention (%)	Reference
Southern Ontario	0.15 – 44.7	90	Vidon and Hill, 2004
Pennsylvania	2.7	44	Newbold et al., 2010
Australia	3.04	77	Woodward et al., 2009
	1.07	98	
Georgia	13.5	95	Lowrance, 1992
Buffer zones in Europe	7	96	Hefting et al. (2005)
	26	58	
France	1.32	100	Pinay and Decamps (1988)
Buffer zones in Europe	1.7	92	Sabater et al. (2003)

Under waterlogged conditions, the breakdown of organic matter is slow because of the absence of  $\text{O}_2$  (D'Angelo & Reddy 1999). Particular groups of ubiquitous heterotrophic facultative anaerobic bacteria are responsible for this process. They utilise the step wise reduction of  $\text{NO}_3^-$  to produce gaseous N ( $\text{N}_2\text{O}$  and  $\text{N}_2$ ) for energy production, during respiration in the absence of  $\text{O}_2$ . These bacteria use alternative electron acceptors such as  $\text{CH}_4$ ,  $\text{NO}_3^-$ ,  $\text{Fe}_3^+$ ,  $\text{Mn}_4^+$ , or  $\text{SO}_4^{2-}$  in place of  $\text{O}_2$  in anaerobic environments (D'Angelo & Reddy 1999; McClain et al. 2003; Mitsch & Day 2006; Wrage et al. 2001). During this process, intermediary compounds are produced that can then be lost from the soil system, such as nitrous oxide ( $\text{N}_2\text{O}$ ) and nitric oxide (NO), which are emitted into the atmosphere, and also that can be reduced to ammonia via nitrate ammonification (Wrage et al. 2001). If  $\text{NO}_3^-$  is not reduced completely to  $\text{N}_2$ ,  $\text{N}_2\text{O}$  and NOx will be the end products of denitrification. Production of  $\text{N}_2\text{O}$  rather than  $\text{N}_2$  is favoured at low pH (Johns et al. 2004), low temperature, high oxygen and nitrate concentrations (Chapin et al. 2004).

Transformations of different forms of N are driven by different microbial functional groups. These microorganisms show a diverse set of genes precursors of synthesis of various enzymes that are responsible for nitrogen cycling reactions. Functional genes (*nirK*, *nirS*, *cnorB*, *qnorB*, *nosZ*) involved in the denitrification pathway have been used as genetic markers for denitrifier's abundance, diversity and

for functional gene expression (Philippot et al. 2007). At conversion nitrite to nitric oxide the corresponding nitrite reductase gene (including nirS and nirK) is often used as a representative molecule denitrifying microorganisms research community structure tag (Ying et al. 2016).

Denitrification has negative implications for soil fertility and productivity in terrestrial ecosystems; however, it can be an important regulator of eutrophication in N-sensitive aquatic ecosystems (Seitzinger et al. 2006). Although denitrification is generally higher under anaerobic conditions it can also occur in soils that are not completely water logged and can also occur within soil aggregates, where oxygen diffusion rates are slow and the soil redox potential is low (Cameron et al. 2013). Considering riparian systems Davis et al. (2008) showed that these ecosystems have characteristics conducive to denitrification, such as high concentrations of organic carbon (orgC) and high soil water content, resulting in the formation of anoxic conditions that are conducive to complete denitrification. However, the effectiveness of riparian forest in N removal varied widely and may be proportional to the length of the riparian buffer. Wide buffers (0.50 m) more consistently removed significant portions of nitrogen entering a riparian zone than narrow buffers (0–25 m). On the other hand, buffers of various vegetation types are equally effective at removing nitrogen but buffers composed of herbaceous and forest/herbaceous vegetation were more effective when wider (Mayer et al. 2007).

### **3. Concluding remarks**

The nitrogen cycling in the ecosystem is a key process to release nutrients, which are proven by plant growth and development system. Environmental characteristics, such as flooding and microbial community living in the soil are the main that drive forces nutrient cycling in flooded soils.

The N retention in riparian ecosystems is of particular environmental importance since this element is regarded as a major pollutant of aquatic environments. Emissions of N compounds such as N<sub>2</sub>O and NO<sub>x</sub> are also considered the major contributors to the greenhouse effect when denitrification process is not complete.

The nitrate concentrations in the riparian forest are generally mitigated by the denitrification process, uptake by vegetation and microorganisms' immobilization. The effectiveness varies according to the hydrological conditions and vegetation. Thus, the

denitrification is the most effective process for removing nitrate and to equilibrate the soil N forms.

This clearly demonstrates the role of the riparian ecosystem in the movement and retention of nutrients, especially those compounds of nitrogen to the adjacent ecosystems. Indeed, forest removal and degraded process to which they are subject can directly affect the flow of dissolved nutrients to the aquatic environment.

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## Chapter 2

### Implications of ammonia accumulation for the riparian forest restoration

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**Title: Implications of ammonia accumulation for the riparian forest restoration**

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***Abstract***

The composition of the microbial community and their functional capacity associated with nitrogen cycle were studied in a restored 6 year-old riparian forest located at Sabará stream, tributary of Rio das Velhas (Minas Gerais State, Brazil), which receive a sewage discharge and has suffered periodic flooding. To evaluate to restore of the experimental site we compared soil fertility, nitrogen dynamics and microbial community of the experimental site (ES) with those of a disturbed site (DS) and a preserved site (PS). The experimental riparian forest (ES) achieved an intermediate stage of restoration as compared to a preserved (PS) and a disturbed (DS) sites in relation to the following parameters: soil organic carbon, nitrate, total nitrogen, humic and fulvic acids, microbial biomass carbon and microbial biomass bacteria, Gram<sup>-</sup> and Gram<sup>+</sup> bacteria, fungi and arbuscular mycorrhizal fungi. Some differences in soil properties and microbial community profiles revealed a connection between available carbon, nitrogen dynamics and the impact of periodic flooding. Higher soil ammonium and lower nitrate contents were registered in ES likely to come from an exogenous source such as the flood waters, which had a lesser impact over PS. However, the diversity and richness of the nitrifying ammonium oxidizing bacteria (AOB) community were similar DS. PS there was a higher soil microbial community carrying the nosZ genes, which perform the complete denitrification pathway, preventing the accumulation of ammonium. Based on these attributes, the ES is evolving towards the PS; however, the forest restoration is slower probably by high ammonium stock. It is expected that with the evolution of ES recovery reach resilience overcoming the disturbance.

***Key words:*** biogeochemical cycle, microbial community, phospholipid fatty acids, riparian buffer recovery, soil quality, temperature gradient gel electrophoresis.

## ***Introduction***

Riparian ecosystems correspond to the portion of forest located on the margins of natural streams of water, connecting aquatic and terrestrial ecosystems gives it an essential role in mitigation of disturbances and maintenance of water resources (Gregory et al. 1991). These buffers are composed by flood tolerant plant species that work in reducing the loss of nutrients, in soil anchorage and stabilization and prevention of erosion through the connecting action of the root system and formation of humic organic matter on the soil, fundamental condition for water percolation and permeability of the flooded forest (Schultz et al. 2004; Kimura et al. 2017).

Decomposition of leaf litter by the action of microorganisms of the soil is the main source of Organic Matter (OM) humified for the system which drives many of the biogeochemical processes in riparian buffers (Dosskey & Bertsch, 1994) such as, nitrogen retention by regulation of denitrification, especially in the promotion of anaerobic conditions via increased microbial respiration (Gift et al. 2010). The humified fractions of OM (humic acids, fulvic acids and humin) still improve the physicochemical and biological characteristics of the soil contributing to its fertility and consequently improving and/or maintaining the sustainability of the ecosystem (Fageria et al. 2012). The OM and other soil properties such as texture, pH, clay content, and the composition of the microbial communities and their functional (metabolic) abilities are crucial to the soil processes and ecosystem productivity by influence of nutrient cycling (Chodak et al. 2016).

Agents of disturbance such as floods are specific of riparian zones (Moore & Richardson 2012) as occurs in Velhas river basin whose riparian forest has been subjected to periodic flooding (Kimura et al., 2017). Although it is a key process and important part of the ecosystem dynamics, it can lead to loss of nutrients by leaching (Mchergui et al. 2014). Furthermore, biogeochemical processes in submerged soils are very different from those drained and aerated soils and these differences affect the prevalent soil microorganisms and related activities as well as the availability and nutrient losses (Patrick 1982). The mineralization process of organic nitrogen – limiting nutrient for plant growth and microorganisms – requires aerated sites what could result in the accumulation of ammonia in the system (Dosskey et al. 2010). However, other processes such as denitrification and Dissimilatory Nitrate Reduction to Ammonium (DNRA) are anaerobic, demanding soils with limitation of O<sub>2</sub> (Pinay et al. 2002).

Structure and size microbial community are considered sensible indicators of soil health by respond to changes in soil properties, soil oxygen availability, and water quality (Šimek & Cooper 2002; Suthar et al. 2010; Reddy & DeLaune 2008; Unger et al. 2009; Zenglin 2015) being useful in evaluating forest recovery processes. Molecular microbial analysis methods have aided in the understanding of diversity and functionality of soil microorganisms. The amplification of DNA fragments of a desired microbial group associated with the technique of TGGE has allowed the identification of the dominant members of microbial community (Dandie et al. 2011; Muyzer & Smalla 1998; Wang et al. 2012) and Phospholipids fatty acid (PLFA) analysis which allows to evaluate the active soil microbial biomass (Buyer & Sasser 2012; Card et al. 2010; Young-Mathews et al. 2010). In this context, an evaluation of the effectiveness of riparian ecosystems recovery process may be made of a multidimensional point of view considering complex links between soil characteristics, microbial processes and the impact of floods on them (Simon & Steinemann 2000).

The aims of this study was to evaluate the progress of the recovery process six years after plantation of an area of forest in the basin of the Rio das Velhas through the environmental services indicators related to biogeochemical cycles and the physical, chemical and biological properties of the soil.

## **Materials and Methods**

### ***Description of the area***

The study was conducted in Sabará stream a tributary of Rio das Velhas, Minas Gerais State, Brazil (19° 53' 11"S and 43° 48' 24" W) (Fig. 1). The Sabará stream receives domestic wastes from the Sabará city and from its tributary Gaia stream which also receives domestic and industrial wastes from different cities. The Experimental site is a riparian site that had been converted into pasture and later subjected to restoration procedures. This site still has suffered annual floods. The regional climate is tropical (Aw), with moist summers, average annual rainfall of 1500 mm, and the flora is typical of Brazilian Cerrado.

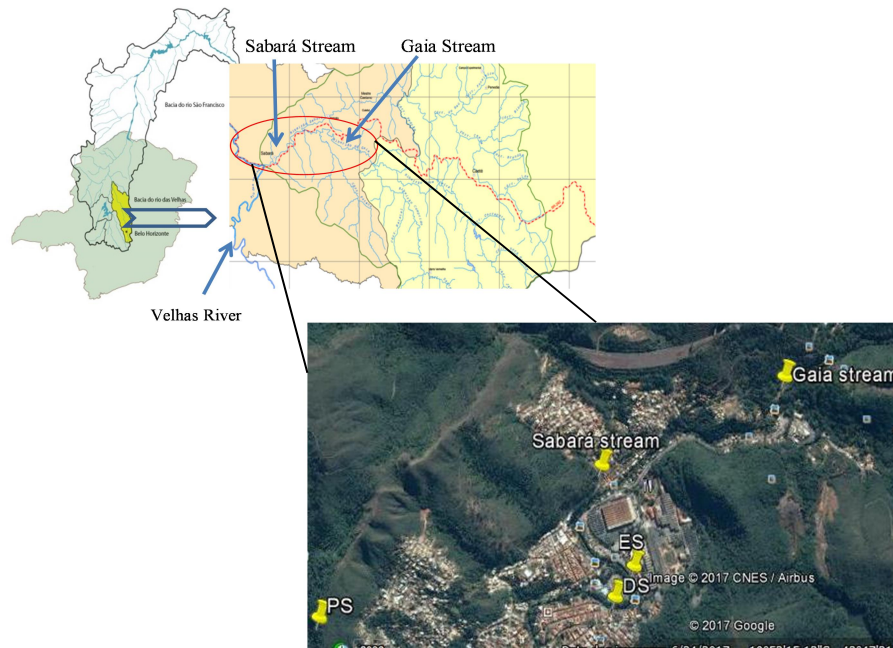


Figure 1. Location of Stream Sabará and Velhas River basin in Minas Gerais state (SEMAM 2004) and study sites (ES: experimental site; PS: preserved site, and DS: disturbed site).

### ***Experimental design and Soil sampling***

The experimental site (120 m along the stream × 30 m along the hill slope = 3600 m<sup>2</sup>) was previously re-vegetated according to the buffer zone model (Kimura et al. 2017; Lowrance et al. 1997; Schultz et al. 2004; Welsch 1991) for recovering the lost functions of drainage, stability and soil fertility. In Zone I (5 m from the river) phreatophyte and flood-tolerant species (eg: *Morus nigra*, *Rapanea guianensis*, *Miconia sp*, *Eugenia uniflora*, *Psidium rufus*, *Inga edulis*, *Croton urucurana*, *Eritrina speciosa*, *Nectandra laceolata* (cf)) were planted. In Zone II (10 m from the margin), woody species (eg: *Croton floribundus*, *Anadenanthera peregrina*, *Centrolobium tomentosum*, *Machaerium villosum*, *Pitadenia gonoacantha*, *Luehea divaricata*, *Samanea tubulosa*) that could produce biomass to improve the litter and organic matter formation were planted. In Zone III (15- m from the margin) herbaceous, shrub and woody species (eg: *Hymenea courbaril*, *Mimosa bimucronata*, *Tabebuia sp*, *Tradescantia sp*, *Helianthus annus*, and *Piper umbelattum*) were planted to ensure not only the drainage but also the control of surface and subsurface erosion (Kimura et al. 2017). The seedlings were transplanted to the field, after 4 months of growth under nursery conditions, maintaining a spacing of 3 × 3 m. Fertilisation was performed according to Somasegaran & Hoben (1985).

A preserved riparian forest (120 m × 30 m) was chosen as a positive reference of biological, physical and chemical integrity. This forest was located near the ES within an Environmental Protection Park. A degraded site (120 m × 30 m) adjacent to the ES without woody vegetation was chosen as a negative control. This area showed soil and environmental conditions similar to the ES before restoration.

Soil samples were collected along three transects (100 m each), crossing each of three riparian zones after 6 years of restoration. The samples were obtained from 0 to 20 cm depth, at each study site (Experimental site, Preserved site and Disturbed site). A total of 27 samples (3 soil samples/transect × 3 transects/site × 3 sites) were collected, which were used for all the physical, chemical and biological analyses. The studies sites suffered annual floods with greater or lesser intensity.

### ***Physicochemical analysis***

The samples were sieved out through a 2 mesh for physical and chemical properties according to Embrapa (1997). The textural class was based on percentages of sand, silt, and clay in the soil. The following soil characteristics were determined: pH in CaCl<sub>2</sub>, total organic carbon, contents of exchangeable bases (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>), available Phosphorus (P), potential acidity (H + Al) measured in SMP buffer, sum of exchangeable bases (SB), cationic exchange capacity (CEC), and base saturation (V%) (Embrapa 1997). Concentrations total nitrogen (TN) were measured according to Schuman et al. (1973), and the fractions ammonium (NH<sub>4</sub><sup>+</sup>-N) and nitrate (NO<sub>3</sub><sup>-</sup>-N) by the colorimetric method according to Coelho et al. (1992). The determination of organic carbon in the HA, FA and H fractions was performed as described by Dabin (1971). The microbial biomass C (*C<sub>mic</sub>*) by the fumigation-extraction method using fumigated (CCl<sub>4</sub>) and non-fumigated soil samples (Vance et al. 1987). Glomalin-related proteins (PRSG-T) were determined by method of Wright and Upadhyaya (1996), and the protein content was estimated by Bradford essay (1976).

### ***DNA extraction and PCR-TGGE***

DNA was extracted (0.25 g soil aliquots) using the Power Soil™ DNA Kit (MO BIO Laboratories Inc, Carlsbad, CA, USA) according to the manufacturer's instructions. PCR was performed using the primers CTO 189f AB-GC/CTO 654R (Kowalchuck et al. 1997) to target gene AOB (Ammonia-Oxidizing Bacteria) and nosZ2F/nosZ2R-GC (Clark et al. 2012) to target denitrifying genes the final stage of denitrification. With

amplicons length expected of 465bp and 268bp, respectively. PCR mixtures contained 2µl of target DNA, 5µl of 10x PCR buffer, 2µl of MgCl<sub>2</sub>, 1µl dNTPs solution, 1µl of forward and reverse primers, 2.5µl of BSA (0.1%) and dH<sub>2</sub>O to complete the volume.

TGGE was performed by the use of the TGGE MAXI System Controller (Biometrics GmbH, Göttingen, Germany). The PCR products (05µl of each) were loaded on 6% polyacrylamide (acrylamide:bisacrilamida = 37:5:1) gels. Electrophoresis were run in 1x Tris-acetate-EDTA (TAE) buffer for 20 hours at 120V with temperature gradient of 45–53 °C to AOB and 46-54 °C to nosZ, and then stained in silver nitrate as described in Chaves (2005). The images were captured by UV light gel transillumination and analyzed using software BIONUMERICS software version 6.6 (Applied Maths, Kortrijk, Belgium). The similarities between profiles were generated by the similarity index of Dice with tolerance of 1% were based on UPGMA method – Unweighted Pair Group Method using Arithmetic Averages (Priest & Austin 1995). The richness of AOB and nosZ was determined by counting of distinguishable bands in polyacrylamide gel and the total number of individuals by the relative intensity of the bands. Bacterial Shannon-Weaver diversity index ( $H'$ ) was calculated according to the formula:  $H_{sw} = \sum - (h_i/H) \log (h_i/H)$ , where  $n$  is the total number of bands in the profile,  $h_i$  is the intensity of individual band,  $i$  and  $H$  are the total intensity of all bands in that profile.

### ***PLFA analysis***

Lipids were extracted from soil, fractionated, and quantified using the procedure described by White et al. (1979). Lipids were extracted with liquid organic solvents and then separated into fractions using column chromatography with Hewlett-Packard chromatograph GC system model 5890 series 2. After the extraction the material was divided in neutral, glyco and phospholipids containing polar lipids (Guckert et al. 1985), then were converted to Fatty Acid Methyl Ester (FAMES) by alkaline methanolysis (White et al. 1979). On average, 40 individual PLFAs were identified and quantified. The PLFAs were designated as A:BωC, with A = number of C atoms, B = number of establishment bonds, and C = position of the first double bond from aliphatic end of the molecule. The suffixes "c" and "t" indicate cis and trans geometry. The suffixes "i", "a" and "Me" refer to iso, anteiso and mid-chain methyl branching, and "cy" refers to cyclopropil rings (Bossio & Scow 1998).



Fatty acid methyl esters reported to be biomarkers of bacteria, gram-positive bacteria (Gram<sup>+</sup>), gram-negative bacteria (Gram<sup>-</sup>), anaerobic bacteria, aerobic bacteria, fungi, arbuscular mycorrhizal fungi (AMF), denitrifying group, algae, unclassified and actinomycetes were used as signatures for these microbial groups (see Vestal & White 1989; Bossio & Scow 1998; Bradley et al. 2006; Balasooriya et al. 2008; Bach et al. 2010; Farrel et al. 2013; Prayogo et al. 2013; Zhang et al. 2016 for details). Stress indicators were calculated based on the proportion of cyclopropyl fatty acids to monoenoic precursors (C19:0cy/C18:1ω7) and the total of monounsaturated to saturated fatty acids (Kieft et al. 1997; Bossio & Scow 1998).

### **Data Analysis**

The averages of the chemical and biological parameters were determined in three soil replicates. All variables (chemical and biological) were evaluated for homogeneity of variance with Levene's test and for normality with Shapiro-Wilk. We performed one-way analysis of variance (ANOVA) tests to comparing the variables among sites. If differences were detected, further analyses Tukey honest significant difference (HSD) post hoc tests. To explore the sources of variability in structure microbial community of forests, we ran a Principal Component Analysis (PCA), then summarizing the gradients of variability and which environmental parameters were most significant in explaining the variation in microbial community (Mingoti, 2007). In addition to species scores, we plotted the centroids of the species scores in the bi-dimensional space of the two main PCA axes. These centroids helped interpret the variation gradients. All statistical analysis was performed using the software R version 3.2.2 and the significance level was set at 5% ( $p \leq 0.05$ ).

### **Results and Discussion**

Some differences in soil properties and microbial community profiles revealed a connection between available carbon, nitrogen dynamics and the impact of periodic flooding.

#### ***Physicochemical properties of soils***

The soil texture of the study sites was characterized with sandy loam, sandy clay loam, and loam sandy, for ES, PS, and DS, respectively. Soil texture as abiotic factor is

important factors that influence distribution of minerals, organic matter retention, microbial biomass and other soil properties (Scott & Robert 2006). Soil chemical properties are shown in table 1. Riparian soils tend to be naturally acidity because of the amount and speed of available organic compounds decomposition which leads to soil acidification (Brady & Weil 2010). This higher soil pH values in the study sites may be explained by an external factor such as the polluted waters of urban Sabará stream that has been considered one of the most polluted rivers in the Velhas basin (Junqueira & Campos 1998). Pollutants of surface waters probably also influenced phosphorus content in this study. Similar results were reported by Carpenter et al (1998) which observed that nonpoint sources are now the dominant inputs of P and N to most surface waters. In relation exchangeable bases, ES showed to be intermediate between others sites. These results reflected in fertility soil, with ES showing values in CEC, SB, and BS twice folds higher than DS. According to Zamir et al. (2015), soils with high CEC and BS have greater ability to retain and exchange nutrients and what evidence the improvement in the quality of the soil in ES.

Table 1. Chemical properties of soils sampled at a disturbed, experimental and preserved sites. Values are mean  $\pm$  SD (n=3).

<i>Parameters</i>	<i>Disturbed site</i>	<i>SD</i> ( $\pm$ )	<i>Experimental site</i>	<i>SD</i> ( $\pm$ )	<i>Preserved site</i>	<i>SD</i> ( $\pm$ )
<b>pH</b>	7.20 <sup>a</sup>	0.06	7.33 <sup>a</sup>	0.22	6.50 <sup>b</sup>	0.15
<b>P (mg/dm<sup>3</sup>)</b>	51.33 <sup>a</sup>	1.86	33.67 <sup>b</sup>	2.03	44.67 <sup>a</sup>	1.45
<b>K<sup>+</sup> (mmol<sub>c</sub>/dm<sup>-3</sup>)</b>	3.77 <sup>a</sup>	0.09	2.20 <sup>b</sup>	0.23	2.52 <sup>b</sup>	0.06
<b>Ca<sup>2+</sup> (mmol<sub>c</sub>/dm<sup>-3</sup>)</b>	56.67 <sup>c</sup>	0.33	154.67 <sup>a</sup>	0.88	146.67 <sup>b</sup>	1.22
<b>Mg<sup>2+</sup> (mmol<sub>c</sub>/dm<sup>-3</sup>)</b>	5.33 <sup>ns</sup>	0.33	8.33 <sup>ns</sup>	1.45	7.67 <sup>ns</sup>	0.67
<b>H+Al (mmol<sub>c</sub>/dm<sup>-3</sup>)</b>	9.67 <sup>ns</sup>	0.33	8.33 <sup>ns</sup>	0.88	8.33 <sup>ns</sup>	0.33
<b>SB (mmol<sub>c</sub>/dm<sup>-3</sup>)</b>	66.00 <sup>c</sup>	0.00	165.33 <sup>a</sup>	0.33	157.00 <sup>b</sup>	1.00
<b>CEC (mmol<sub>c</sub>/dm<sup>-3</sup>)</b>	75.67 <sup>c</sup>	0.33	173.67 <sup>a</sup>	0.88	166.00 <sup>b</sup>	0.58
<b>BS (%)</b>	87.67 <sup>b</sup>	0.33	95.33 <sup>a</sup>	0.88	94.67 <sup>a</sup>	0.33

P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; H+Al: Potential acidity; SB: sum of bases; CEC: Cation exchange capacity; BS: base saturation. Means followed by the same letter in a row are significantly different using Tukey test at  $p < 0.05$ . ns, non-significant.

The highest values of C sequestrated in the PS were found for organic C, humic acid (HA), fulvic acid (FA) and *Cmic* (Table 2). The sequestered C in the ES was intermediate to that was found in the PS and DS. The increase in C content in ES

compared to DS may also have contributed to high CEC (Giesler et al. 2005). High content of FA fraction compared HA was found in this study corroborating with the works of Kimura et al. (2017) and Spaccini and Piccolo (2009) that described the humic substances (HS's) of areas subjected to periodic flooding as formed in their majority by small molecules, less condensed and more water-soluble as FA. PS was characterized by a higher content of *Cmic* followed by ES and DS, respectively, following a similar pattern to organic C content. These results corroborate with literature once that it is well known that soil microbial biomass greatly depends on soil organic matter as substrate; a decrease in SOC causes reduction in soil microbial biomass (Chen et al. 2005).

Concerning nitrogen dynamic, ES was intermediate to that found in the PS and DS related total nitrogen and nitrate ( $\text{NO}_3^-$ ). Surprisingly, ES presented high values of  $\text{NH}_4^+$  similar was found in DS and differing preserved site. These results suggest that the amount of  $\text{NH}_4^+$  in the ES and DS areas comes from floodwaters with lower impact on PS (Adair et al. 2004). Indeed, the alterations in anaerobic/aerobic microbial structures and denitrifying populations promoted by the flooding cycles reinforce this hypothesis, suggesting a distinct degree of resilience among sites. In fact, all the studied areas, particularly DS and ES, are located downstream a sewage discharges which may alter the ammonium and nitrate concentration in the river (Suthar et al. 2010).

With respect to glomalin content, its higher amount in PS and ES reflects the high AMF activity in these soils (Kimura & Scotti 2016). Differently, in DS the organic C reflects the impact on the soil, suggesting an environment not favorable for the development of plants and microorganisms evidenced by the low contents of *Cmic* and higher stress level (Priyanka & Anshumali 2016).

Table 2. Carbon, nitrogen and glomalin concentrations of soils sampled at a disturbed, experimental and preserved sites. Values are mean  $\pm$  SD (n=3).

<i>Parameters</i>	<i>Disturbed</i>	<i>SD</i>	<i>Experimental</i>	<i>SD</i>	<i>Preserved</i>	<i>SD</i>
	<i>site</i>	( $\pm$ )	<i>site</i>	( $\pm$ )	<i>site</i>	( $\pm$ )
<b>Organic C (g/kg)</b>	13.70 <sup>c</sup>	0.90	18.92 <sup>b</sup>	1.07	23.47 <sup>a</sup>	2.16
<b>Cmic (<math>\mu</math>/g)</b>	264.83 <sup>c</sup>	17.0	544.67 <sup>b</sup>	43.93	870.98 <sup>a</sup>	37.38
<b>Humic acid (g/kg)</b>	0.98 <sup>c</sup>	0.28	1.70 <sup>b</sup>	0.21	2.37 <sup>a</sup>	0.18
<b>Fulvic acid (g/kg)</b>	2.21 <sup>c</sup>	0.32	3.22 <sup>b</sup>	0.36	4.08 <sup>a</sup>	0.23
<b>TN (g/kg)</b>	0.98 <sup>c</sup>	0.02	1.23 <sup>b</sup>	0.03	2.03 <sup>a</sup>	0.05
<b>NH<sub>4</sub><sup>+</sup> (mg/kg)</b>	54.85 <sup>b</sup>	0.67	55.55 <sup>a</sup>	7.27	33.60 <sup>c</sup>	2.52
<b>NO<sub>3</sub><sup>-</sup> (mg/kg)</b>	96.09 <sup>c</sup>	2.10	129.09 <sup>b</sup>	12.80	209.58 <sup>a</sup>	10.99
<b>Glomalin (mg/g)</b>	10.88 <sup>b</sup>	1.03	24.02 <sup>a</sup>	1.61	21.87 <sup>a</sup>	1.25

Cmic: microbial biomass carbon; TN: total nitrogen; NH<sub>4</sub><sup>+</sup>: ammonium; NO<sub>3</sub><sup>-</sup>: nitrate. Means followed by the same letter in a row are significantly different using Tukey test at  $p < 0.05$ . ns, non-significant.

### Characterization of soil microbial community

#### TGGE

Analysis of the microbial community fingerprints, using matrices based on either presence/absence or relative abundance of bands/peaks, indicated there is no difference in the AOB community, but showed a significant difference in nosZ community. Counting of the bands resulted in richness of bands and bacterial Shannon-Weaver diversity index ( $H'$ ) observed in Table 3.

Table 3. Shannon-Weaver index ( $H'$ ) and band richness ( $R$ ) of microbial community of soils sampled at a disturbed, experimental and preserved sites. The data were derived from the TGGE profiles. Values are mean  $\pm$  SD (n=3).

<i>Genes</i>	<i>Disturbed</i>	<i>SD</i>	<i>Experimental</i>	<i>SD</i>	<i>Preserved</i>	<i>SD</i>
	<i>site</i>	( $\pm$ )	<i>site</i>	( $\pm$ )	<i>site</i>	( $\pm$ )
<b>Richness (R)</b>						
<b>AOB</b>	19.89 <sup>ns</sup>	3.98	22.00 <sup>ns</sup>	3.20	18.44 <sup>ns</sup>	4.45
<b>NosZ</b>	3.89 <sup>b</sup>	0.60	5.00 <sup>b</sup>	1.12	6.89 <sup>a</sup>	1.76
<b>Shannon-Weaver (<math>H'</math>)</b>						
<b>AOB</b>	1.19 <sup>ns</sup>	0.11	1.24 <sup>ns</sup>	0.08	1.19 <sup>ns</sup>	0.13
<b>NosZ</b>	0.55 <sup>b</sup>	0.05	0.63 <sup>b</sup>	0.07	0.78 <sup>a</sup>	0.11

AOB: ammonium oxidation bacterial; nosZ: denitrifiers bacterial. Means followed by the same letter in a row are significantly different using Tukey test at  $p < 0.05$ . ns, non-significant.

AOB has been found to be very sensitive to the high  $\text{NH}_4^+$  concentration what could explain low  $\text{NO}_3^-$  in ES (Norton & Stark 2011). High concentrations of ammonium have also been found to inhibit the activity of Nitrobacter, particularly in high pH values, probably due to the production of free ammonia (Reddy & Patrick 1984) what also could explain low nitrate in ES compared PS. Denitrifiers community represented by the *nosZ* genes in ES was similar DS, differing from PS. In general, the richness of bands followed the same pattern. However, different to what was observed in quantitative results, similarity analysis for the *nosZ* community samples taken from ES clustered separately from those samples collected from the DS and PS as displayed by the dendrogram (Fig. 2). Indicating that dynamic of denitrification in ES differ the other sites, probably because inundation is selecting the microbial community. It is well known that both duration of inundation (Mentzer et al. 2006) and cycles of wetting and drying (Fierer & Schimel 2002) affect soil microbial community composition and activity.

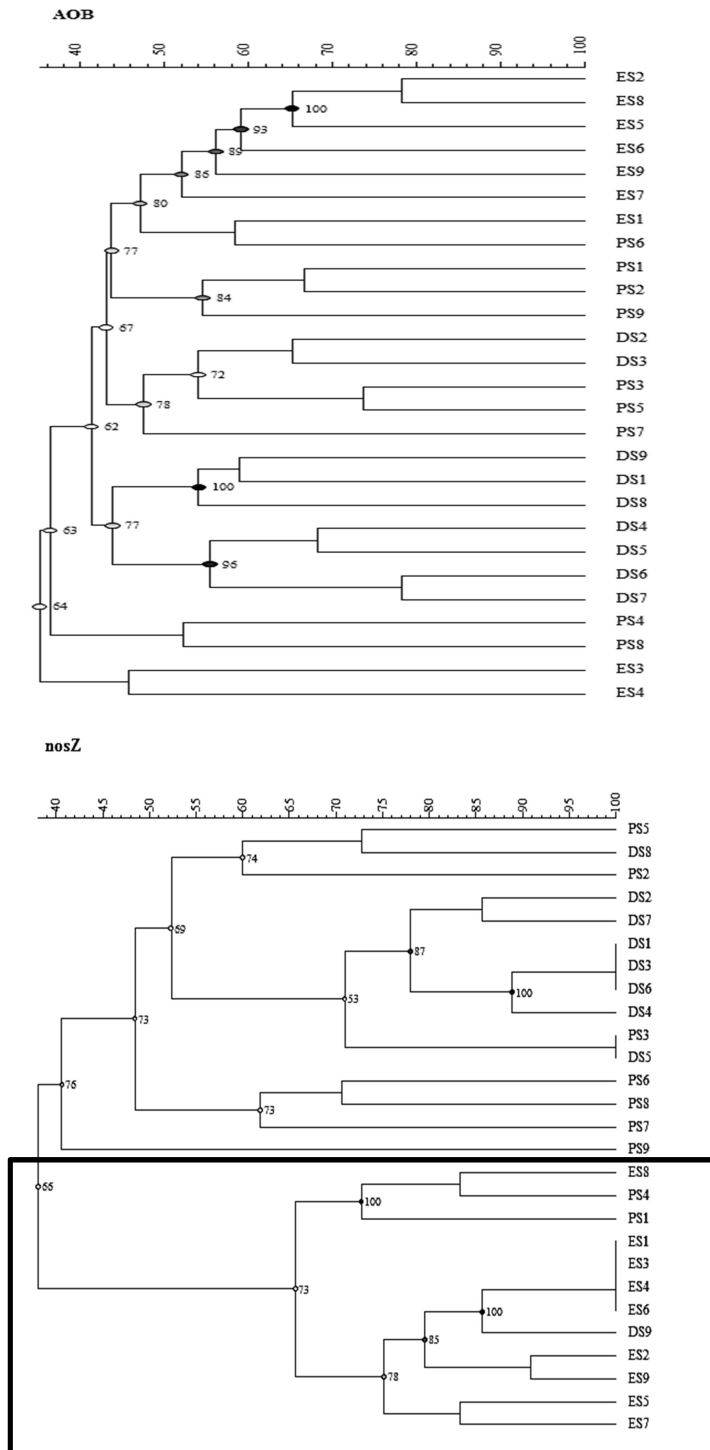


Figure 2. Dendrograms based on TGGE profiles, using UPGMA (Dice coefficient of similarity) of microbial community of soils sampled at a disturbed, experimental and preserved sites. The numbers at the nodes indicate the cophenetic correlation coefficient.

## PLFA

Remarkable differences in the distribution of microbial soil groups on the basis of PLFA concentrations were observed (Table 4). Not all groups responded consistently. Differences found to exhibited relation with the available C and N dynamics and soil flood. Bacterial biomarkers were more expressive than fungi, which consistent with the lowest prevalence of fungi in humid areas (Bossio & Scow 1998; Mentzer et al. 2006; Unger et al. 2009). Biomarkers for bacteria, Gram<sup>+</sup>, AMF, and denitrifying showed ES intermediate between DS and PS. While PLFAs biomarkers for Gram<sup>-</sup>, actinomycetes, fungi, anaerobic and aerobic microorganisms, pose ES as similar to DS.

In the samples, Gram<sup>+</sup> bacteria were usually more abundant than Gram<sup>-</sup>. These patterns suggest that soil moisture conditions maintain an environment favourable to the more anaerobic Gram<sup>+</sup> bacteria (Yu & Ehrenfeld 2010). Gram<sup>+</sup> bacteria (eg. actinomycetes) are known to have a strong association with anaerobic conditions (Sundh et al. 1997), while Gram<sup>-</sup> bacteria are known to associate with aerated conditions (Ponder Jr. & Tadros 2002). Unger et al. (2009) studying the flooding effects on soil microbial communities also observed a decrease in Gram-negative bacterial and anaerobic bacterial markers in soil. Although, in this study this result not reflected at anaerobic group PLFAs that showed lower biomass than aerobic group PLFAs. The anoxic condition would also favor denitrification, increasing ammonium formation, hypothesis not confirmed by denitrifying community (PLFA biomarkers C15:0, C16:0) that was found to be lower in ES than PS (Table 4). Perhaps the denitrifying activity of most denitrifying bacteria in ES could be to suppressed and they cannot catalyze the final step of denitrification (reduction of N<sub>2</sub>O to N<sub>2</sub>) (Baumann et al. 1996).

In general, the concentration of PLFAs of anaerobic organisms done by stress ratio (Cyclopropyl 19/precursory) was lower in ES. Our results also showed that the ratios of saturated to monounsaturated fatty acids were lower ES follows by PS. Bach et al. (2010) also documented that ratios of monounsaturated:saturated PLFAs were highest in stressed soils and decreased in restored soils. The decreases of the ratio imply increased soil resource availability or decreased soil nutrient stress (Yang et al. 2010). Lower ratios in ES and PS are indicative of alleviation of nutrient stress, likely as a result of increased C substrate availability in the soil (Bossio & Scow, 1998).

Respect to AMF community, literature confirms that AMF propagules can tolerate the flooding effect (Harner et al. 2011) and despite the restoration in ES was

recorded intermediary the microbial activity was similar with PS in glomalin content (Table 2), as the glomalin is produced by AMF, supporting Kimura and Scotty (2016). Microorganisms assigned to unclassified existed with a much higher proportion in DS. Unclassified PLFAs occur in both prokaryotic and eukaryotic organisms and can not be classified as being either specifically fungal or bacterial (Mitchell et al. 2010). These results suggest there is a large proportion of microbial community unknown in DS.

Table 4. PLFA concentrations ( $\mu\text{g/g}$ ) of functional groups of microbial community at soils sampled at a disturbed, experimental and preserved sites. Values are mean  $\pm$  SD (n=3).

<i>Functional groups</i>	<i>Disturbed</i>	<i>SD</i>	<i>Experimental</i>	<i>SD</i>	<i>Preserved</i>	<i>SD</i>
	<i>Site</i>	( $\pm$ )	<i>Site</i>	( $\pm$ )	<i>Site</i>	( $\pm$ )
<b>Bacterial biomass</b>	7.20 <sup>c</sup>	1.77	14.36 <sup>b</sup>	2.25	24.40 <sup>a</sup>	14.36
<b>Gram<sup>+</sup></b>	3.26 <sup>c</sup>	1.13	8.20 <sup>b</sup>	0.42	11.48 <sup>a</sup>	1.31
<b>Gram<sup>-</sup></b>	3.55 <sup>b</sup>	0.78	5.76 <sup>b</sup>	0.86	11.25 <sup>a</sup>	2.63
<b>Actino</b>	1.29 <sup>b</sup>	0.30	2.06 <sup>b</sup>	0.26	4.06 <sup>a</sup>	1.09
<b>Fungi</b>	1.30 <sup>b</sup>	0.39	2.38 <sup>b</sup>	0.22	3.92 <sup>a</sup>	0.98
<b>AMF</b>	0.33 <sup>c</sup>	0.20	0.83 <sup>b</sup>	0.07	1.41 <sup>a</sup>	0.35
<b>Algae</b>	0.11 <sup>ns</sup>	0.03	0.07 <sup>ns</sup>	0.01	0.07 <sup>ns</sup>	0.03
<b>Unclassified</b>	2.87 <sup>a</sup>	0.24	0.75 <sup>c</sup>	0.10	1.23 <sup>b</sup>	0.12
<b>Anaerobic</b>	0.98 <sup>b</sup>	0.14	2.54 <sup>b</sup>	0.56	5.08 <sup>a</sup>	1.30
<b>Aerobic</b>	2.18 <sup>b</sup>	0.35	7.19 <sup>b</sup>	0.39	10.70 <sup>a</sup>	1.73
<b>Denitrifying</b>	0.30 <sup>c</sup>	0.10	4.30 <sup>b</sup>	0.25	5.90 <sup>a</sup>	0.80
<b>Bacterial stress ratio</b>	0.45 <sup>a</sup>	0.01	0.25 <sup>c</sup>	0.02	0.39 <sup>b</sup>	0.01
<b>Monounsaturated:saturated</b>	0.70 <sup>a</sup>	0.03	0.39 <sup>c</sup>	0.01	0.50 <sup>b</sup>	0.10

Gram<sup>+</sup>: gram-positive bacteria; Gram<sup>-</sup>: gram-negative bacteria; Actino: actinomycetes; AMF: arbuscular mycorrhizal fungi. Means followed by the same letter in a row are significantly different using Tukey test at  $p < 0.05$ . ns, non-significant.

Another important characteristic of this work is related to denitrification, which is the primary mechanism of removal of  $\text{NO}_3^-$ -N in riparian areas, and considered the reaction responsible for removal of  $\text{NO}_3^-$ -N in PS as shown by the high concentration of denitrifiers in the TGGE and PLFAs profiles (Vidon & Hill 2004). Furthermore, the concentration of denitrifiers PLFAs suggests that denitrification is higher in PS followed by ES and DS, respectively. Unlike what was found in the TGGE, in the PLFAs the denitrifying microorganisms were different among ES and DS. These results associated with the phylogenetic analysis of TGGE profiles suggest there is removal of  $\text{NO}_3^-$  in ES, but this is still different in PS. Therefore, the decrease in nitrate formation



in ES and DS may be attributed to prolonged anaerobic cycles provoked by flooding since the nitrifying community is present. Another factor that may explain the low nitrate concentration in ES and DS is the high ammonium concentration since AOB has been found to be very sensitive to the high concentration of this substrate (Norton & Stark 2011). The anoxic condition would also favor denitrification, increasing ammonium formation, but this hypothesis was not confirmed since the denitrifying community (PLFA biomarkers C15:0, C16:0) was found to be inhibited at the ES. In this way, the ammonium accumulation in these sites is likely to come from an exogenous source as flood waters. Indeed, ammonium-N is extremely soluble and is a major component of raw sewage (Suthar et al. 2010).

### ***Relation between microbial community composition and soil properties***

The PCA shows the variation patterns in PLFA and TGGE profiles between the areas due to soil parameters (Fig. 3). The total variance explained in the first two axes reached 85.35%, and axis 1 represented 73.54% of variation. The properties of the soil NT, NO<sub>3</sub><sup>-</sup>, HA, FA and organic C, except the concentration of NH<sub>4</sub><sup>+</sup>-N, were positively correlated with axis 1 ( $p < 0.05$ ). The PCA1 clearly grouped the areas experimental and preserved, pairing up positively with the data. The disturbed community was strongly influenced by PCA2. Certain PLFAs were strongly associated with the PCA1. C organic content, *Cmic*, NT, and NO<sub>3</sub><sup>-</sup>-N mainly influenced the microbial groups of denitrifying bacteria, *nosZ*, actinomycetes, Gram<sup>+</sup> and Gram<sup>-</sup> bacteria, fungi and AMF, for example.

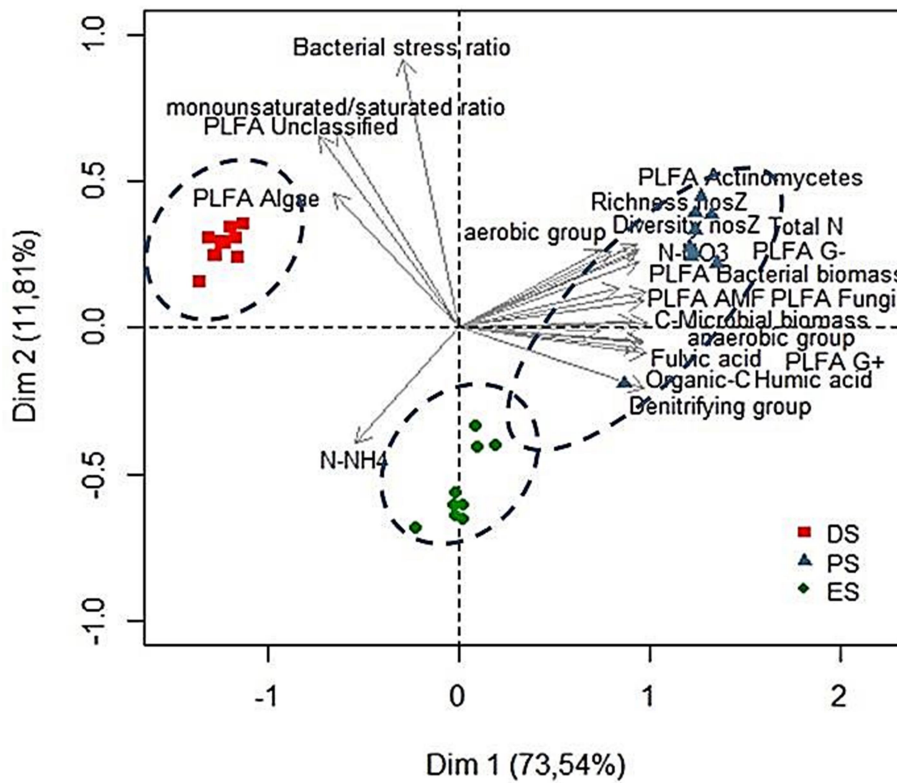


Figure 3. Component principal analysis (PCA) biplot of microbial community's structure fitted predictor variables. Studied areas are abbreviated: Disturbed Site (DS), Experimental Site (ES), and Preserved Site (PS).

It was evident that  $\text{NH}_4^+\text{-N}$  was the most important soil parameter influencing the microbial community in ES and responsible for differentiation between PS and ES. Indeed the external input of this ammonium, probably from the flood events may be modifying the nitrification and denitrification processes, as well as the microbial community related to them (Adair et al. 2004).

In contrast, it was found that a lower concentration of  $\text{NH}_4^+$  in the PS comparative to the other sites, suggesting that besides nitrification, denitrification might be occurring at the preserved site through the traditional or anammox pathways. Anammox pathway produces  $\text{N}_2$  by oxidizing  $\text{NH}_4^+$  with  $\text{NO}_2^-$  reduction under low aerobic conditions in different soils types (Kuypers et al. 2005). The first step of denitrification process consists of the nitrite reduction to  $\text{NO}$  by the nitrite reductase (Nir) enzyme performed by several bacteria (Cutruzzolà 1999) and the final steps are mediated by the nitrous oxide reductase (Nos) enzyme expressed by *nosZ* that reduce  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Zumft & Kroneck 2007) under anaerobic conditions.

The richness and diversity of oxide reductase bacteria (*nosZ*), estimated by TGGE, were increased in PS in comparison to other sites (PS > ES=DS). This result suggests that complete denitrification having N<sub>2</sub> as final product may have prevented the NH<sub>4</sub><sup>+</sup> accumulation in PS. Indeed, under preserved conditions, it was expected a strong stimulation of the entire N cycle. Thus, the high anaerobic denitrifying population in PS, in association with that carrying the *nosZ* gene, was dominant under natural vegetation of preserved site, but did not occur in the managed and disturbed sites. In DS and ES, the soil NH<sub>4</sub><sup>+</sup> accumulation whether by water flooding transportation, by a deficient nitrification or even via an incomplete denitrification (dissimilatory nitrate reduction to ammonium -DNRA) (Cole & Brown 1980), highlight the efficient buffering effect performed by the preserved site in relation to the nitrogen cycle.

Particularly, a significant difference was found between in microbial community of disturbed and the other areas. Besides, this difference was based on the association with algae biomarkers and unclassified and stress indices. Important to realize that soil algae constitute the initial successional stage on substrates which are poor in plant nutrient, and for this reason this group was correlated with degraded soils in this study (Starks & Shubert 1982).

Our results showed that alterations in anaerobic/aerobic microbial structures and denitrifying populations in studied sites were promoted by the flooding and ammonium accumulate in soil, suggesting a distinct degree of resilience among experimental and preserved sites. Therefore, the restoration in ES is progressing towards the PS direction but seems to be retarded by the N-NH<sub>4</sub> accumulation, possibly having as its source the flood waters. Furthermore, a 6 years-old restored site has been considered to be young (Card & Quideau 2010; McKinley et al. 2005). Thus, we still may expect the development of this site to condition similar to PS by overcoming the strong impact of soil NH<sub>4</sub> accumulation and improving resilience.

## **Conclusions**

- Flood affects the variability and bioavailability of organic C and inorganic N in these riparian environments and consequently shape the microbial activity differently.

- Ammonium accumulation in these sites is likely to come from an exogenous source as flood waters. Indeed, ammonium-N is extremely soluble and is a major component of raw sewage.
- Restoration in ES is progressing towards the PS direction but seems to be retarded by the N-NH<sub>4</sub><sup>+</sup> accumulation. We may expect the development of this site to condition similar to PS by overcoming the strong impact of soil NH<sub>4</sub><sup>+</sup> accumulation and improving resilience.

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## Chapter 3

### **Riparian reforestation with a single exotic species restores soil aggregation and porosity but not humic substances**

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# Riparian reforestation with a single exotic species restores soil aggregation and porosity but not humic substances

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## Abstract

A degraded riparian area in the Uberaba River basin (Brazil) was restored by local landowners using a monoculture of the exotic species *Syzygium cumini* (L.) Skeels over 15 years. To assess this riparian restoration, we compared this experimental site (ES) with a disturbed site (DS) and a preserved site (PS) with regard to soil organic matter, humic and fulvic acids (HA and FA), glomalin, soil aggregation and porosity. The results did not show significant differences between ES and PS with regard to soil aggregation and porosity. The cation exchange capacity and the HA and FA contents of ES were significantly different from those in PS and DS. Whereas the HA content of ES was less than that in PS, FA was significantly increased. The order of aromatic composition of humic substances by site was PS > ES > DS and contrasted with the aliphatic composition, which was DS > ES > PS. A principal component analysis based on all variables demonstrated that ES was closer to PS than to DS but that there was no overlap among sites, as PS was driven by humic aromatic substances and the experimental site by fulvic and aliphatic compounds. We concluded that there was a functional recovery of ecosystem services related to soil aggregation and porosity, but the qualitative differences in organic matter formation between the restored and preserved sites were not consistent with ES being fully restored.

**Keywords:** Biodiversity, ecosystem services, fulvic acid, humic acid, restoration, soil aggregation, soil porosity

## Introduction

Ecological restoration is widely used to reverse environmental degradation, conserve biological diversity and mitigate ecosystem service deficiencies (Jackson & Hobbs, 2009). Whereas some authors understand ecosystem services as the result of functional ecosystem relationships promoted by biodiversity across multiple processes over time (Isbell *et al.*, 2014), others consider them to be processes or functions to be consumed or utilized by humanity (Boyd & Banzhaf, 2007). Thus, the concept of ecosystem services may conflict with conventional biodiversity conservation perspectives (Schröter *et al.*, 2014).

Different restoration efforts have been undertaken with the aim of increasing biodiversity and ecosystem services, but the relationship between biodiversity and ecosystem service provisioning remains uncertain (Choi, 2004). Among the recorded cases of successful ecological restoration procedures,

approximately 44% assessed biodiversity rather than ecosystem services, and only 25% focused on the latter (Benayas *et al.*, 2009). Thus, the poor knowledge of ecosystem services does not allow a complete evaluation of restoration success, particularly in riparian zones, where the restoration outcomes remain inconsistent and variable (Balvanera *et al.*, 2006).

The main ecosystem service provided by riparian forests is the protection of both aquatic and terrestrial ecosystems, buffering them against physical, chemical and biotic disturbances (Naiman & Decamps, 1997). Riparian forests ensure water drainage and erosion control through the effects of plant roots and soil aggregation (Bronick & Lal, 2005; Kimura *et al.*, 2017), which also control soil porosity. The latter drives soil water drainage and aeration through macro- and micropore balances (Six *et al.*, 2000).

Soil aggregates are formed by the attachment, flocculation and cementation of humic organic matter with clay and polyvalent cations (Bronick & Lal, 2005). These rearrangements of the primary unit constitute the micro-aggregates (<250  $\mu\text{m}$ ), which, once they are attached to each other by the physical action of plant roots and fungal hyphae and by the cementing

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action of microbial and plant-derived polysaccharides (Six *et al.*, 2000), form macro-aggregates with a size  $>250 \mu\text{m}$  (Tisdall & Oades, 1982). Arbuscular mycorrhizal fungi (AMF) play a special role in micro-aggregate formation not only through the physical actions of their hyphae but also because of the excretion of heat-shock glycoproteins known as glomalin-related soil proteins (PRSGs) (Wright & Upadhyaya, 1998; Rillig *et al.*, 2010).

Humic substances (HSs) composed of humic and fulvic acids are produced via the decay of plants rich in lignin (Di Donato *et al.*, 2016), forming supramolecular associations of heterogeneous molecules held together by hydrophobic interactions (Nebbioso & Piccolo, 2012). According to Di Donato *et al.* (2016), HSs can be classified into the following molecular groups based on the Van Krevelen (1950) diagram: lignin-like molecules, carboxyl-containing aliphatic molecules and condensed aromatic molecules related to black carbon.

Humic acids (HAs) have a higher molecular weight and represent a more condensed fraction of HSs, whereas fulvic acids (FAs) have lower molecular weights and more oxidized substances than HAs (Hertkorn *et al.*, 2002), which are composed of aromatic units with aliphatic chain substituent (Tao *et al.*, 1999). The role of carbohydrates in improving soil structure is variable (Bronick & Lal, 2005), while HAs and FAs are linked to minerals and soil aggregates with aromatic C or aliphatic alkyl-C (Golchin *et al.*, 1994), resulting in a functional role in soil aggregation, porosity and stabilization (Šimanský & Bajčan, 2014). Thus, the quantitative and qualitative variations in HA and FA composition result in changes in the quality of aggregate formation and its role in soil stabilization (Kimura *et al.*, 2017).

Several land management practices (Gupta & Germida, 2015) and alterations in vegetation (Tivet *et al.*, 2013), such as those induced by grazing, can lead to loss of soil carbon (Larreguy *et al.*, 2017), which can modify the soil macro-aggregation, thereby compromising aggregate stability and HA aromaticity (Tisdall & Oades, 1982; Aranda *et al.*, 2011). Conversely, changes in the abundance and diversity of plant species may also lead to soil disaggregation (Gupta & Germida, 2015), losses of soil carbon and HSs (Tivet *et al.*, 2013; Kimura *et al.*, 2017; Larreguy *et al.*, 2017).

The riparian forest in the Paraná River basin located in Uberaba city (Minas Gerais, Brazil) and along its tributary rivers has been converted into pasture for livestock production, resulting in enhanced soil erosion because of the disruption of soil organic matter and structural functions (Valera *et al.*, 2016). Grazing activity has been reported to affect negatively the size of the total soil organic C stock (Larreguy *et al.*, 2017), resulting in the reduction of soil infiltration and acceleration of runoff and erosion (Czeglédi & Radácsi, 2005). Although the Brazilian Forest Code restricts the use of exotic species in the restoration of riparian forests (Soares-Filho *et al.*, 2014), some local landowners have empirically used a fast-growing exotic species *Syzygium cumini*

(L.) Skeels in monoculture systems based on popular reports that this species is useful to improve soil physical properties as soil infiltration and erosion control. Such ecosystem services are particularly influenced by humic substances, soil aggregation and porosity, which have been identified as suitable restoration indicators for riparian sites (Tivet *et al.*, 2013; Kimura & Scotti, 2016; Kimura *et al.*, 2017).

Thus, the specific aim of this study was to evaluate the extent to which some soil properties (e.g. humic substances, soil aggregation and porosity) related to the ecosystem services described above were restored under a low-biodiversity riparian forest planted with a monoculture of the exotic species *Syzygium cumini* (L.) Skeels compared with a native riparian forest with high plant biodiversity. We hypothesized that such soil properties would not be restored under a monoculture with low biodiversity. The wider aim of this study was to provide support for the development of management options to improve ecosystem services in restored areas with single species or exotic species and to shed light on the complex and poorly defined relationships between biodiversity and the provision of ecosystem services.

## Materials and methods

### Study sites

The study area (19°34'07.00"S 47°57'16.41"W) was located in the Uberaba River basin, Minas Gerais State (Figure 1a). The site's geologic profile belongs to the Canastra Group, with conglomerates of Bauru Group and Cenozoic alluvial and colluvial deposits near the streams (Valera *et al.*, 2016). The soil type is dystrophic red and yellow-red latosols (Coutinho *et al.*, 2008; Valera *et al.*, 2016).

The experimental site (ES) belonged to farmland that was used mainly for intensive livestock pasturing and was planted using a monoculture of the exotic species *Syzygium cumini* (L.) Skeels in the first decade of the 21st century. This site was formerly covered by native Cerrado vegetation, similar to that found in PS, which has been cleared for grazing activities, resulting in soil erosion. Based on anecdotal reports of the benefits of *Syzygium cumini* (L.) Skeels for soil erosion control, local landowners have restored the riparian forest, growing this species in monoculture.

The preserved site (PS) hosted a riparian-preserved forest with native vegetation belonging to the Cerrado biome, with a large diversity of plants composed of 35 botanical families and approximately 89 species. The dominant plant species were *Astronium* sp., *Terminalia* sp., *Copaifera langsdorffii*, *Hymenaea courbaril*, *Andira fraxinifolia*, *Machaerium brasiliensis*, *Inga vera*, *Piptadenia gonoacantha*, *Nectandra cissiflora*, *Luehea grandiflora* and *Eugenia florida*.

The disturbed site (DS) was an abandoned riparian area formerly used for grazing, which contained no native vegetation and has been colonized by invasive herbaceous



of the Brazilian Agricultural Research Corporation (Embrapa, 1997).

#### *Fractionation of soil organic matter (SOM) and glomalin-related proteins (PRSG-T)*

Fractionation of sequestered carbon from SOM was performed according to the method described by Dabin (1971) using 15 g of sieved soil/sample to obtain the fraction of carbon represented by humic and fulvic acids. This method is based on acid–base reactions. The addition of alkaline agents to the samples resulted in a soluble alkali fraction, so-called humic substances (HS) and a residue fraction named humin (H). When acidified, the HS fraction produced an insoluble fraction called humic acid (HA) and a soluble fraction known as fulvic acid (FA). The determination of organic carbon in the HA, FA and H fractions was performed as described by Dabin (1971).

Glomalin-related proteins (PRSG-T) were extracted using the method described by Wright & Upadhyaya (1998), and the protein content was estimated using the Bradford method (Bradford, 1976).

#### *<sup>1</sup>H-Nuclear magnetic resonance (NMR)*

HS fractions from each study site (ES, DS and PS) were used for proton NMR with water peak presaturation. Proton NMR spectra were recorded on a Bruker NMR spectrometer operating at a proton NMR frequency of 500.1 MHz. The spectra were run at a 1-ms contact time, 7-KHz spinning speed, 5-s repletion time and a scan period of several hours in a 7-mm OD mass rotor (Kang *et al.*, 2002; Longstaffe *et al.*, 2010).

According to Kang *et al.* (2002) and Longstaffe *et al.* (2010), the H-NMR spectra were divided into three regions: I: 0–3 ppm (aliphatic, aromatic CH<sub>2</sub>, CH<sub>3</sub> and proteins); II: 3–6 ppm (carbohydrates H and lignin-methoxyl); and III: 6–8 ppm (aromatic H, lignin and proteins). The percentages of total intensity for each recalcitrant region were estimated by integrating the <sup>1</sup>H-NMR spectrum within each region, and the compositions of the aromatic, aliphatic and carbohydrate groups were recorded.

#### *Statistical analysis*

The studied variables were compared among sites (PS, DS and ES), and mean comparisons were performed using analysis of variance (ANOVA). The mean comparison among treatments was performed by Tukey's multiple range test. The nonparametric Kruskal–Wallis test (at the 5% probability level) was used when the assumptions of the ANOVA were not met, and multivariate comparisons were

performed using a Nemenyi test. Statistical analyses were carried out in R, version 3.2.2, with a significance level set to 5% ( $P \leq 0.05$ ) for all analyses.

A principal components analysis (PCA) based on the decomposition of a correlation matrix was used to reduce the tested variables (organic C, humic acid, fulvic acid, humin, glomalin, aggregate stability, macro- and microporosity, aromatic, carbohydrate and aliphatic compounds) into the most important predictors of the total data variance (Martens & Naes, 1989). The variables were expressed in terms of the two components that better explained the proportion of the total variance among the study sites. Each component was accompanied by information on the intensity and direction of the variable interrelations through a scatter plot over the PCA created in MINITAB 15.

## **Results and discussion**

### *Soil physical and chemical properties*

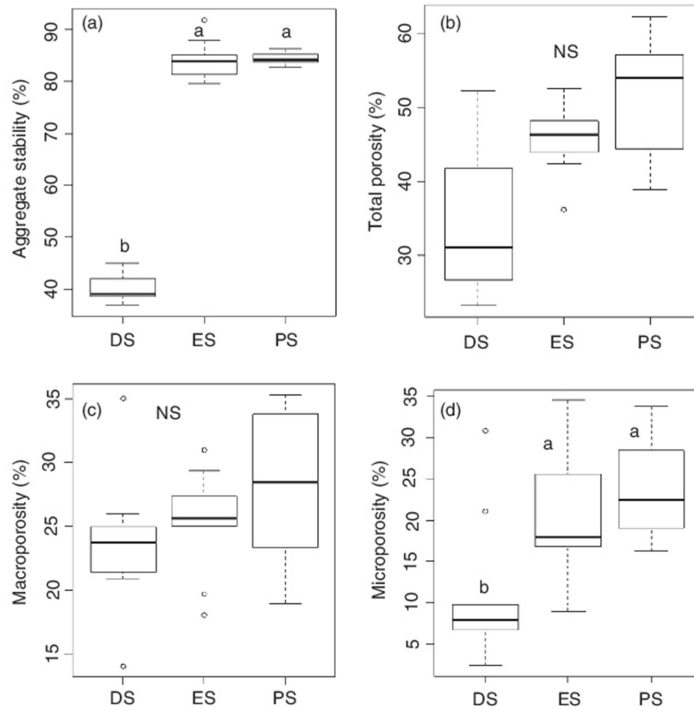
The soil texture of the study sites was characterized by a large sand content relative to that of the clay and silt, with ES having slightly more sand than PS and DS (Table 1). Soil exchangeable Ca, Mg, cation exchange capacity, sum of bases, base saturation and pH were significantly higher in PS compared to both ES and DS. A significantly higher P level at the site was recorded ES than at the other sites. This finding may be attributed to the presence of a fast-growing exotic species with large nutritional demands, as previously demonstrated for *Eucalyptus* species (Correa *et al.*, 2015). These authors showed that fast-growing exotic species stimulate P-related microbial communities such as ecto- and endomycorrhizal fungi and phosphate-solubilizing microorganisms, making P more available in the rhizosphere. ES and PS had similar levels of organic matter (OM), but PS showed a greater CEC, suggesting that the OM produced in PS may be functionally distinct from ES.

Soil structure was evaluated by assessing aggregate stability, total porosity, and micro- and macroporosity (Figure 2). Values of aggregate stability and microporosity in ES differed from those of DS but were similar to those in PS (Figure 2a and d). The total porosity and macroporosity were similar for DS, ES and PS (Figure 2b and c). The lack of differences in total and macroporosity between sites (Figure 2c) can be explained by the large proportion of sand present (Table 1), with a possible increase in percolation capacity. Therefore, the improvement of microporosity in ES may have favoured the restoration of soil drainage, reducing percolation rates. Thus, the similarity of such parameters between the exotic and native forests suggests that the former may provide ecosystem services related to soil structural stability and drainage.

**Table 1** Chemical and physical properties of soils sampled at a disturbed, experimental [i.e. a monospecific plantation of *Syzygium cumini* (L) Skeels] and preserved site

Variables	Sites					
	Disturbed site	SD (±)	Experimental site	SD (±)	Preserved site	SD (±)
Clay (g/kg)	224.67 <sup>NS</sup>	30.7	187.11 <sup>NS</sup>	34.39	191.44 <sup>NS</sup>	35.31
Silt (g/kg)	113.67 <sup>B</sup>	12.58	33.00 <sup>C</sup>	11.28	132.44 <sup>A</sup>	5.89
Sand (g/kg)	661.67 <sup>B</sup>	40.25	780.22 <sup>A</sup>	44.60	676.11 <sup>B</sup>	32.01
P (mg/dm <sup>3</sup> )	5.0 <sup>B</sup>	2.35	18.17 <sup>A</sup>	7.64	8.89 <sup>B</sup>	5.41
K (mmol <sub>c</sub> /dm <sup>3</sup> )	1.04 <sup>NS</sup>	0.40	0.77 <sup>NS</sup>	0.22	1.22 <sup>NS</sup>	0.46
Ca (mmol <sub>c</sub> /dm <sup>3</sup> )	5.11 <sup>C</sup>	2.67	15.67 <sup>B</sup>	6.93	66.72 <sup>A</sup>	47.86
Mg (mmol <sub>c</sub> /dm <sup>3</sup> )	1.67 <sup>B</sup>	0.71	1.94 <sup>B</sup>	0.87	10.67 <sup>A</sup>	4.84
pH	4.27 <sup>B</sup>	0.42	4.14 <sup>B</sup>	0.21	4.81 <sup>A</sup>	0.44
OM (g/dm <sup>3</sup> )	9.44 <sup>B</sup>	4.06	34.33 <sup>A</sup>	7.98	41.44 <sup>A</sup>	20.86
SB (mmol <sub>c</sub> /dm <sup>3</sup> )	7.82 <sup>B</sup>	2.98	18.26 <sup>B</sup>	7.45	76.74 <sup>A</sup>	50.47
CEC (mmol <sub>c</sub> /dm <sup>3</sup> )	61.71 <sup>C</sup>	44.22	85.78 <sup>B</sup>	20.05	121.97 <sup>A</sup>	59.11
BS (%)	19.22 <sup>B</sup>	15.55	22.28 <sup>B</sup>	9.16	54.56 <sup>A</sup>	22.10

BS, base saturation; Ca, calcium; CEC, cation exchange capacity; K, potassium; Mg, magnesium; NS, not significant; OM, organic matter; P, phosphorus; SB, sum of bases. Means followed by same letter in row are not significantly different using Nemenyi test at  $P \leq 0.05$ .



**Figure 2** Comparison of soil variables (a: aggregate stability; b: total porosity; c: macroporosity; d: microporosity) among the sites: DS (disturbed site), PS (preserved site) and ES (experimental site). Different lowercase letters indicate differences at 5% significance (Kruskal–Wallis); NS, no significant differences.

Soil stabilization depends on soil aggregation, which determines soil pore formation and, consequently, water drainage (Six *et al.*, 2000). The formation and stabilization of soil aggregates are driven by soil clay and organic matter

content (Bronick & Lal, 2005) and by microbial communities (Gupta & Germida, 2015), particularly arbuscular mycorrhizal fungi via glomalin production (Six *et al.*, 2000; Rillig *et al.*, 2010).

As soil clay content (Table 1) did not differ among sites, the differences in aggregate stability and microporosity recorded between DS and the other sites (PS and ES) can likely be attributed to the organic matter contribution from plant residues (Table 1) and glomalin production (Figure 3a).

Arbuscular mycorrhizal fungi (AMF), in addition to exerting a physical effect on soil aggregate formation through their hyphae, produce glomalin, which is a special cementing agent for the stabilization of soil aggregates (Wright & Upadhyaya, 1998) and can also be affected by soil management (Rillig *et al.*, 2010). The glomalin content was increased in ES and was comparable to that of PS (Figure 3a), suggesting that AMF were stimulated by the exotic and fast-growing species in the ES, which also explains the high soil P levels at this site (Table 1). Similarly, Kimura & Scotti (2016) related the improvement in the AMF population and glomalin content of the soil with aggregation and stabilization of a restored slope.

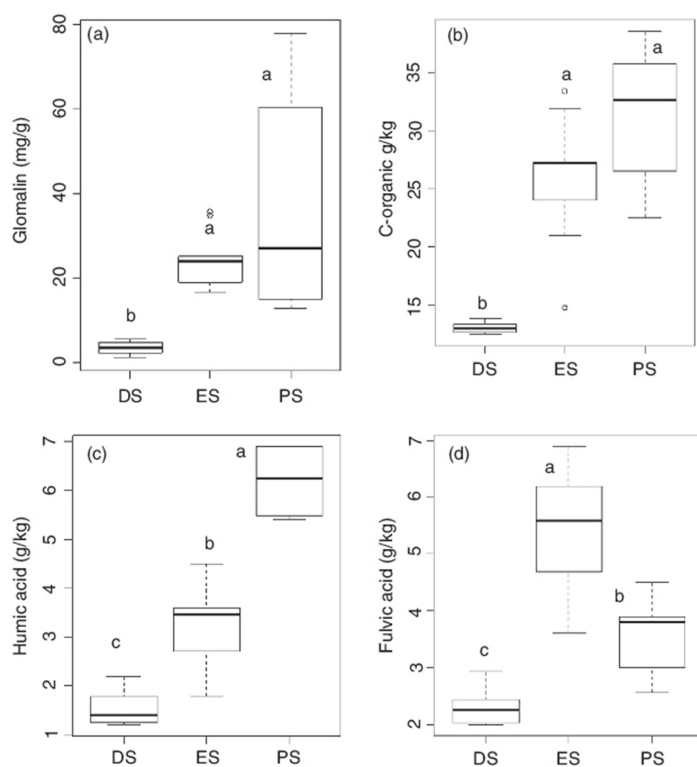
Vegetation type has a strong influence on the decomposition process and its products (Aranda *et al.*, 2011). Although the quantitative SOM formation was similar in ES and PS (Table 1), we may expect qualitative differences as soil organic

matter (SOM) formed from native forest litter may be different from those of monocultures (Lee & Jose, 2003).

#### Humic substances

The chemical profile of SOM may result in different organic stabilizing compounds of HSs (Tisdall & Oades, 1982; Bronick & Lal, 2005). Although soil organic carbon was greater in ES in relation to DS and achieved similar values to PS (Figure 3b), the HA and FA contents of ES were significantly distinct from those of both PS and DS (Figure 3c and d). Whereas there was more HA in PS than in ES, FA was significantly greater in ES than in PS, and the smallest contents of HA and FA were found in DS. Despite these differences between ES and PS in the content of HA and FA, there were no differences between these sites in soil aggregation or porosity.

The formation of aggregates and their stabilization depends not only on the quantity but also on the quality of HS composition (Chizoba *et al.*, 2006; Kimura *et al.*, 2017). HSs show a varied chemical composition with different contributions of C in the aromatic:aliphatic ratio (Golchin *et al.*, 1994), which may result in functional differences in



**Figure 3** Comparison of soil variables (a: glomalin content; b: C-organic content; c: humic acid content; d: fulvic acid content) among the sites: DS (disturbed site), PS (preserved site) and ES (experimental site). Different lowercase letters indicate differences at 5% significance (Kruskal-Wallis); NS, no significant differences.

soil aggregation and porosity (Bronick & Lal, 2005; Šimanský & Bajčan, 2014). While polysaccharides induce a weak and temporary soil aggregation (Tisdall & Oades, 1982), the aromatic groups of HA promote more stable aggregates and microporosity (Chaney & Swift, 1984).

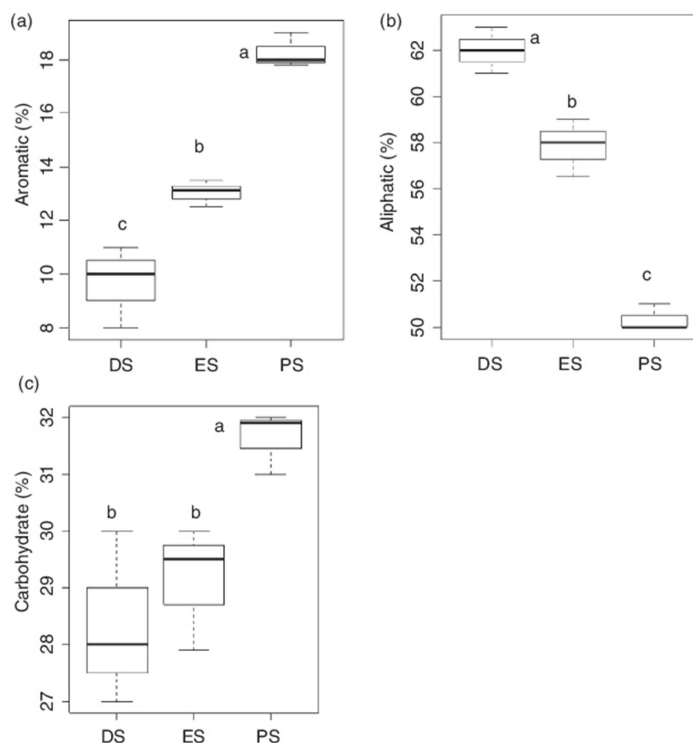
The aromatic composition of HSS at the studied sites (Figure 4a) was found to be in the order PS > ES > DS. In contrast, the aliphatic contribution was in the order DS > ES > PS (Figure 4b). Thus, there was a predominance of recalcitrant aromatic HAs in PS, in contrast with elevated aliphatic compounds in DS. The humic carbohydrate content, although it was greater in PS, was similar in ES and DS (Figure 4c). ES, therefore, appeared in an intermediate position in relation to DS and PS in terms of the quantitative (HA and FA contents, Figure 3b and c) and qualitative composition (aromatic and aliphatic, Figure 4a and b) of HSS. FAs, especially those from aquatic ecosystems, are associated with a little aromaticity (Harvey *et al.*, 1983), with a predominance of aliphatic carboxyl groups (Wilson *et al.*, 1987), whereas aromatic lignin-like and condensed molecules are related to HAs (Tao *et al.*, 1999).

Several land management and environmental factors can modify the aromatic and aliphatic composition of HSS, including (i) changes in vegetation type (Golchin *et al.*, 1997; Tivet *et al.*, 2013), (ii) soil management and amendment

(Aranda *et al.*, 2011), (iii) alteration of the soil microbial community (Gupta & Germida, 2015) and (iv) exotic species management by the enrichment of humic polysaccharide content (Santana *et al.*, 2015).

The dominant vegetation in ES was the exotic species [*Syzygium cumini* (L.) Skeels], while that in PS was composed of native species from the Cerrado biome and that in DS was characterized by a few invasive species. The lower levels of aromatic compounds found in ES compared to PS, which are associated with an increase in FAs, suggest a different humification pattern of the litter from ES. Such differences in the aromaticity of humic substances resulted in a higher recalcitrance in PS than ES and a possible qualitative difference in aggregate formation. In addition, the high sand content found in the soil from ES may have contributed to the formation of rapid and temporary bridges in aggregates favouring unstable and aggregates rich in less recalcitrant FAs. However, the quantitative increase of FA content in ES might have compensated for its lower recalcitrance, resulting in similar aggregation patterns at both sites. Thus, we suggest that the ecosystem services of soil erosion control that are related to aggregation seemed to be similar in ES and PS.

According to Fortun *et al.* (1989), FAs promote a stronger and more rapid soil binding mechanism than HAs do, but



**Figure 4** Comparisons of the contribution of aromatic (a), aliphatic (b) and carbohydrate (c) compounds in the humic sample solutions based on H-NMR spectra. ES, experimental site; PS, preserved site; DS, disturbed site. Different lowercase letters indicate differences at 5% significance (Kruskal–Wallis); NS: no significant differences.



the latter form complexes with clay very slowly, resulting in flocculated and stable aggregates and becoming more resilient to environmental impacts. Indeed, there was a higher HA content in PS, in contrast to ES, which is more susceptible to external factors such as pasturing; this could explain its smaller HA content.

Exotic plant species can quantitatively and qualitatively modify soil biomass inputs compared with native species, favouring a differential chemical selective pressure over the microbial populations involved in the decomposition process (Correa *et al.*, 2015) and its products. While Islam & Weil (2000) observed an increase in SOM, especially FAs, in afforestation systems with the exotic *Acacia* spp. compared to native species, Carrasco-Letelier *et al.* (2004) recorded an increase in humic aliphatic content induced by the conversion of natural prairies to *Eucalyptus* plantations. These alterations highlight the role of litter composition in SOM formation.

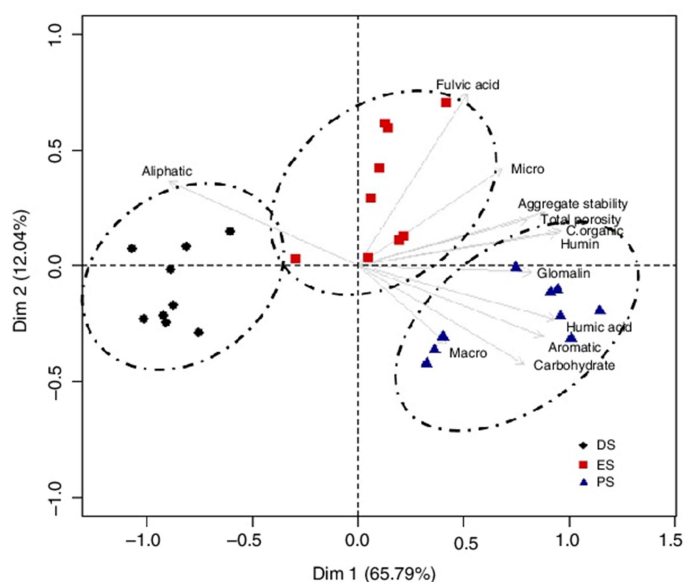
#### Principal component analysis

The first two axes of the principal component analysis (Figure 5) based on the studied variables (organic C, HA, FA, humin, aggregate stability, total porosity, macro- and microporosity, glomalin, carbohydrate, aromatic and aliphatic species) found in each site explained 77.83% of the total variation. Component 1, which was positively related to the variables organic C, HA, humin, aggregate stability, total porosity, macro- and microporosity, glomalin, carbohydrates and aromatic chemical species, explained

65.79% of the variation, while component 2, which was more related to fulvic acid and aliphatic species, explained 12.04%.

The contribution of each variable by site is shown in Figure 5, revealing that samples from each site formed single and separated groups without intersections. The DS samples (black circles) were separated and particularly driven by aliphatic chemical species, in addition to the smaller influence of the other study parameters. The samples from PS (blue triangles) were separated particularly by the HA, aromatic and carbohydrate chemical species contributions. The ES samples (red squares) were under the effect of larger values of FA, in addition to the lesser influence of other parameters. Thus, the dissimilarity of ES and PS was related to the quality of HSS, the main factor segregating the sites in the PCA.

The fact that ES shows intermediate soil properties between DS and PS (Figure 5) indicates that the intervention in ES (plantation of an exotic tree species) has contributed to the restoration of some soil properties, but the chemical soil quality is still clearly different between ES and PS 15 years after the intervention. In contrast, Kimura & Scotti (2016) and Kimura *et al.* (2017), using the same soil parameters to assess restoration of different riparian zones with native species, demonstrated an overlap between samples from ES and PS in the PCA. These authors suggested that the restored sites with native species were progressing towards the preserved site 6 years after restoration. Similarly, McKinley *et al.* (2005) and Card & Quideau (2010) considered that soil quality tends to improve with time, stabilizing 7 years after restoration.



**Figure 5** Principal component analysis based on soil properties measured on disturbed (DS), preserved (PS) and experimental sites (ES). (TP, total porosity; Macro, macroporosity; Micro, microporosity; aliphatic, aromatic and carbohydrate humic compounds; C-organic, fulvic acid; humic acid, aggregate stability).

Because there were no overlaps among the samples from the study sites (Figure 5) and the maturity of the restored forest in ES (15 years old), the study sites PS and ES cannot be said to show the same chemical soil quality. This finding can likely be attributed to differences between PS and ES regarding plant diversity, species composition and age of the vegetation, all of which may have led to differences in soil organic matter quality. As ES achieved a similar pattern to that in PS in relation to soil parameters, such as soil aggregation and porosity, we can assume that the former has been restored from a functional viewpoint. However, it remains distinct from PS based on the quality of SOM as expressed by an increase in fulvic acids and aliphatic compounds with a concomitant reduction in the aromatic fraction of humic acids. Therefore, we did not find a clear relationship between the plant diversity degree and some ecosystem services as measured by soil physical properties in accordance with Choi (2004) and Benayas *et al.* (2009).

### Conclusion

A riparian site with a 15-year-old plantation of *S. cumini* (ES) achieved similar levels of some soil parameters (aggregate stability, porosity, glomalin and organic C) in relation to a native forest but with qualitative differences in soil organic matter (i.e. different amounts of humic and fulvic acids, aromatic, aliphatic and carbohydrate compounds).

Soil aggregation and porosity are related to the ecosystem services of soil stabilization and infiltration, and these soil properties were similar between a diverse native forest and a monospecific tree plantation. No clear correlation was found between plant diversity and these soil properties.

The qualitative differences in soil organic matter between PS and ES indicate that introducing a monospecific tree plantation in a degraded riparian site is insufficient to fully restore soil properties.

To achieve a balance between functional ecosystem services and biodiversity, it is recommended to use more diverse native species assemblages, in this case from the Cerrado biome, in restoration efforts.

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## Conclusões Gerais

Neste estudo demonstramos claramente o papel da vegetação ciliar no movimento e retenção de nutrientes do ecossistema terrestre para o aquático. A remoção ou degradação do ecossistema pode afetar diretamente o fluxo de nutrientes dissolvidos para o ambiente aquático, especialmente para aqueles compostos formados com nitrogênio.

Sua posição estratégica nas margens de cursos d'água torna-as sujeitas a eventos de inundação periódicos o que vem a afetar a variabilidade a biodisponibilidade de nutrientes (ex. carbono e nitrogênio) moldando conseqüentemente a comunidade microbiana do solo ligada a importantes processos biogeoquímicos. A inundação pode também agir como fonte direta de resíduos orgânicos.

Nosso estudo realizado na mata ciliar localizada às margens do Rio das Velhas demonstrou que eventos de inundação embora sejam um fator-chave para este ecossistema atuam como aporte direto de amônio no solo. O acúmulo deste amônio associada aos eventos de inundação parecem estar afetando o tempo de recuperação necessário a floresta e com isso o processo de recuperação está ocorrendo de forma mais lenta.

Nosso estudo desenvolvido em mata ciliar na Bacia do Rio Uberaba, avaliou processo de recuperação de mata ciliar realizado com um plantio de monocultura exótica. Nossos resultados demonstraram que embora a espécie exótica tenha conseguido promover a recuperação de serviços ecossistêmicos relacionados à estabilidade e agregação do solo não houve idêntico resultado quando avaliamos a qualidade da matéria orgânica formada pelo plantio e a comparamos com a matéria orgânica formada em uma floresta ciliar de maior diversidade vegetal. Uma vez que a matéria orgânica do solo participa em diferentes processos essenciais a saúde e manutenção do ecossistema, concluímos que o uso de monocultura exótica não foi suficiente para uma restauração completa das propriedades do solo de uma mata ciliar.