

CONVERSÃO DE PASTAGEM EM PLANTIOS DE LEGUMINOSAS
ARBÓREAS: SERRAPILHEIRA, CARBONO E NITROGÊNIO NO
PERFIL DO SOLO

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UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE
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CAMPOS DOS GOYTACAZES – RJ
MARÇO - 2021

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como parte das exigências para obtenção do
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Orientadora: Prof.^a Deborah Guerra Barroso
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*"Fracassei em tudo que tentei na vida
Tentei alfabetizar as crianças brasileiras, não consegui.
Tentei salvar os índios, não consegui.
Tentei fazer uma universidade séria e fracassei.
Tentei fazer o Brasil desenvolver-se autonomamente e fracassei.
Mas os fracassos são minhas vitórias.
Eu detestaria estar no lugar de quem me venceu."*

Darcy Ribeiro

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RESUMO

SIQUEIRA, David Pessanha, D.Sc., Universidade Estadual do Norte Fluminense Darcy Ribeiro. Março 2021. Conversão de pastagem em plantios de leguminosas arbóreas: serrapilheira, carbono e nitrogênio no perfil do solo. Orientadora: Prof.^a Deborah Guerra Barroso. Co-orientador: Prof. Marcos Vinicius Winckler Caldeira.

A produção e decomposição da serrapilheira exercem um papel importante na ciclagem de nutrientes e no sequestro de carbono (C), influenciando na manutenção e na produtividade dos povoamentos florestais. Grande parte do C fica armazenado no perfil do solo, atuando como um reservatório importante para o ciclo do C. Além do C, o nitrogênio (N) também pode ser armazenado no solo, sendo outro nutriente fundamental para a manutenção dos plantios florestais. Este trabalho objetivou conhecer a produtividade e a composição química da serrapilheira, dinâmica da liberação dos nutrientes e as transformações do C e N no perfil do solo sob plantios homogêneos de leguminosas arbóreas, aos 28 dias após a conversão de pastagem. Para isso, três espécies da família Fabaceae foram selecionados: *Centrolobium tomentosum*, *Hymenaea courbaril* e *Plathymenia reticulata*. Em cada plantio, foram instalados coletores de serrapilheira que foram esvaziados mensalmente durante um ano. A decomposição foi avaliada por meio de “litterbags” ao longo de 240 dias nos plantios de *P. reticulata* e *H. courbaril*. O C e N orgânico e a abundância natural do ¹³C e ¹⁵N do solo foram determinados até 1m de profundidade. A produção de

serrapilheira (fração de folhas das espécies plantadas) foi de 5689 kg ha⁻¹ ano⁻¹ no plantio de *P. reticulata*, quase duas vezes superior a *H. courbaril* e *C. tomentosum*, com 3231 kg e 3117 kg ha⁻¹ ano⁻¹, respectivamente. No entanto, a fração de folhas de outras espécies foi superior no plantio de *H. courbaril* (6407 kg ha⁻¹ ano⁻¹), cerca de três vezes superior à *P. reticulata* (1844 kg ha⁻¹ ano⁻¹). A *P. reticulata* se destacou retornando maior C, N e P para o ecossistema. A decomposição foliar foi similar entre as espécies, entretanto, a liberação de nutrientes foi superior para *P. reticulata* enquanto *H. courbaril* apresentou imobilização de nutrientes, especialmente N. A decomposição das folhas de *P. reticulata* foi provavelmente limitada em P, enquanto *H. courbaril* parece ser co-limitada por N e P. A maior parte da matéria orgânica do solo é proveniente dos plantios florestais atuais e não da pastagem anterior, exceto para *C. tomentosum*. O ciclo do N parece ser diferente entre as espécies já que *P. reticulata* teve maior quantidade de N retornado via serrapilheira e maior enriquecimento do ¹⁵N no perfil do solo em relação às outras espécies, o que indica perdas gasosas do N. *P. reticulata* e *H. courbaril* parecem apresentar utilização eficiente do N pela vegetação, enquanto *C. tomentosum* parece ter sofrido limitação pelo C do solo.

Palavras-chave. Ciclagem de nutrientes; Espécies fixadoras de N; Matéria orgânica do solo; Fracionamento Isotópico

ABSTRACT

SIQUEIRA, David Pessanha, D.Sc., Universidade Estadual do Norte Fluminense Darcy Ribeiro. March 2021. Pasture conversion to nitrogen-fixing tree plantations: litter, carbon, and nitrogen in the soil profile. Prof. Deborah Guerra Barroso. Co-supervisor: Prof. Marcos Vinicius Winckler Caldeira

Litter production and decomposition play an important role in the productivity of tree plantations, nutrient cycling, and carbon (C) sequestration in tropical ecosystems. The soil profile is a crucial sink for C cycle as a great part of its storage takes place in it. Apart from the C, nitrogen (N) is another essential nutrient for the development of forest plantations and also can be stored in the soil. This work aimed to report the litter productivity and its chemical composition, nutrient release dynamics, and C and N transformations in the soil profile under N-fixing tree monospecific plantations 28 after pasture conversion. Three forest species were selected: *Centrolobium tomentosum*, *Hymenaea courbaril* e *Plathymenia reticulata*. Litter traps were installed in each forest stand and emptied monthly over one year. Litter decomposition was assessed for *P. reticulata* and *H. courbaril* for 240 days. Soil organic C and N and the natural abundance of ^{13}C and ^{15}N were evaluated up to 1m soil depth. Litter productivity (leaves of the planted species) was $5.689 \text{ kg ha}^{-1} \text{ yr}^{-1}$ for *P. reticulata*, almost two times higher than *H. courbaril* and *C. tomentosum* with 3.231 and $3117 \text{ kg ha}^{-1} \text{ yr}^{-1}$, respectively. On the other hand, other species' leaves fraction was higher for *H. courbaril* (6.407 kg ha^{-1}

yr⁻¹) ca. three times higher than *P. reticulata* (1.844 kg ha⁻¹ yr⁻¹). *P. reticulata* stood out due to higher C, N, and P returning to the system owing to higher leaves productivity. The mass loss displayed similar pattern between species, however, a greater nutrient release was observed for *P. reticulata* whereas *H. courbaril* showed N immobilization. *P. reticulata* decomposition was very likely P limited while *H. courbaril* was co-limited by P and N. Most of the current soil organic matter is from the tree plantations instead of the previous pasture, except for *C. tomentosum*. N cycle appears to be different among species as *P. reticulata* had higher N returned via litterfall and similar ¹⁵N enrichment in the soil profile compared to the other species indicating gaseous loss of N. *P. reticulata* and *H. courbaril* seemed to be N-efficient whereas *C. tomentosum* appears to be hindered by soil C-limitation.

Keywords. Nutrient cycling, N-fixing species, Soil organic matter, isotopic fractionation

1. INTRODUÇÃO

O bioma Mata Atlântica possui alta diversidade de espécies e elevado grau de endemismo, sendo identificado como um “*hotspot*” mundial de biodiversidade (Myers et al., 2000). Apesar da relevância ecológica, a Mata Atlântica é um dos biomas mais ameaçados pelo desflorestamento, onde apenas 9-12% da vegetação original ainda existe em pequenos fragmentos (Ribeiro et al., 2009; SOS Mata Atlântica, 2020).

Devido à grande área perdida para o desmatamento, a restauração da Mata Atlântica é urgente, e, se bem manejadas, as novas áreas revegetadas poderão também contribuir para atender às demandas de produtos madeireiros e não madeireiros do mercado consumidor, reduzindo intervenções antrópicas em áreas naturais (Barroso et al., 2018).

No contexto da alta biodiversidade da Mata Atlântica, a experiência com as espécies florestais nativas, sua ciclagem de nutrientes e interações com a fertilidade do solo é limitada, e se tratando de espécies tropicais fixadoras de nitrogênio (N), o conhecimento é ainda mais escasso (Mayer et al., 2020; Peng et al., 2020). Através da fixação biológica do N, espécies fixadoras podem se associar com bactérias diazotróficas e converter o N₂ atmosférico em amônia (NH₃) para o solo e este fato tende a estimular a ciclagem de outros nutrientes, alterando o ciclo biogeoquímico dos sistemas florestais (Chaer et al., 2011; Mendes et al., 2021).

Assim, as espécies fixadoras de N, com potencial para restaurar o C e N do solo para os níveis encontrados sob uma floresta nativa em um curto período (Macedo et al., 2008; Chaer et al., 2011), devem ser priorizadas em projetos de reflorestamento já que a melhoria da fertilidade do solo poderá ser crucial para a produtividade dos plantios e favorecer o crescimento de espécies mais exigentes nutricionalmente (Chaer et al., 2011; Forrester et al., 2013; Mayer et al., 2020).

A ciclagem dos nutrientes é fundamental em ecossistemas florestais, sendo a principal fonte de nutrientes, o que reflete na capacidade produtiva e no potencial de recuperação ambiental dos plantios (Caldeira et al., 2007; Grugiki et al., 2017). Os processos de ciclagem podem também favorecer outras culturas em sistemas consorciados, reduzindo a necessidade de insumos (Duarte et al., 2013).

O suprimento de nutrientes via ciclagem da serrapilheira (folhas, galhos, flores e frutos) é ainda mais relevante em ecossistemas tropicais e seus solos altamente intemperizados, com baixa fertilidade. Isso torna importante a compreensão da produção e composição química da serrapilheira das diferentes espécies, parâmetros importantes para escolha das espécies mais adequadas aos objetivos dos plantios e à realidade de cada sítio.

A composição química da serrapilheira pode ser considerada de “alta qualidade”, caracterizada por alta concentração de N, baixa relação C:N e baixo teor de lignina e fenóis ou de “baixa qualidade”, quando apresenta características opostas. A serrapilheira rica em N e baixos teores de lignina e fenóis, comum em espécies fixadoras de N, pode favorecer o aporte de matéria orgânica (MO) do solo, devido à maior eficiência no uso do substrato pelos decompositores (Cotrufo et al., 2013; Castellano et al., 2015).

De maneira geral, as espécies fixadoras de N vêm sendo apontadas como favorecedoras do acúmulo de carbono (C) no solo, quando comparadas às espécies não fixadoras (Luo et al., 2004; Forrester et al., 2013; Mayer et al., 2020), elevando média +11,8% o C do solo (Nave et al. 2009). Para explicar este fato, Resh et al. (2002) apontam o maior aporte de C via serrapilheira e a retenção do C antigo no solo. No entanto, os mecanismos por trás desses processos permanecem pouco conhecidos (Peng et al., 2020). Estes efeitos podem variar entre as espécies fixadoras de N, conforme verificado por Hoogmoed et al. (2014) para duas espécies do gênero *Acacia* spp..

Para melhor compreender o destino do C e do N da serrapilheira e os “pools” no perfil do solo após mudanças de uso da terra, o fracionamento de isótopos estáveis tem sido aplicado como uma ferramenta importante (Hobbie e Ouimette 2009; Liu et al., 2018; Paul et al., 2019). A técnica é também efetiva para rastrear os efeitos da mudança do uso da terra na origem da matéria orgânica (MO) do solo (Conen et al., 2008; Vicente et al., 2016; Carvalho et al., 2017).

Isso se dá devido a diferenças nos ciclos fotossintéticos, no qual plantas C_3 contribuem com a MO empobrecida em ^{13}C , enquanto plantas C_4 tipicamente contribuem com MO enriquecida em ^{13}C . O ^{13}C é o átomo mais pesado do C e pode permanecer na MO por mais tempo, devido ao “consumo” preferencial do isótopo mais leve pelos decompositores (Conen et al., 2008; Liu et al., 2018).

Em meta-análise realizada por Zheng et al., (2021), baseada em dados isotópicos da serrapilheira e do solo, os autores concluíram que 24% do C e 58% do N contidos na serrapilheira foram recuperados pelo solo, independente das condições experimentais. Ainda, os autores concluíram que, proporcionalmente, maior quantidade de C e N foram transferidos para o solo quando a serrapilheira foi decomposta lentamente, descrevendo a relação dos nutrientes na serrapilheira com os nutrientes no solo.

Neste contexto, foi hipotetizado que plantios florestais de espécies fixadoras de N de mesma idade podem diferir quanto à produtividade e qualidade da serrapilheira, liberação de nutrientes e, conseqüentemente, quanto às mudanças do C e do N orgânicos no perfil do solo. É importante ressaltar que não há relatos na literatura destas informações em áreas após a conversão de pastagem em plantios monoespecíficos de espécies tropicais fixadoras de N, o que redobra a importância deste estudo.

Vale ainda salientar que para comparar informações sobre a ciclagem de nutrientes e C e N do solo sob diferentes espécies, um sítio de estudo que elimine a influência da idade da vegetação, clima e tipo de solo tem um papel crucial, mas ainda são extremamente raros (Vesterdal et al., 2008). Ademais, sítios de estudo que possuem essas características frequentemente não tem repetições, como pode ser observado em Oostra et al., 2006, cujos dados resultaram em grande contribuição para compreensão do impacto de plantios florestais sobre o C do

soo em regiões temperadas. Assim, devido à singularidade dessas áreas, elas não devem ser desconsideradas.

2. REVISÃO DA LITERATURA

2.1. Espécies florestais fixadoras de nitrogênio

As espécies florestais da família Fabaceae, popularmente conhecidas como leguminosas, são adaptadas às condições de alta temperatura e umidade. É uma família com elevada biodiversidade, com registro de aproximadamente 19.000 espécies (Doyle e Luckow, 2003).

Grande parte das espécies da família Fabaceae são capazes de formar associações simbióticas com algumas bactérias diazotróficas, também conhecidas como rizóbios, sendo as bactérias principalmente dos gêneros *Rhizobium*, *Bradyrhizobium* e *Azorhizobium*. Essas associações são responsáveis pela conversão do N atmosférico (N_2) para a amônia (NH_3), forma assimilável pelas plantas, resultando em incremento no crescimento vegetal (Alemneh et al., 2020; Sindhu et al., 2020) e alterações nas relações planta-solo (Barroso et al., 2018).

Devido à alta adaptabilidade, rápido crescimento e melhorias para a fertilidade do solo, diversas espécies desta família podem ser escolhas promissoras para recuperação de áreas degradadas, plantios silviculturais puros ou consorciados (Barroso et al., 2018; Chaer et al., 2011; Mayer et al., 2020). O estímulo desses plantios resultará na redução da pressão antrópica em áreas de floresta natural e proverá matéria prima para o mercado consumidor, que apresenta aumento constante da demanda por madeira de espécies nativas.

Dentre as espécies da família Fabaceae, algumas já são conhecidas por seu potencial para uso madeireiro e não madeireiro, destacando a importância de estudos que contemplem tais espécies. Dentre elas, foram selecionados três plantios homogêneos para que sejam explorados quanto o fluxo de nutrientes e as interações com o C e N no solo, informações, até então, não abordadas em trabalhos anteriores.

2.1.1. *Centrolobium tomentosum* Guillem ex. Benth

O araribá (*Centrolobium tomentosum*) é uma árvore caducifólia, que apresenta queda total das folhas no inverno. Sua altura varia entre 10 e 20 m e diâmetro à altura do peito (DAP) entre 30 e 60 cm, podendo um indivíduo adulto atingir 35 m de altura e 100 cm de diâmetro. Em povoamento com 21 anos de idade Barroso et al., (2018) observaram indivíduos com altura média de 12 m e DAP médio de 13 cm, em espaçamento 3 x 3 m. A espécie comporta-se como secundária inicial, ocupando clareiras e bordas de matas. É uma espécie heliófila, que tolera sombreamento na fase juvenil e na fase adulta é exigente em luz (Carvalho, 2003).

O araribá é recomendado para uso em arborização e nos sistemas integrados com culturas anuais e arborização de pastos. A capacidade de nodulação da espécie já foi descrita por Pagano (2008). A madeira é considerada densa, apresentando $0,73 \text{ g cm}^{-3}$ (Castro et al., 2016), podendo ser usada na construção civil e naval, obras externas, tacos, carpintaria, marcenaria de luxo, móveis finos, torneados, lambris, postes, mourões e esteios. Também produz lenha e carvão de boa qualidade. Suas raízes e cascas fornecem corante e suas sementes são apreciadas para o consumo humano, sendo descrita com gosto similar ao amendoim. Além disso, a madeira é apreciada para construção de barris para o envelhecimento de cachaças, conferindo elevadas concentrações de diferentes ácidos orgânicos (Bortoletto et al., 2016). O valor do m^3 da madeira em pé variou entre US\$ 366,68 e US\$ 1317, de acordo com o guia de árvores com valor econômico (Campos Filho e Sartorelli, 2015).

As cascas e as folhas do araribá, devido ao tanino que apresentam, são usadas na medicina popular, agindo como forte adstringente para o tratamento de feridas e contusões (Silva et al., 2013). Os mesmos autores citam que há maior

rendimento na extração de tanino das folhas em relação ao casco e lenho. Além disso, as cascas podem também ser usadas como adstringente (Erbano e Duarte, 2012).

Em uma área de mata ciliar do Rio Jacaré-Pepira, São Paulo, Aidar e Joly, (2003) avaliaram a quantidade e a qualidade de serrapilheira produzida pelo araribá. Os autores concluíram que a espécie foi responsável por grande contribuição da ciclagem de nutrientes. O retorno de N foi de 74,4 g indivíduo⁻¹ seguido pelo Ca, com 73,9 g indivíduo⁻¹. O nutriente com menor contribuição foi o P com 6,1 g indivíduo⁻¹.

O trabalho desenvolvido por Aidar e Joly, (2003) é o único relato na literatura em relação a serrapilheira produzida pela espécie, embora o sítio de estudo não seja uma área de plantio homogêneo, o que irá alterar as interações planta-solo. Ainda, os autores recomendam o araribá como uma boa opção para recuperação de áreas degradadas ciliares no Sudeste do país, corroborando Sujii et al., (2017) e Pagano, (2008).

2.1.2. *Hymenaea courbaril* Linnaeus

O jatobá (*Hymenaea courbaril*) é uma espécie semidecídua, podendo apresentar altura de 15 m e 13 cm de DAP, aos 21 anos após o plantio, em espaçamento 3 x 3 m (Barroso et al., 2018), comportando-se como uma espécie secundária. É uma espécie semi-heliófila, podendo ser plantada em bordas de mata, clareiras e também sob dossel fechado (Lorenzi, 2002; Carvalho, 2003). O jatobá não tolera limitações hídricas nem ambientes encharcados e temperaturas muito elevadas prejudicam seu crescimento (Locosselli et al., 2019). De acordo com Martins et al., (2020), o jatobá apresenta grande potencial para cultivo em diferentes regiões do estado de Minas Gerais, apontando a espécie como sendo de boa plasticidade, entretanto, o crescimento inicial da espécie é prejudicado sob disponibilidade de água abaixo de 50% da capacidade de campo (Nascimento et al., 2011), corroborando Locosselli et al., (2019).

Em plantio visando à restauração de área degradada, Moraes Junior et al. (2020) observaram boa sobrevivência ($\cong 70\%$) e crescimento em altura e diâmetro satisfatórios do jatobá aos 42 meses após o plantio, sendo recomendado pelos autores a produção de mudas em sacolas plásticas (1177 cm³).

A madeira do jatobá é considerada densa $0,75 \text{ g cm}^3$ (Silva et al., 2015), podendo ser usada na construção civil e carpintaria, em acabamentos internos (vigas, caibros, ripas, batentes de portas, assoalho), cabos de ferramentas e implementos agrícolas. Também pode ser usada em áreas externas como postes, dormentes, cruzetas e esquadrias, carroçarias, vagões, engenhos e tonéis. De acordo com o guia de árvores com valor econômico, o valor do m^3 da madeira em pé ficou entre US\$ 458,64 e 2029,2 na média 2014/2015 (Campos Filho e Sartorelli, 2015). Já a prancha do jatobá na região de Bauru, São Paulo alcançou preço do m^3 em torno de R\$ 4500 (Cepea, 2020).

O jatobá também apresenta usos florestais não madeireiros, por exemplo: a resina pode ser usada no tratamento de bronquite, asma, deficiência pulmonar e laringite (Tamayo et al., 2008), para tratamento de problemas renais, anemia e leucemia (Cartaxo et al., 2010) e a farinha do fruto pode ser usada na alimentação humana, sendo uma fonte rica em cálcio, magnésio e fibras (Costa et al., 2016; Caldeirón-Peralta et al., 2017).

Os extratos das sementes do jatobá também apresentaram propriedades anti-melanomas e atividades anti-oxidantes em células de ratos (Spera et al., 2019). Ademais, outros metabólitos secundários produzidos pelo jatobá foram recentemente descritos por Tiago et al., (2020) (ex.: esteroides, flavonoides, compostos fenólicos e saponinas), sendo apontados como relevantes para controle de herbivoria, sendo necessários novos estudos.

O jatobá pode ainda ser usado em sistemas integrados com pastagens, pois de acordo com Veras et al. (2010), a sombra da copa não alterou a produção de matéria seca do capim-andropogon, em uma densidade de 100 indivíduos ha^{-1} .

2.1.3. *Plathymenia reticulata* Benth

O vinhático (*Plathymenia reticulata*) é uma espécie considerada pioneira ou tardia, dependendo do bioma em que se localiza. Apresenta comportamento decíduo e quando a espécie se apresenta no Cerrado, em geral, tem porte diminuído, variando de 6 a 12 m de altura e 30 a 50 cm de DAP. Na Mata Atlântica, Barroso et al. (2018) observaram altura de 15 m e 25 cm de DAP, aos 21 anos após o plantio, em espaçamento 3 x 3 m. É uma espécie adaptada a terrenos pobres, com potencial para atender à demanda para recuperação de

áreas degradadas e adequação das propriedades rurais (Lacerda et al., 2001; Carvalho, 2009). A capacidade de se adaptar a sítios nutricionalmente pobres e o rápido crescimento da espécie podem ser, em parte, explicados pela associação com fungos micorrízicos arbusculares e bactérias diazotróficas, já descritos por Siqueira et al., (2019).

O vinhático possui madeira com boa aptidão para exploração comercial. É considerada moderadamente densa (0,50 a 0,55 g cm³), de fácil trabalhabilidade, podendo ser utilizada em mobiliários de luxo, lâminas faqueadas decorativas, painéis, construção civil, acabamentos internos (molduras, persianas, rodapés, forros, tacos e tábuas para assoalho), confecção de tonéis de vinho (Lorenzi, 2002). O m³ da madeira em pé atingiu valores entre US\$ 366,68 e 1317,2 de acordo com o guia de árvores com valor econômico (Campos Filho e Sartorelli, 2015).

Além disso, o vinhático é conhecido pela riqueza em compostos químicos como taninos e flavonoides, podendo atuar como anti-inflamatório e antimicrobiano (Fernandes et al., 2005), anti-hemorrágico quando ocasionado por picada de cobra (Della Torre et al., 2011; De Moura et al., 2015), e até como dieta protetiva para peixes sob exposição crônica ao mercúrio (Gombeau et al., 2019), sendo classificada como a espécie de maior utilidade do Cerrado brasileiro (Almeida et al., 1998).

2.2. Serrapilheira e decomposição

Os solos tropicais são considerados ácidos e de baixa fertilidade, em função do elevado grau de intemperismo, principalmente devido às características do clima. Plantios florestais para fins silviculturais ou ambientais em tais solos dependem da ciclagem de nutrientes, em fase adulta, quando o *turnover* de nutrientes passa a ser dependente da própria floresta (Folster e Kanna, 1997; Selle, 2007).

A ciclagem de nutrientes em ecossistemas florestais pode ocorrer na forma de três ciclos, conforme Switzer e Nelson, (1972): ciclo geoquímico que é caracterizado por trocas de elementos minerais entre o ecossistema e seus componentes externos (intemperismo da rocha matriz, deposição de poeira, chuva química, erosão e lixiviação); ciclo bioquímico que é caracterizado pela

mobilização do nutriente no interior dos tecidos vegetais; ciclo biogeoquímico que se refere às trocas químicas entre o solo e a planta por meio da absorção de nutrientes pela planta e retorno desses ao solo, via deposição da serrapilheira e decomposição.

A serrapilheira pode ser definida como material de origem vegetal, como folhas, caules, cascas, flores e frutos, e de origem animal como carcaças e fezes, em diferentes estados de decomposição, que se acumulam sobre o solo, servindo como fonte de energia e nutrientes para os microrganismos decompositores e para a vegetação (Brun et al., 2001).

Estudos da ciclagem de nutrientes via serrapilheira são fundamentais para o conhecimento da estrutura e funcionamento dos ecossistemas florestais (Vital et al., 2004), onde a dinâmica da serrapilheira é responsável pelo maior volume de nutrientes que percorrem o sistema solo-planta (Machado et al., 2012).

Os estudos que abordam a serrapilheira em plantios florestais monoespecíficos são limitados a poucas espécies, entre as quais podemos citar: *Araucaria angustifolia* (Schumacher et al., 2004), *Eucalyptus* spp. (Vieira et al., 2014), *Parkia platycephala*, *Libidibia Ferrea* e *Samanea saman* (Machado et al., 2012), *Tectona grandis* (Rosa et al., 2017), *Mimosa caesalpinifolia* (Freire et al., 2010), *Acacia mearnsii* (Schneider et al., 2005) e *Hevea brasiliensis* (N'Dri et al., 2018). Entretanto, quanto às espécies tropicais fixadoras de N, a literatura é escassa.

A deposição sazonal está diretamente ligada aos elementos climáticos, principalmente fotoperíodo, precipitação pluviométrica e temperatura. Estes fatores além de influenciarem a queda das folhas, estão diretamente relacionados à decomposição da serrapilheira sob o piso florestal. A decomposição também é influenciada pela composição química da serrapilheira, estágio sucessional, idade e época de coleta (Caldeira et al., 2007).

A concentração dos nutrientes, a qualidade do carbono (teor de lignina, celulose e hemicelulose, por exemplo) e as relações estequiométricas entre esses elementos variam de acordo com as espécies e são apontados, em diversos casos, como os maiores influenciadores do processo de decomposição da serrapilheira (Bradford et al., 2016; Lanuza et al., 2018; Zhang et al., 2018).

Nesta perspectiva, a concentração dos nutrientes está relacionada à qualidade da serrapilheira. A serrapilheira de “alta qualidade” é caracterizada por

alta concentração de N, baixa relação C:N, baixo teor de lignina e fenóis, enquanto que a serrapilheira de “baixa qualidade” apresenta características opostas (Castellano et al., 2015). Essa definição é principalmente devido à disponibilização dos nutrientes, em que a serrapilheira com alto teor de N e baixa lignina, por exemplo, em geral, se correlaciona com taxas de decomposição elevadas (Hobbie, 2015).

A grande influência da qualidade da serrapilheira na taxa de decomposição se dá, principalmente, devido à limitação no fornecimento de nutrientes para a comunidade de decompositores, podendo, por exemplo, ser limitada em energia quando a serrapilheira é rica em carbono recalcitrante, como lignina e tanino (Hättenschwiler e Jørgensen, 2010). Além da limitação de C, em ecossistemas tropicais a limitação por fósforo é comumente observada, devido aos solos tropicais serem naturalmente pobres (Vivanco e Austin, 2006; Cassart et al., 2020).

O esquema a seguir proposto por Bradford et al., (2016) sumariza os fatores controladores do processo de decomposição, evidenciando que a microbiota decompositora não exerce um papel independente, estando suscetível às alterações no clima e na composição química da serrapilheira (Figura 1).

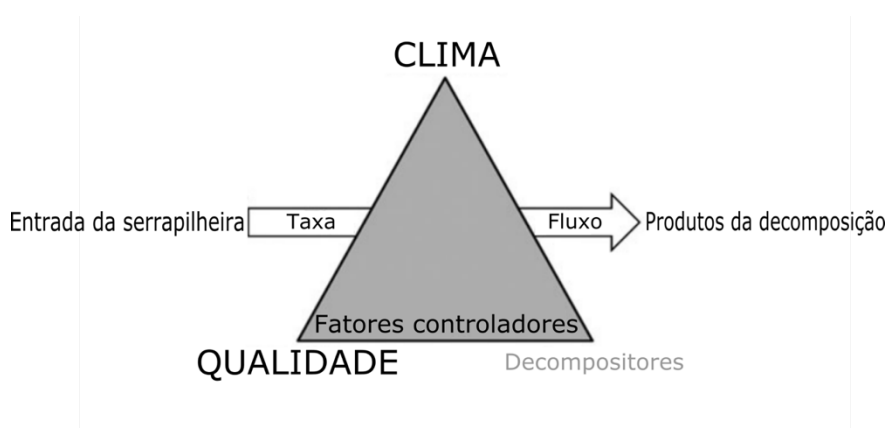


Figura 1- Principais fatores na regulação da taxa de decomposição foliar. A atividade dos microrganismos decompositores é influenciada pelo clima e pela composição química da serrapilheira. Fonte: Bradford et al., (2016) modificado.

A produtividade da serrapilheira, o conteúdo de nutrientes que são aportados ao solo pela vegetação e a taxa de decomposição/mineralização devem ser amplamente estudados, pois irão refletir na capacidade produtiva e no potencial de recuperação ambiental, tendo em vista as alterações que irão

provocar nas características físicas, químicas e biológicas do solo (Schumacher et al., 2004; Caldeira et al., 2007; Grugiki et al., 2017).

Para avaliar a decomposição da serrapilheira, as bolsas de decomposição “*litterbags*” propostas por Bockock e Gilbert, (1957) são amplamente utilizadas ao redor do mundo, sendo preenchidas, em geral, com material foliar seco e avaliação da perda de massa ao longo do tempo. As folhas são comumente selecionadas para preencher os *litterbags* por serem responsáveis pela maior contribuição na produção total da serrapilheira (Witschorek e Schumacher, 2013; Carvalho et al., 2017).

2.3. Carbono e nitrogênio do solo

O ciclo do C nos ecossistemas florestais engloba especialmente a biomassa vegetal (parte aérea e raízes), em que o acúmulo de C ocorre por meio da fotossíntese e o que se acumula no solo, é majoritariamente proveniente da produção e decomposição da serrapilheira acima e abaixo do solo (Roscoe e Machado, 2002).

O C orgânico configura-se como indicador de qualidade do solo, em virtude de suas características como fonte e dreno de nutrientes do ecossistema, sendo os sistemas florestais considerados grandes reservatórios de C (Denardin et al., 2014). Nesse sentido, o manejo do solo e a ação de fatores bióticos (microrganismos decompositores) e abióticos, como radiação, temperatura e umidade, influenciam na degradação da MO e, conseqüentemente, na concentração de C. Ademais, maiores deposições e maiores perdas de C ocorrem nas camadas superficiais, sob maior efeito dos múltiplos fatores que regulam as atividades microbianas (Denardin et al., 2014).

As espécies presentes em cada sítio vão também influenciar na quantidade e qualidade do C do solo, principalmente devido às diferenças quanto à produtividade e qualidade da serrapilheira e as interações das árvores com o solo (Vesterdal et al., 2012; Callesen et al., 2013). Vicente et al. (2016) observaram que plantios de eucalipto com diferentes idades, seringueira e uma floresta secundária diferiram em relação ao C no perfil do solo, em que o plantio de seringueira apresentou maior C orgânico do solo em relação aos plantios de eucalipto (nas diferentes idades) e à floresta secundária.

Estudo em uma área de floresta convertida em plantios florestais (seringueira e palma) revelou que perdas de C do solo ocorreram após a conversão, principalmente nas camadas superficiais do solo (Guillaume et al., 2015), corroborando Denardin et al., (2014). Este fato é amplamente reportado e é explicado devido ao consumo do C do solo nos anos iniciais de crescimento do novo plantio após a mudança de uso da terra.

Alterações na MO e no C do solo tendem a ocasionar mudanças concomitantes no conteúdo de N (Conant et al., 2005). Isso ocorre devido a elos entre os processos que ocorrem no solo, que podem conduzir para mudanças coordenadas entre o C e o N (Nel et al., 2018). O N é também um nutriente essencial para o crescimento e desenvolvimento vegetal e é apontado como não limitante em sítios tropicais e subtropicais (Martinelli et al., 1999). Devido à abundância de N, especialmente em sítios de plantio formados por espécies fixadoras de N, torna-se fundamental conhecer as particularidades desses ecossistemas, que afetam a produtividade e sustentabilidade dos plantios.

Embora efeitos coordenados entre o C e o N possam ser esperados (Nel et al., 2018; Zhang et al., 2021), a conversão da floresta nativa em pastagem promoveu redução do C orgânico do solo, contudo, não houve alteração do N, em estudo realizado por Cardoso et al. (2010), atribuído ao fato da decomposição do N ser mais rápida na área de floresta em relação a pastagem. As perdas de C, em longo prazo, podem resultar em elevada degradação ambiental, uma vez que, a MO está associada a maiores reservatórios de nutrientes e energia nesses ecossistemas (Cardoso et al., 2010).

Em contrapartida, Han et al. (2020) observaram reduções para o C e N do solo após a conversão de área de floresta em plantios agrícolas ou pasto, especialmente nas camadas superficiais. Ademais, as variações foram coordenadas entre o C e N orgânicos no perfil do solo, ou seja, pouca variação na relação C/N, sendo observados pelos autores padrões similares nas diferentes mudanças no uso da terra. De acordo com Hicks Pries et al. (2017), embora o N apresente maior mobilidade vertical, o movimento do C proveniente da serrapilheira é mínimo no perfil do solo, o que também justifica as alterações serem observadas com maior clareza nas camadas superficiais do solo.

O conhecimento da flutuação do C e N no perfil do solo é fundamental para a caracterização e monitoramento de diferentes plantios florestais em

relação à qualidade do solo, podendo auxiliar ações de manejo e tomada de decisão quanto à escolha de espécies. Além da quantidade de C e N, o fracionamento isotópico tem auxiliado a compreender as transformações que ocorrem ao longo do tempo, especialmente em áreas que sofreram mudanças no uso da terra.

2.3.1. Fracionamento isotópico do carbono (^{13}C) e nitrogênio (^{15}N)

A abundância natural do ^{13}C tem sido utilizada como um indicador dos processos ambientais que impactam o armazenamento de C no solo, como a mudança de uso da terra, e, conseqüentemente, um melhor entendimento do ciclo do C em ecossistemas florestais, diretamente relacionado com os gases do efeito estufa e o aquecimento global (Ngaba et al., 2019).

Estas diferenças podem ser detectadas, principalmente em função da variação no ciclo fotossintético da vegetação predominante de cada área. As plantas que fixam CO_2 através da rubisco (ciclo fotossintético C_3) têm composição isotópica (^{13}C) que varia de -24 a -34‰ ; empobrecida em ^{13}C , enquanto as plantas que fixam CO_2 pela PEPcase (ciclo fotossintético C_4) a composição é de -6 a -19‰ ; enriquecida em ^{13}C (Smith e Epstein, 1971). A MO enriquecida em ^{13}C é constituída pelo isótopo mais pesado e tende a permanecer na MO do solo por mais tempo (Conen et al., 2008).

A conversão da área de floresta em pastagem, na região sul do Brasil contribuiu para mudanças nas proporções de C oriundo da floresta (vegetação C_3), no entanto, após 50 anos da conversão da área, boa parte do C detectado ainda era oriundo da floresta (Dortzbach et al., 2015). Araújo et al., (2011) observaram substituição de grande parte da MO do solo já aos 20 anos após a conversão de floresta em pastagem, no estado do Rio de Janeiro, o que demonstra que o tempo de alteração da MO do solo após a mudança do uso da terra em relação a outros fatores pode variar largamente.

Com relação ao N, a dinâmica que envolve seu ciclo é complexa e envolve diversos processos de redução e oxidação (nitrificação, desnitrificação, lixiviação). A variação na razão entre os isótopos do N mais pesado e o mais leve (e mais abundante) fornece informações sobre as transformações e os fluxos de N nos ecossistemas florestais (Callesen et al., 2013).

Para o N, a variação da concentração de ^{15}N oriundo de fontes naturais, tem como padrão o ^{15}N do ar (0,3663%), que está entre -10 a +10‰, sendo estas variações atribuídas aos processos fisiológicos que ocorrem nas plantas, às associações com microrganismos (retenção do isótopo enriquecido em ^{15}N nos micélios de plantas com associações micorrízicas), às diferentes fontes de N utilizadas na agricultura e à demanda de N pelas plantas (Högberg, 1997; Högberg et al., 2011)

A análise da relação dos isótopos estáveis de C e N do solo em conjunto tem sido uma ferramenta importante para avaliar o grau de desenvolvimento do solo e para estudar mudanças históricas de vegetação (Krull et al., 2003). As mudanças na vegetação irão influenciar a quantidade e a qualidade da MO do solo. Assim, Balasubramanian et al. (2020) listaram as principais razões que explicam as alterações no C e no N do solo: (1) mudanças na taxa de retorno de nutrientes devido à substituição da comunidade decompositora (2) variações na quantidade e qualidade da serrapilheira acima e abaixo do solo e (3) aumento das taxas de decomposição do C e N lábeis.

Como as mudanças que ocorrem no solo tendem a ser coordenadas em relação às concentrações de C e N, quando avaliadas em conjunto, as alterações de enriquecimento e empobrecimento de ^{13}C e ^{15}N também tendem a seguir trajetórias similares no perfil do solo, conforme observado por Peri et al., 2012 e Han et al., 2020. É esperado o enriquecimento de ^{13}C e ^{15}N com o aumento da profundidade do solo e uma das explicações é o “consumo” (decomposição) preferencial dos isótopos mais leves, levando ao enriquecimento em camadas mais profundas ao longo do tempo (Boström et al., 2007; Nel et al., 2018).

Embora as mudanças coordenadas possam ser esperadas em uma escala global (Nel et al., 2018), este padrão não pode ser assumido como uma regra, já que mudanças de uso da terra podem ocasionar enriquecimento do ^{13}C e empobrecimento do ^{15}N , por exemplo (Balasubramanian et al., 2020).

Informações quanto à abundância natural de ^{13}C e ^{15}N e a dinâmica no perfil do solo após conversão de pastagens em plantios florestais de espécies fixadoras de N ainda são desconhecidas, o que reafirma a importância de estudos que contemplem esses ecossistemas.

3. TRABALHOS

3.1. LITTER DECOMPOSITION AND NUTRIENT RELEASE OF TWO TROPICAL N-FIXING SPECIES, RIO DE JANEIRO BRAZIL¹

Abstract Litter decomposition plays a major role in forest productivity, nutrient cycling, and carbon sequestration in tropical woody ecosystems. However, nutrient release and leaf litter stoichiometry in tropical legume tree plantations are poorly explored or even unknown in the long term after outplanting. Better knowledge is needed to improve our understanding of the pattern of changes in the decomposition of N-fixing leaf litters and their possible impact on carbon storage. This study aimed to report the decomposition patterns and nutrient release of two tropical legume species and to evaluate the leaf litter stoichiometry involved in the processes. Based on a litterbag experiment, we measured the nutrient release and leaf litter stoichiometry during the litter decomposition of two tropical legume tree species (*Plathymenia reticulata* and *Hymenaea courbaril*), 27 years after outplanting. Decomposition rates were similar between the species, however, nutrient

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release was greater in *P. reticulata*, while *H. courbaril* showed immobilization, especially that of nitrogen (N) which increased by almost 20% in the early phase of decomposition followed by gradual release. N-fixing species did show different nutrient chemistry over time, this was not surprising given that initial nutrient concentrations varied broadly, except for carbon (C) and phosphorus (P). Most of the nutrient concentrations increased as the remaining litter mass decreased in both species, except for C and potassium. The C:N and N:P ratios differed between the species, but N:P did not correlate to mass loss. Both species had N-rich leaves, but *P. reticulata* decomposition was very likely P-limited while *H. courbaril* appears to be co-limited by N and P. It shows different patterns in nutrient cycling and the stoichiometry involved in the decomposition dynamics of tropical N-fixing species. Both species may help re-establishing nutrient cycling in disturbed ecosystems, especially *P. reticulata*, and these findings may guide fertilization managements.

Keywords: Nutrient dynamics; Legume species; Litter chemistry; Ecological stoichiometry; Tropical Forest

Introduction

Several factors drive organic matter mineralization and nutrient release in forest ecosystems, but recent studies have shown that chemical litter quality can be the major influencer of decomposition rates (Bradford et al. 2016; Bhatnagar et al. 2018; Lanuza et al. 2018; Vivanco and Austin 2019; Bo et al. 2020). The decomposition process plays a fundamental role in forest productivity, given that it is a fundamental source of organic matter and nutrients. Among the fluxes occurring during litter decay, carbon (C) turnover is also directly related to climate warming, especially in tropical ecosystems where the C turnover is more rapid (Villela et al. 2012; Martinelli et al. 2017; Ochoa-Hueso et al. 2019).

To better comprehend the litter decomposition, ecological litter stoichiometry, which is defined as the balance of nutrients and how this balance is related to the

environment (Ågren and Weih 2020; Yu et al. 2020), helps as a powerful tool (Zhang et al. 2018). Nutrient release from decaying litter is also the main factor providing the nutritional requirements of soil fauna (Barantal et al. 2014; Liu et al. 2019).

Although the decomposition patterns have been explored worldwide over the past years, little is known about decomposition of tropical tree plantations and the stoichiometry regulating their dynamics through nutrient availability (Cornwell et al. 2008; Hättenschwiler and Jørgensen 2010; Cassart et al. 2020). In addition, information about N-fixing species in the long term after outplanting is even scarcer. Better knowledge of tropical ecosystems locally, especially in the threatened Atlantic forest biome (Myers et al. 2000) is fundamental, as this information supports proposals for forest restoration and guides decision-making when choosing species for different managements (Duarte et al. 2013; Austin et al. 2014; Caldeira et al. 2019; Vitória et al. 2019).

Among N- fixing species belonging to the Fabaceae family, *Plathymenia reticulata* Benth and *Hymenaea courbaril* Linnaeus are commonly chosen for plantations due to their interesting attributes, such as good timber production (Diameter at breast height with 24.0 and 12.5 cm, respectively; 21 years after outplanting) and canopy cover and can be used for forestry plantation, as well as recovery of degraded areas and/or intercropping systems (Barroso et al. 2018). Apart from that, *P. reticulata* and *H. courbaril* were successfully used for different medical purposes (Cartaxo et al. 2010; De Toledo et al. 2011; Nicole M et al. 2011). Their potential for multiple purposes highlights the importance of better knowing the ecology of these species related to nutrient cycling. It is also noteworthy that the knowledge provided by studies focusing on these species will help to mitigate the impacts of natural forest exploitation (Martins et al. 2020).

The main objectives of this work were to: (1) assess the pattern of decomposition and nutrient release of two important tropical legume species, and (2) understand the ecological stoichiometry involved in the decomposition of each species.

Material and Methods

Site description

The study was carried out in the mountain region of Rio de Janeiro, Brazil, within the municipal limits of Trajano de Moraes. The site was previously a pasture that had experienced erosion and unplanned fires, and in 1992, Atlantic Forest species were planted to recover the area. The plantation was done as minimum tillage, with manual weeding, 0.40 x 0.40 x 0.40 m planting pits, and use of cattle manure (10 L per plant) and 10-28-06 NPK fertilizer (100 g per plant), both only at planting time. A total of 49 seedlings per tree species were planted in single plots and spaced at 3 x 3 m (21 x 21 m each stand). The seedlings were produced from seeds collected in the region that contained no description of the matrices collected. In total, the site has 23 species planted in single plots (at the same age), and during the first year after planting, ants were controlled with baits. To our knowledge, there is no record of these Atlantic Forest plantations in the long-term after outplanting.

In light of the higher survival rates among the planted species evaluated by Barroso et al. (2018), we selected two of them belonging to the Fabaceae family: *Plathymenia reticulata* Benth and *Hymenaea courbaril* Linnaeus. The climatic and physical soil properties at the site are in Table 1, whereas the study site map, additional planting details, and the topsoil chemical properties were detailed by Barroso et al. (2018). For monthly precipitation, humidity, and air temperature see Fig S1.

Table 1. Climatic and physical soil properties (0-10 cm depth) at the study site.

Altitude	Rainfall	Air Temperature	Relative Humidity	Sand	Silt	Clay	Bulk density
m	mm yr ⁻¹	°C	%	-----	g kg ⁻¹	-----	g cm ⁻³
720	1000	16 - 24	86 - 99	340	119	541	1.07

Litter collection and decomposition

Fresh leaf litter was collected in each plantation for one year. In each plantation, litter traps were fixed to the ground at 1.5 m height for litter collection. The litter traps were emptied monthly, and the leaves from the common gardens were separated from the other fractions and oven-dried for 72 hours at 65°C.

To evaluate leaf decomposition, we used the litterbags methodology described by Bock and Gilbert (1957). The experiment was installed in September 2018 for *P. reticulata* (time 0) and in December 2018 for *H. courbaril* (time 0), according to the natural peak of leaf litter productivity of each species. The litterbags using nylon mesh 2 mm and sized 20 x 20 cm, containing 10 g of leaf litter were randomly placed to the soil. Leaves with pathogenic symptoms and/or herbivory were avoided. A total of 96 litterbags (48 in each species) were installed and extracted at eight dates (10, 30, 60, 90, 120, 150, 180, and 240 days after incubation – 6 litterbags removed at each time).

The collected litterbags were cleaned by eliminating insects, soil particles, and other undefined materials. Then, the samples were oven-dried for 72 hours at 65° C, weighed (0.01 g), and ground (1 mm mesh) to determine chemical concentrations. The C concentrations were measured according to Tedesco et al. (1985), N was measured with the Kjeldahl method after sulphuric acid digestion, P determined colorimetrically (Braga and Defelipo 1974), K using fusion-flame photometry, calcium (Ca), magnesium (Mg), and manganese (Mn) were determined by atomic absorption spectroscopy (Bataglia et al. 1983). Lignin and cellulose were measured only at time 0 and determined by the acid detergent fiber method (Van Soest and Wine 1967).

Calculations and data analysis

For the lignin and cellulose data, we calculated the confidence interval together with the Students T-test to illustrate the initial variation between the leaf litters data. Nutrient release patterns of C, N, P, K, Ca, Mg, and Mn were calculated as the percent of

each nutrient loss based on the original content (nutrient concentration multiplied by dry mass at each point in time and multiplied by 100) during the decomposition.

The mass loss data (expressed as a percentage; dry mass at each point in time multiplied by 100 and divided by 10 as the initial dry mass) were submitted to regression adjustments and analysis of variance (ANOVA) of the adjusted models. To verify if the data met ANOVA assumptions, they were submitted to the diagnostic plots (normality, linearity, and homoscedasticity of residuals) and maximum likelihood function (Box Cox test) MASS package, Venables and Ripley (2002).

The confidence interval ($p < 0.05$) of the predicted adjusted models were calculated. We calculated the decomposition rate constant (k) based on the model proposed by Olson (1963):

$$W_t/W_0 = \exp(-kt)$$

Where W_0 = initial mass of the litter, W_t = mass remaining after time t (days), and k = decomposition constant.

When investigating the ecological stoichiometry relationships as a function of mass lost over the decomposition period, the same procedure was applied prior to regression adjustments, but only phosphorus concentration from *H. courbaril* required log transformation.

Principal component analysis (PCA) was conducted to evaluate the variability among the nutrient concentrations and litter decay in each forest species. For this analysis, data were standardized through z-scale transformation to avoid scale influence and plotted using ggbiplot package (Vu 2011) in R environment (R Core Team 2019).

Results

Initial litter quality

With regard to the initial litter quality, *P. reticulata* had higher lignin content compared to *H. courbaril*. However, the cellulose and Lignin:N ratio were almost similar between species (Table 2).

Table 2. Initial leaf litter lignin cellulose, and Lignin:N ratio \pm confidence interval of tropical *Plathymenia reticulata* and *Hymenaea courbaril* species, Southeast Brazil

Parameter (g kg ⁻¹)	<i>P. reticulata</i>	<i>H. courbaril</i>
Lignin	595.00 \pm 16.29	358.67 \pm 101.96
Cellulose	123.67 \pm 12.76	198.00 \pm 84.90
Lignin:N	30.89 \pm 1.56	27.98 \pm 4.03

Litter decay and nutrient release

The nutrients had seemingly not been exhausted (Fig 1) until the end of the experiment. Over the litter incubation period, we observed a linear decrease in the remaining mass for both species (Fig 2a), confirmed by the overlapped adjusted decay models; At the end of the incubation period (240 days), the remaining mass was similar between the species; 59% for *P. reticulata* and 61% for *H. courbaril* litters. The decomposition rate constant (k day⁻¹) also was similar between *P. reticulata* and *H. courbaril*, 0.0021 and 0.0020, respectively.

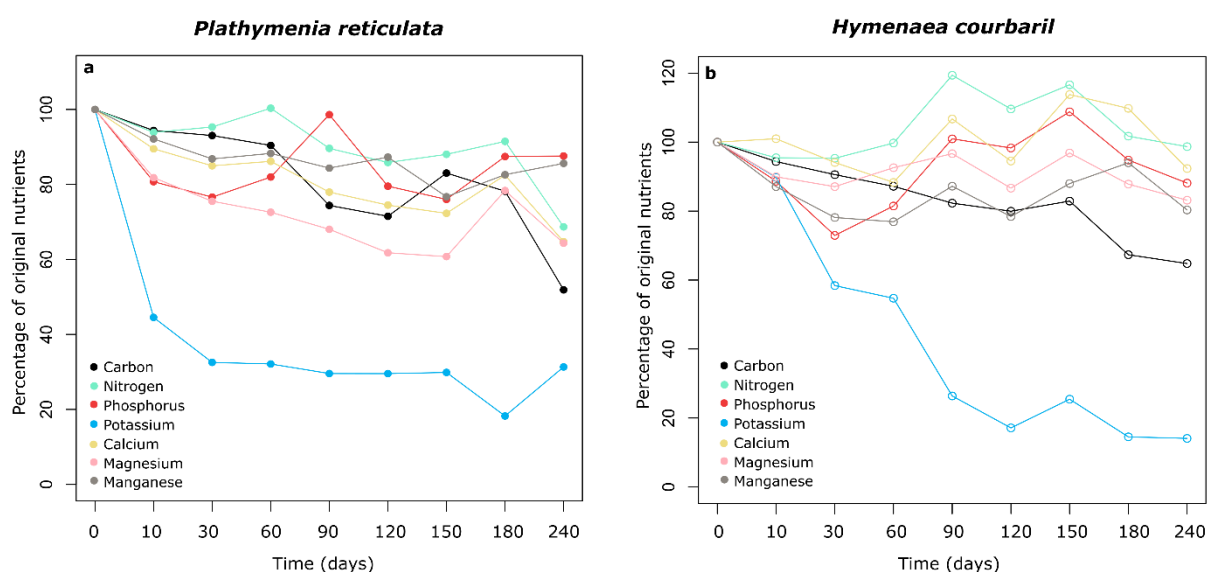


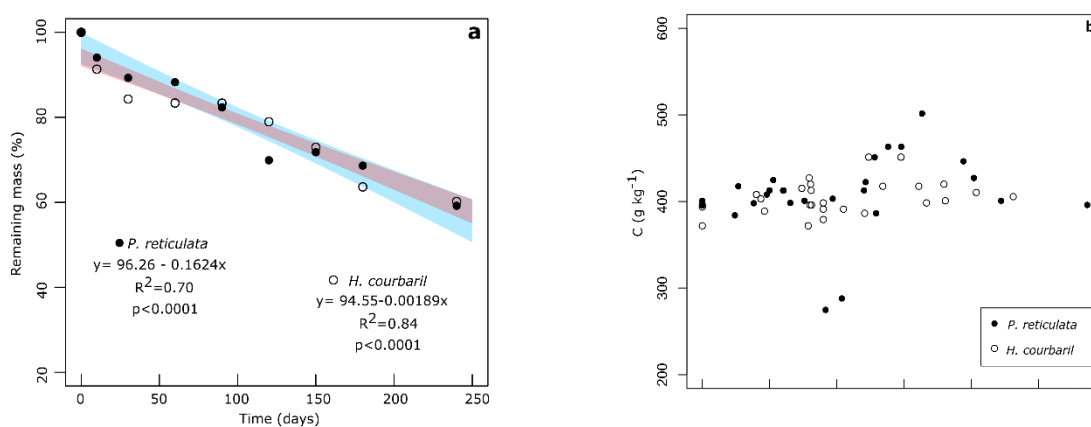
Fig 1. Percentage of original nutrients content (%) as a function of time during the decomposition of tropical *Plathymenia reticulata* (a) and *Hymenaea courbaril* (b) tree species over a period of 240 days, Southeast Brazil.

In the first 10 days, we observed mass loss and release for all nutrients, except for Ca in the *H. courbaril* litter (Fig 1a and b). Potassium, P, and Mn showed a greater drop from their original contents in *P. reticulata*, while the other nutrients' releases were a little less steep. Nutrient accumulation was observed, especially in *H. courbaril*, where N content was increased by almost 20% in the early stage of decomposition followed by gradual release.

Carbon, N, Ca, and Mg had a more pronounced decrease in *P. reticulata* leaves 180 days after the start of the experiment, whereas *H. courbaril* did not show a strong pattern for nutrient release up to 240 days.

Nutrient relations in litter

Linear correlations with similar slopes were observed when *H. courbaril* nutrient concentrations were plotted against the mass lost for all nutrients, except C. However, *P. reticulata* did not show correlations to C, Ca, and Mg, while K had a nonlinear correlation (Fig 2b-h).



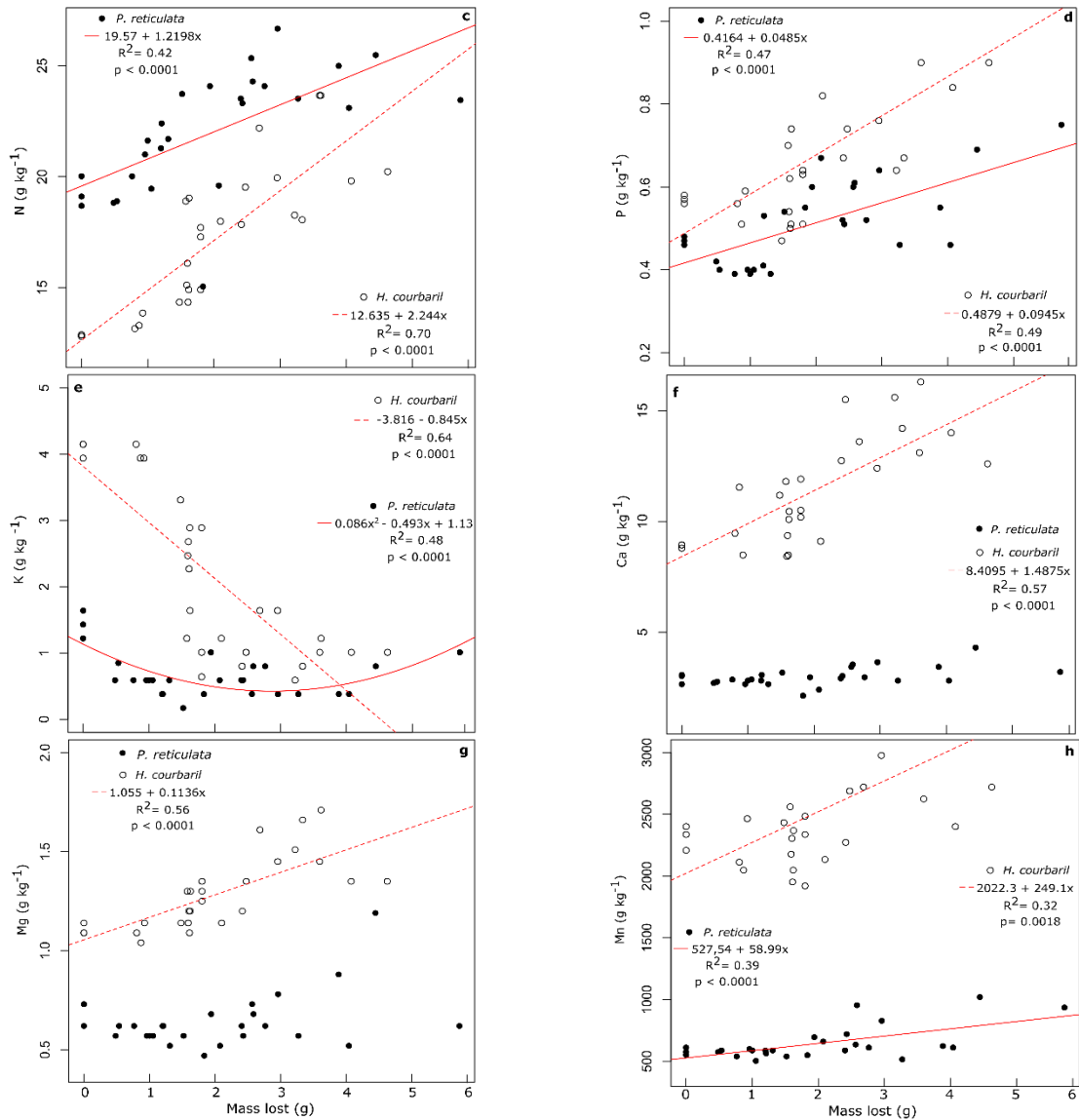


Fig 2. Changes in the concentration of C, N, P, K, Ca, Mg, and Mn as a function of the mass lost during the decomposition of tropical *Plathymenia reticulata* and *Hymenaea courbaril* tree species over a period of 240 days, Southeast Brazil. Confidence Interval of the adjusted model of the *P. reticulata* in blue and of the *H. courbaril* in purple.

Linear relationships for C:N and C:P ratios were observed, and both decreased over the decomposition period. The C:P slope was similar in both species, whereas *H. courbaril* had a higher C:N drop. No correlations were found when plotting the N:P ratio as a function of the mass lost for both species (Fig 3a-c).

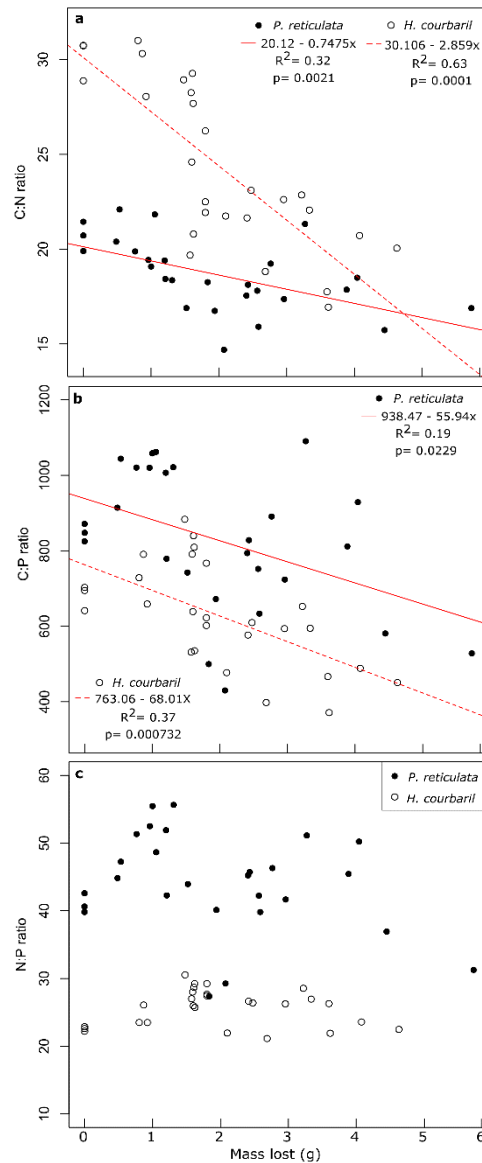


Fig 3. Changes in C:N, C:P, and N:P ratios as a function of the mass lost during the decomposition of tropical *Plathymentia reticulata* and *Hymenaea courbaril* tree species over a period of 240 days, Southeast Brazil

The PCA of the *P. reticulata* species (78.9% explanation of the data variability) showed a direct association between litter decay and N concentrations with PC1 (Fig 4a), and all nutrients had a negative correlation to PC1. Phosphorus, Mn, K, Mg, and Ca showed a positive correlation to PC2, but C and C:N, C:P, and N:P ratios had negative correlations.

The PCA of the *H. courbaril* species (79% explanation of the data variability) revealed an indirect correlation among litter decay, nutrient concentrations, and ratios with

PC1 (indicated by the arrows in opposite directions), except for C and N:P ratio, which were negatively related to PC2 (Fig 4b). On the other hand, *P. reticulata* PCA did not show variables directly negatively correlated to mass loss.

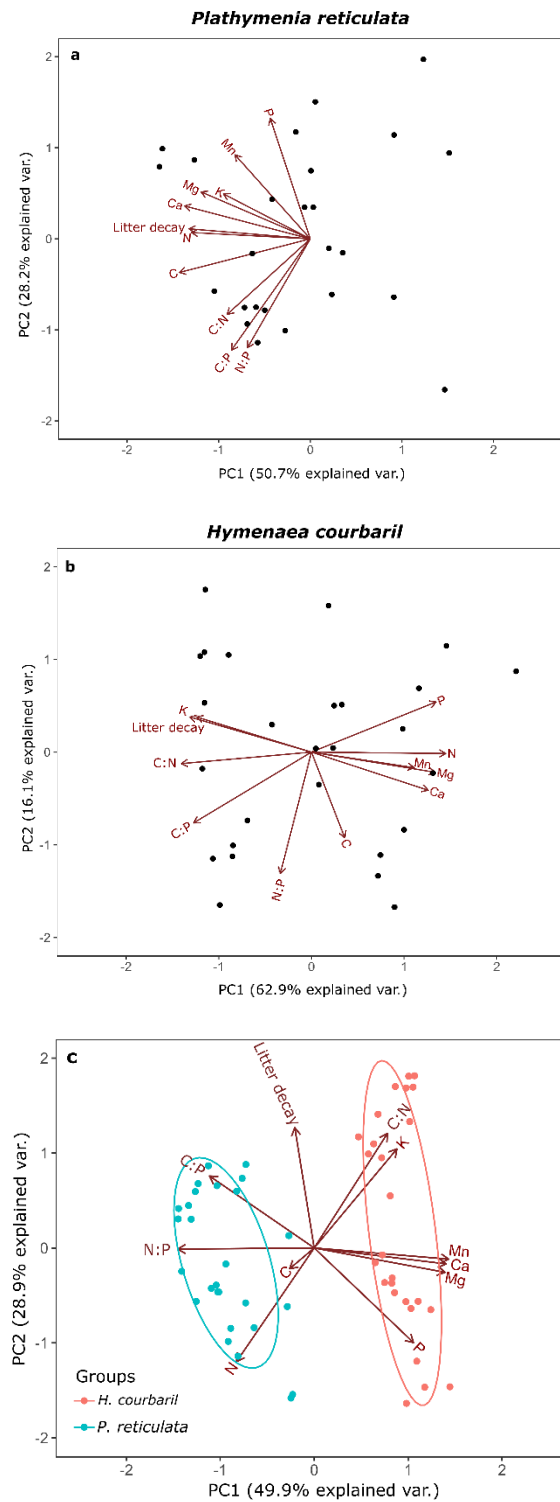


Fig 4. Principal component analysis of the litter decay, C, N, P, K, Ca, Mg and Mn concentrations and C:N, C:P, and N:P ratios for *Plathymenia reticulata* (a),

Hymenaea courbaril (b) species, and a comparative between both species (c), Southeast Brazil. The area of the ellipses was determined according to the normal distribution of the data (ellipses level = 68%).

With respect to the relation of species and nutrient concentrations, the species were organized in different groups in the PCA (78.9% explanation of the data variability), where *P. reticulata* is more associated to N, C, N:P, and C:P ratios and *H. courbaril* with C:N ratio, K, Mn, Ca, Mg and P (Fig 4c).

Discussion

Litter decay and nutrient release

Litter mass loss was not significantly affected by the species, regardless of the litter traits and sampling date. The decomposer communities developing upon the litter between sites, as observed by Pandey et al. (2007), might adapt to leaf litter chemistry of each species. It might explain the lack of differences between litter decays rates. The decomposition rate constant (k) was similar to a tropical N-fixing species (*Inga subnuda*), but lower than other leguminous species (*Erythrina verna* and *Senna macranthera*) that had higher decay rates (Duarte et al. 2013).

In our site, we did observe a weak variation between average air temperature and humidity over time (Fig S1). Furthermore, climate may strongly affect decomposition rates in low temperatures, but other factors might play a role in regulating decomposition rates when the temperature raises (Bradford et al. 2016), such as physical and chemical traits of leaves.

Physical leaf traits, e.g. specific leaf area and toughness, have been reported to influence mass loss (Pérez-Harguindeguy et al. 2000; Güsewell and Verhoeven 2006). Although we did not assess these traits, *P. reticulata* had clearly higher leaf specific area and tenderer leaves than *H. courbaril*, see Fig S2 for leaves overview. Therefore, a faster decomposition could be expected in *P. reticulata*, yet this pattern was not observed.

Likewise, litter quality has been described as an important factor regulating the decomposition process mainly due to the maintenance of the decomposers through resources availability (Lanuza et al. 2018; Ochoa-Hueso et al. 2019; Bo et al. 2020).

Litter decomposers produce enzymes to assimilate C from the complex organic matter and use it as an energy source (Waring et al. 2014). In accordance with Hättenschwiler and Jørgensen (2010), energy deprivation may drive decomposition processes. Carbon showed a similar pattern in mass loss across both species and a stable C concentration over time was observed. Apart from that, declines in the decomposition rate are expected when recalcitrant C takes place during litter decay (Duarte et al. 2013). It suggests that probably the only easily accessible C was released jointly with the mass loss over the experiment.

Concerning N content, the accumulation over the decomposition period may be explained by external factors, e.g. atmospheric deposition. We observed that the N accumulation peaks occurred in the months with higher precipitation records (November for *P. reticulata* and March and May for *H. courbaril* – Fig S1).

Moreover, N accumulation can be increased by other factors e.g. biological nitrogen fixation and decomposers (fungi and bacteria) that assimilate the required nutrients and remain stuck in the leaves (Heal et al. 1997); the latter can also explain P accumulation in both species. The same pattern for N and P accumulations was already observed by Parsons and Congdon (2008); Bachega et al. (2016); Lanuza et al. (2018). On the other hand, N and P accumulation may help in nutrient conservation in nutrient-poor sites (Singh et al. 1999).

The fast K decrease can be explained by the high solubility presented by the nutrient, which makes interpretation complex. The same pattern was already observed in other studies (Osono et al. 2008; Lanuza et al. 2018). K is an important nutrient as it might

influence organic matter degradation and ecosystem productivity (Fay et al. 2015; Ochoa-Hueso et al. 2019).

Calcium release occurs mainly in the late decomposition phases because it is a major component of cell structures, which will be degraded lastly (Osono and Takeda 2004). Evaluating the decomposition for longer periods would clarify this point, as this pattern was not clearly revealed for both species.

Manganese, P, and Mg had a fast release in the first 10 days in *P. reticulata* decomposition, while Mg was only slower than K. Mg tends to be released faster (Osono and Takeda 2004) and it is essential in fauna support (Makkonen et al. 2012). The same pattern was not observed in *H. courbaril*, which varied more largely over time. Additionally, cations presence is related to litter acidity and decomposers consumption (Cornelissen et al. 2003).

Nutrient relations in litter

Carbon leaf concentrations did not correlate to mass lost in both tree species, as they had small variation over decomposition time. It supports our prior assumption that only easily accessible C was released, e.g. cellulose, following the mass loss (Moorhead et al. 2013; Bachega et al. 2016) and providing the decomposers with energy.

Both species had high initial lignin concentration, especially *P. reticulata*, when compared to other species (Mendonça and Stott 2003; Tripathi et al. 2006; Duarte et al. 2013), which could lead to energy starvation in late stages of decomposition when it regulates the processes more (Berg and Matzner 1997; Chapin et al. 2002). The recalcitrance of this compound, in which few organisms are capable to degrade (Sigoillot et al. 2012) may almost stop decomposition rates (Marchante et al. 2019), which leads to a thick litter layer accumulation (Marchante et al. 2008). Large recalcitrant fraction in the litter can be important for C sequestration in the long term (Leblanc et al. 2006), especially in *P. reticulata* leaf litter.

The higher N initial litter concentration might increase the mass loss during the early stages of decay (Hättenschwiler and Jørgensen 2010; Hobbie 2015a), but this pattern was not observed in the present study. However, Ochoa-Hueso et al. (2019), in turn, found that in high N availability conditions, the decomposers may be impaired due to harmful effects on respiration capability.

Our species differed broadly regarding N:P ratio; *P. reticulata* ranged from 40 to 60 while *H. courbaril* from 20 to 30. This ratio reflects which parameter could be limiting litter decomposition: N-limited when litter has low N:P ratio (<14) and P-limited when high N:P ratio (>16) (Tessier and Raynal 2003). It suggests, that both species' litter decomposition was likely P-limited, but in *H. courbaril* the P-limitation appears to be weaker. Moreover, P-limitation in plant litter was reported as common in tropical sites (Aerts 1997; Vivanco and Austin 2006; Cassart et al. 2020) including tropical leguminous trees (Mendonça and Stott 2003; Duarte et al. 2013).

Concerning C:P ratio, when above 300, as a general rule, the litter decomposition is P-limited (Stevenson and Colle 1999; Duarte et al. 2013), which was observed in the present study for both species over the decomposition period. The same pattern for another tropical legume tree species (*Inga subnuda*) was observed by Duarte et al. (2013), which could suggest a broad N-fixing effect that needs to be investigated more closely. Although we considered the critical N:P and C:P ratios proposed by Tessier and Raynal (2003) and Stevenson and Colle (1999), the nutrient limitations may vary depending on the groups of organisms in each site (Güsewell and Gessner 2009).

N-limitation may occur with N concentrations below 11.3 g kg⁻¹ (Güsewell and Verhoeven 2006); both studied species had greater concentration than that threshold. Indeed, *P. reticulata* PCA (Fig 4a) indicated a weak correlation between litter decay and P concentrations, but in *H. courbaril* the negative relation between the variables was more robust (Fig 4b).

Several elements, such as C, N, and P exert a critical role in the decomposition, which commonly led to co-limitation during the processes (Fanin et al. 2013). The co-limitation in *H. courbaril* may also explain the high N accumulation (Fig 1b), given that N-limited microbes immobilize N more efficiently (Güsewell and Gessner 2009).

Regarding the C:N ratio, a relation of 25:1 is considered ideal for microorganisms maintenance and organic matter decomposition, and lower microbial activities could be associated with lower (Bachega et al. 2016) or higher C:N ratios (Berg and Matzner 1997; Hättenschwiler and Jørgensen 2010), leading to N mineralization or limitation, respectively.

Our results did not indicate N starvation for *P. reticulata* litter, so N mineralization may be occurring, and the species might be a good choice to re-establish N cycling in forest ecosystems. However, *H. courbaril* had a higher initial C:N ratio (about 30:1) which suggests N-limited decomposition even with N concentration above the threshold mentioned by Güsewell and Verhoeven (2006). In line with Vivanco and Austin (2019), sometimes N can be trapped in complex plant polymers, but other nutrients, such as manganese, might facilitate N release.

Little attention has been addressed to micronutrients, e.g., Manganese, which is directly involved in the lignin degradation through the enzyme manganese peroxidase. This fact attributes an important role to Mn in the decomposition processes of lignified litters (Berg et al. 2007, 2015), as *P. reticulata* leaf litter. *H. courbaril* had almost fourfold Mn than *P. reticulata* did, and lower lignin, but C concentration remained similar over the incubation period. Since easily degradable C remained available, Mn did not play a key role until that point.

This study reported for the first time the nutrient release and decomposition decay rates of two tropical N-fixing species, and it may help to understand the ecological role of each species. However, some topics related to nutrient release and the decomposition

processes remain unclear and need further tests for a greater ecological overview of the tropical N-fixing species. Now this study leads to a new question: Did stoichiometrically distinct litters affect soil microbiota and their metabolic capabilities e.g., fungi:bacteria ratio and enzymatic activities?

Conclusion

Both species had similar decay rates, but *P. reticulata* had a greater nutrient release compared to *H. courbaril*. It suggests that tropical N-fixing species have different nutrient release patterns. *P. reticulata* decomposition was primarily limited by phosphorus, but *H. courbaril* was co-limited by nitrogen and phosphorus.

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Competing interests

The authors declare they have no competing interest.

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3.2. NUTRIENT RETURN AND SOIL ORGANIC CARBON AND NITROGEN AFTER PASTURE CONVERSION TO TROPICAL N-FIXING TREE PLANTATIONS²

Abstract

Tropical ecosystems play a key role in global warming and remain underestimated in the global biogeochemical balances and this paper pioneers the discussion about litter productivity and quality, soil carbon, and nitrogen in tropical N-fixing tree plantations after pasture conversion. We aimed to answer the following questions: (1) Is the nutrient return similar among the three tropical legume species? (2) Did the conversion of the pasture to the tropical N-fixing plantations change the organic matter dynamic? (3) Do different tropical N-fixing tree species develop different ¹⁵N patterns in soil profile? For this, three tropical N-fixing species were selected: *Plathymenia reticulata*, *Hymenaea courbaril*, and *Centrolobium tomentosum*. Litter productivity was assessed monthly over one year. Soil organic carbon, nitrogen, C/N ratios and the natural abundance of ¹³C and ¹⁵N have been evaluated in the soil profile. Litter fractions had different contributions and nutrients returned among species. However, soil organic carbon and nitrogen were similar across species and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not follow similar trajectories. Soil organic matter appears to be relatively undecomposed even in deeper soil layers and most of the carbon originates from the planted trees instead of the former pasture, except under *C. tomentosum*. There is high support for gaseous N loss, but *P. reticulata* and *H. courbaril* exhibited N efficiency utilization. N utilization in *C. tomentosum*'s plot appears to be hindered by C-limitation.

Keywords: Legume species, nutrient cycling, isotope fractionation, soil organic matter

1. Introduction

Litter productivity and quality play a major role in nutrients supply in the forest ecosystems. That is especially the case in highly weathered soils of low fertility, such as tropical soils, where litter supplies most

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of the rapidly mineralizable nutrients through soil-plant system (Meier et al. 2005; Waring et al. 2014; Ludvichak et al. 2016).

The vegetation cover associated with long-term land use changes will modify the dynamics of nutrient turnovers and composition of organic matter since litter productivity and quality differ among species (Chen et al. 2005; Guo et al. 2016). Likewise, changes in the vegetation modify carbon (C) fluxes and soil C and it is also a key factor for current climate warming. The natural abundance of $^{13}\text{C}/^{12}\text{C}$ ratio in the soil profile has helped study the dynamics, stabilization, and ecological functions of soil organic matter (SOM), and has provided insights into soil C cycle (Martinelli et al. 2017; Paul et al. 2019; Balasubramanian et al. 2020), and several studies have already reported land-use changes effects on SOM origin and stability (Conen et al. 2008; Vicente et al. 2016; Carvalho et al. 2017; Ngaba et al. 2019).

This is mainly due to differences in the photosynthetic pathways, in which, C_3 photosynthetic pathway contributes with ^{13}C -depleted and C_4 photosynthetic pathway typically supplies with ^{13}C -enriched organic matters. The latter constitutes the heavier atom of C and remains in SOM for a longer time due to preferential decomposition of lighter C isotope (Conen et al. 2008; Liu et al. 2018). It means that through isotopic fractionation we can track C losses from previous pastures and ongoing fresh inputs from the current forest vegetations through ^{13}C enrichment or depletion (Chen et al. 2005; Krull et al. 2006; Vicente et al. 2016; Liu et al. 2018).

Nitrogen is another essential nutrient in forest ecosystems and it is known as a non-limiting nutrient in tropical and subtropical areas (Martinelli et al. 1999). To better understand N transformations and pools, the natural abundance of $^{15}\text{N}/^{14}\text{N}$ ratios in the soil has been used as a crucial tool (Hobbie and Högberg 2012; Craine et al. 2015). Regarding soil organic nitrogen (SON) fractionation, tropical forests are known to have elevated $\delta^{15}\text{N}$ values. Enrichment of ^{15}N in that sites has correlated to N fluxes mainly due to gaseous loss of ^{14}N by nitrification and denitrification (Houlton et al. 2006; Hobbie and Ouimette 2009; Peri et al. 2012). Yet, ^{15}N depletion might occur due to other N processes (Craine et al. 2015; Rivero-Villar et al. 2018) and mycorrhizal plant interactions with host plants (Hobbie and Ouimette 2009).

Apart from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values being assessed individually, they both may display similar patterns with soil depth with similar trajectories (Nel et al. 2018), which indicates coupling effects (Peri et al. 2012; Han et al. 2020). Comparisons with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values along with C and N concentrations and C/N ratio are indicative of SOM stability (Balasubramanian et al. 2020; Han et al. 2020) and turnover (Peri et al. 2012). It happens due to links among soil processes which may lead to coordinate shifts in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Nel et al. 2018).

The decreasing C/N ratio with soil depth might be associated with the degree of decomposition, microorganisms colonization and humification of SOM (Han et al. 2020) in which a higher C/N ratio reflects fresh and incompletely decomposed organic matter (Kramer et al. 2017). Therefore, a decreasing trend from the upper to bottom soil layers would be expected (Han et al. 2020).

In general, SOM decomposition is more rapid in tropical ecosystems (Villela et al. 2012; Ochoa-Hueso et al. 2019). However, tropical sites are poorly explored and information about tropical N-fixing tree plantations is even scarcer. To our knowledge, information about pasture conversion to tropical N-fixing species in the long term after outplanting is so far unknown and this study addresses for the first time the nutrient turnover and soil organic carbon (SOC) and nitrogen (SON) dynamics in the soil profile beneath three N-fixing species.

This way, the aim of this study was to answer the following questions: Is the nutrient return similar among three tropical legume species? Did the conversion of the pasture to the tropical N-fixing tree species change the dynamic of the SOM? Do different tropical N-fixing tree species develop different $\delta^{15}\text{N}$ patterns in soil profile?

2. Materials and methods

2.1. Study site description

The study was carried out in the mountain region of Rio de Janeiro, Brazil, within the municipal limits of Trajano de Moraes (22° 04' 32" S 42° 03' 51" W) in the Atlantic Rainforest Biome domain. The average temperature ranges between 18 and 24 °C and annual precipitation of 1100 mm. The altitude is 700 m above sea level. The soil is a Typic Haplohumults (Soil Survey Staff, 2014) with low activity clay.

The site was previously a pasture (no information for how long) that had experienced erosion and unplanned fires. In 1992, Atlantic Forest species were planted to recover the area. The plantation was done as minimum tillage, with manual weeding, 0.40 x 0.40 x 0.40 m planting pits, use of cattle manure (10L per pit), and 10-28-06 NPK fertilizer (100 g per plant) at planting time.

A total of 49 seedlings per tree species were planted in single plots and spaced at 3 x 3 m (21 x 21 m each stand). Surrounding the plots, there are equidistant plantations and a natural forest fragment that could provide seeds for understory regeneration. Additionally, floristic inventory and diversity assessments did not display presence of grasses in any of the forest plots (unpublished data) which indicates that the stands are composed mainly of C₃ vegetation.

We selected three forest species belonging to the Fabaceae family: *Plathymenia reticulata* Benth, *Hymenaea courbaril* Linnaeus, and *Centrolobium tomentosum* Guillem ex Benth. The species had survival rate of $\geq 90\%$, average height ranging from 13 to 15 m and the diameter at breast height ca. 13 cm for *H. courbaril* and *C. tomentosum* and 24 cm for *P. reticulata* (Barroso et al., 2018). The species also have a great environmental and economic interest in timber and non-timber products (Cartaxo et al. 2010; Della Torre et al. 2011; Erban and Duarte 2012; Calderón-Peralta et al. 2017; Gombeau et al. 2019).

Yet, a 50-years-old unfertilized pasture composed of *Brachiaria decumbens* and a Secondary Forest (upper canopy logged approximately 35 years ago) were considered as references for C₄ and C₃ vegetation covers, respectively, and used for calculations with soil ¹³C fractionation data beneath the aforementioned species. These areas are also located in the Atlantic Rainforest biome (21° 07' 50" S 42° 21' 59" W) and were evaluated by Vicente et al., (2016).

2.2. Litter productivity and nutrient turnover

For litter sampling, litter traps were built using PVC pipes and a 2.0 mm nylon mesh measuring 0.75 x 0.75 m and fixed to the ground at 1.5 m height. A total of 20 litter traps were installed in each forest stand and emptied monthly for one year.

Samples from each month were hand-sorted into three fractions as following: leaves from the planted forest species; hereinafter referred to as “leaves”, leaves from other species (without distinction among species); hereinafter referred to as “other species”, and miscellanea (e.g., barks, fruits, branches, and flowers). Each fraction was oven-dried at 65°C for 48h. Then, the leaves from the planted species were analyzed for C, N, P, K, Ca, and Mg (20 samples per forest plantation were pooled together and six aliquots were chemically analyzed). This way, the annual litterfall fluxes (kg ha⁻¹yr⁻¹) could be estimated by calculating the sum of the litterfall's productivity of each month and the nutrients returned to the soil system in each forest stand (nutrient concentration multiplied by annual litterfall flux).

The N was measured with the Kjeldahl method, and P determined colorimetrically, K was determined using fusion-flame photometry, and both Ca and Mg were determined by atomic absorption spectroscopy (Bataglia et al., 1983). Carbon was measured as described by Tedesco et al. (1985).

2.3. Soil organic carbon and nitrogen in soil profile

Mineral soil was sampled in the central planting rows between trees (approximately 40 cm from the trunk basis) with a soil core auger in the following depths: 0-10 cm; 10-20 cm; 20-30 cm; 30-40 cm; 40-50 cm; 50-75 cm and 75-100 cm, with four replicates per depth in each forest stand. Soil samples from 0-10

and 10-20 cm soil depths were considered individually beneath each forest stand for physical characterization.

However, for deeper soil layers, one trench was dug up to 100 cm and we considered the same samples from each depth for all the forest stands given the soil similarity beneath them. Samples were air-dried and sieved (2 mm sieve) for soil particle size determination. The volumetric ring method was used to determine soil bulk density (Embrapa, 1997). The soil physical characterization is described in Table 1.

Table 1. Particle size fractions and soil bulk density up to 100 cm in different tropical N-fixing tree species in the Southeast Brazil

Depth (cm)	Particle size fraction (%) and bulk density -BD- (g cm ⁻³)	Forest species		
		<i>C.tomentosum</i>	<i>H. courbaril</i>	<i>P. reticulata</i>
0-10	Sand	45	40	38
	Silt	23	24	22
	Clay	32	36	40
	BD	1.130	1.202	1.010
10-20	Sand	45	42	32
	Silt	14	13	10
	Clay	36	44	57
	BD	1.320	1.320	1.201
20-30	Sand	32	32	32
	Silt	10	10	10
	Clay	57	57	57
	BD	1.098	1.098	1.098
30-40	Sand	26	26	26
	Silt	9.6	9.6	9.6
	Clay	63	63	63
	BD	1.159	1.159	1.159
40-50	Sand	26	26	26
	Silt	8.2	8.2	8.2
	Clay	65	65	65
	BD	1.105	1.105	1.105
50-75	Sand	26	26	26
	Silt	6.7	6.7	6.7
	Clay	66	66	66
	BD	1.124	1.124	1.124
75-100	Sand	26	26	26
	Silt	5.9	5.9	5.9
	Clay	68	68	68
	BD	1.121	1.121	1.121

The soil organic carbon and nitrogen were determined by dry combustion in an automated elemental analyzer (CHNS/O analyzer). The natural abundance of ¹³C and ¹⁵N were determined using the Isotope Ratio Mass Spectrometer Delta V Advantage (IRMS - Thermo Scientific) coupled with Organic Elemental Analyzer (Thermo Scientific), and the results were expressed in (‰) relative to the Pee Dee Belemnite (PDB) and atmospheric N₂ International standards, respectively, calculated using the following equation:

$$3. \quad \delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (\text{R}_{\text{sample}} - \text{R}_{\text{reference}}) / \text{R}_{\text{reference}}$$

where $\text{R}_{\text{sample}} = {}^{13}\text{C} / {}^{12}\text{C}$ ratio of the sample and $\text{R}_{\text{reference}} = {}^{13}\text{C} / {}^{12}\text{C}$ ratio of the reference samples

To distinguish the proportion of C derived from the previous pasture (C₄ vegetation) and from the current forest stands (C₃ vegetation) we used the equation proposed by Vitorello et al., (1989):

$$4. \quad \%C-C_4 = [(\delta - \delta_a) - (\delta_p - \delta_a)] * 100,$$

where δ = natural $\delta^{13}\text{C}$ abundance in the samples; δ_a = natural $\delta^{13}\text{C}$ abundance in the soil samples without C₄ plant residue (Secondary forest from Vicente et al., (2016) used as reference) ; δ_p = natural $\delta^{13}\text{C}$ abundance of the pasture plant material (-12.65‰).

Lastly, the following equation was used to obtain the % of the C₃ carbon:

$$\%C - C_3 = 100 - \%C - C_4$$

4.1. Data analysis

Considering that the site has no statistical design and no fill requirements for parametric analysis, litter productivity, canopy openness, and nutrient return data were submitted to descriptive analysis and compared by Confidence Interval ($p < 0.05$) by Student's T-test (Rmisc package, Hope, 2013) through R software (R Core Team, 2019). The comparison was based on the overlapping of the Confidence Interval limits (or lack thereof), which allows to differ means with high statistical support.

The coupling effect of $\delta^{13}\text{C}$ relative to $\delta^{15}\text{N}$ was tested by regression adjustments and analysis of variance (ANOVA) of the adjusted models. To verify if the data met ANOVA assumptions, they were submitted to the diagnostic plots (normality, linearity, and homoscedasticity of the residuals) and maximum likelihood function (Box-Cox test) MASS package, Venables and Ripley (2002).

Principal component analysis (PCA) was conducted using soil data assessments to evaluate a possible grouping pattern among species. For this analysis, data were standardized through z-score transformation to avoid scale influence and plotted using ggbiplot package (Vu 2011).

5. Results

5.1. Litter productivity and nutrient return

Leaves fraction in *P. reticulata* stand was almost two times higher than *C. tomentosum* and *H. courbaril* but an opposite trend was shown by the other species fraction (Figures 1A and B). Total litter productivity (all fractions) was higher in *H. courbaril* stand owing to the other species and miscellanea fractions (Figures 1C and D). The species differed regarding seasonal leaves productivity as *H. courbaril* had higher production in the beginning of the rainy season (from October to December) and *P. reticulata* in the

dry season (from June to September). Meanwhile, *C. tomentosum* did not markedly display a seasonal pattern over the year and the same was observed for the other fractions across species.

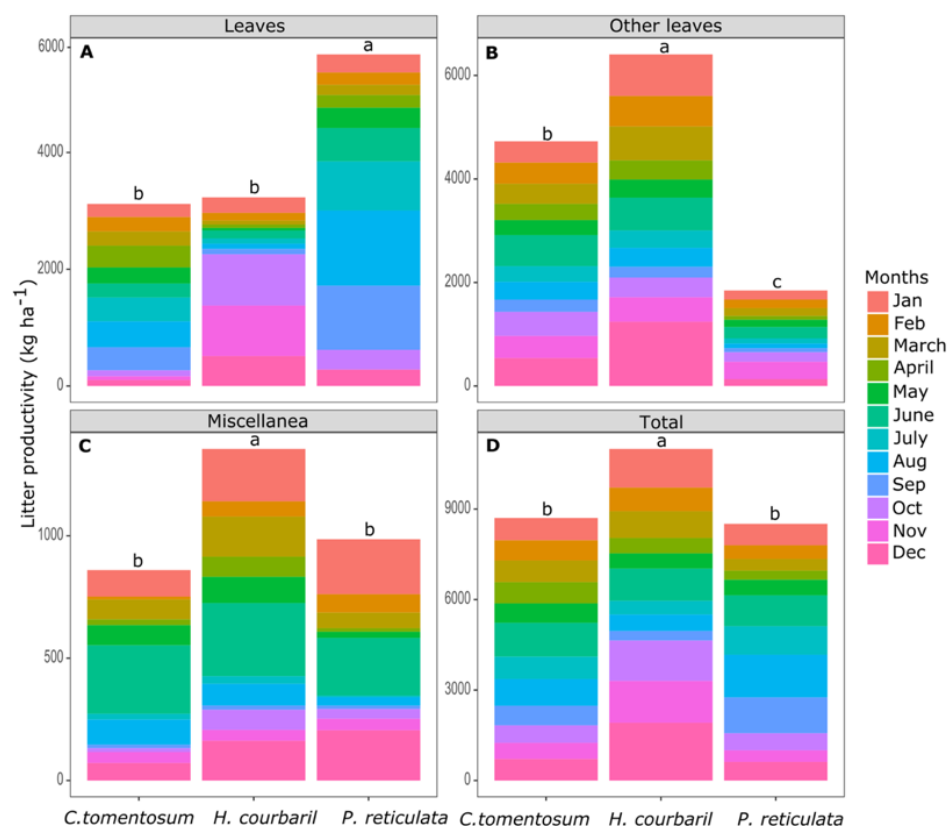


Fig 1. Leaves litter (A), other species litter (B), miscellanea (C) and total litterfall productivity (D) in *Centrolobium tomentosum*, *Hymenaea courbaril*, and *Plathymenia reticulata* stands over one year. *Means differed by Confidence Interval ($p < 0.05$)

Carbon, N, and P contents in *P. reticulata* leaves were the highest among species, whereas showing the smallest K content. *H. courbaril* had the higher Ca content and *C. tomentosum* the higher Mg (Table 2).

Species	C	N	P	K	Ca	Mg
	kg ha ⁻¹ yr ⁻¹					
<i>P. reticulata</i>	1881.0±260.9	90.97±13.4	2.21±0.17	6.75±2.89	13.78±4.25	3.28±1.12
<i>H. courbaril</i>	1044.6±141.47	34.69±4.0	1.53±0.22	11.01±1.96	23.87±2.17	2.98±0.49
<i>C. tomentosum</i>	945.5±382.6	33.62±26.5	1.12±0.27	14.04±1.95	16.34±1.56	7.42±2.97

Table 2. Nutrients turnover from each forest stand ± confidence interval ($p < 0.05$)

5.2. Soil organic carbon and nitrogen in soil profiles

Regardless of the C and N returned by litterfall, SOC and SON showed the same pattern among species with most of the loss occurring in the uppermost ~30cm soil layer followed by a slight increase

(mainly for *P. reticulata*) and stable decline with greater soil depths. However, *C. tomentosum*, in turn, had slightly lower SOC (Figure 2A and B).

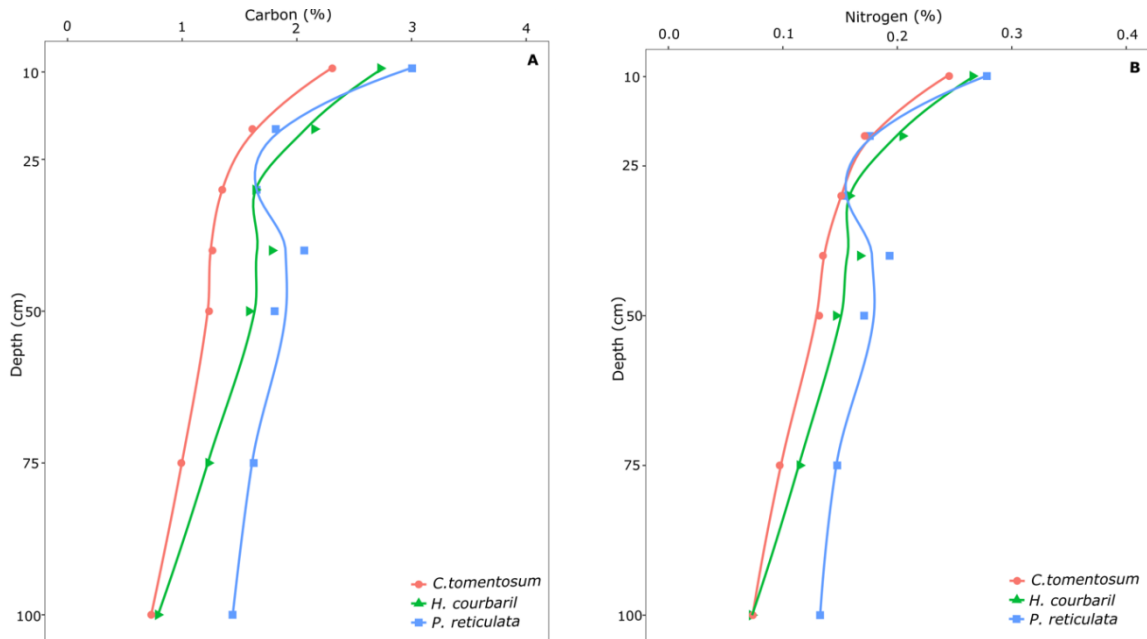


Fig 2. Changes in soil organic carbon (A) and soil organic nitrogen (B) in the soil profile beneath *Centrolobium tomentosum*, *Hymenaea courbaril* and *Plathymenia reticulata* forest stands.

With respect to C/N ratio, *P. reticulata* and *H. courbaril* showed a stable response with soil depth and varied from 11.9 to 12.9 whereas *C. tomentosum* irregularly fluctuate with decreases followed by increases with greater soil depth ranging from 10.3 to 11.9 (Figure 3).

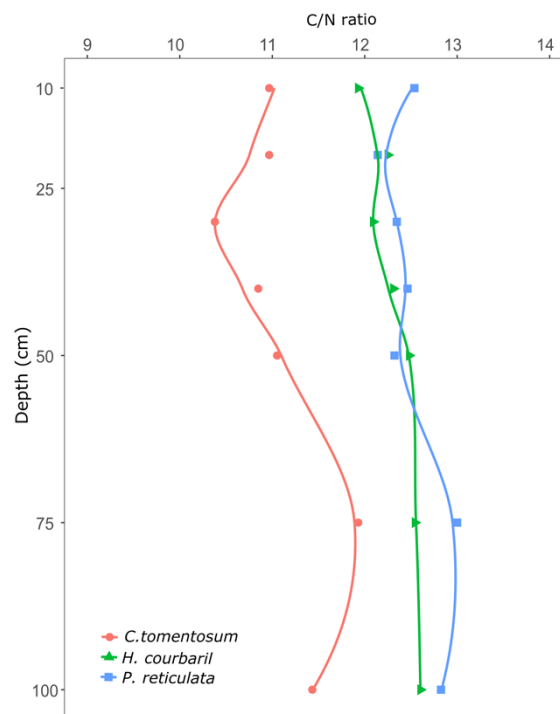


Fig 3. Changes in soil C/N ratio in the soil profile beneath *Centrolobium tomentosum*, *Hymenaea courbaril* and *Plathymenia reticulata* forest stands.

The natural abundance of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were similar among forest stands with most of the C originating from the current planted species in *P. reticulata* and *H. courbaril* stands, equally as the unmanaged secondary forest. Conversely, the soil under *C. tomentosum* still had a great contribution to the SOM from the previous pasture (Figure 4A). $\delta^{13}\text{C}$ of the organic matter showed relative ^{13}C -depleted values, consistent with the C_3 vegetation in the forest stands.

The soil $\delta^{15}\text{N}$ values did not follow the same trajectories as $\delta^{13}\text{C}$ values with soil depth. We observed relatively ^{15}N -depleted N in the upper layers, but $\delta^{15}\text{N}$ values became more ^{15}N -enriched with depth. This pattern did not follow until deeper layers but only at intermediate depths. (Figure 4B). Vertical changes in $\delta^{15}\text{N}$ values were around $\sim 2\text{‰}$ under *P. reticulata*, approximately two times more than *C. tomentosum* and *H. courbaril* stands.

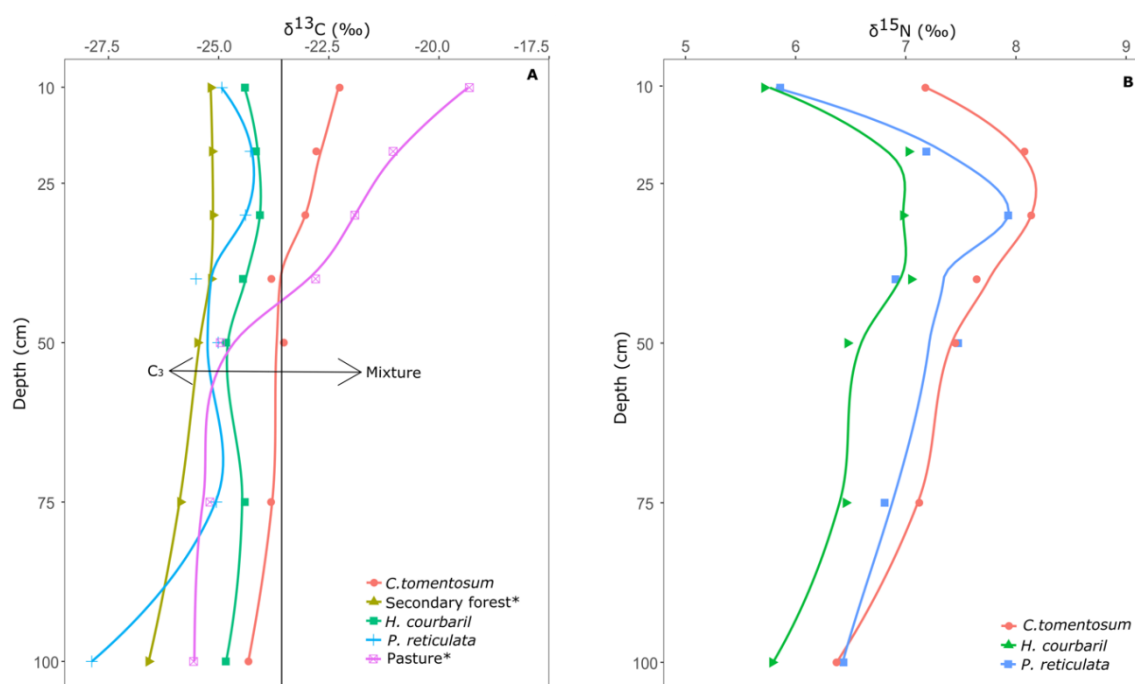


Fig 4. Changes in $\delta^{13}\text{C}$ values (A) and $\delta^{15}\text{N}$ values (B) in the soil profile of *Centrolobium tomentosum*, *Hymenaea courbaril* and *Plathymenia reticulata* forest stands. *Secondary forest and pasture $\delta^{13}\text{C}$ data are from Vicente et al. (2016).

The $\delta^{13}\text{C}$ values showed higher contribution of C_4 vegetation under *C. tomentosum* even in the upper soil depths (0-10, 10-20, and 20-30 cm) when compared to *H. courbaril* and *P. reticulata* (Fig. 5). Nevertheless, the accumulated C- C_3 at 0-10 cm depth was lower to the Secondary Forest (28.5 Mg ha^{-1})

when compared to the N-fixing species where the values ranged from 33.8 Mg ha⁻¹ to 46.1 Mg ha⁻¹, but higher than *P. reticulata* at 10-20 cm soil depth.

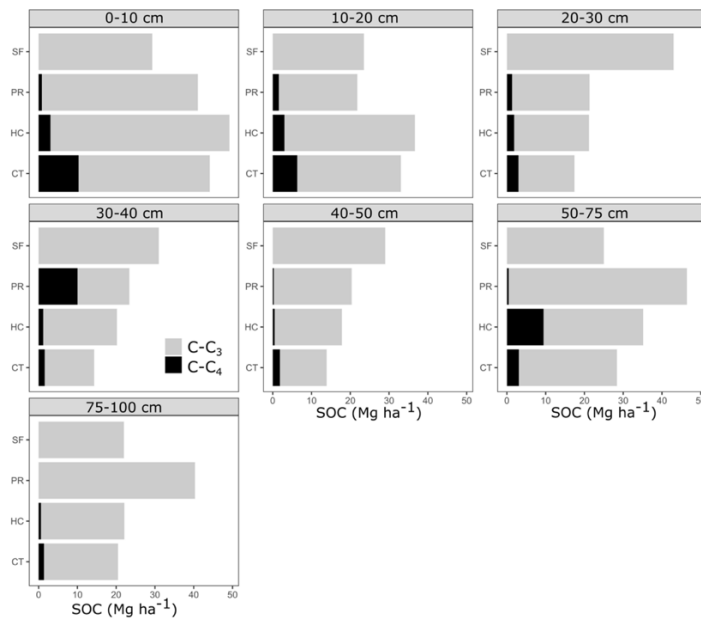


Fig 5. SOC divided into C3 plants (tree)-derived and C4 plants (pasture)-derived in the soil profiles beneath different tropical N-fixing tree plantations and the Secondary Forest (SF) from Vicente et al., (2016); PR – *Plathymenia reticulata*; HC – *Hymenaea courbaril*; CT – *Centrolobium tomentosum*.

The coupling relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed linear adjustment for *H. courbaril* and *P. reticulata* but *C. tomentosum* did not have a credible adjustment (Figure 6A). The PCA (85.1% explanation of the data variability) showed a direct association between soil organic C and N, and a negative correlation to PC1. The isotopic fractionation of ^{13}C and ^{15}N is indirectly correlated to soil C:N ratio indicated by the arrows in opposite directions (Figure 6B). The species were organized in different groups, where *C. tomentosum* is more associated to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fluctuations and *P. reticulata* and *H. courbaril* associated together to soil C, N and C:N ratio.

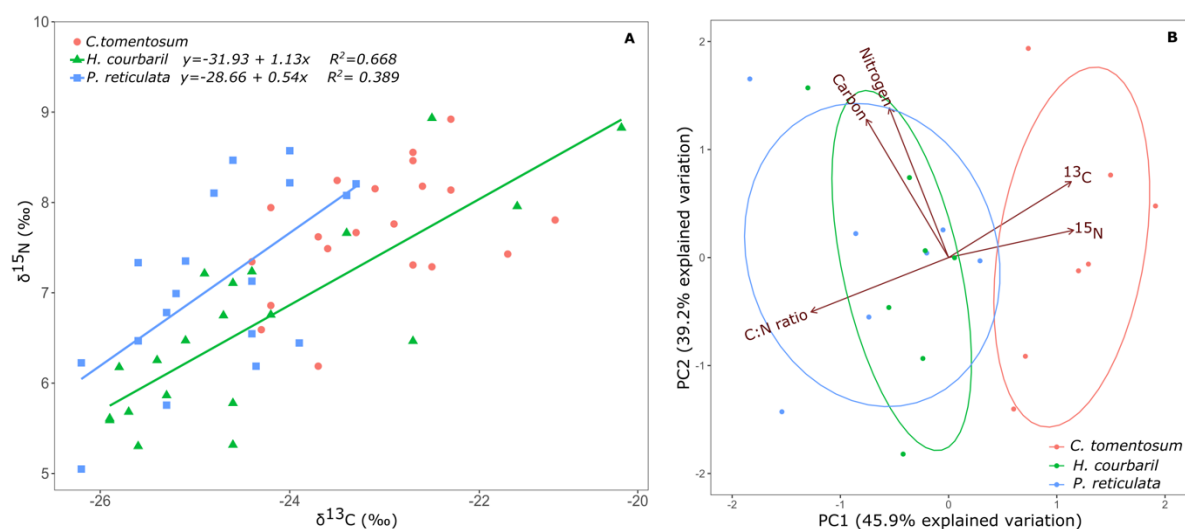


Fig 6. Relationship between $\delta^{13}\text{C}$ values and $\delta^{15}\text{N}$ values from soil profile (0-50cm) of *Centrolobium tomentosum*, *Hymenaea courbaril* and *Plathymania reticulata*. The adjustment of *C. tomentosum* isotopic fractionation was not credible ($p > 0.05$) (A), and principal component analysis of soil variables comprising all soil layers. The area of the ellipses was determined according to the normal distribution (B).

6. Discussion

6.1. Litter productivity and nutrients return

The total litterfall productivity in the forest stands is consistent with the overall mean across Atlantic Forest sites (Martinelli et al. 2017), albeit the aforementioned literature review has not included N-fixing plantations. The N returned to the soil system, especially in *P. reticulata* stand, indicates that the species might be suitable for pasture systems, since there are many degraded pastures due to N-limited soils (de Freitas et al. 2013). *P. reticulata* canopy cover allowed high light entrance (Figure A2), which could be favorable, along with N turnover, when intercropping.

Tropical soils are also often P-limited, which explains its low concentration in the litter across species as already reported by van der Sande et al. 2018 and Caldeira et al. 2019. Moreover, P has high mobility in the phloem of plants and can be translocated to young plant tissues during senescence (Ludvichak et al. 2016). The highest leaf P concentration (Table A1) was found in *H. courbaril*, however, in light of the increased leaves productivity, *P. reticulata* had the highest P turnover and soil P as well (Barroso et al. 2018c). The higher P level (in the context of the observed species) in the soil-plant system and *P. reticulata* growth rate suggest that the species is likely using this nutrient efficiently.

Although K varied among the species, this nutrient is very soluble and easily leached from the leaves (Osono and Takeda 2004; Lanuza et al. 2018). This fact leads to abrupt changes in a short period,

making discussions difficult. Higher Ca and Mg contents were found in *H. courbaril* and *C. tomentosum*. The nutrient balance is related to the diversity of the area (Gama-Rodrigues and Barros 2002), where other species may also have exerted a strong influence in the soil nutrients and hence in their fluxes. Surprisingly, other species productivity was higher than the leaves in *H. courbaril* and *C. tomentosum* stands and considering that a relation between the nutrients in the plant-soil system is expected (Hobbie 2015b), the nutrient returning from other litter fractions must be further investigated

Despite C concentrations being similar among species (Table A1), the leaves litter fraction also increased the total C returned in *P. reticulata* stand. Soil organic C accumulation and stability have been suggested by Ngaba et al. (2019) to be strongly influenced by litter quality in which soil carbon isotope fractionation can be a tool for soil carbon status.

6.2. Soil organic carbon and nitrogen in soil profiles

Since no fertilization was done during species growth – only at planting time, 27 years before soil sampling (Barroso et al. 2018c), we assumed that SOC, SON, C/N ratios, and isotopic fractionation are mainly on account of the litter productivity and quality (Ludwig et al. 2015). The high accumulation of litterfall in undisturbed soil conditions in tropical forest resulted in higher C and N within SOM (Balasubramanian et al. 2020). However, there is no support for the higher C and N contents returned via *P. reticulata* leaves litter to the SOC and SON. It might be explained due to limited soil capacity for SOM stabilization when the litter quantity and quality have no longer effects on the SOM (Castellano et al. 2015). Yet, *P. reticulata* had high concentration of recalcitrant litter compounds (Siqueira et al. *in press*), which might have affected the substrate use efficiency by microbes and therefore SOM stabilization (Marschner et al. 2008; Cotrufo et al. 2013).

In accordance with Guillaume et al. (2015), the effect of land-use change on C and N contents in the subsoil also depends on the time after conversion as the authors observed similar C and N in tropical forest and tree plantations two decades after conversion similar to our results almost three decades after pasture conversion.

Soil C/N ratios are commonly used as indicator of the decomposition degree and tend to decrease with greater soil depth due to increased accumulation of decomposed SOM in deeper soil layers after C loss to respiration (Krull and Skjemstad 2003; Kramer et al. 2017). The potential for SOM loss by respiration is known as unstable organic matter (Sollins et al. 1996). However, *P. reticulata* and *H. courbaril* C/N ratios slightly varied with soil depth while *C. tomentosum* had irregular fluctuations. Dislocation of different decomposition degree of SOM in the soil profile was also observed by Han et al. (2020), and *P. reticulata*

and *H. courbaril* stands were consistent to the undisturbed forest evaluated by the authors. C/N ratio between 11 and 12 mainly represents fresh organic matter (Krull and Skjemstad 2003), which indicates that undecomposed and unstable SOM is dominant even in deeper soil layers across species.

The microbial decomposition of the SOM increases $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as the lighter isotopes are consumed first (Nel et al. 2018) and a pattern observed for the *P. reticulata* and *H. courbaril* plots showed the values of isotopic composition approximate to those found in the secondary forest. Larger accumulation of ^{13}C values in the subsoil was reported by several studies (Krull and Skjemstad 2003; Balasubramanian et al. 2020; Han et al. 2020). The C/N ratio, in turn, showed undecomposed organic matter across species and it is supported by the steady ^{13}C enrichment/depletion in the soil profiles after 35cm to *C. tomentosum* and the pasture.

Soil $\delta^{13}\text{C}$ values were similar to Brazilian native forests (Dortzbach et al. 2015; Carvalho et al. 2017), Amazon rainforest (Araújo et al. 2011), and *Hevea brasiliensis* stand at 35 years which was previously a pasture as well (Vicente et al. 2016), similar to our forest stands. However, the soil beneath *C. tomentosum* had enriched ^{13}C values (about 1.6‰) in the uppermost ~40 cm explained by the mixing of fresh and old (from the previous pasture) organic matters (Balasubramanian et al. 2020).

It is frequently reported a relatively rapid shift from C₄-derived C to C₃-derived C in tropical regions mainly due to weather conditions where the warm and humid environment accelerates the organic matter decomposition rates mostly in the upper soil layers (Maggiotto et al., 2014; Villela et al., 2012). The soil C₄-C derived C losses are decreasing at a slower rate under *C. tomentosum* than *P. reticulata* and *H. courbaril* in the topsoil layers even though the temperature and humidity are the same. It suggests that species-specific traits such as litter chemical composition and microbial community are driving the SOC budget and organic matter decomposition rates more than weather conditions.

The fresh litter is relatively labile compared to organic matter in deeper soil layers and it explains ^{15}N -depleted N across species in the soil surface (Hobbie and Ouimette 2009; Ngaba et al. 2019). Despite the large differences between N returned via litterfall, SON was similar across species. It indicates that substantial N losses might be occurring, especially in the soil under *P. reticulata*, which frequently happens in N-saturated tropical systems due to soil capacity limitations (Hobbie and Högberg 2012).

The ^{15}N enrichment at intermediate depths support our assumption of N loss of ^{14}N by gaseous nitrification and denitrification, which is known to strongly favor loss ^{14}N in tropical ecosystems (Houlton et al. 2006; Hobbie and Ouimette 2009; Peri et al. 2012; Nel et al. 2018). Stands with an open N cycle (large pools and fluxes) seem to cause soil ^{15}N enrichment (Callesen et al. 2013) as we observed across our N-

fixing species. Moreover, *P. reticulata* $\delta^{15}\text{N}$ values varied about $\sim 2\text{‰}$ and it was about two times more than *C. tomentosum* and *H. courbaril*, which can be partially explained by the higher light transmission through canopy (Figure A2) and tendency for higher soil temperatures. Nitrification in weathered soils, as tropical and subtropical sites, leads to large nitrite leaching, which also needs to be investigated more closely in our study site as it might be an environmental problem (Yu et al. 2017).

Apart from nitrification and denitrification Callesen et al., (2013) based on Högberg et al., (2011) and Hobbie and Colpaert, (2003) hypothesized in European common gardens, that N depletion can also be caused by retention of ^{15}N enriched N in mycorrhizal mycelium fungi and a preferential transfer of ^{15}N depleted N to host plants (Hobbie and Högberg 2012). This may also have contributed to the higher ^{15}N enrichment of N in *P. reticulata* soil profile as the natural mycorrhizal association was already described by Siqueira et al. (2019) for the species.

Soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values had the majority of their enrichment/depletion occurring in the upper $\sim 40\text{cm}$ and they did not follow the same trajectories with soil depth as proposed by Nel et al. (2018). On the other hand, *P. reticulata* and *H. courbaril* had a linear coupling relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation. The positive relationship between C-N isotope fractioning, which means ^{13}C and ^{15}N synchronized enrichment, is evidence of high N utilization efficiency due to plant absorption of ^{15}N -depleted with SOM mineralization (Han et al. 2020).

The lack of a credible adjustment in the C-N fractionation in the *C. tomentosum* soil profile reflects an unbalanced N utilization efficiency during SOM mineralization where $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values enriched more than the other species in the soil profile (up to 50 cm). Despite the lower SOC and C/N ratio, the SON was similar across species and the isotopic fractionation showed the highest $\delta^{15}\text{N}$ values (about 8‰) in *C. tomentosum* stand. It suggests that C shortage in the organic matter is provoking an imbalanced C and N relationship while the organic matter is decomposed. This fact indicates that some tropical N-fixing species may develop inefficient N utilization owing to C deficit.

Although the high evidence of N losses mostly by nitrification and denitrification across species, *P. reticulata* and *H. courbaril* still showed high N utilization efficiency. Meanwhile, the opposite pattern displayed by *C. tomentosum* was, indeed, supported by the PCA analysis (Figure 5B), revealed that tropical N-saturated ecosystems could be primarily constrained by C-limitation. However, a broad effect of tropical N-fixing tree species on SOC, SON, and isotopic fractionation that includes more species needs to be investigated more closely.

7. Conclusions

Regardless of the higher amounts of C and N returned via litterfall in the *P. reticulata* stand, soil organic carbon and nitrogen was similar across species, but *C. tomentosum* had slightly lower soil organic carbon.

Afforestation with tropical 27-year-old N-fixing tree plantations was able to restore soil C and N to similar rates as in forest sites after less hostile disturbances. However, *C. tomentosum* still had higher C₄-C derived left in the upper 0-30 cm soil layer. It seems that tropical N-fixing tree species differ in C and N sequestration rates within mineral soil.

N cycling seems to be different concerning N turnover via litterfall and soil N status among species which warrants further investigations. There is high evidence of ¹⁴N gaseous loss by nitrification and denitrification and *P. reticulata* and *H. courbaril* are still very likely N efficient. N utilization efficiency in tropical N-fixing species might be constrained by C shortage.

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Supplementary material

Table A1. Nutrient concentrations in leaf litter from each tropical common garden \pm confidence interval ($p < 0.05$)

Species	C	N	P	K	Ca	Mg
	g kg ⁻¹					
<i>P. reticulata</i>	398.4 \pm 3.12	19.27 \pm 0.88	0.47 \pm 0.02	1.43 \pm 0.2	2.91 \pm 0.3	0.69 \pm 0.08
<i>H. courbaril</i>	387.2 \pm 17.12	12.85 \pm 0.05	0.57 \pm 0.02	4.08 \pm 0.1	8.89 \pm 0.12	1.10 \pm 0.04
<i>C. tomentosum</i>	364.8 \pm 8.25	12.76 \pm 2.46	0.42 \pm 0.16	5.54 \pm 1.5	6.17 \pm 2.05	2.89 \pm 0.63

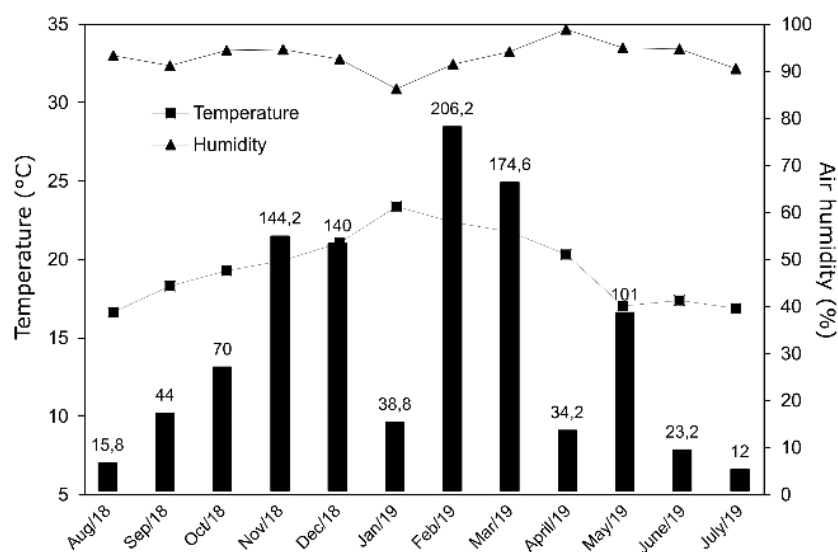
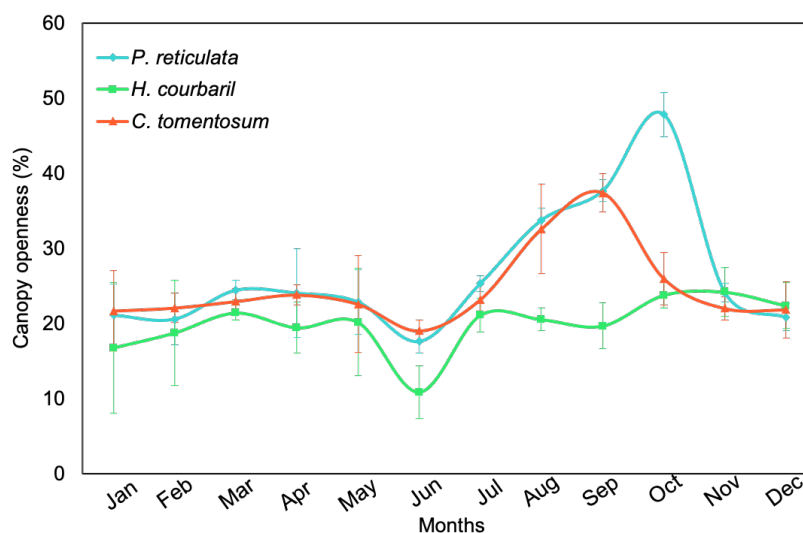


Fig A1. Detailed temperature, air humidity and precipitation (mm) in the study site.

Fig A2. Canopy openness under *Centrolobium tomentosum*, *Hymenaea courbaril* and *Plathymenia reticulata* stands over one year. Bars indicate Confidence Interval ($p < 0.05$)

4. RESUMO E CONCLUSÕES

No primeiro trabalho foi realizada a avaliação da dinâmica da decomposição e liberação dos nutrientes ao longo do tempo, bem como a estequiometria envolvida no processo para o plantio de *P. reticulata* e *H. courbaril*. Os resultados mostraram que, embora a perda de massa tenha sido similar entre as espécies, a liberação dos nutrientes foi diferente, sendo, de maneira geral, maior para as folhas de *P. reticulata*, especialmente para o carbono (C), nitrogênio (N) e fósforo (P). A imobilização de nutrientes foi verificada para ambas as espécies, no entanto, com maior intensidade em *H. courbaril*.

As diferentes concentrações de nutrientes no material foliar levaram a variações estequiométricas (relação C:N:P), o que está diretamente relacionado ao fornecimento de energia para a microbiota decompositora. As relações dos nutrientes indicam que a decomposição de *P. reticulata* foi provavelmente limitada em P, enquanto *H. courbaril* parece ser co-limitada por N e P.

No segundo trabalho, o foco principal foi a produção de serrapilheira, o retorno de nutrientes e a dinâmica do C e N no perfil do solo. Nesta etapa, foram consideradas três espécies: *Plathymentia reticulata*, *Hymenaea courbaril* e *Centrolobium tomentosum*. Os resultados revelaram que a produção de serrapilheira (fração de folhas das espécies plantadas) foi de 5689 kg ha⁻¹ ano⁻¹ no plantio de *P. reticulata*, quase duas vezes superior a *H. courbaril* e *C. tomentosum*, com 3231 kg e 3117 kg ha⁻¹ ano⁻¹, respectivamente. No entanto, a fração de folhas de outras espécies foi superior no plantio de *H. courbaril* (6407 kg ha⁻¹ ano⁻¹), três vezes superior a *P. reticulata* (1844 kg ha⁻¹ ano⁻¹). A maior

produção da serrapilheira no plantio de *P. reticulata* ocasionou maior retorno de C, N e P. Apesar disso, o C e N orgânicos do solo foram similares, exceto para *C. tomentosum* que teve %C ligeiramente inferior.

O fracionamento isotópico do ^{13}C provou que o plantio de *C. tomentosum* ainda apresenta grande contribuição da MO proveniente da pastagem anterior ao plantio, mesmo após 28 anos. No entanto, para *P. reticulata* e *H. courbaril*, a matéria orgânica é majoritariamente dos plantios florestais, similares à floresta secundária. O enriquecimento isotópico dos valores de $\delta^{15}\text{N}$ nas camadas intermediárias do solo indica que há grande perda gasosa de ^{14}N (nitrificação e desnitrificação). Quando considerada a discriminação isotópica coordenada do ^{13}C e ^{15}N , o plantio de *P. reticulata* e *H. courbaril* têm uso eficiente do N pela vegetação, o que não foi observado para *C. tomentosum*, provavelmente devido à limitação do C.

Em conclusão, as espécies florestais fixadoras de N apresentaram diferenças quanto à produção de serrapilheira e liberação de nutrientes, o que refletiu na dinâmica do C e N do solo. Embora sejam necessários novos estudos para compreender os fatores não abarcados que influenciaram a ciclagem de nutrientes, este estudo descreve pela primeira vez o comportamento de espécies tropicais fixadoras de N após a conversão de pastagem, sendo importante para tomada de decisão na escolha de espécies para fins ecológicos e/ou silviculturais. Além disso, traz informações para o ciclo biogeoquímico de nutrientes em áreas tropicais, que ainda permanecem pouco exploradas.

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