UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-graduação em Ecologia, Conservação e Manejo de Vida Silvestre

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ESTUDO DE INDICADORES BIOLÓGICOS DE RECUPERAÇÃO DE MATA CILIAR ÀS MARGENS DO RIO DAS VELHAS.

Belo Horizonte
Janeiro de 2016

Andrei Kimura Marcondes de Castro

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Versão final

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Orientadora: Profa. Dra. Maria Rita Scotti Muzzi

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Andrei Kimura Marcondes de Castro

Ao vigésimo nono dia do mês de janeiro do ano de dois mil e dezesseis, às quatorze horas, na sala 259, Bloco L 2, do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, teve lugar a defesa de Doutorado no Programa de PG ECMVS, de autoria do Doutorando Andrei Kimura Marcondes de Castro, intitulado: **"Estudo dos indicadores biológicos de recuperação de mata ciliar as margens do Rio das Velhas."** Abrindo a sessão, a Presidente da Comissão, Profa. Dra. Maria Rita Scotti Muzzi, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra para o candidato para apresentação de seu trabalho. Estiveram presentes a Banca Examinadora composta pelos Professores: Profa. Dra. Maria Aparecida de Rezende Stoianoff (UFMG), Profa. Dra. Maria Giovana Parizzii (GEOLOGIA/UFMG), Dra. Izabel Chaves (Pós-Doc/UFMG); Profa. Dra. Danielle Letícia Silva (Pós-Doc/UFMG) e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do candidato. Após a argüição, apenas os Srs. Examinadores permaneceram na sala para avaliação e deliberação acerca do resultado final, sendo a decisão da banca pela:

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Nada mais havendo a tratar, a Presidente da Comisão encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 29 de janeiro de 2016.

Comissão Examinadora	Assinatura
Profa. Dra. Maria Aparecida de R. Stoianoff	Maria Aparecida de Rezende Stoianoff
Profa. Dra. Maria Giovana Parizzi	Maria Giovana Parizzi
Dra. Izabel Chaves	Izabel Chaves
Dra. Danielle Letícia Silva	Danielle Letícia da Silva
Profa. Dra. Maria Rita Scotti Muzzi	Maria Rita Scotti Muzzi

À minha amada filha Cecília.

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Resumo

Com o objetivo de reabilitação de áreas de mata ciliar às margens do Rio das Velhas, tendo como fim último o controle de erosão, estabilidade e permeabilidade frente ao fator inundação, foi feita a implantação de uma vegetação protetora do talude (margem esquerda – Capítulo 2) e de uma mata ciliar inundável (margem direita – Capítulo 3). A evolução da reabilitação foi monitorada através do estudo de indicadores físico-químicos e biológicos. As variáveis monitoradas e utilizadas como indicadores da recuperação foram aqueles relacionados com o controle da erosão e favorecimento da permeabilidade, tais como conteúdo da matéria orgânica húmica do solo, qualidade da matéria orgânica do solo, agregação e porosidade do mesmo. Além disso foram estudados os parâmetros relacionados com a formação de agregados do solo tais como: população microbiana do solo com especial ênfase para fungos micorrízicos e sua produção de glomalina. Estas variáveis estudadas na área experimental foram comparadas com dados obtidos de uma área preservada e uma área impactada contigua àrea experimental.

Na reabilitação do talude (Capitulo 2) registramos uma significativa contribuição da matéria orgânica do solo e dos fungos micorrízicos para agregação e estabilização dos agregados na área experimental. Apesar da inundação ocorrida foi possível registrar o papel da glomalina neste processo de agregação. A inundação ocorrida na área experimental não comprometeu a estabilidade da área e nem o plantio, o que indica o sucesso do procedimento de reabilitação e o valor dos indicadores bióticos utilizados na aferição da recuperação. Na reabilitação da mata ciliar inundada (Capítulo 3) foi possível estabelecer novos indicadores de recuperação e de estabilização da floresta. Estes indicadores se relacionaram especialmente com a qualidade da matéria orgânica húmica que influenciou significativamente o processo de estabilização e drenagem da mata ciliar. Baseando-se nestes indicadores foi possível concluir que houve recuperação funcional da floresta.

Palavras-chave: Fungos micorrízicos arbusculares, glomalina, ácido húmico, alteração de solo, biomassa microbiana, estabilização de margens de rio.

Abstract

With the objective of rehabilitating riparian forest areas on the banks of the Rio das Velhas, with the ultimate aim of controlling erosion, stability and permeability in the face of the flooding factor, a protective vegetation of the slope was implemented. (left bank - Chapter 2) and a flooded riparian forest (right bank - Chapter 3). The evolution of rehabilitation was monitored through the study of physical-chemical and biological indicators. The variables monitored and used as recovery indicators were those related to erosion control and permeability favoring, such as soil humic organic matter content, soil organic matter quality, aggregation and soil porosity. In addition, parameters related to the formation of soil aggregates were studied, such as: soil microbial population with special emphasis on mycorrhizal fungi and their production of glomalin. These variables studied in the experimental area were compared with data obtained from a preserved area and an impacted area adjacent to the experimental area.

In the rehabilitation of the slope (Chapter 2) we recorded a significant contribution of soil organic matter and mycorrhizal fungi for aggregation and stabilization of aggregates in the experimental area. Despite the flooding, it was possible to record the role of glomalin in this aggregation process. The flooding in the experimental area did not compromise the stability of the area or the planting, which indicates the success of the rehabilitation procedure and the value of the biotic indicators used in the assessment of recovery. In the rehabilitation of the flooded riparian forest (Chapter 3) it was possible to establish new indicators of forest recovery and stabilization. These indicators were especially related to the quality of the humic organic matter that significantly influenced the process of stabilization and drainage of the riparian forest.

Based on these indicators, it was possible to conclude that there was a functional recovery of the forest.

Keywords**:**. Arbuscular mycorrhizal fungi, glomalin, humic acids, landscape alteration, microbial biomass, river bank stabilization.

Sumário

Capítulo 1

1 – Introdução Geral

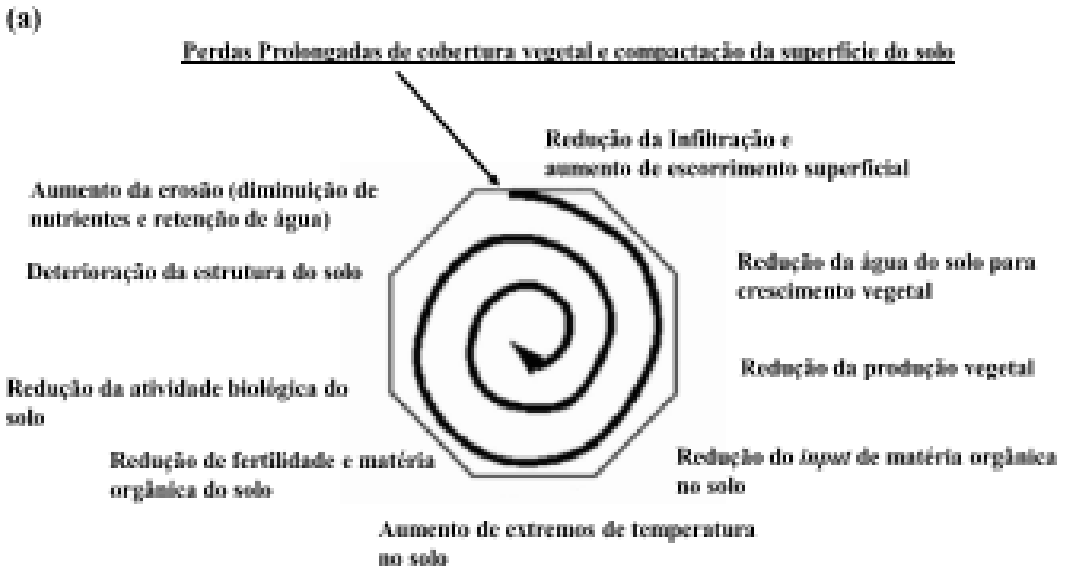
Recuperação de áreas Degradadas

Inúmeros fenômenos físicos e biológicos estão envolvidos na complexidade dos processos de degradação ou recuperação de áreas. Por isso a recuperação destas áreas depende de um conjunto de ações interdisciplinares planejadas e executadas por diferentes profissionais. A gravidade das alterações nas matas ciliares tem preocupado governantes e pesquisadores para criação de sistemas que tamponem o movimento superficial do solo e dos agroquímicos promovidos pela lixiviação do mesmo. Tais sistemas incluem: corredor arbóreo, barreiras físicas e vegetacionais e faixas de permeação. A mata ciliar por si é um sistema florestal tampão capaz de remover nutrientes, sedimentos, matéria orgânica solúvel, pesticidas e demais agro-químicos presentes no material de erosão superficial ou na água subterrânea.

A impermeabilização do solo está entre os grandes desafios da ciência causados pelo crescimento dos centros urbanos. A principal consequência deste impacto foi o aumento da vazão e da velocidade água nos rios receptores, resultando em grandes enchentes com elevado poder de destruição. Segundo Schueler (1995) este aumento da vazão de águas e das enchentes contribuem para a instabilidade das margens dos rios que resulta no aumento da erosividade o que, por sua vez, causa assoreamento comprometendo a biota aquática, a atividade pesqueira e a navegabilidade. A degradação ambiental está relacionada à perturbação funcional do ecossistema.

Whisenant (1999, 2002) propôs um modelo conceitual mostrando que o processo de degradação ambiental é composto por perdas ambientais sucessivas caracterizando um processo helicoidal dinâmico e interativo que

resulta na degradação dos componentes do ecossistema (Figura 1). De acordo com o modelo, cada uma dessas mudanças, provoca outras mudanças, que geram uma espiral de declínio da função e estrutura do ecossistema (Figura 1A). Esse modelo também pode ser interpretado em conjunto com uma matriz de estrutura/função de componentes bióticos/abióticos que em direção inversa conduziria à recuperação. Quando associamos os dois modelos, cada uma das etapas da espiral de Whisenant (1999, 2002) pode ser classificada como estrutural ou funcional, envolvendo componentes bióticos ou abióticos (Figura 1B). Baseando nos dois modelos observa-se que mudanças na estrutura acarretam mudanças na função e vice-versa, assim como, mudanças no componente abiótico alteram o meio biótico e vice-versa. De acordo com o modelo de Whisenant (1999, 2002) se o fator estressante persistir e continuar gerando degradação, o ecossistema será comprometido cada vez mais com perdas de sua estrutura e função, gerando rupturas progressivas dos elementos bióticos e abióticos.A perda prolongada da vegetação (estrutura biótica) resulta na perda da matéria orgânica do solo (estrutura abiótica), modificando sua porosidade. A redução da porosidade resulta no



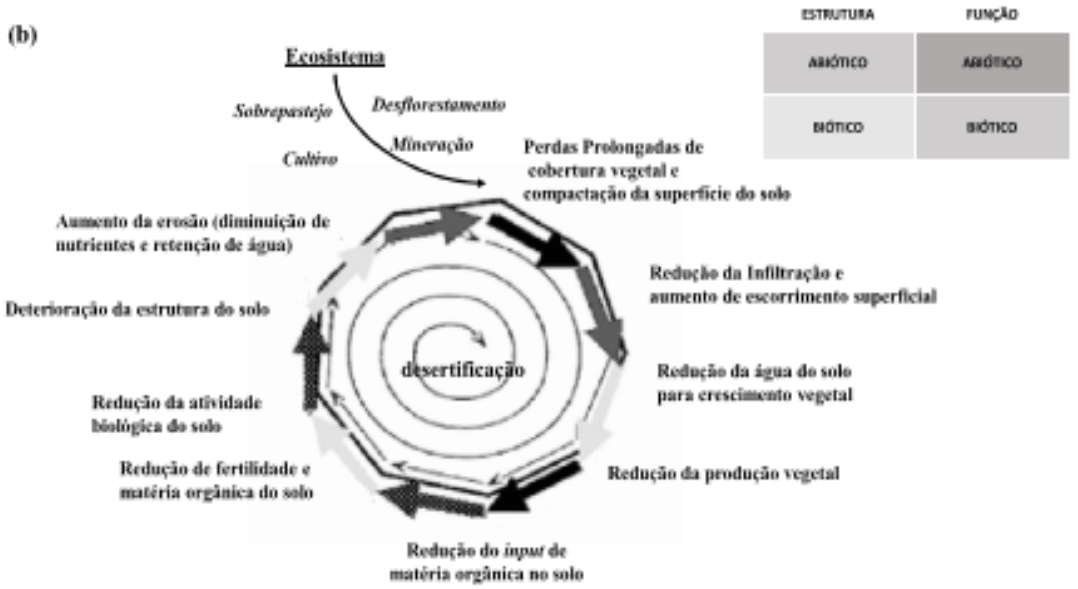
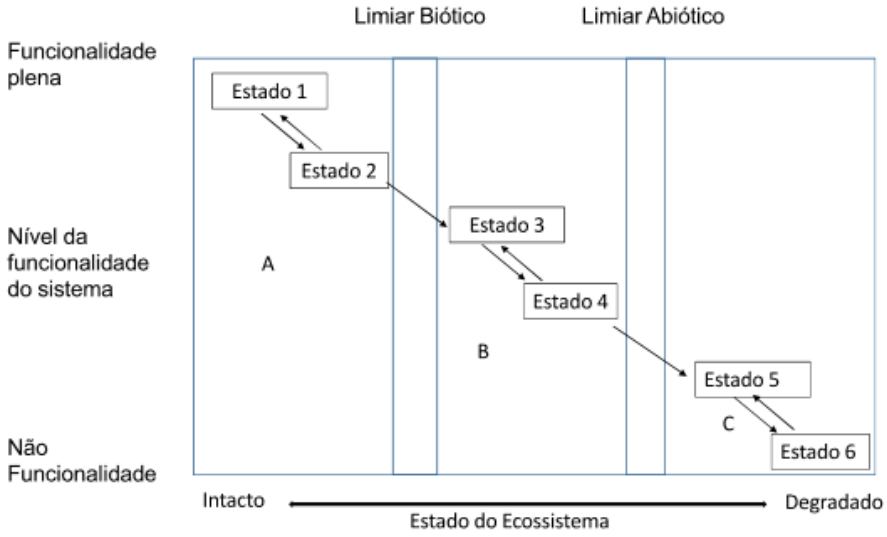


Figura 1: Etapas envolvidas no processo de degradação de um ecossistema e seu potencial de resilencia: (a) degradação do solo caracterizada por etapas com feedback, resultando em uma degradação em espiral e (b) classificação neste modelo em estrutural e funcional, biótico e abiótico comprometimento da permeabilidade do solo (função abiótica) e da atividade biológica do solo (funções bióticas) o que configura o impedimento dos níveis de sucessão (biótico), deflagrando um processo erosivo (abiótico). Inicia-se, assim, um perigoso ciclo de perda de fertilidade do solo com diminuição dos níveis de humificação da matéria orgânica do solo, que é a substância agregante e cimentante, estruturadora da porosidade do solo. A desagregação do solo, em seus extremos, pode significar erosão e/ou desertificação. A persistência do impacto agrava e amplifica a magnitude do problema que, em um processo em espiral, culmina com a desertificação ao longo do tempo.

A partir deste entendimento surgiu o conceito de limiares abióticos e bióticos, proposto por Whisenant (1999, 2002) e Hobbs e Harris (2001) (Figura 2). Há três principais etapas de degradação, com limites ou barreiras ecológicas que as separam. No diagrama, da esquerda para a direita, na primeira fase (A), a função biótica é degradada, mas o sistema tem ainda a capacidade de

recuperação autógeno (resiliência) se a causa da degradação for removida. Se a degradação persistir, o primeiro limiar ecológico é rompido o que representa perdas da função biótica. Se um ecossistema já ultrapassou este limiar, fase (B) haverá perda de resiliência e o ecossistema não retorna aos padrões funcionais originais (A). Se o ecossistema não sofrer intervenções e se permanecer o efeito do agente degradante ocorre a ruptura do terceiro limiar que é o abiotico (C). Nesta terceira fase do diagrama (C), os processos abióticos foram comprometidos e as funcções abioticas serão progressivamente perdidas ao longo do tempo (Figura 2) podendo resultar na instalação de um processo de desertificação.



(Fonte: adaptado de Hobbs e Cramer, 2008. Hobbs, 2007).

Figura 2- Níveis de degradação funcional de um ecossistema

A proposta para recuperação de uma área degradada dependerá do diagnóstico ou níveis de degradação A, B ou C aferidos (Whisenant 1999, 2002; Hobbs e Harris 2001). Baseando-se nos conceitos propostos por por Hobbs e Cramer (2008) e aqueles apresentados por Chazdon (2008) (Figura 3) os modelos de recuperação podem ser resumidos da seguinte forma:

No nível A: Existe perturbação da área sem ruptura das funções bióticas e abióticas e em geral ocorre resiliência. Nesse caso a recuperação se faz naturalmente ou com simples enriquecimento da área e manejo, ocorrendo retorno às condições originais. Este tipo de recuperação é conhecida como RESTAURAÇÃO ou Regeneração. Corresponde ao nível de recuperação 3 proposto por Chazdon (2008) (Figura 3)

(Fonte: adaptado de Chazdon ; 2008)

Figura 3 - Níveis de recuperação de uma área degradada

No nível B: Existe perda da atividade biológica em diferentes graus com ruptura do limiar biológico. Em geral a recuperação biológica não ocorre naturalmente e quando ocorre é extremamente lenta com graus de eficiência imprevisíveis. Assim, torna-se imprescindível a recuperação biológica que se baseia na revegetação e na recuperação das atividades bióticas do solo/água. A recuperação funcional é, em geral, muito bem sucedida podendo ser equiparável a área primitiva (referência) mas o retorno às condicões primitivas não ocorre. Este tipo de recuperação vem sendo denominada revegetação ou reflorestamento. Corresponde ao nível de recuperação 2 proposto por Chazdon (2008) (Figura 3).

No nível C: Não há resiliência e ocorrem diferentes graus de perda física que culminam com o processo de desertificação. A recuperação se baseia na criação de **um novo ecossistema** funcional baseada em intervenções abióticas e bióticas pois esses 2 limiares foram rompidos, procura-se uma recuperação funcional sem obrigatória referência à área original. A recuperação é mais complexa devido à ruptutra dos limiares abióticos e bióticos. Este tipo de recuperação é denominado REABILITAÇÃO. Corresponde ao nível de recuperação 1 proposto por Chazdon (2008) (Figura 3).

Numerosos atributos podem ser considerados quando pretendemos definir metas de recuperação (Hoobs e Harris, 2001), por exemplo, a combinação de práticas de engenharia com critérios ecológicos tem efeito imediato, além de promover uma estruturação do solo em longo prazo possibilita o desenvolvimento da vegetação na área (Ming-Han Li e Eddleman, 2002). As técnicas de revegetação têm avançado muito com a utilização de modelos elaborados, baseados nos conceitos de sucessão secundária e na composição da vegetação original impactada (Rodrigues e Gandolfi, 1998; Sutili, 2007).

A recuperação de uma área com processo erosivo consiste na estabilização do solo e na recuperação da função de permeabilidade e aeração. Para tanto será necessário aumentar a agregação do solo, que por sua vez, depende da qualidade da matéria ôrganica húmica. A qualidade do litter proveniente da vegetação e da atividade biológica decompositora sobre o mesmo são os elementos que regem a dinâmica da agregação do solo. Desta forma, o tipo de vegetação ciliar pode ser fator determinante na estabilização de solos e por conseguinte para o ecossistema prosperar (Ming-Han Li e Eddleman,, 2002).

Quando se pensa na recuperação desses ambientes surge uma questão de ordem prática: existe alguma maneira de se conhecer o grau de degradação ou se o ecossistema ultrapassou os limiares de degradação? A literatura mostra que muitos pesquisadores têm desenvolvido diferentes metodologias para avaliação do grau de funcionalidade dos ecossistemas por meio da avaliação de diferentes indicadores, (Tongway e Hindley, 2004 e Pyke et al. 2009). Entretanto, torna-se imprescindível a definição de indicadores que explicitem o

sinergismo entre os processos funcionais abióticos e bióticos. Dessa forma, torna-se necessária a busca de tais indicadores.

2 - Revisão de literatura

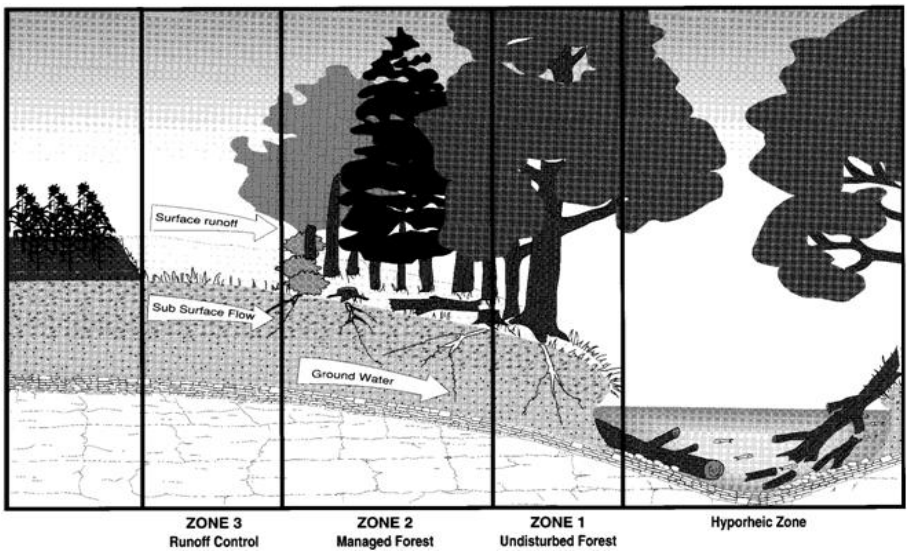
2.1 - Modelo de Recuperação de Mata Ciliare

Quando se trata de reabilitação de uma mata ciliar o aspecto sucessional não é a preocupação imediata. Nesse caso o sucesso da recuperação depende da recuperação das carcaterísticas físicas, quimicas e biológicas do solo. A recuperação fisica da mata ciliar consiste na implantação de procedimentos para contenção do processo erosivo e do assoreamento. O uso de componentes vegetais associados a técnicas de engenharia tem surtido bons resultados no controle de processos erosivos, como por exemplo os enrocamentos, que são, entre vários, métodos de proteção das margens dos rios, também chamados de RSP (Rock Slope Protection) ou rip-rap (Holanda et al., 2008; 2009).

Welsch (1991), propôs uma orientação funcional baseada na capacidade da Mata Ciliar que especifica o sistema tampão constituído de três zonas funcionais responsáveis pela remoção e controle do excesso de nutrientes, sedimentos, matéria orgânica, pesticidas, metais pesados e demais poluentes e capacidade de drenagem e corporação. Segundo este autor as zonas de tamponento ripárias devem ser divididas em três faixas funcionais (Figura 4).

As funções destas regiões dependem a princípio da vegetação selecionada para cada uma destas onde a biomassa produzida em termos de qualidade e quantidade alteram esta função (Grime 1998; Grman et al. 2010; Sasaki e Lauenroth 2011).

A escolha das espécies e das intervenções físicas na recuperação da mata tendo em vista a funcionalidade ecológica é decisiva. Assim sendo a compreensão dos processos funcionais envolvidos nas zonas é condição de aferição da efetividade da recuperação (Qiua e Dosskey 2012).



Fonte: (Welsch, 1991)

Figura 4 – Esquematização da distribuição das zonas.

Segundo Gonzalez et al. 2015, raramente o monitoramento de resultados é aferido, recomendando que se deve incorporar conceitos ecológicos de restauração nos procedimentos de recuperação funcional.

3 – Erosão

Citam Bertoni e Lombardo Neto (1990) que a erosão é o processo de desprendimento e arraste acelerado das partículas do solo, causado pela água e pelo vento. No entanto, ações antropogênicas podem acelerar esse processo em intensidade (Vilela Filho, 2002).

Para contenção do processo erosivo sob o ponto de vista físico, a literatura descreve diversas técnicas mecânicas, sendo que as mais aplicadas são: gabiões, sacos de aniagem, “rip-rap’s”, mantas, paliçadas, enrocamento, retaludamento, dentre outras.

Dentre as utilizadas neste trabalho destacam-se o enrocamento e (Figura 5) constituindo de pedras material proveniente do resíduo de construção civil assim como estacas de madeira. O enrocamento é muito utilizado na

regularização de margens de rio, construção de barragens e na proteção da face de montante de barragens de terra, servindo, nesse caso, como proteção contra a erosão em virtude de sua resistência.

<table>
<tr><th colspan="4">Enrocamento</th></tr>
<tr><th colspan="2">Revestimento
(proteção contínua)</th><th colspan="2">Diques ou espigões
(proteção não contínua)</th></tr>
<tr><th colspan="2">Flexíveis</th><th colspan="2">Flexíveis</th></tr>
<tr><th>Natural</th><th>Sintético</th><th>Natural</th><th>Sintético</th></tr>
<tr><td rowspan="3">Lançado

Arrumado</td><td>Bolsas de concreto

Bolsas de solo-cimento

Bolsas de argamassa

Blocos de cimento</td><td rowspan="5">Lançado

Pilares de concreto ou madeira</td><td rowspan="5">Bolsas de concreto

Bolsas de solo-cimento

Bolsas de argamassa

Blocos de cimento</td></tr>
<tr><td colspan="2">Rígidos</td></tr>
<tr><td colspan="2">Argamassados

Com injeção de consolidação</td></tr>
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Figura 5 : Tipos de enrocamento.

3.1 – Importância da (M0) do solo no processo erosivo

O interesse na matéria orgânica no solo (MOS), segundo Sollins et al. (2007), tem crescido com a preocupação dos aumentos dos níveis atmosféricos de CO_2. Os maiores níveis de carbono sequestrado no planeta são encontrados na matéria orgânica do solo, o que é economicamente importante como fonte de crédito de carbono nos manejos florestais.

A MOS apresenta uma função central no funcionamento do ecossistema como reserva de nutrientes devido a sua alta capacidade de troca catiônica (CTC), influência sobre o pH, a porosidade do solo e sua drenagem (Zech et al.,1997) e especialmente a estabilidade do solo através da agregação. A capacidade agregante da MOS permite a formação de poros responsáveis pela capacidade de aeração e filtração de um solo, afetando a produtividade do mesmo. A MOS é a fonte de nutrientes para a biota, estimulando processos fisiológicos e bioquímicos relacionados com o metabolismo celular (Canellas et al.,1999). Portanto, a preservação da SOM é crucial para a sustentabilidade do ecossistema (Denef et al. 2007).

A microbiota do solo é a principal responsável pela decomposição dos resíduos orgânicos, pela ciclagem de nutrientes e pelo fluxo de energia dentro do solo, exercendo influência tanto na transformação quanto na estocagem do carbono e nutrientes minerais, (Jenkinson e Ladd, 1981; Gregorich et al.,1997) estando envolvida nos processos de formação de ácidos húmicos, (Stevenson, 1985). A biomassa microbiana se modifica com o substrato presente, sendo a resultante do manejo do solo, ou seja, mudanças funcionais (Gregorich et al.,1997).

A MO contém 80 a 90% do carbono total do solo e pode ser dividida em fração lábil não humificada (restos vegetais e animais pouco decompostos) e fração humificada recalcitrante (resistente ao ataque microbiano, denominada substâncias húmicas), como descrito por Theng *et al*. (1989). Os ácidos húmicos (AHs), constituintes das substâncias húmicas estáveis resistentes à degradação, constituem substâncias ionizadas capazes de estabelecer reações químicas com argila, areia e silte, (Stevenson,1994), e juntamente com os

exopolisacarideos bacterianos e hifas fúngicas formam agregados estáveis. A humificação se inicia com a decomposição da lignina cuja única fonte são os vegetais. Seguem-se reações bioquímicas para formação de compostos orgânicos mais complexos em um processo de óxido-redução, baseada na síntese e/ou resíntese dos produtos da mineralização da lignina. A literatura mostra que o ciclo que se processa a humificação da matéria orgânica do solo varia de 8 a 12 meses (Stevenson,1994).

A estrutura do solo é definida como o tamanho e arranjo de partículas e poros no solo (Six *et al*. 2000). Solo com boa estrutura apresenta um equilíbrio de seus poros que permitem aeração e drenagem adequada para o crescimento vegetal. Além disto, solo com boa estrutura apresenta estabilidade dada pelos seus agregados. Os agregados são formados pelas interações físico-quimicas entre matéria orgânica, areia e argila (An *et al*. 2010). Estas forças interativas são estabilizadas pelos polissacarídeos e mucilagens microbianas e hifas fúngicas. Duas categorias de agregados são formadas: Os macroagregados (> 250 µm) e microagregados (< 250µm) asseguram a resistência do solo contra as forças de ruptura da água e outros fatores. (Six *et al*. 2000) devido a ligações químicas catiônicas fortes e multivalentes que formam pontes entre coloides e argilas (Barthè e Roose, 2002; An *et al.,* 2010).

3.2 – Importância dos fungos micorrízicos arbúsculares (AMF) no processo erosivo

Dentre os fungos do solo, os fungos micorrízicos arbusculares (AMF) formam associações mutualísticas com cerca de 80% das plantas terrestres. Os AMF desempenham funções múltiplas nos ecossistemas, favorecendo o crescimento das plantas, participando da decomposição da matéria orgânica do solo, agregando essa matéria orgânica pelo efeito físico das hifas e pela produção de substâncias agregantes como a glomalina (Cardoso e Kuyper 2006).
Esses fungos em associação com as várias espécies vegetais influenciam seus hospedeiros (Johnson *et al.*,1997), favorecendo a aquisição de nutrientes, especialmente aqueles com baixa mobilidade no solo, como o P e Zn, e

recebem em troca carboidratos como fonte de energia (Smith e Read, 1997). Dessa forma, auxiliam no estabelecimento e crescimento das plantas (Marques *et al*., 2001; Scotti e Correa, 2004), na diversidade e sustentabilidade em diferentes ecossistemas (Van der Heijden et al., 1998).

Como mencionado, a eficiência na aquisição de nutrientes varia entre as espécies de AMF e, por isso, a população nativa apresenta uma variabilidade de efeitos no crescimento vegetal. Portanto, a composição de espécies de AMF nas plantas e no solo deve ter conseqüências importantes para a produtividade vegetal (Van der Heijden *et al.*,1998; Marques *et al*. 2001).

As ligações eletrostáticas entre todos os elementos do solo se fazem por meio das cargas geradas na ionização da matéria orgânica do solo e pelos exopolissacarídeos microbianos, responsáveis, em última análise, pela estruturação dos agregados e pela porosidade do solo. A população fúngica tem um papel importante na atividade decompositora da matéria orgânica contribuindo para estruturação dos agregados formados (Marques *et al*. 2003; Aristizábal *et al*., 2004).

A melhoria da estrutura e de agregação do solo influencia diretamente a estabilização do solo (Wright e Upadhyaya,1998). Este efeito se faz não só através da trama de hifas no solo aumentando a agregação de partículas como os demais fungos (Nichols, 2003), mas também por uma característica impar dos AMF que é a capacidade de produzir na parede do seu micélio externo uma glicoproteína com forte capacidade iônica (Wright e Upadhyaya, 1996). A glomalina apresenta elevado conteúdo de carbono, características hidrofóbicas, recalcitrante, sendo uma molécula muito estável capaz de proteger as hifas das perdas de nutrientes e água (Wright e Upadhyaya, 1998). A glomalina tem uma vida média entre 6 a 42 anos, (Rillig *et al*., 2001) e vem sendo correlacionada com a estabilidade dos agregados (Wright e Upadhyaya,1998; Rillig *et a*l., 2001).

A utilização de espécies nativas inoculadas com rizóbio e AMF, em substituição à adubação química, vem sendo especialmente interessante para recuperação de áreas degradadas, (Marques *et al.*,2001; Scotti e Correia, 2004; Duarte *et al*. 2006).

Os principais obstáculos à pesquisa com AMF são a incapacidade de se conseguir culturas axênicas e a dificuldade na identificação de espécies, principalmente quando elas estão presentes somente na fase vegetativa (micelial). A maior parte do conhecimento sobre AMF no ambiente se baseia em contagens de esporos e hifas, porém a população de esporos no solo pode não refletir a composição de espécies presentes na rizosfera (Husband *et al*., 2002).

3.3 – Importância da agregação do solo para o processo erosivo

A estrutura do solo é o fator chave para o seu funcionamento, na sua capacidade de suportar a vida animal e vegetal por armazenamento de nutrientes e permeamento de ar e água. Um dos indicadores da estrutura do solo é a estabilidade dos agregados (Six *et al*., 2000, AN *et al.,* 2010). A agregação de partículas depende da capacidade de arranjo das mesmas, assim como de floculação e cimentação. A capacidade de floculação depende do tipo de partícula (capacidade iônica) e a capacidade cimentante depende da matéria orgânica (raízes, exopolissacarídeos bacterianos, eficiência das hifas fúngicas no seu papel físico agregante. A agregação interfere diretamente na porosidade e, portanto, na capacidade de retenção de água e aeração do solo. A importância da estabilidade do solo, durante o processo de recuperação de um sistema perturbado, demonstra a necessidade de implementação de estratégias que promovam a formação de agregados, como o uso de AMF (Ramos-Zapata e Guadarrama 2004).

Segundo Lugato *et al.* (2008) a acessibilidade microbiana à matéria orgânica do solo é determinante na quantificação, repartição e qualidade da matéria orgânica seqüestrada ou fixada no solo na forma de húmus o que é fator determinante da agregação do solo e, consequentemente, modificador de sua porosidade.

A estabilidade dos agregados depende de dois fatores preponderantes: das características físicas do solo e da natureza da matéria orgânica húmica (Sá *et al*., 2000). Por outro lado, a formação da SOM depende não só da qualidade e da quantidade da matéria orgânica vegetal disponibilizada no solo mas também da atividade microbiana do mesmo. O nível de decomposição do material orgânico vegetal é modificado pela composição química vegetal que, por sua vez, seleciona a população decompositora.

Como citado por López-Bucio, Cruz-Ramirez e Herrera-Estrella (2003) existem três processos principais que afetam a arquitetura dos sistemas radiculares: primeiro, a divisão celular do meristema primário da raiz (i.e., nas células iniciais) que permite o crescimento intermediário pela adição de novas células à raiz; segundo, a formação de raízes laterais a capacidade exploratória do sistema radicular; e terceiro, a formação de pelos radiculares aumenta a superfície total das raízes primária e laterais. A alteração de qualquer um destes três processos pode ter profundos efeitos na arquitetura do sistema radicular e a capacidade das plantas crescerem em solos nos quais as fontes de nutriente são limitadas. Assim, as plantas podem aumentar sua aquisição de nutrientes pela ativação de programas de desenvolvimento que alterem a arquitetura do sistema radicular. A interação da raiz com o solo visa a busca de nutrientes os quais estão retidos na SOM dos agregados e também na fração química disponível do solo, assim, juntamente com a microbiota as raízes promovem a estabilidade dos agregados.

3.4 - MO húmica do solo

Segundo Christensen (1996a, b) a SOM pode estar livre ou associada fracamente às partículas do solo, sendo denominada de matéria orgânica não complexada (MONC) ou pode estar ligada fortemente às partículas minerais do solo, formando os complexos orgâno-minerais (COM) contendo a matéria orgânica humificada com baixas taxas de decomposição ou recalcitrante. As substâncias húmicas são formadas durante a decomposição da matéria vegetal rica da lignina, cutina e suberina pela ação de microrganismos do solo (Tao *et*

al., 1999; Adani *et al*., 2007). De acordo com os estudos recentes (Nebbioso e Piccolo, 2012 Nebbioso *et al*., 2014a), as substâncias húmicas (HS) são descritas como moléculas pequenas, heterogêneas e estabilizadas por forças hidrofóbicas e pontes de H, formando uma estrutura supramolecular (Piccolo, 2002). Como apontado por estes estudos proteômicos, os ácidos húmicos e humina representam as frações de maior peso molecular e mais condensadas, enquanto os ácidos fúlvicos são compostos por frações de menor peso molecular e mais oxidadas (Hertkorn *et al*., 2002 Nebbioso *et al.*, 2015). As substâncias húmicas variam quanto a sua composição estrutural dependendo da origem vegetal e dependendo da sua ligação à fração mineral. Nos agregados podem conter proporções variáveis de compostos de carbono aromáticos ou mais ricos em compostos C- alifáticos ou ainda C- alquila (Golchin *et al*., 1994). A recalcitrância depende do tipo de lignina e da habilidade em formar grupos hidrofóbicos nos agregados com pouco oxigênio, que não são acessados pela água ou pelos microrganismos (Nebbioso *et al*., 2014 a, b). Estas variações podem modificar a qualidade e as funções da matéria orgânica húmica e consequentemente as funções de agregação e fertilidade do solo. (Baigorri *et al*., 2009) assim como a estabilização do solo.

Quimicamente as substancias húmicas são agregados moleculares contendo açúcar, ácidos graxos, polipeptídios, cadeias alifáticas e anéis aromáticos (Simpson et al., 2002). De acordo com Golchin *et al*. (1997), a análise de ^{13}C dos espectros de ressonância magnética nuclear (NMR) de amostras do solo podem ser divididas em 4 regiões correspondentes aos ou grupos químicos : 0–46 ppm (alquila C), 46–110 ppm (O-alquila C), 110–165 ppm (aromático C) e 165–210 ppm (carbonila C). Tao et al. (1999) encontraram resultados similares no espectro de NMR de ácidos húmicos e fúlvicos os quais foram divididos em 4 regiões: I: 0–50 ppm, II: 51–105 ppm, III: 106–160 ppm and IV: 161–200 ppm, correspondendo aos grupos das regiões: I: alifáticos, II: carboidratos, III: aromáticos e IV: carboxila.

Outro tipo de ressonância magnética nuclear muito usada é baseada no deutério ou hidrogênio e de acordo com Kang *et al*. (2002) e Longstaffe *et al.* (2010), o espectro de ácidos húmicos com H-NMR pode ser dividido em 3

regiões : I: 0–3 ppm (alifáticos, aromáticos - CH_2, CH_3 e proteínas), II: 3–6 ppm (H -carboidratos e lignina-metox) e III: 6–8 ppm (H-aromático, lignina e proteínas).

A desagregação do solo promovida pela erosão é um indicador do declínio da estrutura do solo (Six *et al*., 2000), e a modificação dos espécimes químicos nos micros e macro agregados podem ser considerados como indicadores de impacto. (Six e Paustian, 2014).

Além do tipo de matéria orgânica formada, o tipo de solo, o conteúdo de argila e a umidade podem modificar a agregação do solo (Yoo et al., 2011) e impactos ambientais como as enchentes afetam diretamente os processos de decomposição e formação da matéria orgânica húmica e consequentemente a estabilização dos agregados (Glazebrook e Robertson, 1999; Kögel-Knabner *et al*., 2010).

4 – MATERIAIS E METODOLOGIA

4.1 – Área de Estudo

Bacia do Rio das Velhas:

Histórico:

O projeto de Recuperação das Matas Ciliares do Rio das Velhas do Projeto Manuelzão, inserido na Meta 2010, vem sendo elaborado desde 2004, quando foi feito um diagnóstico geral da Bacia do Rio das Velhas, através de expedições fluviais, aéreas e terrestres. Essas expedições permitiram constatar um elevado grau de degradação das matas ciliares do Rio das Velhas especialmente no trecho entre Itabirito - MG e Jaboticatubas - MG.

O relatório da expedição científica ao Rio das Velhas apontou como principais causas de degradação da Bacia:

1- Uso inadequado do solo com desmatamento e aterros,

2- Canalizações dos rios e impermeabilização do solo,

3-Atividades agropecuárias com ênfase nas pastagens,

4-Poluição: Presença de resíduos sólidos, esgotos e efluentes.

A destruição das matas ciliares, além da canalização dos rios afluentes e impermeabilização do solo, são as principais causas de alteração do volume, regime e vazão dos rios constituindo a principal causa de erosão. Estes impactos causados especialmente pela canalização dos rios, impermeabilização do solo, aterros e uso inadequados do solo não podem ser retratados; portanto novas soluções devem ser adotadas para minimizar seu efeito.

Impactos em diferentes trechos do Rio das Velhas: A expedição científica constatou que vários pontos de mata ciliar do Rio das Velhas se encontram em grau de degradação avançado, com intensa erosão e com assoreamentos volumosos provocados por violenta escavação das margens, afetando áreas agrícolas, pastagens e áreas urbanas. Nenhuma ação de recuperação foi registrada nestes trechos, o que sugere a necessidade de uma avaliação e estudo cuidadoso. Na literatura nacional, não foi encontrada nenhuma referência sobre aplicação de estruturas protetoras contra erosão na Bacia do Rio das Velhas. Assim, considerando que não existem estudos aprofundados para recuperação das Matas Ciliares na Bacia do Rio das Velhas, e também o grande número de processos erosivos ao longo do rio, torna-se premente o estabelecimento de tecnologias para a contenção destes processos erosivos e estabelecimento de modelos a serem replicados.

Impacto no trecho selecionado para estudo:

Na área experimental (Fotografia 1) foi possível verificar que o curso de água, na busca do equilíbrio, escava uma das margens e depositava sedimentos na outra, causando problemas de erosão, assoreamento e instabilidade das margens. O tipo de erosão encontrado era de escorregamentos rotacionais profundos onde grandes massas de solo são desprendidas, devido especialmente a escavações de pé de talude, como o solapamento provocado por correnteza do “talweg“ (ou tualegue) próximo ao talude na margem direita. Este processo criava uma inclinação negativa no talude cada vez mais

acentuada o que, por sua vez, gera mais desmoronamentos com queda de grandes blocos individualizados, ou desmoronamentos de conjunto de blocos por combinação desfavorável de planos estruturais da rocha com plano do talude de corte, ameaçando as residências de famílias moradoras da rua adjacente de modo progressivo.

A pedido da comunidade local, a equipe do Projeto Manuelzão junto com os pesquisadores do Departamento de Botânica/ICB/UFMG e Escola de Engenharia

Hidráulica/UFMG elaboraram um estudo visando a identificação de métodos e técnicas para contenção do processo erosivo citado.

Por outro lado, a margem esquerda sofria inundações que atingiam o frigorífero que funciona no local. Uma intervençaão na margem esquerda iria gerar uma desestabilização da margem direita. A equipe optou-por trabalhar a estabilização das duas margens.

Fotografia 1 – Área experimental

Área 1

A área estudada está situada à margem esquerda do Rio Velhas em Belo Horizonte - MG, Bairro Beija-Flor/Capitão Eduardo, coordenadas: S 19.8388º/O 43.8653º. A vegetação dominante é o Cerrado segundo Rizzini (1997), o clima é tropical (Aw) com temperatura média entre 22 e 23ºC e

chuvas predominantes no verão e invernos secos, segundo a classificação de Köppen. O índice pluviométrico varia de 1.300 a 1.400mm/ ano. Os dados médios de precipitação e temperatura, no período de estudo, foram medidos na estação meteorológica da Pampulha (Instituto Nacional de Meteorologia – INMET).

Em 2008 foi construído na área um talude, pois por ser uma área de degradação intensa, a chuva e o rio estavam erodindo as margens. Esta ação foi posterior à estabilização da margem direita do rio; contudo, com a proteção na margem direita, a colocava-se a necessidade margem esquerda, cujo processo erosivo já era significativo, seria ainda mais forte, tornou-se premente a proteção dos processos erosivos da margem esquerda através da implantação de espigões e posterior revegetação do talude.

O referido talude esteve sujeito a intenso solapamento de sua base na margem esquerda do Rio das Velhas, ameaçando sua estabilidade , e as residências e ruas do referido bairro. Após um estudo da dinâmica do rio no local (efetuado pelo Departamento de Engenharia Hidráulica da UFMG) concluiu-se pela necessidade de construção de espigões na margem esquerda visando a criação de bacias de sedimentação na base do mesmo. Porém, esta intervenção geraria um impacto sobre a outra margem (direita) do rio em função do desvio da direção do fluxo da água no rio que, além de causar o processo erosivo, depositaria material do outro lado. Por este motivo foi criada uma mata ciliar no leito maior do rio (área de inundação), oposto ao talude, tendo a sua margem reforçada com enrocamento. O mesmo foi reforçado com enrocamento na sua base visando receber os espigões. Após estas intervenções o talude será revegetado. A introdução de espécies vegetais nesta área visou, especialmente, a contenção do processo erosivo (Fotografias 2).

A comunidade vegetal é reconhecidamente importante no processo de estabilização do solo devido ao seu efeito de agregação do solo pelo sistema radicular e pelo efeito da rizosfera. A instalação da comunidade vegetal promove o “input” de matéria orgânica aumentando a diversidade biológica de

microrganismos do solo, como fungos micorrízicos arbusculares, através do efeito rizosférico sendo fundamentais no processo de agregação.
Neste contexto, o presente trabalho teve por objetivo avaliar o efeito do plantio e da inoculação micorrízica na estabilidade dos agregados visando indicar o tratamento mais eficiente no controle da erosão.

Area 2

A área experimental consiste de mata ciliar, localizado na margem direita do Rio das Velhas na cidade de Sabará, Estado de Minas Gerais, Brasil. Esta mata ciliar pertence à um frigorífico abatedouro (‘19°50’20.33’ S; 53°51’59.13’ W’), que perdeu sua área florestal pois foi degradada (Fotografia 1).
A vegetação predominante é savana tropical (Cerrado Brasileiro). A temperatura media anual esta entre 22-23ºC, com ventos secos e chuvas durante o verão. A precipitação anual é de 1200 mm. No entanto, este local sofria inundações anuais que afetavam as dependências do abatedouro. Durante as inundações na bacia do Rio São Francisco, o fluxo aumenta de 2810 m^3/s para 8000 m^3/s (Godim-Filho *et al*., 2004). Assim sendo, para resolver este problema, um projeto foi proposto para o reestabelecimento da mata ciliar (120 m × 45 m = 5400 m^2) com a reestruturação da área inundável que pode exercer uma função natural de drenagem (área experimental). Uma mata ciliar preservada (120 x 45 m) foi escolhida como testemunha positiva, íntegra e de referência biológica, física e química (Stoddard *et al.*, 2006), localizada próxima àrea experimental (50 km) em um parque de proteção ambiental (19°52'47’19°52'34’S. 44°07'44’–43°47'30’W) com uma floresta de mata ciliar tropical (Savana), enquanto a área degradada (120 x 45 m) é adjacente àrea experimental, aonde a vegetação predominante é pioneira e de espécies invasoras (*Brachiaria decumbens* e *Ricinus communis),* sem vegetação lenhosa. Na área de estudo foi realizado tanto o levantamento topográfico quanto batimétrico que tornou possível o estabelecimento e a calibração de um modelo hidráulico, usando o softwere HEC-RAS (River

Analysis System). O modelo permitiu a definição da área de inundamento em diferentes períodos e as forças de arraste associadas (Vieira, 2008).

Estabilização Física das Margens

Após o estabelecimento da área de inundação, foi feito o nivelamento do solo (Cia. Eco Máquinas) e, como mencionado anteriormente, a estabilização (Fotografias. 1A, 1B e C) foi feita com fixação de estacas transversais associadas com “riprap” (Cia Deflor) como mostra a Fotografia 2.

Fotografia 1 A – Estaqueamento de mudas em conjunto com Berma longas e enrocamento (riprap) na base

Fotografia 1 B – Detalhes da colocação de estacas de plantas e Timbergrab de toras

Fotografia 2 – Berma longa, enrocamento e colocação de estacas

5 – Objetivos da Pesquisa

A Mata Ciliar do Rio das Velhas se encontra com elevado grau de degradação e por isso o projeto Manuelzão e o Instituto Biológico da UFMG vêm estudando estratégias e modelos para recuperação dessas áreas. Uma das áreas críticas se encontra na região metropolitana de Belo Horizonte onde um talude situado às margens deste Rio, estava sofrendo intenso solapamento, ameaçando uma rua e as residências de moradores locais as quais se localizam no topo do talude. Foi feito um retaludamento e um reforço da base do talude (espigões) para contenção do processo de solapamento. Além disso, foi feito o plantio do talude usando diferentes de modelos de plantio e tratamentos diferenciais de inoculação visando o controle da erosão. Na outra margem (direita) foi feita uma estabilização da margem com enrocamento e madeira (“timber cribbing cross-section”) e após rebaixamento da área esta foi recuperada com o plantio de floresta inundável.

Visando a avaliar a estabilidade e recuperação das duas áreas foi feito estudo usando-se indicadores bióticos (matéria orgânica, biomassa microbiana, fungos micorrízicos e glomalina) e abióticos (agregação, porosidade e fertilidade) do solo visando aferir a estabilidade das áreas sob inundação e o grau de recuperação atingido 4 e 6 anos pós plantio.

5.1- Objetivo Geral

Avaliar o efeito do plantio sobre a estabilidade do solo e recuperação funcional das áreas de estudo às margens do Rio das Velhas.

5.2 – Objetivos Específicos

Avaliar a recuperação das áreas de estudo com base nos seguintes indicadores bióticos e abióticos:

- Conteúdo de matéria orgânica humificada do solo;
- Qualidade da matéria orgânica do solo;
- Agregação e porosidade do solo;
- Fungos micorrízicos;
- Biomassa microbiana;
- Glomalina do solo;
- Crescimento e sobrevivência vegetal

Capítulo 2

Soil aggregation and arbuscular mycorrizal fungi as indicators of slope rehabilitation in the São Francisco river basin (Brazil)

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Abstract

Anthropogenic activity along the Velhas River (São Francisco River basin) has destabilized the banks of the river channel across an urban fragment. To improve the physical stabilization, the base of a slope was filled with urban construction waste. After this, the slope was re-vegetated with native species and arbuscular mycorrhiza fungi (AMF) inoculation was applied with positive landscape changes. This study aims to evaluate the role of the AMF population in the soil aggregation and stabilization of the re-vegetated slope. The soil aggregation was higher in the experimental site than the disturbed site, especially under AMF inoculation. This aggregates improvement was accompanied by an increase of soil humic acid and soil glomalin content at 24 months after transplantation, even with flooding impact at 12 months after transplantation. A scatter plot based on Principal Components analysis of aggregates showed that the preserved site was grouped with the some samples from the inoculated plots, the other soil samples were located between the preserved and disturbed sites. This result shows that the recovering site is evolving toward the conditions of the preserved site and that the rehabilitation process is in an intermediate phase related to aggregate formation. The AMF inoculation of woody species was indicated in rehabilitation procedures.

Keywords:. Arbuscular mycorrhizal fungi, glomalin, Humic acids, landscape alteration, microbial biomass, river bank stabilization

INTRODUCTION

The Velhas River is the principal tributary of the São Francisco River, and its channel is open and shallow. Therefore, its flow depends on the flooding of the

riparian area in the rainy season. It is considered the most important disturbed river of the São Francisco Basin due to several anthropogenic factors, such as inappropriate land use for agriculture, pasture, industrial and mining activities. Moreover, other factors, such as the flood proofing of urban tributary rivers and the high sewage load, have contributed to severe impacts on the Velhas River. Over time, all of these disturbances have altered the hydrology of the landscape, resulting in erosion and sedimentation. As a result, mass-wasting processes have destabilized the channel banks and widened the channel. In certain portions of the Velhas River, the height and angle of the bank can exceed the critical conditions for stabilization, resulting in shear deformation of the material and washouts at the base of the slope. When erosion reaches a level that produces a loss of resilience, resulting in destabilization and erosion, the ecosystem becomes maximally degraded (KINGS e HOBBS 2006).

To stabilize against superficial erosion, especially in slope areas, vegetation appears to be the chief means of structural protection due to the role of plant roots (REUBENS *et al.* 2007. GHOLAMI e KHALEGHI 2013) by either hydrological or mechanical mechanisms (NILAWEERA e NUTALAYA 1999). Hydrological stabilization is related to the role of the vegetation, facilitating evapotranspiration and soil porosity, which, in turn, control soil infiltration. Mechanical stabilization refers to the anchoring effect of coarse and fine roots (NILAWEERA e NUTALAYA 1999).

However, the aggregation of soil is the fundamental property that determines resistance to erosion and degradation as well as productivity (SIX *et al*. 2000). Soil-water stable aggregates (WSAs) are the best indicator of the ability of a soil

to resist erosion (BARTHÈ e ROOSE 2002; AN *et al.* 2010). Aggregate stability is a highly complex parameter influencing a wide range of soil properties. including carbon stabilization, soil porosity, water infiltration, aeration, compression, water retention, hydraulic conductivity, resistance to erosion by water and overland flow (AN *et al.* 2010). Therefore, aggregate stability has been considered an indicator of soil quality.

Aggregate formation and stabilization depend on the formation of soil organic matter (SOM). The principal basis of the aggregation process is the ability of humic acid polymers to link different particles, and the clay-humic complex is the primary unit of aggregation (primarily due to humic acid) that improves aggregate stability (PICCOLO e MBAGWU 1994). Humic acid interacts with clay and forms an organo-metal complex (EDWARDS e BREMNER 1967) with a size of <250 µm. These stable micro-aggregates, in turn, are bound together into macro-aggregates (>250 µm) by the transient and temporary action of fungal hyphae and roots as well as by polysaccharides produced by microorganisms and plants. Macro-aggregate stability is correlated with the relative content of humic substances (ŠIMANSKÝ e BAJČAN 2014)

Arbuscular mycorrhizal fungi (AMF) have been considered as an important instrument for the reclamation stressed sites due their significant role for plant establishment even in the early stages of the revegetation. AMF also play an important role in soil aggregation (RILLIG *et al.* 2010.), not only through the physical action of their hyphae but also by their ability to produce heat-stable proteins, called Glomalin-Related Soil Protein – PRSG (WRIGHT e UPADHYAYA 1996). PRSGs are small hydrophobic proteins found in the

hyphae of many types of mycorrhizal fungi. These proteins contribute to the insolubility and hydrophobicity of aggregates (RILLIG *et al*. 2003).

In particular, soil aggregate formation promoted by humic acids modifies the quantity and size of the pores (macro and microporosity), which ensures soil aeration and drainage (STEVENSON 1994).

To produce physical stabilization of a slope with a negative angle on the bank of the Velhas River, urban concrete construction wastes were used as fill at the base of the slope to a depth of 8 m in the river. The slope was re-vegetated after this physical intervention. Stability of soil aggregates is an appropriate parameter for predicting the potential risk of water erosion in this re-vegetated slope and as an indicator of soil erodibility (AN *et al*. 2010). Besides, vegetation and their mycorrhizal associations could modify qualitative and quantitative soil aggregate formation.

Therefore. this study aims to evaluate the soil aggregation of the re-vegetated area and the soil abiotic and biotic factors related to aggregation as indicators of the stability of the soil and the success of the rehabilitation process.

MATERIAL AND METHODS

Experimental site

The experimental area consisted of a slope (Fig 1.1), located on the left bank of the Velhas River in the city of Belo Horizonte, State of Minas Gerais, Brazil. This urban site belongs to the neighborhood Beija-Flor (“19º50’20.33”S. 53º51’59.13” W”). The predominant vegetation is tropical savanna (Brazilian Cerrado). The annual temperature mean is 22 to 23ºC, with rainfall primarily during the summer, with dry winters. The total annual rainfall is 1200–mm. The

slope suffered a flood at 12 months after the transplantation. During the flood event in São Francisco basin resulted an increase of flow rate from 2810 m^3/s to 8000 m^3/s (GODIM- FILHO *et al*. 2004)

STODDARD *et al.* (2006) proposed the term reference condition for biological integrity (RCBI) to preserved site whose functional pattern would be known to allow the identification of disruptions or losses (biotics and abiotics) in areas under degradation process (KING e HOBBS. 2006). These losses should be recovered during rehabilitation process. Therefore, a preserved riparian forest located in an Environmental Protection Park (19º52'47" –19º52'34" S. 44º07'44" – 43º47'30" W) was chosen as a positive reference area or protected site near the experimental site (50 m). The native vegetation is the tropical savanna (Cerrado) found in preserved riparian forest. A riparian area without vegetation adjacent to the base of the slope in the degraded portion of the Velhas River was chosen as a control site.

Experimental design

After the physical stabilization of a linear slope with urban construction wastes (material generated from demolition of urban buildings), the slope showed an angle of approximately 45^0 (performed by Eco Maquinas Company) (Fig.1.2 A and 1.2B) and it was re-vegetated (Fig 1.2 C) and the flood reached the experimental site 12 months after the transplantation (Fig 1.2 D). The experiment was conducted as a randomized block design, with an area of 0.2160 ha (135 x 16 m = 2160 m^2) divided into three blocks of 0.072 ha each (45 x 16 m = 720 m^2). Each block was divided into two treatments (Plots un-

inoculated and inoculated with arbuscular mycorrhizal fungi) of 0.036 ha (22.5 x 16 m = 360 m^2), with a 1 m border on each side (Fig 1.3).

Planting

The area was planted with seedlings of shrub and tree species: *Psidium guajava, Eugenia uniflora, Croton urucurana*, *Morus nigra,*. *Inga edulis*, *Erythrina speciosa*, *Jacaranda mimosifolia, Hymenaea courbaril,*. *Piptadenia gonoacantha*, *Samanea inopinata* and *Mimosa bimucronata.* After four months of growth under nursery conditions at vegetation house belongs to Integration Plant Microorganism Laboratory in Biological Science Institute of Federal University of Belo Horizonte, the seedlings were transplanted to the field.

The inoculation of 50% of the species was performed under nursery conditions. A spacing of 3 x 3 m was maintained, and fertilization was performed according to Somasegaran and Hoben (1985). After transplantation of native woody species seeds of commercial herbaceous species were planted between the rows to surface erosion control along the first year:

Inoculants

The shrubs and trees were inoculated with the spores of AMF (arbuscular mycorrhizal fungi) of the species *Gigaspora margarita,Acaulospora scrobiculata* and *Glomus etunicatum* from the collection of the Laboratory of Interaction of microorganism and plants. The plants were grown at the greenhouse of Biological Science Institute from Federal University of Minas Gerais (ICB-UFMG) and inoculated in a plant nursery. A total of 1 ml of a suspension of 50 spores of each type of AMF (150 spores per pot) was used for

the inoculation. Commercial herbaceous seeds were inoculated using mixture of seeds with soil containing spores of the abovementioned species (220 spores /100 g of soil) before sowing in the soil. This mixture was sow in the soil between the rows where woody species were planted. Therefore, inoculated plots received both plants and seeds which were inoculated with AMF.

Plant growth and establishment

The soil coverage was estimated using a 1 m² quadrant that was subdivided into 100 identical cells of 10 x 10 cm (TOLEDO e SCHULTZE-KRAFT 1982), which were placed in each plot. The coverage in each cell was recorded. Three quadrant samples were collected per plot or treatment/block at 24 months (3 replicates x 2 treatments x 3 blocks), and the plant presence /quadrant was estimated (%).

Soil sampling

The samples were collected from 0-20 cm at 6 and 24 months after transplantation. In each plot (360 m^2), 6 soil samples/plot were collected. There was a total of 18 samples/treatment/time or 36 samples/time (6 soil samples x 2 treatments x 3 blocks) which were used for all of the physical, chemical and biological analyses.

Similar sampling was performed in the adjacent preserved area (the preserved riparian forest used as a reference site) and at the disturbed site on the Velhas River.

Soil analysis

A - Textural and soil fertility analyses

Samples of the soil were sieved with 2 mm mesh and analyzed for physical-chemical properties at 6 and 24 months after planting (EMBRAPA 1997). Besides textural analysis, the experimental site was compared with preserved site in relation to the cation content, cation exchange capacity, base saturation, organic matter content, soil pH among others parameter, using Tuckey multiple-range test at the 5% confidence level ($P \leq 0.05$).

B- Determination of soil aggregation and porosity

Analyses were performed on soil samples sieved at 2 mm. Soil aggregation measurements were carried out to measure the proportion of water-stable aggregates (WSAs), using standard methods (KEMPER e ROSENAU 1986). Undisturbed soil samples from the studied sites were collected in cylinders of a predetermined volume and sent for porosity analysis according to the Brazilian Agricultural Research Corporation (EMBRAPA 1997).

C- Microbial biomass (C_{mic}*). soil population of AMF and glomalin content*

The C_{mic} analysis was performed with the fumigation-extraction method according to VANCE *et al*. (1987) using fumigated and non-fumigated (CCl_4) soil samples.

The mycorrhizal spores were recovered from the soil by the sieving and decanting method according to GERDEMANN e NICOLSON (1963), and the data were expressed as the number of spores/ gram of dry soil. Healthy spores were counted. Each spore type was mounted sequentially in PVLG (polyvinyl-lacto-glycerol) and Melzer's reagent (MORTON 1988) for identification according to the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM – -http://invam.caf.wvu.edu).

Glomalin-related proteins (PRSG-T), were extracted with the WRIGHT e UPADHYAYA method (1996 and 1998), and the protein content was estimated by the Bradford method (BRADFORD 1976).

D- Fractionation of soil organic matter

The fractionation of sequestered carbon from soil organic matter was performed according to DABIN (1971) using 15 g of sieved soil/treatment/block to obtain the fraction of carbon represented by humic acid and fulvic acid.

Statistical Analysis

The results of the samples with normal distribution were subjected to a one-way ANOVA using MINITAB software version 13.2, and the means of the treatments were compared using Tukey's multiple-range test at the 5% confidence level ($P \leq 0.05$). Spearman correlation and a principal component analysis (PCA) based on a variance analysis was used to select the most significant variables from the candidate variables of soil organic matter, microbial biomass, AMF spores, glomalin, humic acid, fulvic acid, humin and soil porosity. This method allowed the variables to be evaluated for their ability to modify the aggregation of the soil. The analysis served to express the variables in terms of two components that explained the total variability associated with soil aggregation. Each component was accompanied by information about the intensity and direction of the correlation of the variable to the component. The results were illustrated in dispersion and loading-plot graphics created using MINITAB 15 software.

RESULTS AND DISCUSSION

The soils from the experimental site showed a textural composition between sandy loam and silty loam, whereas the soil of the preserved site was predominantly loamy. Soil fertility, based on cation and organic matter availability in the experimental site, was low prior to transplantation in comparison to the preserved site (Table 1). The content of soil organic matter in the soil increased over time, regardless of the treatment, and had significantly low values in the disturbed site. Similarly, the soil CEC increased at 24 months after transplantation, reaching values equal to those found at the preserved site. This increase can be explained only by functional changes in SOM with the input of plant biomass as showed in literature (SAMEC *et al*. 2014).

Indeed, at 24 months after transplantation, the inoculated plots showed a higher plant coverage than the non-inoculated plots (Figure 2A), especially with herbaceous species and grasses. Therefore, inoculation with arbuscular mycorrhizal fungi favored the establishment of vegetation at the experimental site and this effect occurred in detriment of flooding event. Survival of individuals depends on flooding tolerance (WILLIAMS 2005) and as predicted the woody species occupation was not modified by inoculation procedures but the inoculated woody species associated with the herbaceous plants may have played an important role in soil aggregation and in the control of laminar soil erosion, as described in the literature (RILLIG *et al*. 2003).The rehabilitation procedures increased the macro-aggregate or larger aggregates production (Figure 2B) in the experimental site, which may be an indicator of erosion control (BARTHÉS e ROOSE 2002). Micro-aggregates are faster and more easily taken away by erosion processes than larger macro-aggregates

(ŠIMANSKÝ 2011). In the first 6 months after transplanting, micro-aggregates or small aggregates were predominant at the experimental site. In spite of the flooding impact, a change in the aggregate profile occurred at 24 months after transplantation, with a significant improvement in the abundance of aggregates >250 µm in inoculated plots. No aggregates were found at the disturbed site, and largest aggregates (>250 µm) were found to be higher in abundance at the preserved site (Figure 2B).

The total spore number was not significantly different between inoculated treatments at 24 months after transplantation (Figure 3A) suggesting a flood effect on AMF establishment. However, the spore number of both the Acaulosporaceae and Glomaceae AMF families increased in the inoculated plots (Figure 3A). Literature confirms that AMF propagules can tolerate flooding effect (HARNER *et al*. 2011). The significant increase of Acaulosporaceae and Glomaceae spores speaks in favor of the flood tolerance of these families. AMF can favor not only plant growth but also the input of litter and microbial decomposers (SCOTTI e CORREA 2004). However, the microbial biomass did not differ between the inoculated and un-inoculated plots (Figure 3B), as well as total AMF population. Although bacteria population are considered a transient agent of aggregation, roots and hyphae from fungal community, particularly arbuscular mycorrhizal hyphae, are considered as temporary binding agents that are able to persist for months and years, binding micro-aggregates to form macro-aggregates (SIX *et al*. 2000; RILLIG *et al.* 2010). On the other hand, the glomalin produced by AMF hyphae was significantly higher in inoculated than un-inoculated plots (Figure 3C). This glycoprotein is considered an especial

agent of soil aggregate stability (WRIGHT e UPADHYAYA 1998) which is affected by soil management (NICHOLS e. MILLAR 2013). Therefore, the increase in the population of some AMF families and soil glomalin content after 24 months in the experimental site could be related to an improvement in soil aggregation.

The rehabilitation procedure improved soil organic matter (SOM) content but there was not difference between the inoculated and un-inoculated plots (Figure 4A). The soil organic matter content is not always related with soil aggregation and even a negative correlation was recorded between SOM and macroaggregates (ROKOSCH *et al*. 2009). However, the persistent binding agents as humic acids and glomalin are correlated to aggregate stability cementing the inter-particle bonds of micro-aggregates (EDWARDS e BREMNER 1967; WRIGHT e UPADHYAYA 1998).

The recalcitrant or humified fraction is the most stable final product of the decomposition of lignin and is able to improve aggregate stability (PICCOLO e MBAGWU 1994). Our results showed that cultivation promoted humic acid (Figure 4B) formation at 24 months after transplantation with the significant contribution of AMF inoculation. The fulvic acid content (Figure 4C) was also improved in both the inoculated and un-inoculated plots compared to the disturbed site. The lack of treatment effect can be associated with the flooding effect. In general, humic substances (HS) from a river or wet sites under periodic flooding are comprised of molecules of smaller size, less condensed and more water soluble as fulvic acids (SPACCINI e PICCOLO 2009).

In fact, the improvement in soil aggregation indicates a considerable contribution of humic acid and glomalin especially in inoculated plots where an increase of plant ocupation was also registered.

The treatment-independency of some studied variables as soil organic matter, total AMF spores, microbial biomass, and fulvic acid content could be attributed to the flooding effect characterized by the mixture of soluble elements among treatments and plots. Flooding can also alter the dynamics of all soil biological cycles and their products, therefore modifying not only the number but also the function of microbial species (GLAZEBROOK e ROBERTSON 1999). These results reinforce the hypothesis of tolerance and stability of soil aggregates to flooding effect and the role of their forming agents as soil glomalin and humic acid. The mycelium of AMF fungus can maintain water-stable soil aggregates trough increased soil water repellency of their hyphae (RILLIG *et al.* 2010) what can explain the improvement of flood tolerance in inoculated plot .

While in the preserved site it was found a balanced distribution between microporosity and macroporosity (24 and 25% respectively), in the disturbed site the contribution of macroporosity to total porosity was twice the microporosity (15: 36.3% respectively). The same tendency was found in the experimental site 6 months after transplantation where the proportion of macroporosity was also twice the microporosity (39:13% respectively). The cultivation procedure favored the desired distribution of macro- and micro-porosity at the experimental site 24 months after transplantation when the proportion between macroporosity and microporosity reached 27:21%, respectively. This increase in microporosity could be attributed to aggregation

formation. However, this improvement was independently of the inoculation treatment. This balance in soil porosity is essential to drain the water into soil under flood. Moreover, soil aggregates control the porosity and therefore aeration and soil water drainage. There is a direct relationship between the quantity of macro- and micro-pores and soil water infiltration (EYNARD *et al.* 2004). The improvement in micro-porosity in the experimental site under flooding effect, suggests once again the stability of aggregates.

The results of PCA confirm that all of the studied variables (soil organic matter, AMF community, humic acids, glomalin and microbial biomass) contributed to explain 55% of the variation in soil aggregate formation, in addition to the vegetation effect. Therefore, the improvement in soil aggregation was a result of all of the studied variables under natural conditions. Including the second component (soil porosity), 72% of the variability in soil aggregation was explained.

The scatter plot (Figure 5) was generated based on the variables that modified aggregation such as component 1 (organic matter, humic acids, AMF fungi, glomalin and microbial biomass) against to the porosity variable (component 2). Based on the soil aggregates formed (Figure 5), several samples from the experimental site were grouped close to the samples from the preserved site. In contrast, other samples were placed between the preserved and disturbed sites and were influenced by both components 1 and 2. Another group formed by samples from the inoculated and un-inoculated plots was clustered separately. The latter could be the result of the flooding impact because there was a strong

influence of soil porosity (component 2), similar to the disturbed site that formed another separated group.

These results showed that the characteristics of the recovering site are evolving toward those of the preserved site and that the rehabilitation process has attained an intermediate phase of restoration. This state of the process can be understood as a result of soil macro-aggregate stabilization formed by humic acid and glomalin action and all of them can be considered indicators of slope rehabilitation.

CONCLUSIONS

Arbuscular mycorrhizal fungi inoculation favored the establishment of native species during slope rehabilitation and improved of soil occupation as well as soil aggregates formation. Soil aggregates was considered an important indicator of slope rehabilitation when compared to the reference site which was able to withstand the environmental impacts of flooding. The soil aggregation improvement can be attributed to the glomalin and humic acid contributions especially in inoculated plots which can be used to indicate the direction of restoration evolution. Therefore, the AMF inoculation of woody and herbaceous species could be recommended in rehabilitation procedures.

ACKNOWLEDGMENTS:

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LEGENDS

Table 1. :Chemical analyzes of soil treatments plots and reference site 6 and 24 months after transplantation. *OM: Organic matter. SB: Sum of Bases. CEC: Cation Exchange capacity. V(%): Base saturation.

Figure 1.1 : Localization of experimental site in the google map (red arrow) . Figure **1 2** : Slope rehabilitation. **A:** Urban concrete construction wastes were used as fill at the base of the slope. **B:** Slope before re-vegetation. **C:** Slope 12 months after re-vegetation. **D :** Slope under flooding. Figure **1 3:** Experimental design of slope revegetation: 1: Inoculated plots with Arbuscular Mycorrhizal Fungi. 2- Uninoculated plots.

Figure 2 A Occupation index of plants (%) in experimental area 24 months after transplantation and **B**- Soil aggregates distribution (%) in the experimental area at 6 (T1) and 24 (T2) months after planting compared with Preserved site considering all classes > 250 µm and ≤ 250 µm. Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($p \leq 0.05$).

Figure 3 Soil AMF community : Number of spores total, Acaulosporaceae and Glomaceae families **(A)** Microbial biomass (**B**) and Soil glomalin content **(C**) from Experimental soil (AMF Inoculated and Un-inoculated plots) and reference sites 6 and 24 months after transplantation. Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($p \leq 0.05$).

Figure 4 Soil organic matter (**A),** soil Humic acid **(B)** and Fulvic acid **(C)** of experimental and reference sites at 6 and 24 months after transplantation. Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level ($p \leq 0.05$).

Figure 5- Scatter plot of two main components explaining the variation in soil aggregation based on variables of soil organic matter and their fractions. Microbial biomass (C mic) mycorrhizal fungi and soil glomalin (Component 1) and porosity (Component 2), stratified by treatments: Preserved site, Inoculated plots, Uninoculated plots and Disturbed site.

Table 1: Chemical analyzes of soil treatments plots and reference sites 6 and 24 months after transplantation

	pH		H+Al		O.M*.		K		Ca^{2+}		Mg^{2+}		P		SB*		CEC*		V%*	
	H_2O		mmol/dm³		%		mg/dm³		mmol/dm³		mmol/dm³		mmol/dm³		mmol/dm³		mmol/dm³			
Treatments /months	6	24	6	24	6	24	6	24	6	24	6	24	6	24	6	24	6	24	6	24
Un-inoculated	7.70	6.83	7.5 ^{ab}	7.33 ^{b}	15.16^{b}	24.33^{ab}	2.34^{ab}	1.83^{b}	47.73^{ab}	97.33^{ab}	1.63^{c}	3.8^{bc}	107.00^{a}	23.67^{b}	51.76^{b}	107^{ab}	54.16^{b}	114.66^{ab}	87.71^{ab}	92.66^{ab}
Inoculated	7.15	7.26	7.90^{ab}	8.67^{ab}	13.12^{b}	21.75^{ab}	1.39^{b}	2.60^{ab}	38.45^{b}	99.67^{ab}	1.8^{c}	3.7^{bc}	53.00 ^{b}	21.67^{b}	41.67^{b}	112^{ab}	49.6^{b}	120.67^{ab}	83.23^{b}	92.33^{ab}
Preserved site	6.9		8.00 ^{ab}		27.00^{a}		2.60^{ab}		149 a		7.3^{a}		42 b		159 a		167 a		95 a	
Disturbed site	7.35		10.00^{a}		15.00^{b}		3.90^{a}		56^{b}		6^{ab}		50^{b}		66^{b}		76^{b}		87^{ab}	

- Means with different letters are significantly different as determined by Tuckey multiple-range test at the 5% confidence level (P ≤0.05). (NS = Not significant).

Figure 1

.1

.2:

A

B

C

D

3:

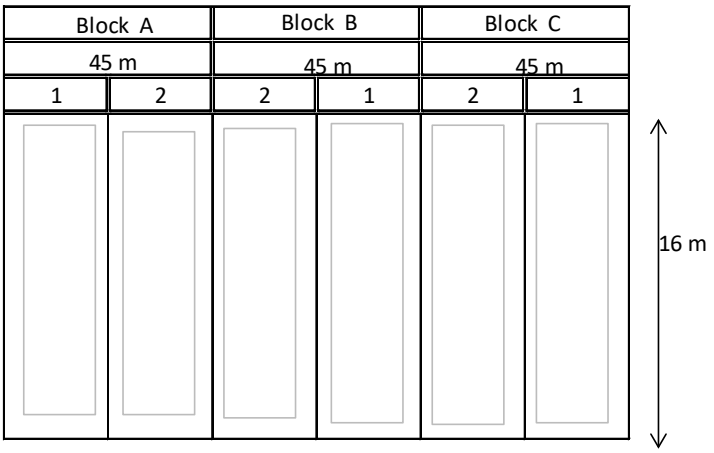
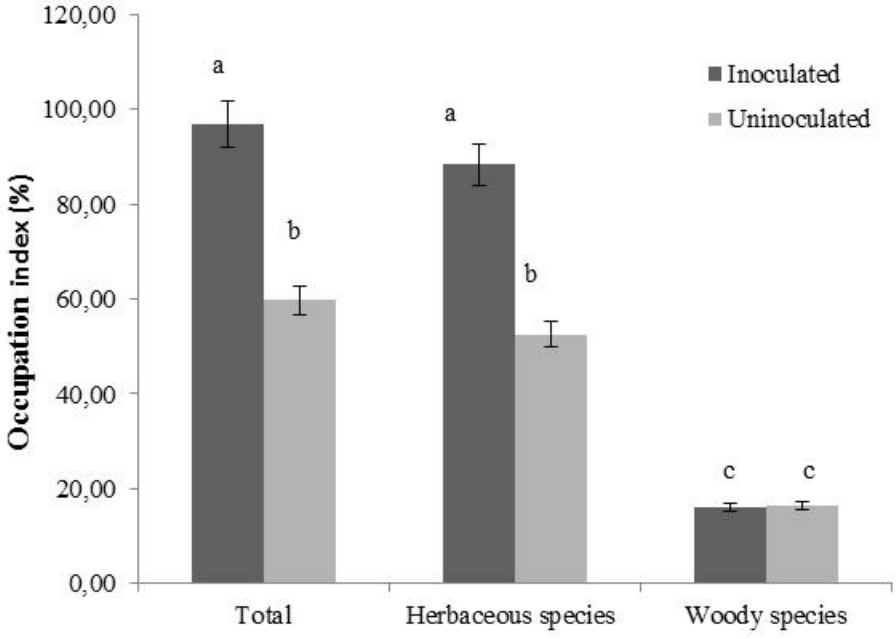


Figure 2

A


120,00
100,00
80,00
60,00
40,00
20,00
0,00
Occupation index (%)
a
b
a
b
c
c
Inoculated
Uninoculated
Total
Herbaceous species
Woody species

B

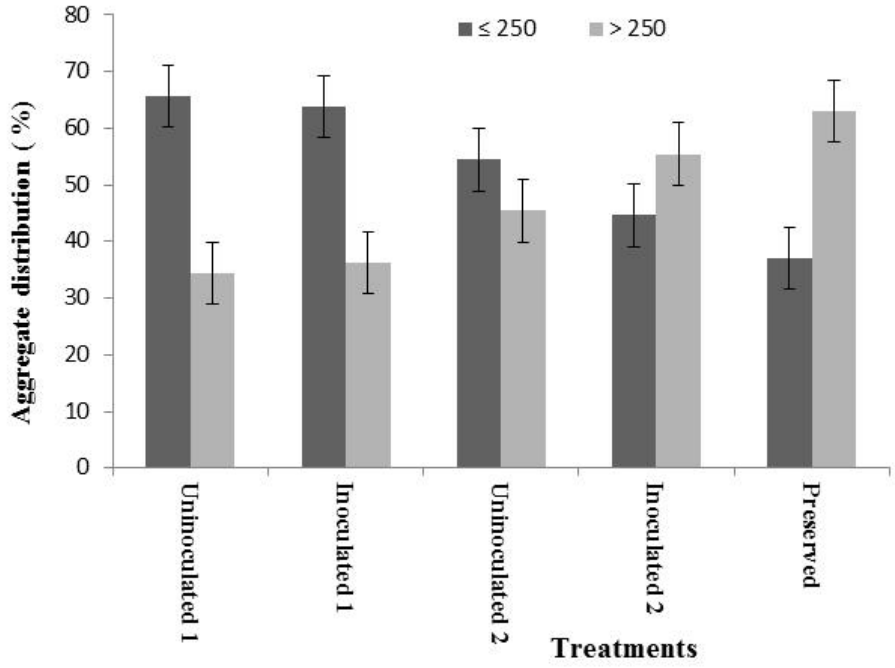

80
70
60
50
40
30
20
10
0
Aggregate distribution (%)
≤ 250
> 250
Uninoculated 1
Inoculated 1
Uninoculated 2
Inoculated 2
Preserved
Treatments

Figure 3

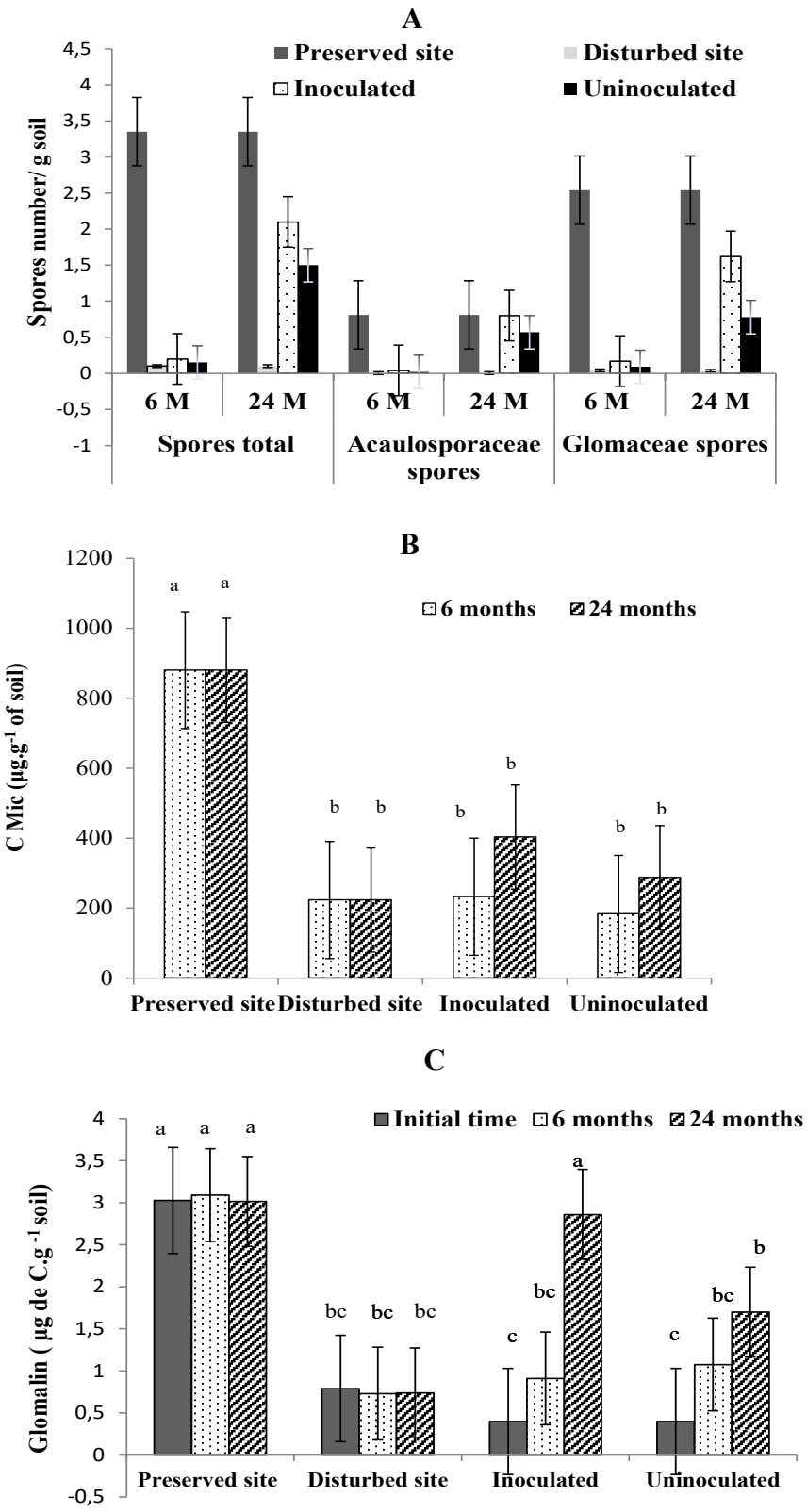

A
Preserved site
Disturbed site
Inoculated
Uninoculated
Spores number/ g soil
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
-0,5
-1
6 M
24 M
6 M
24 M
6 M
24 M
Spores total
Acaulosporaceae spores
Glomaceae spores
B
6 months
24 months
C Mic (µg.g-1 of soil)
1200
1000
800
600
400
200
0
a a
b b
b b
b b
Preserved site
Disturbed site
Inoculated
Uninoculated
C
Initial time
6 months
24 months
Glomalin (µg de C.g -1 soil)
4
3,5
3
2,5
2
1,5
1
0,5
0
-0,5
a a a
bc bc bc
c bc a
c bc b
Preserved site
Disturbed site
Inoculated
Uninoculated

Figure 4

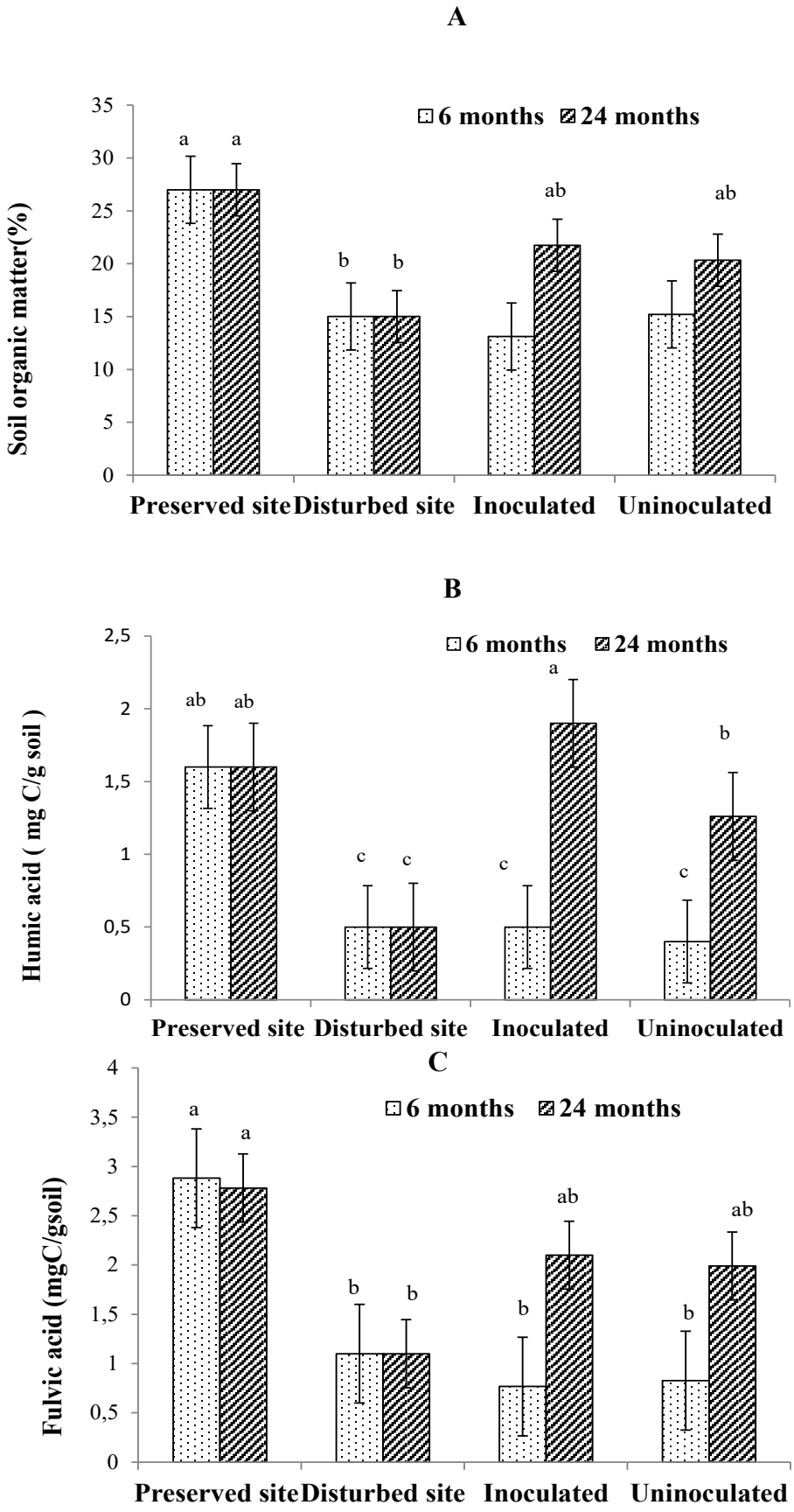
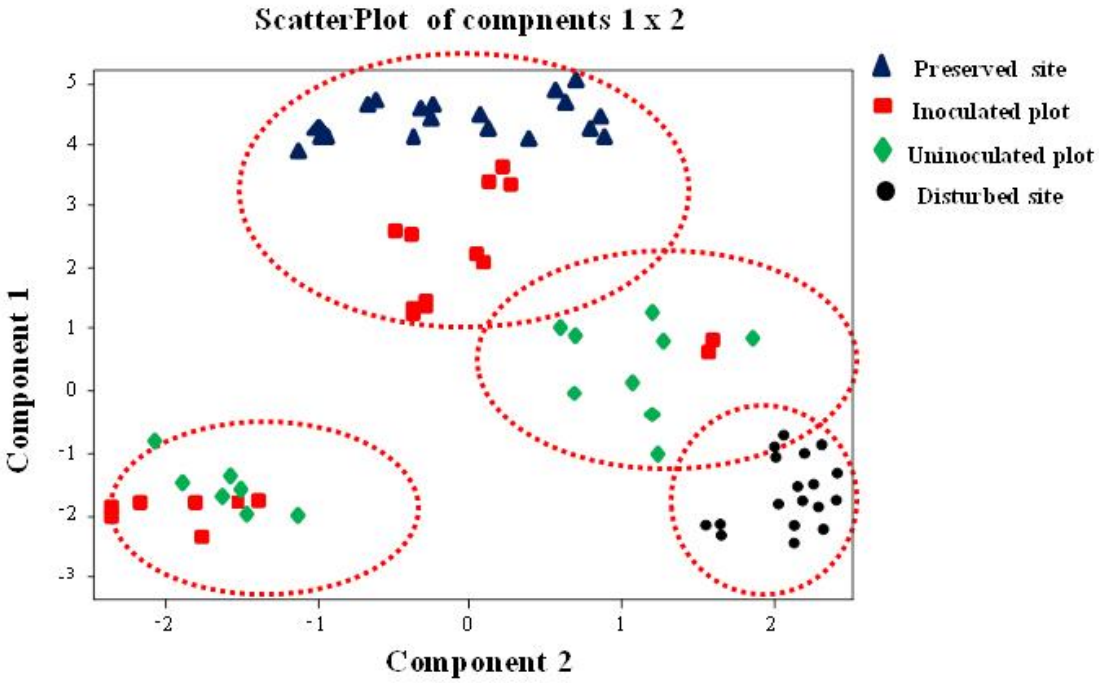

A
6 months
24 months
Soil organic matter(%)
35
30
25
20
15
10
5
0
a a
b b
ab ab
Preserved site
Disturbed site
Inoculated
Uninoculated
B
6 months
24 months
Humic acid (mg C/g soil)
2,5
2
1,5
1
0,5
0
ab ab
c c
c
a
c
b
Preserved site
Disturbed site
Inoculated
Uninoculated
C
6 months
24 months
Fulvic acid (mgC/gsoil)
4
3,5
3
2,5
2
1,5
1
0,5
0
a a
b b
b
ab
b
ab
Preserved site
Disturbed site
Inoculated
Uninoculated

Figure 5


ScatterPlot of compnents 1 x 2
Preserved site
Inoculated plot
Uninoculated plot
Disturbed site
Component 1
Component 2
5
4
3
2
1
0
-1
-2
-3
-2
-1
0
1
2

Capitulo 3

Soil humic acid and aggregation as restoration indicators of a seasonally flooded riparian forest under buffer zone systems

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12 – Introdution

Riparian forest refers to the transition zone between the aquatic and terrestrial environments (Lowrence et al., 2001), and this floodplain area is known as riparian forest buffer system because of its ability to intercept surface runoff, wastewater, subsurface flow and deeper groundwater flows from upland sources for the purpose of removing or buffering the effects of nutrients, sediments, organic matter and pesticides, non-point source pollution (NSP) or other pollutants (Lowrence et al., 1997). Under this functional point of view, a riparian buffer system is said to consist of three functional zones plus the hyporheic zone (Welsch, 1991; Lowrence, 1997; Shultz et al., 2004). The hyporheic zone consists of the groundwater area where a bidirectional flux between the stream and groundwater occurs (Triska, 1993). Zone I is composed by a permanent woody vegetation, immediately adjacent to the stream bank, whose primary function is stabilisation of the margins. Zone II refers to the strip upslope from Zone I consisting of preserved or managed forest, where the litter biomass input should be prioritised to allow its buffering function. Zone III is the last transition of the riparian terrestrial ecosystem, which includes woody and herbaceous species that controls of surface erosion (Welsch, 1991; Lowrence, 1997; Shultz et al., 2004).

The movement of soil and water/air in the soil is sustained by soil structure, which comprises the arrangement of solids and void components (Bronik and Lal, 2005) that help in soil stabilisation. This function is promoted by trees and their root systems (Gholami and Khaleghi, 2013) and by soil aggregation that is achieved via the attachment, flocculation and cementation of organic matter particularly the soil humic substances, by biota, with clay, ionic bridging, and polyvalent cations (Duiker et al., 2003) which forms the micro aggregates (<250 μm) that once combined with other particles form the macroaggregates (>250 μm) (Edwards and Bremner, 1967 Tisdall, 1996).

Humic substances (HS) are considered to be formed during the decay of plants rich in lignin (lignin theory), cutin and suberin by microbial action, whose

residues are resistant to biodegradation (Tao et al., 1999 Adani et al., 2007). HS were more recently described as small heterogeneous molecules held together by mainly hydrophobic and H bonding forces in supramolecular associations (Piccolo, 2002) as pointed by the proteomic studies (Nebbioso and Piccolo, 2012 Nebbioso et al., 2014a). Humic acids and humin represent a higher molecular weight and a more condensed fraction of HS, whereas fulvic acids are composed of lower molecular weight and more oxidised substances than humic acids (Hertkorn et al., 2002 Nebbioso et al., 2015). Humic substances vary widely in structural composition, and the fraction linked to minerals in aggregates show high proportions of recalcitrant compounds with aromatic C or aliphatic alkyl-C (Golchin et al., 1994). The recalcitrance was attributed to the association of single small molecules into hydrophobic domains separed from water and decomposing microorganisms, as well as oxygen (Nebbioso et al., 2014 a and b). These variations result in differences in the quality and functions of humic matter in soil aggregation and stabilisation (Baigorri et al., 2009).

On the other hand, vegetation loss, long-term cultivation and soil management can reduce the humic acids aromaticity (Aranda et al. 2011) favouring the soil disaggregation (Oaedes, 1984, Elliott, 1986) affecting particularly macro-aggregates (Gupta and Germida, 1988 , 2015) .This effect was attributed to the transient and temporary microbial community associated with the aggregates (Gupta and Germida, 2015).

Besides soil cultivation, clay content and soil moisture can modify soil aggregation (Yoo et al., 2011) and environmental impacts as floods have a strong effect on the soil microorganisms, decomposition processes, humic acid formation and consequently on formation and stabilisation of soil aggregate (Glazebrook and Robertson, 1999; Kögel-Knabner et al., 2010).

According to Whisenant (1999, 2002), the maximum degradation is reached with the decline of soil structure comprising the loss of aggregation and increase of erosion, which can culminate in the process of desertification (King and Hobbs, 2006). According to Six et al. (2000), disaggregation and erosion are the indicators of soil structure decline, and the loss of carbon content into

micro aggregates and macro-aggregates may be considered as indicators of the impact (Six and Paustian, 2014).

The Velhas River is the primary tributary of the São Francisco River, one of the most important rivers of Brazil, whose riparian forest has been lost due to the great anthropic impact – hydrologic and geomorphological - that has destabilised the banks of the river channel across an urban fragment (Guimarães et al. 2014). The study site of the present investigation is a riparian forest in the Velhas River that was re-vegetated after the loss of vegetation. As a consequence, this area suffered an intense erosion process with destabilisation of the margins that was aggravated by the annual flood.

To improve the physical stabilisation of the margins, a timber cribbing cross-section and a system of groynes, a riprap were installed before the re-vegetation process, which was performed using the zones model. This study aims to evaluate the effect of the zones of the planted forest on soil HS composition, soil aggregation and their relationship with the rehabilitation process.

13 - Material and Methods

The experimental area consisted of riparian site, located on the right bank of the Velhas River in the city of Sabará, State of Minas Gerais, Brazil. This riparian site belongs to a slaughterhouse (‘19°50’20.33’ S; 53°51’59.13’ W’), which had lost the forest and was degraded. The predominant vegetation is tropical savanna (Brazilian Cerrado). The mean annual temperature is 22–23°C, with dry winters and rainfall during the summer. The total annual rainfall is 1200 mm. However, this site suffered annual floods that affected the slaughterhouse dependencies. During the flood event in the São Francisco River basin, the flow rate increased from 2810 m^3/s to 8000 m^3/s (Godim-Filho et al., 2004). To solve this problem, a project was proposed for the establishment of a riparian forest (120 m × 45 m = 5400 m^2) with the reconstruction of a floodplain river that could perform the natural function of water drainage (Experimental site). A preserved riparian forest (120 m × 45 m) was chosen as the positive reference of biological, physical and chemical integrity (Stoddard et al., 2006), which was

located near the experimental site (50 m) in an Environmental Protection Park (19°52'47'19°52'34'S. 44°07'44'–43°47'30'W) with a riparian tropical forest (Savanna) while the degraded site (120 m × 45 m), is adjacent to the experimental site, where the dominant vegetation was pioneer and invasive species (*Brachiaria decumbens* and *Ricinus communis)* without woody vegetation.

In the study area were realised two topographic and bathymetric campaigns that made possible the establishment and the calibration of a hydraulic model, using the HEC-RAS software (River Analysis System). The model allowed the definition of the flooded area for different return periods (Fig 1.1) and the calculation of the associated drag forces (Vieira, 2008),

13.1 - Physical stabilisation of banks

After establishment of the flood area, it soil levelling was conducted (Eco Maquinas Company) and, as mentioned earlier, stabilisation (Fig 1.2 A, B and C) was achieved with a timber cribbing cross-section associated to a riprap (Deflor company) as shown in Figure 1.2.

13.2 - Experimental design and planting (Afforestation)

After the physical stabilisation (Fig 1.2 B, C and D), the study site (120 m × 45 m) was re-vegetated according to the zones model (Lowrence, 1997; Shultz et al., 2004; Welsch, 1991) for recovering the lost functions such as drainage, stability and fertility (Fig 1.2 E). In Zone I (15 m), a phreatophyte species and flood-tolerant species that could survive and improve the stabilisation of the margins were planted (*Morus nigra, Rapanea guianensis, Miconia* sp.*, Eugenia uniflora, Psidium rufus, Psidium guayava, Inga edulis, Inga vera, Croton urucurana*, *Eritrina speciosa, Virola surinamensis, Tabebuia umbellata, Nectandra laceolata* (cf) and *Ocotea* sp.). Zone II (15 m) was cultivated with woody species that could produce biomass to improve

the contribution of litter and the formation of humic acid. Thus, the target was to increase the soil aggregation and porosity, ensuring the stability and drainage (*Rolinia laurifolia, Prothium heptaphyllum, Croton floribundus, Anadenanthera peregrina, Centrolobium tomentosum, Copaifera langsdorffii, Lonchocarpus* sp*, Macherium villosum, Pitadenia gonoacantha, Platymiscum floribundum, Luehea divaricata, Myrcia* sp*, Guapira opposita, Roupalla brasiliensis* and *Cupania* sp). Zone III (15 m) was cultivated with herbaceous, shrub and woody species to ensure not only the drainage but also the control of surface and subsurface erosion (*Hymenea courbaril, Mimosa bimucronata, Samanea tubulosa, Sterculia* sp*, Ceiba speciosa, Acrocomia aculeata, Tabebuia* sp*, Tradescantia* sp*, Helianthus annus,* and *Piper umbelattum*). After 4 months of growth under nursery conditions, the seedlings were transplanted to the field (Fig 1.2 E). A spacing of 3 × 3 m was maintained, and fertilisation was performed as suggested by Somasegaran and Hoben (1985). Post-transplantation of the native woody species, seeds of commercial herbaceous species were planted between the rows for surface erosion control during the first year. The study site suffered annual flood for 6 years post-transplantation when the forest was annually submerged at least for 1 week (Fig.1.2 F and G).

13.3 - Soil sampling

Soil samples were collected on three transects (100 m each), crossing the three riparian zones after 6 years of transplantation from 0 to 20 cm, in each zone plot (5400 m^2) per study site (Experimental site, Preserved site and Disturbed site). A total of 27 samples per site (9 soil samples/zone × 3 zones) were collected, which were used for all the physical, chemical and biological analyses. Similar sampling was performed in the adjacent preserved area (the preserved riparian forest used as a reference site) and in the disturbed site.

13.4 - Soil analysis

13.4.1 - Textural and soil fertility analyses

The soil samples were sieved with a 2-mm mesh and analysed for physical and chemical properties after planting (Embrapa, 1997). Besides textural analysis, the experimental site was compared with the preserved site in terms of the cation content, cation exchange capacity, base saturation, organic matter content and soil pH, among other parameters.

13.4.2 - **Determination of soil aggregation and porosity**

Soil aggregation measurements were carried out with the sieved soil samples to measure the proportion of water-stable aggregates (WSAs), using standard methods (Kemper e Rosenau, 1986). Undisturbed soil samples from the studied sites were collected in cylinders of a predetermined volume and sent for porosity analysis according to the Brazilian Agricultural Research Corporation (Embrapa 1997).

13.4.3 - **Fractionation of soil organic matter**

The fractionation of sequestered carbon from soil organic matter was performed according to the method of DABIN (1971) using 15 g of sieved soil/treatment/block to obtain the fraction of carbon represented by humic acid and fulvic acid.

13.4.4 - **Nuclear magnetic resonance (NMR)Spectroscopy** –

C-1 ^{13}C-NMR spectra from whole soil

These analyses were performed using mixed samples from each site. CPMAS ^{13}C-NMR spectra were recorded with a 360-1 System operating at the ^{13}C frequency of 363.335 MHz. The spectra were run with the following settings: 1

ms contact time, 7 kHz spinning speed, 3 s repetition time, scan time a little above 1 h, decoupling field about 60 KHz, 7 mm rotors and a sample size of 200 mg. (Preston, 1996)

C-2 ^{1}H-NMR spectra from humic acid solution

Humic acid fractions from the experimental site (Zones I, II and III) and mixed fractions from the preserved and disturbed sites were used for Proton NMR. Proton NMR spectra were recorded on a Bruker NMR Spe**ctro**meter operating at Proton NMR frequency of 500.1 MHz. The spectra were run at 1 ms contact time, 7 KHz spinning speed, 5 s repletion time and scan time of several hours in a 7 mm OD mass rotor.(Kang et al. 2002, Longstaffe et al. 2010, Fernández–Romero et al. 2015)

13.5 - Statistical analysis

The studied variables were compared among the sites (preserved, impacted and experimental sites) and among the zones using descriptive means and mean comparison that were performed using regression analysis likelihood method (Wedderburn, 1974). The level of significance of contrasts was corrected by Bonferroni method. The correlations were verified using Spearman analysis.

Principal component analysis (PCA) based on a variance analysis was used to select the most significant variables from the candidate variables: oxidised C, humic acid, fulvic acid, humin, soil aggregation and porosity, aromatic vinyl and aliphatic compounds that were related among them with each site. The analysis served to express the variables in terms of the two components that explained the total variability among the sites. Each component was accompanied by information about the intensity and direction of the correlation of the variable to the component. A perceptual map was plotted via PCA (Mingoti, 2007) and created using MINITAB 15 software. The significance level was set at 5% ($P \leq 0.05$) for all analyses.

14 - Results and Discussion

14.1 - **Soil analysis**

From hydrosedimentological's point of view, the results were very positive considering that with the stabilization of banks and revegetation were restored new conditions of morphological balance, consistent with the changes occurred in the basin and in the stretch.

As expected, the disturbed site and zone I of the studied sites had the highest sand content (Table 1) but it was predominant in the experimental site. A low nutrient status was found in the disturbed site, highlighting the organic matter, P, Ca^{2+} and CEC in comparison to the experimental and preserved sites, and the exception was Zone I. The textural and chemical characteristics of Zones II and III were similar between the preserved and experimental sites. These results can be attributed to the strong flood effect over Zone I of the study sites, especially over the disturbed site that had no woody vegetation. Although the flood had reached Zone II, the primary impact was over Zone I, unless changes were noted in Zone I of the preserved area even under flood.

14.2 - **Soil C species, aggregates and soil porosity**

Table 2 shows that in the preserved site, the largest values of C sequestrated in soil were found as oxidised C, C-humic and humin, which were dominant in Zones II and III. In the experimental site, the same result was observed. In disturbed site these values were lower than those of the other sites. In contrast, fulvic acid was especially high in Zone I at all sites.

These results suggest that HS of the experimental site can be different from those of the disturbed and preserved sites. Vegetation types, producing

different litters, can modify the chemistry of soil organic matter (Laird et al., 2008; Golchin et al., 1997).

Soil aggregation was not observed in the disturbed site. Microaggregates were formed in the preserved and experimental sites. However, a high concentration of macro-aggregates (>0.5 mm) was found in all the zones of the preserved site and in Zone II of the experimental site. While macroporosity was dominant in Zone I, microporosity was high in Zone II, especially in the preserved and experimental sites (Table 2).

The zone effect on carbon species (Table 3) showed that the experimental site did not differ from the disturbed or preserved site in Zone I (Table 3A), Zone II (Table 3B) and Zone III (Table 3C) exept the C –oxidized. However, the C species of the preserved site were different from those of the disturbed site in all zones.

In contrast, soil aggregation in the experimental site was different from that in the preserved and disturbed sites in all zones (Table 3 A, B and C). Regarding porosity, only the variable microporosity of the experimental site was different from that of the preserved and disturbed sites in Zones I and II (Tables 2A and 2B). However, in Zone III (Table 2C), the microporosity was similar in both the experimental and preserved sites. These results showed that the restoration procedures modified the functions of the riparian forest with regard to environmental services as soil aggregation and porosity.

Soil aggregation is one of the most important soil quality indicators because of its association to soil porosity and, therefore, to aeration and water drainage (Bronick and Lal, 2005). The formation of aggregates and their size and stabilisation depend on the soil type and humic acid formation (Roseta and Chinyere, 2006). Microaggregates (<250 μm) are formed from recalcitrant and labile HS attached to clay and polyvalent cations to form other particles that together form the macroaggregates (>250 μm).

In this way in the studied sites the type of humic acid formed rather than its have an influence on aggregation as showed by Tisdall (1996).

Humic and fulvic acids can differ in quantity and chemical composition, depending on several variables such as climate, original soil (Aranda et al.

2011), altitude, soil age (Baglieri et al., 2012), vegetation type (Singhal and Sharma, 1983) and soil management (Golchin et al., 1997, Miglierina and Rosell, 1995). Considering that the experimental and preserved sites show the same soil origin and altitude and similar woody vegetation, the variables that may be distinguishable are flood intensity, herbaceous vegetation type and age of the soil organic matter. The vegetation type of the experimental and preserved sites was different from that of the disturbed site, where the pioneering and invasive plants were dominant. Vegetation succession can enhance the accumulation of soil carbon and the quality of humic acid (Shen et al., 2014), especially in the riparian forest (Zhanga et al., 2012). The vegetation type can modify the humification and acid aromaticity indices of organic as showed by Abakumov et al. (2013) and Yao et al. (2009). These results speak in favour of the idea the chemical composition of HS as a potential indicator of restoration as described by Pietrzykowski and Chodak (2014) using infrared spectroscopy.

14.2.1 - **Humic carbon species**

Despite the vast research on humic acid structure, the detailed nature of HS is still not completely understood. From a chemical point of view, the HS are molecular aggregates consisting of sugar, fatty acids, polypeptides, aliphatic chains and aromatic rings (Simpson et al., 2002). According to Golchin et al. (1997), the NMR spectra from soil samples could be divided into four chemical shift regions based on the chemical types of carbon, as follows: 0–46 ppm (alkyl C), 46–110 ppm (O-alkyl C), 110–165 ppm (aromatic C) and 165–210 ppm (carbonyl C). Tao et al. (1999) found the same NMR spectra in humic and fulvic acid samples that were divided into four regions: I: 0–50 ppm, II: 51–105 ppm, III:106–160 ppm and IV: 161–200 ppm, corresponding to the chemical groups:I: aliphatic, II: carbohydrate, III: aromatic and IV: carboxyl regions. This spectra distribution was confirmed by Knicker and Lüdermann (1995), Adani et al. (2007) and Laird et al. (2008).

The samples of whole soil (Fig. 2) showed very broad and unresolved signals, due to which it was not possible to determine the types of carbon

species. All the samples showed saturated carbons in the 60–0 ppm region, which are considered as the conspicuous components of all terrestrial humic acids (Hatcher, 1980). These results could suggest a contribution of aliphatic organic matter, especially alkyl C, from the water in the riparian forest soils in all the studied sites. The unsaturated:saturated ratio was about 1:4.

The H-NMR spectra of the humic acid fraction samples (Fig. 3) showed a difference among them in terms of the number and position of the peaks. According to Kang et al. (2002) and Longstaffe et al. (2010), the H-NMR spectra can be divided into three regions: I: 0–3 ppm (aliphatic, aromatic CH_2, CH_3, proteins), II: 3–6 ppm (carbohydrates H and lignin-methoxy) and III: 6–8 ppm (aromatic H, lignin and proteins). A comparison of the samples from the different sites showed that the spectra consist of wide peaks in the 3–6.0 ppm region (region 2) in all zones with dominance in the carbohydrates region. The percentages of total intensity for each recalcitrant region estimated by integrating the ^{1}H-NMR spectrum within each region and the composition of aromatic and aliphatic groups are listed in Table 4. The presence of aromatic compounds was verified in all samples. Considering the 8.0-6.0 (region III) and 2.0-0 ppm (region I) regions, a higher contribution of aliphatic peaks was found in the experimental (Zone I) and disturbed sites with the following distribution: disturbed site > experimental site Zone I > experimental site Zone II > preserved site > experimental site Zone III. Similar spectra were obtained by Chefetz and Xing (2009) and Stuermer and Payne (1976) with humic acid from sediments.

When all the variables were compared by the likelihood method (Table 5B), it was observed that the aromatic and aliphatic content of the experimental site was also different from those of preserved and disturbed sites, similarly to humic acid content and soil aggregates. These results confirm the differences of humic substances among studied sites. However, the fulvic acid content of the experimental site was similar to those of the disturbed and preserved sites.

Considering that the studied sites suffered annual flooding for 6 consecutive years (Fig. 1.2 G), a dominance of the more leachable molecules with smaller size and less aromatic rings as that of fulvic acid could be expected

(Kang et al., 2002), which can explain the similarity of fulvic acid content among the sites.

Despite the flooding effect, humic acid was produced in the experimental site, although it was quantitatively and qualitatively different from that in the disturbed and preserved sites, which suggests that the studied sites are under the influence of different carbon sources. Indeed, different land management procedures can induce changes in the aromaticity of humic acids (Aranda et al., 2011) due the presence of other non-lignin carbon sources (Bartuska et al., 1980). These data favor the hypothesis of interference of an environmental impact factor, such as flooding, over the humic acid formation in the experimental site.

Bartuska et al. (1980) showed by using ^{13}C-NMR spectra of different lignin sources that no resonances were found in the 0–50 ppm region for unsubstituted aliphatic carbons, in contrast to that observed in the soil humic acid fractions from the studied sites. Thus, the unsubstituted aliphatic carbons in the humic acids must originate from other sources such as microbial exudates and soil algal lipids (Hatcher et al., 1981). Therefore, the high contribution of aliphatic peaks in the studied sites could be understood as a contribution of the flooding, as observed by Graham et al. (2002).

14.3 - Spearman correlation and PCA

The correlation data (Table 5B) showed that humic acid formation in the studied sites favoured the soil aggregation promoted by macro-aggregates as well as it improved the microporosity. In other words, the formation of humic acid is the primary factor for soil stability and water infiltration. However, fulvic acid was not related to the environmental services in the studied area and the aliphatic species were not related to fulvic acid.

The PCA results (Fig. 4A) confirm that all of the studied variables (oxidised C, humic acid, fulvic acid, humin, soil aggregation and porosity, aromatic species: vinyl and aliphatic) were related to each site and explained 79% of the results. Component 1 comprising the aliphatic and macroporosity explained 10% of the

results and the second component comprising oxidised C, humic acid, fulvic acid, humin, soil aggregation, microporosity, aromatic species: vinyl explained 68.8% of the results. The PCA confirms that the primary contribution was done by humic acid. The contribution of relevant variables for each component was plotted and showed the distribution of analysis per zone (Fig. 4A). The samples from the disturbed site (red) formed a separate group related to component 1 (macroporosity and aliphatic chemical species). The samples from the experimental (green) and preserved (blue) sites formed very closed groups. Some samples from Zone 1 of the experimental (green square) and preserved (blue square) sites grouped separately from each other, suggesting a distinct profile, especially of C species.

In Zone I, the flood impact on humic acid formation that affected the environmental services of soil aggregation and porosity, particularly in the disturbed and experimental sites. However, in the preserved site, this damage was more buffered by the preserved humic acid soil and vegetation. In Zone I, the aliphatic composition and macroporosity were the primary indicators. Zone II and Zone III appeared to be more resilient to the flooding effect in both the experimental and preserved sites what can be related to soil aggregation and humic acid formation and its aromatic: vinyl composition.

Despite the qualitative and quantitative differences in the humic acid formation among the experimental and preserved sites, the humic acid produced in the experimental site was able to carry out the environmental services of aggregation and porosity. Thus, although the samples from Zone I of the experimental site have strongly contributed for the dissimilarities between the experimental and preserved sites, the restoration in the former can be considered for the latter. Reinforcing this idea, the dendrogram shown in Fig. 4B confirmed that samples from the experimental site clustered with those from the preserved site and formed a separate cluster from the disturbed site samples.

In conclusion, in spite of the flood effect, the humic acid formed in the experimental site allowed an improvement of soil aggregation and porosity in Zones II and III. The acquisition of these environmental services was responsible for the similarities between the preserved and experimental sites.

Thus, it can be said that the restoration is evolving towards those of the preserved site and that the rehabilitation process has attained an intermediate phase of restoration. The aliphatic contribution to humic acid formation was a determinant for the separation of zones under the flood effect, which allow us to elect Zone II as the primary indicator of environmental services in the restoration process of a riparian forest. The qualitative and quantitative humic acid composition together with soil macro-aggregation could be considered as a strong indicator of riparian forest restoration, especially in Zone II.

Legends

Table 1: Analysis of soil chemical characteristics of whole Oxisol samples from the preserved site (PS), disturbed site (DS) and experimental site (ES) and each of the divisions in Zone I, Zone II, and Zone III.

Table 2: Physical and chemical soil attribute analysis among the sites and zones per mean of studied variable by likelihood regression.

Table 3: Comparison of soil variables among the sites in Zone I (A), Zone II (B), and Zone III (C) by likelihood method at 5% significance level with Bonferroni adjustment.

Table 4: Contribution of aromatic (including vinyl protons) molecular species and aliphatic compounds in the humic acid sample solutions, considering the regions of H-NMR spectra. ES: Experimental site Zones (I, II and III), PS: Preserved site, DS: Disturbed site.

Table 5 A**:** Comparison of soil variables among the sites by likelihood method at 5% significance with Bonferroni adjustment. **B**: Analysis of Spearman correlations among the variables.

Figure 1.1: : Localization of experimental site in the google map (yellow arrow)

Fig 1.2. Experimental site. A: Before transplantation, B: timber cribbing cross-section (DEFLOR company), C: Rip Rap (DEFLOR company), D: Aerial view, E: Transplantation, F: Forest 4 years post-transplantation, G: Forest 4 years after transplantation under flooding.

Figure 2: Solid-state ^{13}C-NMR spectra of whole soils from the study sites with different vegetation cover.

Figure 3: ^{1}H-NMR spectra of humic acid samples from the preserved site (P), disturbed site (D), experimental site Zone I, experimental site Zone 2 II, experimental site Zone III.

Fig **4 A**: Principal component analysis and scatter plot based on the mean of studied variables in each zone of the sites and **B**: Dendrogram (ES: Experimental site, DS: Disturbed site, PS: preserved site).

Figures

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Fig 1.2. Experimental site. A: Before transplantation, B: timber cribbing cross-section (DEFLOR company), C: Rip Rap (DEFLOR company), D: Aerial view, E:

Transplantation, F: Forest 4 years after transplantation, G: Forest 4 years after transplantation under flooding.

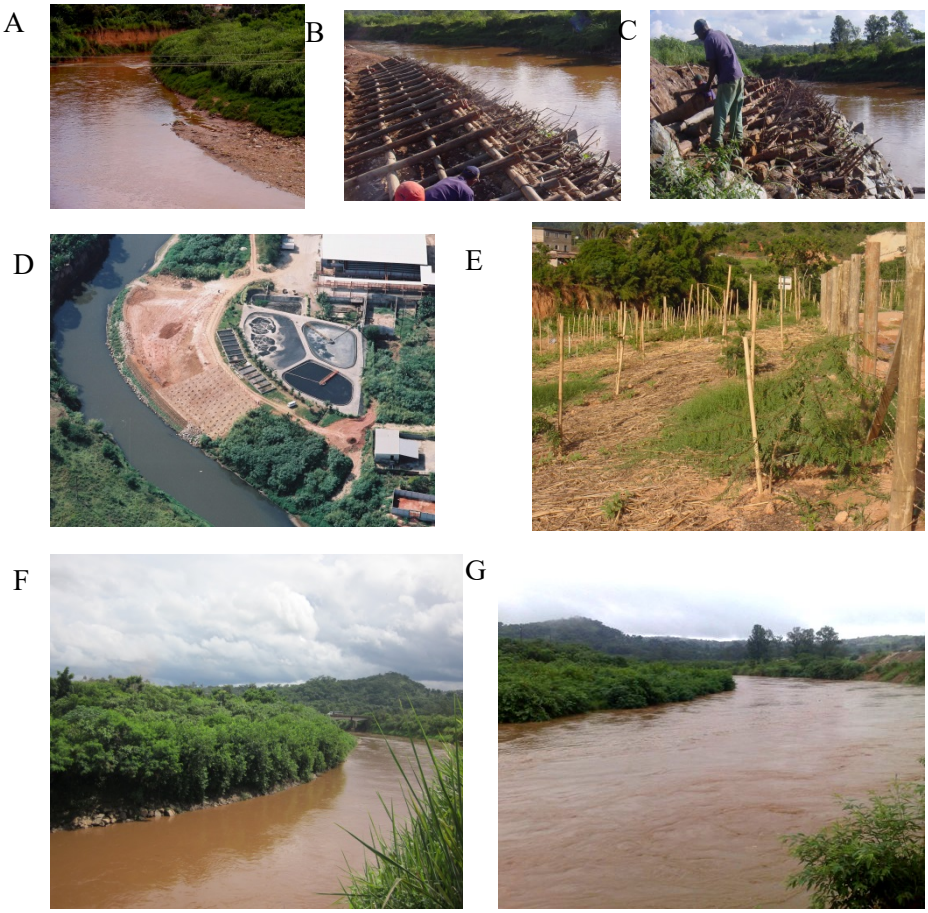
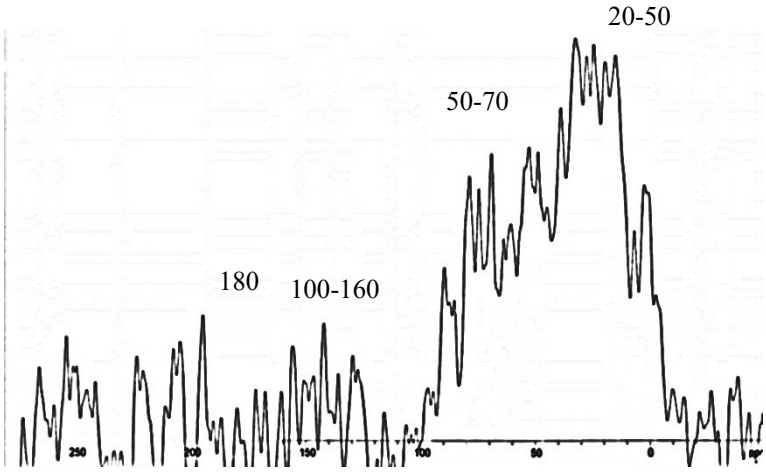
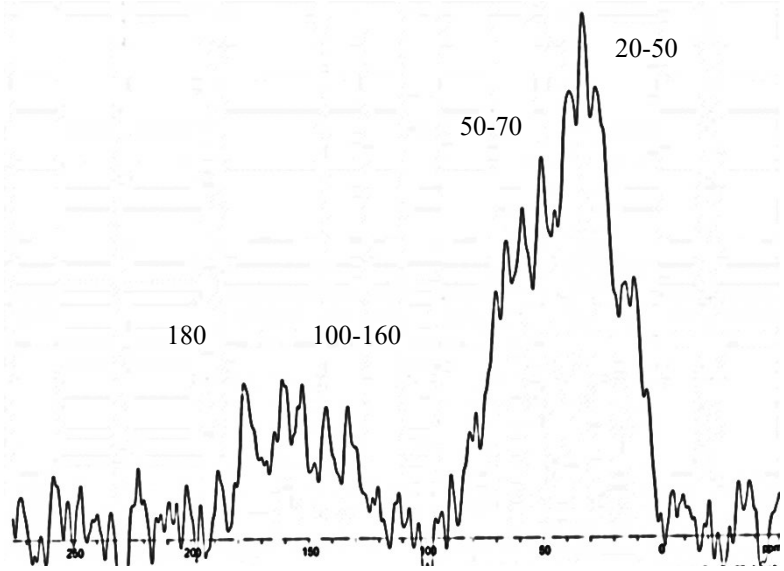


Figure 2: Solid-state ^{13}C-NMR spectra of whole soils from the study sites with different vegetation cover.

Disturbed site



Experimental site



Preserved site

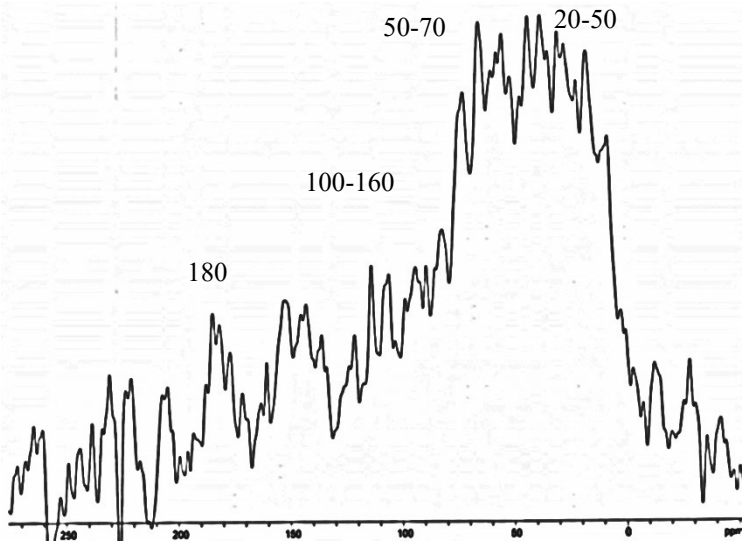


Figure 3:

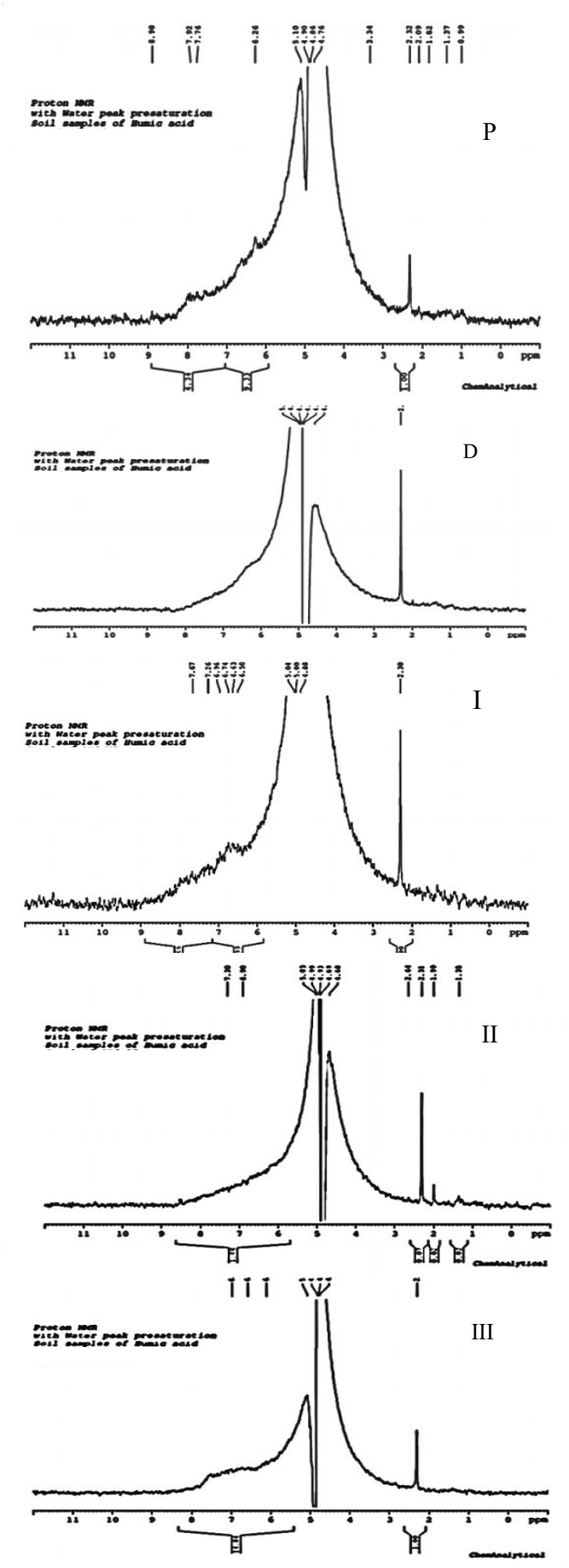
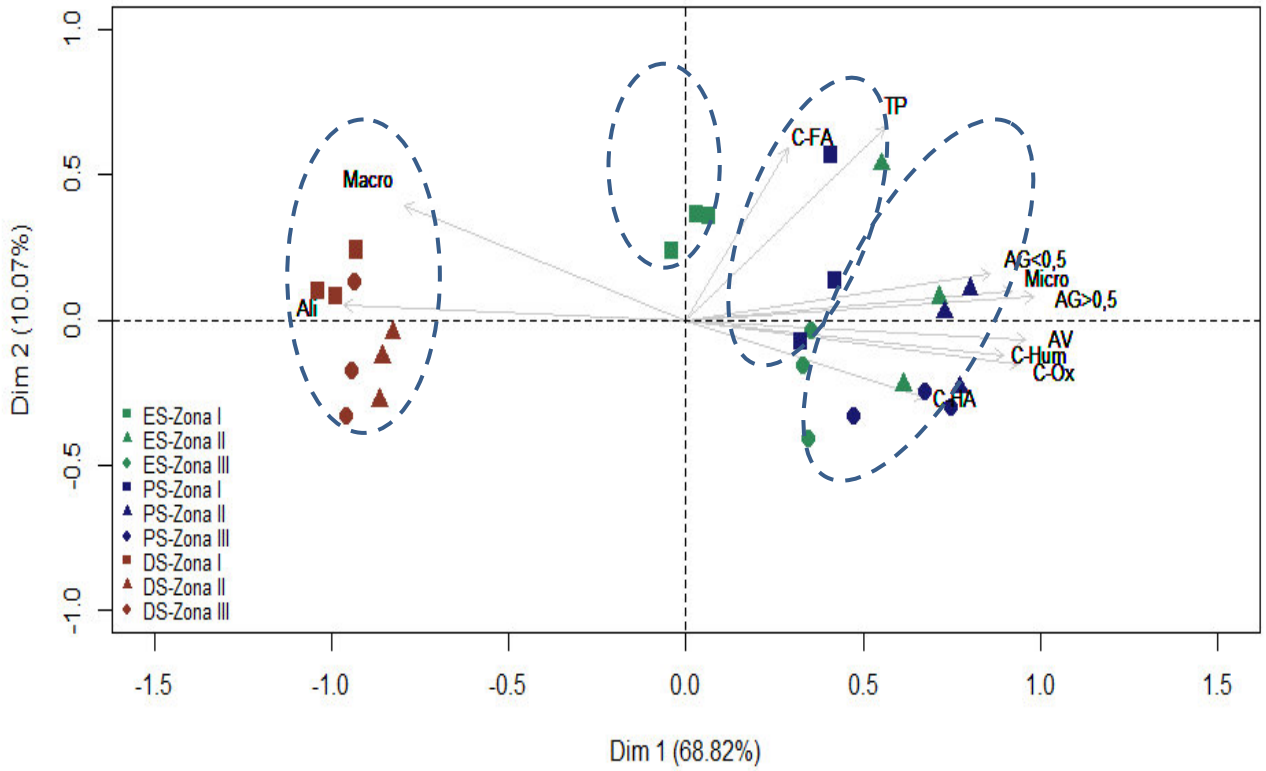

Proton NMR
with Water peak presaturation
Soil samples of Humic acid
P
D
I
II
III
ppm
Chemalytical

Fig **4 A**:


Dim 2 (10.07%)
Dim 1 (68.82%)
Macro
Ali
TP
C-FA
AG<0,5
Micro
AG>0,5
AV
C-Hum
C-Ox
C-HA
ES-Zona I
ES-Zona II
ES-Zona III
PS-Zona I
PS-Zona II
PS-Zona III
DS-Zona I
DS-Zona II
DS-Zona III

4 B

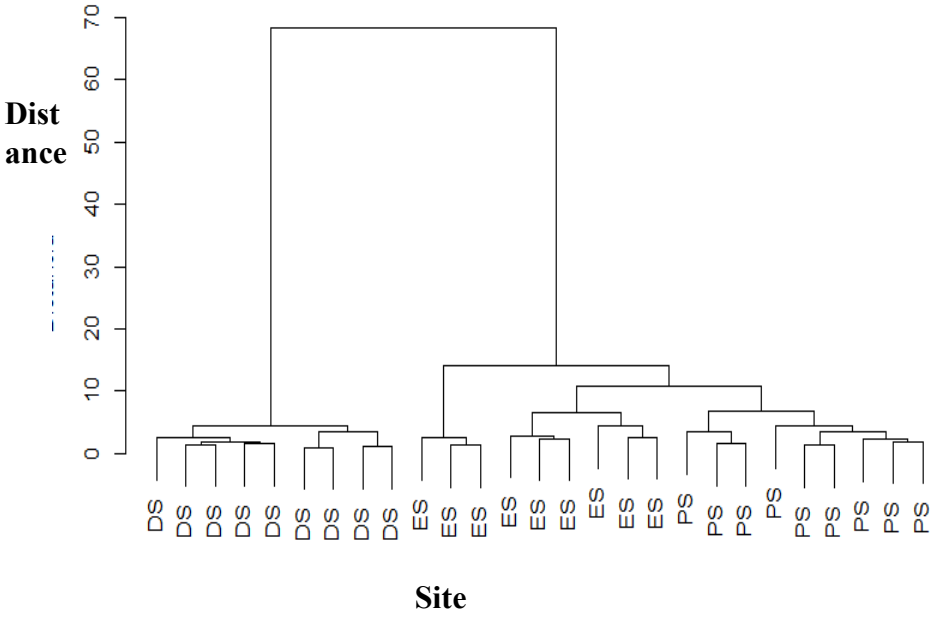

Dist
ance
Site
DS
ES
PS

Table 1 : Soil analysis of chemical characteristics of whole Oxisol samples from Preserved Site (PS) , Disturbed site (DS) and Experimental site (ES) and each of the divided in Zone I , Zone II and Zone III.

Samples	**pH**	**OM**	**P**	**K^+**	**Ca 2+**	**Mg 2+**	**SB**	**CEC**	**Clay**	**Silt**	**Total sand**
		(mg/dm^3)	(mg/dm^3)	$Mmol/dm^3$					%		
ES ZI	7.6	16	30	2,6	156	6	164,6	152	23,7	25,9	50,4
	7,5	16	27	2,8	150	8	160,8	154	24,4	24	51,5
	7,6	15	31	2,5	161	5	168,5	150	23,15	26,86	49,99
ES ZII	7,2	30	47	2,4	145	9	156,4	165	28,4	31,9	39,7
	7.1	29	51	2,2	147	12	161,2	167	26,6	33	40,4
	7.2	28	45	2,9	145	7	154,9	163	31,2	29	39,8
ES ZII	6.9	28	94	2,2	155	8	165,2	175	23,5	34,6	41,9
	6,8	27	61	2,4	156	11	169,4	163	32,4	25,6	42
	6.9	28	68	2	153	7	162	168	29,6	29	41,4
PS Z1	6.9	27	42	2.6	149	8	157	167	18,2	39,7	42,1
	6.8	25	44	3,1	144	8	155,1	161	18,4	40,2	41,4
	6.8	26	40	2,3	142	7	151,3	163	18,5	39,5	42
PS Z2	6,7	30	45	3,1	156	9	168,1	169	17,5	41,1	41,4
	6,6	31	42	3	161	10	174	170	17,7	41	41,3
	6,8	28	44	2,9	157	8	167,9	165	18,3	41,9	39,8
PS Z3	6,7	26	45	2,7	142	8	152,7	163	19,3	42,1	40,6
	6,9	28	42	3,1	148	7	158,1	169	18,3	42,1	39,6
	6,8	28	40	2,4	151	11	164,4	165	19,3	40,1	40,6
D S Z1	7,4	13	28	3,1	52	3	58,1	75	3,7	10,2	86,1
	7,5	11	27	3,5	51	2	56,5	74	2,1	9,6	88,3
	7,8	15	25	3,6	46	4	53,6	70	8,4	11	80,6
DS Z2	7,6	15	30	3	54	4	61	77	5	12	83
	7,5	16	29	2,9	65	3	70,9	79	5	11	84
	7,4	15	29	3,1	61	3	67,1	76	4	14	82
DS Z3	7.3	15	30	2,9	56	4	62,9	76	4,5	11,4	84,1
	7.5	16	29	2,8	47	4	53,8	74	5	10	85
	7,2	17	31	3,2	55	3	61,2	78	4,5	13,5	82

Table 2: Physical –Chemical soil attributes analysis among sites and zones per mean of studied variable via likelihood regression SD: standard deviation *: DS: Disturbed site, ES: Experimental site, PS: preserved site

Variables		DS*			ES*			PS*		
		Zone I	Zone II	Zone III	Zone I	Zone II	Zone III	Zone I	Zone II	Zone III
C-oxidized	Mean	0.14	0.23	0.22	0.94	1.81	1.55	1.36	2.50	2.10
	SD	0.05	0.08	0.11	0.14	0.68	0.05	0.11	0.26	0.06
C- humic acid	Mean	0.02	0.02	0.03	0.08	0.16	0.11	0.20	0.41	0.60
	SD	0.02	0.01	0.04	0.03	0.06	0.02	0.01	0.07	0.33
C- Fulvic acid	Mean	0.22	0.21	0.16	0.25	0.22	0.14	0.32	0.23	0.24
	SD	0.03	0.02	0.07	0.02	0.04	0.02	0.04	0.03	0.02
C-Humine	Mean	0.03	0.19	0.10	0.85	1.62	1.24	0.72	2.10	1.40
	SD.	0.03	0.03	0.03	0.60	0.41	0.04	0.20	0.21	0.26
Aggregate class (mm) ≥ 0.5	Mean	0.00	0.00	0.00	49.60	59.42	52.10	62.80	65.60	64.50
	SD	0.00	0.00	0.00	1.00	1.33	1.50	0.90	0.50	0.51
Aggregate class (mm) < 0.5	Mean	0.00	0.00	0.00	50.33	40.58	47.87	37.20	34.37	35.50
	SD	0.00	0.00	0.00	1.10	1.33	1.55	0.90	0.45	0.50
Macroporosity	Mean	0.34	0.30	0.33	0.33	0.24	0.27	0.26	0.26[c]	0.25
	SD	0.01	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.01
Microporosity	Mean	0.18	0.18	0.17	0.22	0.34	0.26	0.27	0.30	0.27
	SD.	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.01
Total porosity	Mean	0.52	0.49	0.50	0.55	0.58	0.53	0.53	0.56	0.51
	SD	0.01	0.02	0.01	0.01	0.05	0.02	0.04	0.02	0.01

Table 3 Comparison of soil variables among the sites in Zone I (A), Zone II (B), and Zone III (C) by likelihood method at 5% significance level with Bonferroni adjustment.

A

Variables	Zone I					
	DS – ES		DS - PS		ES – OS	
	Mean	Valor-P[1]	Mean	Valor-P[1]	Mean	Valor-P[1]
C-oxidized	-0,80	**0,004**	-1,22	**0,000**	-0,42	0,181
C- humic acid	-0,06	1,000	-0,18	0,211	-0,12	0,640
C- Fulvic acid	-0,03	0,944	-0,11	**0,005**	-0,08	0,050
C-Humine	-0,82	**0,006**	-0,69	**0,019**	0,13	1,000
Aggregate class (mμ) > 250	-49,60	**0,000**	-62,80	**0,000**	-13,20	**0,000**
Aggregate class (mμ) < 250	-50,33	**0,000**	-37,20	**0,000**	13,13	**0,000**
Macroporosity	0,01	1,000	0,08	**0,000**	0,07	**0,003**
Microporosity	-0,04	**0,002**	-0,09	**0,000**	-0,05	**0,000**
Total porosity	-0,03	0,611	-0,01	1,000	0,02	1,000

B

Variables	Zone II					
	DS - ES		DS - PS		ES – OS	
	Mean	Value-P[1]	Mean	Value-P[1]	Mean	Value-P[1]
C-oxidized	-1,58	**0,000**	-2,27	**0,000**	-0,69	**0,012**
C- humic acid	-0,14	0,484	-0,39	**0,002**	-0,25	0,055
C- Fulvic acid	-0,01	1,000	-0,02	1,000	-0,01	1,000
C-Humine	-1,43	**0,000**	-1,91	**0,000**	-0,48	0,143
Aggregate class (mμ) > 250	-59,42	**0,000**	-65,60	**0,000**	-6,18	**0,000**
Aggregate class (mμ) < 250	-40,58	**0,000**	-34,37	**0,000**	6,22	**0,000**
Macroporosity	0,06	**0,005**	0,04	0,067	-0,02	0,791
Microporosity	-0,15	**0,000**	-0,12	**0,000**	0,04	**0,004**
Total porosity	-0,09	**0,001**	-0,07	**0,005**	0,02	1,000

C

Variáveis	Zone III					
	DS - ES		DS - PS		ES – OS	
	Média	Valor-P[1]	Média	Valor-P[1]	Média	Valor-P[1]
C-oxidized	-1,33	**0,000**	-1,88	**0,000**	-0,55	**0,050**
C- humic acid	-0,07	1,000	-0,56	**0,000**	-0,49	**0,000**
C- Fulvic acid	0,02	1,000	-0,08	**0,042**	-0,10	**0,009**
C-Humine	-1,15	**0,000**	-1,30	**0,000**	-0,16	1,000
Aggregate class (mμ) > 250	-52,10	**0,000**	-64,50	**0,000**	-12,40	**0,000**
Aggregate class (mμ) < 250	-47,87	**0,000**	-35,50	**0,000**	12,37	**0,000**
Macroporosity	0,06	**0,008**	0,09	**0,000**	0,03	0,302
Microporosity	-0,09	**0,000**	-0,10	**0,000**	-0,01	0,540
Total porosity	-0,03	0,578	-0,01	1,000	0,02	1,000

Table 4 Contribution of aromatic (including vinyl protons) molecular species and aliphatic compounds in the humic acid sample solutions, considering the regions of H-NMR spectra. ES: Experimental site Zones (I, II and III), PS: Preserved site, DS: Disturbed site.

Site	Peak areas (arbitrary units)-%	
	Aromatic and vinyl	Aliphatic
ES ZI	87,7	12.3
ES ZII	90.1	9.9
ES ZIII	93.5	6.5
PS	93.2	6.8
DS	74,6	25.4

Table 5 A: Comparison of soil variables among the sites by likelihood method at 5% significance with Bonferroni adjustment. **B**: Analysis of Spearman correlations among the variables.

A

Variables	DS – ES*		DS – OS		ES – OS	
	Mean	Pvalue	Mean	P Value	Mean	P Value
C-oxidized	-1,24	**0,000**	-1,79	**0,000**	-0,55	**0,003**
C- humic acid	-0,09	0,488	-0,38	**0,000**	-0,29	**0,000**
C- Fulvic acid	-0,01	1,000	-0,07	**0,003**	-0,06	0,007
C-Humine	-1,13	**0,000**	-1,30	**0,000**	-0,17	0,891
Aggregate class (mμ) > 250	-53,71	**0,000**	-64,30	**0,000**	-10,60	**0,000**
Aggregate class (mμ) < 250	-46,26	**0,000**	-35,69	**0,000**	10,57	**0,000**
Macroporosity	0,05	**0,003**	0,07	**0,000**	0,03	0,127
Microporosity	-0,09	**0,000**	-0,10	**0,000**	-0,01	1,000
Total porosity	-0,05	**0,003**	-0,03	0,067	0,02	0,622
Aromatic and vinyl	-13,20	**0,000**	-15,33	**0,000**	-2,13	**0,000**
Aliphatic	13,87	**0,000**	16,00	**0,000**	2,13	**0,002**

.*: DS: Disturbed site, ES: Experimental site, PS: preserved site,

B

*C-ox: C-oxidized, C-hu: humic acid, C- fu:- Fulvic acid. CHun: Humine. Macrag: Aggregate class (mm) > 0.5.,

*Variables	C-ox.	C-hu	C-fu	C-Hum	Macrag	Micrag	Macrp	Micrp	T.Pr	ArV	Aliph
C-ox.	1.00										
C-hu	**0.87**	1.00									
C-fu	0.15	0.38	1.00								
C-Humn	**0.91**	**0.83**	0.10	1.00							
Macroag	**0.88**	**0.95**	0.36	**0.81**	1.00						
Microag	**0.48**	**0.40**	0.17	**0.52**	**0.39**	1.00					
MacroP	**-0.79**	**-0.78**	-0.13	**-0.72**	**-0.77**	-0.36	1.00				
MicroP	**0.81**	**0.81**	0.26	**0.83**	**0.84**	**0.52**	**-0.80**	1.00			
T.Por.	**0.45**	**0.44**	0.29	**0.56**	**0.50**	**0.51**	-0.14	**0.68**	1.00		
Aro:V.	**0.85**	**0.79**	0.01	**0.76**	**0.79**	**0.58**	**-0.70**	**0.66**	0.32	1.00	
Aliph	**-0.85**	**-0.79**	-0.03	**-0.78**	**-0.79**	**-0.58**	**0.73**	**-0.68**	-0.30	**-0.98**	1.00

Micrag: Aggregate class (mm) < 0.5., Macrp: Macroporosity, Micrp: Microporosity,. T Pr: Total porosity,. Ar.V : Aromatic and vinyl., Aliph: Aliphatic

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