

RAFAEL COSTA PINHEIRO

**AVALIAÇÃO DA DISTÂNCIA MÁXIMA DE ABSORÇÃO DE NUTRIENTES EM
FORMAÇÕES FLORESTAIS TROPICAIS USANDO ^{15}N COMO MARCADOR**

Botucatu

2019

RAFAEL COSTA PINHEIRO

**AVALIAÇÃO DA DISTÂNCIA MÁXIMA DE ABSORÇÃO DE NUTRIENTES EM
FORMAÇÕES FLORESTAIS TROPICAIS USANDO ^{15}N COMO MARCADOR**

Tese apresentada à Faculdade de
Ciências Agronômicas da Unesp Câmpus
de Botucatu, para obtenção do título de
Doutor em Ciência Florestal.

Orientador: Jean-Paul Laclau

Coorientador: Iraê Amaral Guerrini

Botucatu

2019

P654a Pinheiro, Rafael Costa
Avaliação da distância máxima de absorção de nutrientes em
formações florestais tropicais usando ^{15}N como marcador /
Rafael Costa Pinheiro. -- Botucatu, 2019
108 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp),
Faculdade de Ciências Agrônômicas, Botucatu
Orientador: Jean-Paul Laclau
Coorientador: Iraê Amaral Guerrini

1. Absorção de água. 2. Raízes (Botânica). 3. Isótopos. 4.
Eucalipto. 5. Cerrados. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da
Faculdade de Ciências Agrônômicas, Botucatu. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: AVALIAÇÃO DA DISTÂNCIA MÁXIMA DE ABSORÇÃO DE NUTRIENTES EM
FORMAÇÕES FLORESTAIS TROPICAIS USANDO ^{15}N COMO MARCADOR

AUTOR: RAFAEL COSTA PINHEIRO
ORIENTADOR: JEAN-PAUL LACLAU
COORIENTADOR: IRAÊ AMARAL GUERRINI

Aprovado como parte das exigências para obtenção do Título de Doutor em CIÊNCIA FLORESTAL,
pela Comissão Examinadora:

Prof. Dr. JEAN-PAUL LACLAU

UMR Eco&Sols / Centre de Coopération Internationale En Recherche Agronomique Pour Le Développement -
Videoconferencia

Pesquisador Dr. CLAYTON ALCARDE ALVARES
/ Suzano Papel e Celulose

Prof. Dr. JOSÉ LEONARDO DE MORAES GONÇALVES
Ciências Florestais / Escola Superior de Agricultura Luiz de Queiroz (ESALQ)

Prof. Dr. VLADIMIR ELIODORO COSTA
Física e Biofísica / Instituto de Biociências de Botucatu - UNESP

Prof.ª Dr.ª VÂNIA REGINA PIVELLO
Ecologia / Universidade de São Paulo

Botucatu, 02 de abril de 2019

Aos meus amados pais, Sevane e Fernando (saudades), por me dar a vida, por tudo que sempre me ensinaram e me incentivaram a fazer, e por todo amor e dedicação;

Aos meus queridos avós, principalmente à minha avó Ivone (saudades),
minha segunda mãe;

À minha querida e amada irmã, por todo carinho, companheirismo e incentivo;

E à minha querida e amada esposa Livia, por toda cumplicidade, amor e apoio incondicional;

Dedico esta árdua pesquisa, com muito amor e muita gratidão.

AGRADECIMENTOS

A Deus.

À minha esposa Lívia, pela ótima convivência, compreensão, companheirismo, amizade, incentivo e amor durante todo o curso; além disso, por todas as ajudas no campo, no laboratório e nas análises dos dados referentes ao meu estudo.

À minha mãe, Sevane, minha inspiradora, a qual devo toda a minha vida e meus princípios e ensinamentos, e à minha irmã Lívia, pela amizade, carinho, apoio, amor, momentos de descontração e lazer.

Aos outros membros da família: cunhados, tios, primos, etc., e aos amigos de longa data, pelos incentivos e momentos de confraternização.

À Faculdade de Ciências Agrônômicas de Botucatu – Universidade Estadual “Júlio de Mesquita Filho” (UNESP) pela oportunidade de cursar o doutorado por meio do Programa de Pós-graduação em Ciência Florestal e pela estrutura e apoio fornecidos ao longo do curso.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pelo apoio financeiro, concedido por meio de bolsa regular de doutorado no país (Processo nº 2015/25946-0) e também pela Bolsa Estágio de Pesquisa no Exterior (BEPE) durante o período de seis meses em Montpellier, na França (Processo nº 2018/06335-8).

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela bolsa de estudos concedida durante 4 meses do curso de doutorado.

À empresa Suzano Papel e Celulose e todos os seus funcionários, em especial aos engenheiros José Luiz Stape, Clayton Alvares, Dileto e Gava, pela parceria nesse trabalho por meio de apoio financeiro, técnico e pela disposição de alguns de seus talhões de eucalipto para servirem como área de estudo desse trabalho.

Ao Instituto de Pesquisas e Estudos Florestais (IPEF) pela prestação de serviços na coleta de folhas das árvores de eucalipto mais altas, por meio de seus escaladores profissionais Edison e sr. Cardoso.

Ao Jardim Botânico Municipal de Bauru (JBMB), em especial aos funcionários Luiz, Jonas, Viviane, Rafael e “Ed”, pela parceria nesse trabalho por meio da disposição da área de Cerrado utilizada e do grande e valioso auxílio para a marcação de parcelas na área e para a coleta de folhas das árvores.

À empresa Floragro, na figura do Eder como dono e dos funcionários Dirlei (Alemão), Gilmar, Donizete, Adriano (Lobão) e outros, que tiveram um grande e árduo trabalho para realizar a perfuração dos vários e muito profundos buracos no solo onde foi feita a aplicação do marcador isotópico. Pelas risadas e momentos de descontração em meio a tudo isso!

À Estação Experimental de Ciências Florestais de Itatinga (ESALQ/USP) e seus funcionários Rildo, Lourival e Elaine, por disponibilizar a estrutura do horto para que eu pudesse realizar a lavagem do solo e a separação de raízes finas.

Ao Centro de Isótopos Estáveis do Instituto de Biociências de Botucatu, pela colaboração no projeto: professores Ducatti (*in memorian*) e Vladimir, Evandro, Cibele, Nádia, Mariana, obrigado por todo o auxílio e os conhecimentos passados em relação aos procedimentos de análises isotópicas de ^{15}N ; às alunas Bárbara e Natália, que me ajudaram a pesar a maior parte das amostras a serem analisadas.

Ao CIRAD – La Recherche Agronomique pour le Développement, pelo auxílio financeiro ao projeto, mais especificamente pelo fornecimento do fertilizante enriquecido em ^{15}N e pela oportunidade de participar no Congresso IUFRO Eucalyptus 2018.

Ao pesquisador e professor Dr. Jean-Paul Laclau, meu orientador desde o curso de mestrado, pelos muitos conhecimentos passados, pela amizade, pela paciência e pela atenção prestada, mesmo com a distância física entre nós.

Aos professores Dr. Iraê e Dra. Magali, pela orientação, ensinamentos, conselhos, auxílio nos trâmites administrativos relacionados ao curso e pela amizade.

Aos demais pesquisadores do CIRAD que estiveram relacionados direta ou indiretamente ao estudo: Jean-Pierre, Joannès, Agnès, Yann, Guerric.

Aos companheiros de república Deivid, Leonardo (Zé Butina), Matheus e Darlin, pelas conversas, conselhos, companheirismo, churrascos e cervejas.

Aos amigos desde a turma de graduação que também estiveram presentes nessa fase, Zé de Deus, Lucas (Macarrão), Ivanka, Raoni (Cabeça), Vic.

Aos outros amigos e colegas feitos em Botucatu, envolvidos no PPG Ciência Florestal, Rita, Cris, Lívia, Camila, Amandine, Marianne, Raquel, Aline, Gláucia, Rafael Barroca, Túlio, Rafael Pará, Jhuan, Thaís, Mônica, entre outros que posso ter omitido.

Aos estagiários de graduação que me ajudaram muito, tanto nas atividades de campo como nas de laboratório, principalmente aos Augustos, vulgos “Mega” e “Liberato”.

Ao CEFE (Centre d'Ecologie Fonctionnelle et Evolutive), na figura da pesquisadora Dr^a. Catherine Roumet, e à UMR Eco&Sols, Centros Cooperativos de pesquisa em Montpellier (França), onde pude realizar um rápido experimento, além de conviver e aprender com todos os seus funcionários. Foi uma experiência profissional e pessoal muito rica!

À “família brasileira” que conviveu em Montpellier durante esse período: Livia, Dany, Alberto, Ana Luísa, Bruna e Ivanka. A companhia de vocês foi especial durante todo o tempo que estive por lá. Que sigamos em frente com essa amizade, sempre que for possível.

À família “Le Maire”: Guerric, Albane, Camille, Alix, Héloïse e Martin por acolher a mim e à minha esposa Livia tão bem em Montpellier. Por toda atenção, ajuda, carinho e momentos de descontração nesse período de adaptação.

Às amigas do “bureau du Bâtiment 13”, Amandine, Sara e Ivanka, pelas conversas, aprendizagem do francês, momentos de confraternização e apoio.

Aos funcionários da UMR Eco&Sols, que me ajudaram bastante tanto com questões administrativas, como com atividades relacionadas ao meu experimento: Cassandre, Farid, Didier Arnal, Carlos, Christophe Jourdan, Claude Passard, Philippe Benezech, Patrícia Laclau, entre alguns outros.

Frase de motivação (ARNAL, Didier.): “Il n’y a pas de problème, il n’y a que des solutions!”

Muito obrigado a todos!

Merci beaucoup à tous!

“Cabe ao homem compreender que o solo fértil onde tudo que se planta dá, pode secar. Que o chão que dá frutos e flores pode dar ervas daninhas. Que a caça se dispersa e a terra da fartura pode se transformar na terra da penúria e da destruição. O homem precisa entender que de sua boa convivência com a natureza depende sua subsistência e que a destruição da natureza é sua própria destruição, pois a sua essência é a natureza, a sua origem e o seu fim”.

Elizabeth Jhin (Telenovela “Amor Eterno Amor”)

RESUMO

As mudanças climáticas têm causado alterações na temperatura e também nos regimes de precipitação do planeta, aumentando a probabilidade de ocorrência de secas mais severas, o que, no cenário florestal, pode resultar na mortalidade de florestas ao redor do mundo. Assim, é necessário um conhecimento mais detalhado sobre a dinâmica de absorção de água e nitratos pelas raízes das árvores, que explique o comportamento das árvores sob extrema seca. O objetivo do estudo foi avaliar a distância máxima do tronco e a profundidade máxima de absorção de nutrientes e água pelas raízes das árvores em florestas crescendo sobre Neossolos Quartzarênicos muito profundos, utilizando o ^{15}N como marcador. As consequências do status social das árvores, comparando árvores dominantes e dominadas do mesmo genótipo numa floresta monoclonal foram estudadas em plantios de um clone híbrido de *Eucalyptus urophylla* x *Eucalyptus grandis* durante a estação seca. Os efeitos da sazonalidade (comparando as estações seca e chuvosa) foram estudados em três espécies do Cerrado: i) *Xylopia aromatica* (Lam.) Mart.; ii) *Coussarea hydrangeifolia* (Benth.) Müll.Arg.; and iii) *Miconia albicans* (Sw.) Steud. Além disso, foi avaliada a existência de uma complementaridade de nicho no solo entre essas espécies do Cerrado, com uma absorção do marcador em camadas diferentes. O marcador NO_3^- - ^{15}N foi aplicado em talhões de eucalipto com 7 meses, 1,2 anos, 2,2 anos e 6,4 anos de idade, a várias profundidades do solo em 60 pontos, sendo 3 repetições por profundidade. No Cerrado, o marcador NO_3^- - ^{15}N foi aplicado em 36 pontos: 2 estações do ano (seca e chuvosa), 6 profundidades (até 12 m) e 3 repetições. Todas as parcelas foram alocadas a uma distância maior que 50 m entre elas. O marcador foi aplicado em apenas um ponto no centro de cada parcela. Cinco meses após a aplicação do marcador, o ^{15}N foi determinado a partir do valor de $\delta^{15}\text{N}$ nas folhas amostradas em árvores a diferentes distâncias de cada área de injeção. Os resultados mostram que os fertilizantes em plantios comerciais de eucalipto devem ser aplicados próximos aos troncos para serem absorvidos por todas as árvores (dentro de 2 m), independentemente da idade do povoamento e do status social das árvores. A fertilização (e o controle de ervas daninhas) em metade das entrelinhas de plantios de eucalipto durante a estação chuvosa pode ser suficiente para permitir que todas as árvores absorvam os nutrientes aplicados, o que poderia reduzir o custo de uma

adubação comercial convencional. Os íons de nitrato lixiviados em Latossolos profundos podem ser absorvidos pelas raízes das árvores a uma profundidade de 6 metros entre 0,6 e 2,5 anos após o plantio. Entretanto, a velocidade de exploração das camadas profundas do solo depende do status social das árvores. Na área de Cerrado, o nitrato marcado foi absorvido em diferentes profundidades e distâncias do tronco, dependendo da espécie estudada, o que sugere uma complementaridade de nicho em todo o perfil do solo até uma profundidade de pelo menos 6 metros. Apenas as árvores com um diâmetro à altura do peito maior que 5 cm foram capazes de absorver o marcador injetado em profundidades de mais de 1,5 metros. Inesperadamente, as árvores não absorveram os nitratos marcados mais profundamente na estação seca do que na estação chuvosa.

Palavras-chave: Absorção de água. Raízes finas. Nitrato marcado. Plantios de eucalipto. Cerrado.

ABSTRACT

Climate changes have caused alterations in temperature and in precipitation regimes around the world. This increases the probability of exceptional drought periods, which may result in tree mortality over large areas. Therefore, it is necessary to improve our knowledge about the the dynamics of water and nitrate uptake by the roots that explain the tree behaviour under extreme drought. This study aimed to assess the maximum distance of the trunk and the maximum depth of nutrient and water uptake by tree roots in forests growing on very deep Ferralic Arenosols through ^{15}N tracer. The consequences of the social status of trees, comparing dominant and suppressed trees of the same genotype in a monoclonal forest, were studied in plantations of the same clone of the *Eucalyptus urophylla* x *Eucalyptus grandis* hybrid over the dry season. The effects of seasonality (comparing dry and rainy seasons) were studied in three native savanna (Cerrado) species: i) *Xylopia aromatica* (Lam.) Mart.; ii) *Coussarea hydrangeifolia* (Benth.) Müll.Arg.; and iii) *Miconia albicans* (Sw.) Steud,. Furthermore, the existence of a niche complementarity in the soil among the three studied Cerrado species was evaluated, with the analysis of the tracer uptake in different soil layers, depending on the species. The NO_3^- - ^{15}N tracer was applied in 7-month-old, 1.2-year-old, 2.2-year-old and 6.4-year-old eucalypt stands at several soil depths, with three repetitions per depth at 60 positions. In the Cerrado, NO_3^- - ^{15}N tracer was applied at 36 positions: 2 seasons (dry and rainy), 6 depths (down to 12 m) and 3 repetitions. All the plots were allocated at a distance > 50 m between them. The tracer was applied at only one location in the centre of each plot. Five months after tracer application, ^{15}N was determined from the $\delta^{15}\text{N}$ values in leaves sampled in dominant and suppressed trees at different distances from each injection area. The results show that fertilizers must be applied close to the trunks (within 2 m) in *Eucalyptus* plantations to be taken up by all trees, regardless of stand age and tree social status. Fertilization (and weed control) in half of the inter-rows during the rainy season could be sufficient to make it possible for all the trees to take up the applied nutrients which could reduce the cost of a conventional commercial fertilization., Nitrates leached in deep sandy soils can be taken up by tree roots at a depth of 6 m between 0.6 and 2.5 years after planting in commercial *Eucalyptus* plantations. However, the velocity of exploration of deep soil layers depends on the social status of the trees. The labelled nitrates were taken up at

different depths and distances from the trunk depending on the species in the Brazilian savanna studied, which showed a niche complementarity throughout the soil profile down to a depth of at least 6 m. Only trees with a DBH of more than 5 cm were able to take up the NO_3^- - ^{15}N tracer injected at depths of more than 1.5 m. Unexpectedly, trees did not take up the labelled nitrates more deeply in the dry season than in the rainy season.

Keywords: Water uptake. Fine roots. Labelled nitrate. Eucalypt plantations. Cerrado.

SUMÁRIO

INTRODUÇÃO GERAL	19
 CAPÍTULO 1 - DISTANCE AND DEPTH OF UPTAKE OF LABELLED NITRATE FOR DOMINANT AND SUPPRESSED TREES THROUGHOUT THE ROTATION IN BRAZILIAN EUCALYPTUS PLANTATIONS	34
ABSTRACT	34
1.1. INTRODUCTION	35
1.2. MATERIAL AND METHODS.....	38
1.3. RESULTS.....	44
1.4. DISCUSSION	49
REFERENCES	54
APPENDIXES	60
 CAPÍTULO 2 - NICHE COMPLEMENTARITY BETWEEN PLANT SPECIES TO TAKE UP LABELLED-NITRATE DOWN TO A DEPTH OF 12 M IN A BRAZILIAN SAVANNA	63
ABSTRACT.....	63
2.1. INTRODUCTION	64
2.2. MATERIAL AND METHODS.....	66
2.3. RESULTS.....	74
2.4. DISCUSSION	83
2.5. CONCLUSION.....	87
REFERENCES	89
APPENDIXES	93
 CONSIDERAÇÕES FINAIS.....	97
 REFERÊNCIAS	101

INTRODUÇÃO GERAL

Mudanças climáticas

Neste século, as magnitudes e as taxas de mudança climática associada aos cenários de média e alta emissão de gases de efeito estufa apresentam alto risco de mudança abrupta e irreversível em escala regional na composição, estrutura e função dos ecossistemas terrestres e ecossistemas de água doce, incluindo as zonas úmidas (IPCC, 2014). Espera-se que o aumento das emissões de gases de efeito estufa eleve a temperatura média global entre 2 e 7 °C até o final do século XXI (RIGNOT et al., 2009). As alterações nos regimes de precipitação e temperatura como um resultado das mudanças climáticas devem agravar a intensidade e a frequência de futuras secas e, conseqüentemente, aumentar a mortalidade de florestas em todo o mundo (WU et al., 2011). Prevê-se que as variações e as mudanças climáticas ofereçam riscos à sustentabilidade florestal e à consecução dos objetivos de manejo florestal (LIMA et al., 2012). Anormalidades climáticas nos últimos anos estão colocando sob estresse muitas plantações de eucalipto devido a maiores temperaturas médias anuais, eventos mais intensos de chuvas, períodos de estresse hídrico mais severo e eventos climáticos mais frequentes e extremos (GONÇALVES et al., 2017).

Os efeitos da variabilidade climática local e de grande escala no crescimento das árvores foram detectados em estudos de longo prazo (VENEGAS-GONZÁLEZ et al., 2016). Um dos maiores desafios hoje e nas próximas décadas será a adaptação de genótipos à mudança climática e a condições mais longas de estresse hídrico em regiões onde há novas áreas de reflorestamento (GONÇALVES et al., 2017).

Mecanismos de respostas morfofisiológicas de plantas

Compreender melhor os mecanismos estruturais e fisiológicos pelos quais as árvores mantêm a hidratação do tecido e a fotossíntese em resposta ao déficit hídrico é de importância primordial para predizer os efeitos das mudanças climáticas na sobrevivência de árvores, no sequestro de carbono e no uso da água nos ecossistemas florestais (MCDOWELL et al., 2013; KLEIN et al., 2014). Estratégias como a hidrosolidariedade estão se tornando cada vez mais importantes no manejo de plantações florestais porque a competição pela água está aumentando devido às mudanças climáticas (LIMA et al., 2012). Estudos abrangentes, avaliando o impacto

da seca no uso de água ao nível de povoamento, ainda são escassos em florestas tropicais e subtropicais (MCDOWELL et al., 2008).

O clima dos ecossistemas de savana do Brasil Central (Cerrado) é caracterizado por cinco meses com pouquíssimos eventos de precipitação e um longo período com altas precipitações. Árvores que crescem em ambientes tropicais caracterizados por estações chuvosa e seca distintas devem ajustar sazonalmente suas características morfofisiológicas para lidar de forma exitosa com as mudanças na disponibilidade de água no solo (BUCCI et al., 2008). Em resposta à disponibilidade limitada de água em um ambiente sazonal forte, as plantas reduzem a transpiração pela limitação estomática e/ou pela queda das folhas, para diminuir ainda mais a perda de água por evaporação durante a estação seca. Ou seja, a demanda por evaporação é substancialmente maior durante a estação seca (maio a setembro) por causa da menor umidade relativa do ambiente.

A combinação de maior demanda evaporativa e baixa precipitação durante a estação seca faz do Cerrado um ambiente potencialmente estressante para as plantas vasculares (BUCCI et al., 2008). Apesar das condições estressantes durante a estação sem chuva, muitas espécies de árvores que crescem em florestas tropicais secas são decíduas, mantendo uma safra ativa de folhas durante este período e produzindo novas folhas, antes do início da estação chuvosa (BORCHERT, 1994; RIVERA et al., 2002; FRANCO et al., 2005; LENZA E KLINK, 2006). As suas taxas de crescimento sazonal estão bem acopladas às mudanças sazonais na disponibilidade do principal recurso limitante: a água (BUCCI et al., 2008). As taxas de crescimento do caule aumentam, após os primeiros grandes pulsos de chuva na estação úmida e as altas taxas de crescimento são mantidas até dois a três meses antes do final da estação chuvosa no início de março (SARACENO, 2006). É provável que os fotoassimilados, neste momento, sejam direcionados para melhorar o crescimento das raízes, em vez do crescimento do caule, para garantir a absorção de água durante a estação seca. A produção de novas folhas por árvores de savana durante o período mais desfavorável para o crescimento pode ser uma resposta adaptativa à baixa disponibilidade de nutrientes no solo (SARMIENTO et al., 1985).

A manutenção das folhas, bem como a produção de novas folhas durante a estação seca, gera um custo de carbono para as árvores de savana. Elas têm que lidar com o custo mais alto de manter raízes longas e profundas para explorar a água de fontes de água abundantes e mais estáveis disponíveis em profundidade (SCHOLZ

et al., 2008). Embora a maioria das árvores do Cerrado tenha mais de 50% de sua biomassa abaixo do solo (SARMIENTO, 1985; CASTRO E KAUFFMAN, 1998), algumas espécies lenhosas do Cerrado possuem sistemas radiculares relativamente superficiais (SCHOLZ et al., 2008). São possíveis diferentes tipos de ajustes fisiológicos e morfológicos que permitem que as árvores mantenham os potenciais hídricos foliares acima do ponto de perda do turgor durante a estação seca.

Um entendimento básico dos efeitos da nutrição nos mecanismos envolvidos na resposta da árvore à seca é essencial no âmbito de um clima mais seco no futuro (BATTIE-LACLAU et al., 2014). Tanto o aumento do dióxido de carbono atmosférico (IVERSEN, 2010; NIE et al., 2013) quanto as secas prolongadas (GERMON et al., 2019) devem aumentar a exploração de camadas profundas do solo pelas raízes das plantas no futuro. Picos de crescimento radicular têm sido mostrados em camadas profundas do solo durante períodos de seca para árvores de eucalipto e de seringueira em solos tropicais (MAEGHT et al., 2015; LAMBAIS et al., 2017). Estratégias de tolerância à seca são comuns em plantas herbáceas, enquanto estratégias de prevenção à seca são frequentemente adotadas por árvores em florestas tropicais perenes (BRUNNER et al., 2015).

A profundidade máxima das raízes é uma característica importante para selecionar um material vegetal resistente a secas prolongadas, em um contexto atual de mudanças climáticas (COMAS et al., 2013; PINHEIRO et al., 2016). Nesse sentido, esse trabalho tem potencial de fornecer informações indiretas sobre estas características em florestas de eucaliptos e no Cerrado a partir das profundidades de absorção de ^{15}N no final da estação seca.

Absorção de água e profundidade de raízes

A dinâmica de raízes em camadas muito profundas do solo, principalmente abaixo de cinco metros de profundidade, é um assunto pouco estudado em espécies arbóreas em regiões tropicais (JACKSON et al., 1997; CHRISTINA et al., 2011; LACLAU et al., 2013; MAEGHT et al., 2013), porém as informações disponíveis sugerem que o papel destas raízes é subestimado em florestas tropicais (FREYCON et al., 2015).

As florestas tropicais exercem uma forte influência em climas regionais, atuando como uma bomba biótica que desloca quantidades consideráveis de água do

solo à atmosfera (SPRACKLEN et al., 2012). Essa bomba é de particular importância na estação seca na Amazônia, por exemplo, quando a maior parte da transpiração das árvores vem da água retirada de camadas profundas do solo pelas raízes (NEPSTAD et al., 1994; BRUNO et al., 2006) e também em plantios de eucalipto (CHRISTINA et al., 2011; BATTIE-LACLAU et al., 2014). Um estudo recente no estado de São Paulo mostrou que o comportamento de raízes finas em Latossolos profundos é semelhante para sementes e clones de várias espécies de eucalipto utilizadas em plantios comerciais (PINHEIRO et al., 2016).

Modelos baseados em princípios ecofisiológicos, usando entrada de dados climáticos reais, levam em consideração variações entre sítios nas características do clima e do solo, e podem prontamente ser aplicados a novos sítios (MARSDEN et al., 2013). Esses modelos ecofisiológicos são cada vez mais usados por empresas do setor florestal para estimar a produção de florestas plantadas (ALMEIDA et al., 2010), podendo ser consideravelmente melhorados, levando em consideração o papel das raízes muito profundas (MARSDEN et al., 2013). Entretanto, os modelos usados na escala de árvores para estudar os processos acima do solo (CAMPOE et al., 2013; CHRISTINA et al., 2015) são estabelecidos na escala do povoamento no solo (DUURSMA e MEDLYN, 2012), porque a variabilidade da capacidade de absorção de água e nutrientes no solo entre árvores de um mesmo povoamento ainda não é muito bem conhecida.

Em florestas estruturadas, a densidade de raízes é maior se comparada a plantios florestais uniformes devido à separação de nicho (BOLTE et al., 2013; JENSEN et al., 2012), a qual tem mostrado conduzir a absorção de água complementar a partir de diferentes camadas do solo em florestas temperadas dominadas de faias com uma grande participação de coníferas (GROSSIORD et al., 2014). Tal complementaridade pode amenizar o estresse pela seca em escala de povoamento porque a água é absorvida de maneira mais homogênea (METZ et al., 2016).

Um enraizamento profundo é especialmente importante em ambientes sazonalmente secos, como savanas neotropicais, pois isso possibilita às plantas buscarem a água armazenada em camadas profundas no solo durante períodos de pouca disponibilidade de água e alta demanda evaporativa, e permitindo-lhes manterem a transpiração e a fixação de carbono (OLIVEIRA et al., 2005). Canadell et al. (1996) salientaram que savanas tropicais são o bioma com a maior profundidade

de enraizamento médio ($15 \pm 5,4$ m) e também possuem o enraizamento mais profundo já registrado (68 m). Embora o hábito de enraizamento profundo de algumas plantas do Cerrado seja reconhecido (JACKSON et al., 1999; FRANCO, 2002; OLIVEIRA, 2004), muito pouco se sabe sobre sua significância funcional e sua contribuição à aquisição de água (OLIVEIRA et al., 2005), inclusive no Brasil, onde há uma vasta área coberta desse bioma.

No Cerrado, a vegetação arbustivo-arbórea destaca-se por sua riqueza de tipos fenológicos e morfológicos, o que provavelmente implica os diferentes padrões de distribuição do sistema radicular, assim como diferentes estratégias de uso da água (FRANCO, 2005). Apesar das diferenças na extensão do sistema radicular (JACKSON et al., 1999), as espécies lenhosas do Cerrado são caracterizadas por investirem muito em estruturas subterrâneas (ABDALA et al., 1998; HOFFMANN e FRANCO, 2003), o que afeta consideravelmente o balanço de carbono e que, segundo Franco (2005), representa um dreno importante dos produtos fotossintéticos que poderiam ser investidos em desenvolvimento da parte aérea.

Uma das características marcantes da vegetação do Cerrado é a enorme heterogeneidade de sua estrutura, a qual engloba formações majoritariamente campestres, tal como o campo sujo e formações florestais como o cerradão, havendo uma diferença na composição florística e fitossociológica (GOODLAND e FERRI, 1979). Essas fisionomias diferentes resultam em uma exploração especializada da água que está disponível ao longo do perfil do solo (FRANCO, 2002).

Com respeito à biomassa, plantas do Cerrado, nos estágios iniciais de seu desenvolvimento, investem bastante em seu sistema radicular, havendo uma razão de biomassa de raiz/biomassa da parte aérea entre 1 e 9 para plantas entre 5 e 7 meses de vida (PAULILO e FELIPPE, 1998; MOREIRA e KLINK, 2000). Entretanto, esse alto investimento em raízes não significa que essas raízes atinjam grandes profundidades no solo (FRANCO, 2005).

De acordo com dados revisados por Rizzini (1979), a maior parte de árvores do Cerrado mantém suas raízes até 0,5 metros de profundidade no primeiro ano de vida, não ultrapassando o primeiro metro de profundidade aos dois anos de idade. Outros estudos (MOREIRA e KLINK, 2000) mostram uma pequena profundidade atingida pelo sistema radicular de espécies lenhosas do Cerrado nos primeiros meses de idade.

Os indivíduos adultos continuam a investir bastante em raízes. Em escala de ecossistema, a razão de biomassa de raiz/biomassa da parte aérea varia de 1 a 8, dependendo do tipo de fisionomia do Cerrado (CASTRO e KAUFFMAN, 1998; ABDALA et al., 1998). Profundidades do solo superiores a 6 - 7 m, camadas onde a disponibilidade de água é mais estável ao longo do ano, podem ser atingidas pelas raízes (ABDALA et al., 1998; JACKSON et al., 1999). Todavia, alguns estudos sobre a distribuição do sistema radicular de plantas do Cerrado mostraram que a exploração do perfil do solo é complexa, dependendo também da espécie (FERRI e COUTINHO, 1958), o que resulta na extração de água ao longo de todo o perfil do solo (JACKSON et al., 1999).

A forma de investimento no sistema radicular de determinada espécie do Cerrado pode depender de sua fenologia. Dessa forma, espécies decíduas teriam necessidade de reservas de carboidratos estocadas na raiz para rebrotar e desenvolver a nova copa. Estudos complementares relacionando o desenvolvimento inicial do sistema radicular com a fenologia e a capacidade de sobreviver a queimadas são necessários (FRANCO, 2005).

Em ecossistemas como a Floresta Tropical Amazônica, por exemplo, a água armazenada em solos profundos é crucial na manutenção de copas fisiologicamente ativas durante períodos secos (NEPSTAD et al., 1994). Plantas com enraizamento profundo podem contribuir significativamente ao balanço hídrico dos ecossistemas do Cerrado, e o ciclo hidrológico deste bioma poderia mudar conforme a substituição gradativa da vegetação lenhosa por gramíneas exóticas e culturas agrícolas (OLIVEIRA et al., 2005).

Status social das árvores

A função da estrutura florestal, isto é, a mistura de árvores com diferentes idades e tamanhos, na mitigação ou no aumento da suscetibilidade ao estresse hídrico não tem chamado muita atenção (BRANG et al., 2014; FLOYD et al., 2015). Isso é surpreendente até certo ponto, porque se sabe que árvores de diferentes tamanhos e posições relativas dentro de uma floresta variam em propriedades bioquímicas, fisiológicas e morfológicas que, por sua vez, afetam a eficiência da assimilação do carbono e a suscetibilidade à falha hidráulica (BINKLEY et al., 2013).

Modelos de desenvolvimento florestal deveriam diferenciar a resposta das árvores não apenas pelo tamanho, mas também pelo status social (tamanho de uma árvore em comparação com suas vizinhas) ou grau de sombreamento para ser apropriadamente sensível às decisões de manejo (GROTE et al., 2016). No sistema de inventário florestal de Quebec, por exemplo, o status social é classificado visualmente no campo e leva em consideração a altura relativa da árvore no povoamento (andar) e sua posição em relação a suas vizinhas (grau de isolamento da copa). A mudança no status social de árvores dominantes ou codominantes significa principalmente que a árvore anteriormente classificada como dominante ou codominante agora tem uma altura inferior a dois terços da altura dominante do povoamento (BLAIS et al., 1996).

O impacto da seca na mortalidade em modelos de crescimento de estrutura de florestas ou é negligenciado, determinado por relações determinísticas ou estocásticas (BIRCHNER et al., 2015), ou calculado indiretamente da assimilação líquida de carbono (ADAMS et al., 2013). Aclimação a longo prazo, diferenciação hidráulica com o tamanho da árvore, efeitos de cavitação ou efeitos de reporte que poderiam mitigar ou melhorar o estresse e a mortalidade geralmente não são considerados (VAN DER MOLEN et al., 2011).

A mortalidade das árvores é induzida pela competição por recursos. Assim, as árvores menos competitivas para os recursos mais limitados são mais sensíveis a estresses adicionais e são provavelmente as primeiras a morrerem (GROTE et al., 2016). Se o fornecimento de água é suficiente, geralmente são as árvores dominadas que sofrem com deficiências de luz (LUO e CHEN, 2011).

Experimentos comparando parcelas com todas as árvores plantadas em um único dia (tratamento uniforme), com parcelas em que o plantio foi espalhado por 80 dias (tratamento heterogêneo), mostraram que o status social das árvores é estabelecido muito cedo nas plantações monoclonais de eucalipto. Nos primeiros meses após o plantio, as árvores dominadas permaneceram suprimidas durante toda a rotação (BINKLEY et al., 2010).

Deste trabalho, surgiram três implicações notáveis para a silvicultura. As árvores subordinadas interceptaram menos luz e usaram-na com menos eficiência do que as árvores dominantes; com isso os regimes de desbaste que concentram o uso de recursos em árvores maiores devem ser particularmente eficazes nas plantações de eucalipto. A segunda implicação é que o declínio relacionado à idade

no crescimento do povoamento resulta desproporcionalmente do declínio do crescimento, do uso de recursos e da eficiência do uso de recursos pelas árvores. As árvores dominantes de eucalipto mantiveram uma alta eficiência do uso da luz no final da rotação, e as rotações alongadas provavelmente mostrariam altas taxas sustentadas de crescimento nessas árvores. A implicação final é que os povoamentos altamente uniformes tendem a crescer melhor como resultado de uma maior radiação ativa fotossinteticamente absorvida e de uma maior eficiência do uso da luz. Todas essas características merecem uma investigação mais aprofundada, incluindo os efeitos de desbaste e do espaçamento na eficiência do uso da luz, padrões em povoamentos mais velhos e padrões resultantes de interações de suprimentos limitantes de água, nutrientes e luz (BINKLEY et al., 2010).

Se a água está em demanda, o estresse hídrico pode se tornar o fator dominante para a mortalidade. Portanto, em ambientes secos, a densidade de povoamento é geralmente baixa. Em florestas fechadas, entretanto, os benefícios de árvores de diferentes tamanhos crescendo juntas (i. e., proteção contra radiação excessiva, geada e vento) vêm com a retribuição de um aumento de competição, também por recursos subterrâneos (GROTE et al., 2016). De fato, uma alta mortalidade de árvores, após períodos secos, tem sido observada tanto em florestas tropicais assim como temperadas, onde a morte preferencialmente de árvores dominantes indica uma desvantagem em competição por água (FLOYD et al., 2015; ROWLAND et al., 2015). Em geral, um maior risco de mortalidade em árvores dominantes parece ser um fenômeno comum (BENNETT et al., 2015).

Em um estudo em povoamentos de carvalho (*Quercus petraea*) do norte da França, Trouvé et al. (2015) verificaram que o crescimento máximo em altura segue uma resposta de um único modo, típica à idade do povoamento. Em povoamentos plantados abertamente, com menor competição entre as árvores, o aumento do déficit hídrico no solo durante o verão reduziu o crescimento em altura em relação ao crescimento radial, sugerindo restrições hidráulicas ao crescimento em altura. Um padrão semelhante foi encontrado em povoamentos fechados com a magnitude do efeito, porém diminuindo de árvores dominadas para dominantes. A densidade de povoamentos e o status social das árvores foram os principais condutores das variações na alocação do crescimento, enquanto o estresse hídrico teve um efeito prejudicial na altura da alocação da relação entre crescimento em altura e crescimento em circunferência (TROUVÉ et al., 2015).

A descoberta de uma mortalidade menos induzida pela seca em árvores do sub-bosque é suportada por estudos que medem um maior declínio no crescimento em árvores dominantes do que em árvores dominadas em resposta a eventos de seca (MARTÍNEZ-VILALTA et al., 2012; ZANG et al., 2012). Entretanto, assumindo que o declínio no crescimento é, na verdade, um indicador signifiicante da mortalidade das árvores (BERDANIER e CLARK, 2016), deveria ser observado que as árvores maiores não são sempre as mais afetadas; exceções existem a partir de um padrão geral que parece colocar as árvores dominantes em desvantagem (GROTE et al., 2016). Em alguns casos, os eventos de seca parecem diminuir o crescimento em diâmetro mais das árvores dominadas do que das árvores dominantes (MERLIN et al., 2015; PICHLER e OBERHUBER, 2007). Então, é necessário avaliar as causas fundamentais da relação geral, não apenas para explicar o padrão geral encontrado recentemente, mas também para indicar razões para as exceções (GROTE et al., 2016).

Estudos com ^{15}N e outros marcadores isotópicos

Compreender a distribuição de isótopos estáveis do nitrogênio da planta pode ser complicado, porque o nitrogênio da planta pode ser absorvido pelas raízes, através de associações micorrízicas dos fungos, ou simbioticamente, através de bactérias em nódulos radiculares. As plantas que derivam nitrogênio dos solos tendem a usar íons de amônio ou nitrato, e as mesmas fontes de nitrogênio no solo são usadas quando as associações micorrízicas adquirem nitrogênio no solo para suas plantas hospedeiras (EHLERINGER, 2017). Enquanto as variações do $\delta^{15}\text{N}$ das plantas estão associadas às diferenças das fontes de nitrogênio do solo, a fixação simbiótica de nitrogênio atmosférico resulta em valores de $\delta^{15}\text{N}$ nas plantas que são independentes dos valores de $\delta^{15}\text{N}$ do solo (EVANS, 2001; ROBINSON, 2001; CRAINE et al., 2009). Dentro da planta, valores de $\delta^{15}\text{N}$ tendem a ser relativamente homogêneos, próximos do valor atmosférico que é zero, mas as diferenças das raízes até as folhas podem ser tão grandes quanto 3-7 ‰ em plantas com associações de fungos ectomicorrízicos e aumentarem em regimes de alta fertilização (CRAINE et al., 2015; HOBBIIE, 2015). Assim, a compreensão da natureza das raízes e dos processos abaixo do solo é importante para a interpretação dos valores de $\delta^{15}\text{N}$ dos vegetais folhosos, das sementes e dos grãos (EHLERINGER, 2017).

Os valores de $\delta^{15}\text{N}$ de plantas não fixadoras de nitrogênio que crescem sob condições não fertilizadas tendem a variar de -5 a +8 ‰, dependendo das condições do solo. Craine et al. (2015) resumiu uma variedade de estudos, os quais mostraram que os valores de $\delta^{15}\text{N}$ das folhas tendem a ser similares aos valores de $\delta^{15}\text{N}$ de nitrogênio inorgânico no solo. Portanto, os valores de $\delta^{15}\text{N}$ das plantas não fixadoras são uma boa indicação das fontes de nitrogênio do solo. Entretanto, para as plantas com fixação biológica de nitrogênio, por meio de interações de bactérias simbióticas em nódulos radiculares (tais como as leguminosas), os valores de $\delta^{15}\text{N}$ das plantas tendem a variar de 0 a +3 ‰ e são independentes dos valores de $\delta^{15}\text{N}$ do solo (ROBINSON, 2001; CRAINE et al., 2015).

Se os valores de ^{15}N do solo, principalmente, influenciam os valores de ^{15}N das plantas, o que então conduz as variações de valores de ^{15}N no solo? Muitos estudos têm concluído que os valores de ^{15}N do solo representam um balanço entre as entradas de nitrogênio e as perdas deste, especialmente perdas gasosas que tendem a enriquecer os valores de ^{15}N do solo porque isotopicamente moléculas gasosas mais leves em ^{15}N são, preferencialmente, desprendidas durante a degradação microbiana (KOBAYASHI et al., 1998; EVANS, 2001; CRAINE et al., 2009; CRAINE et al., 2015; HOBBS, 2015). Em sistemas agrícolas, o conhecimento dos valores de ^{15}N dos fertilizantes é imprescindível na compreensão das variações nos valores de ^{15}N das plantas.

A variação natural do $\delta^{15}\text{N}$ tem sido amplamente utilizada em estudos ecológicos de ciclagem de N (PARDO et al., 2007; ROBINSON et al., 2001), pois os valores nas plantas e no solo são os resultados líquidos de muitos processos biogeoquímicos que discriminam ^{15}N , podendo fracionar ou enriquecer os valores de $\delta^{15}\text{N}$. É possível usar o ^{15}N para investigar a ciclagem de N em grandes áreas, porque as informações coletadas em uma única amostragem fornecem informações diretas sobre a ciclagem de N passada, fornecendo um registro do efeito líquido das transformações de N (SHENG et al., 2014).

Da Silva et al. (2011) mostraram que as raízes absorvem ^{15}N quando há absorção de água nas camadas profundas do solo e os valores de $\delta^{15}\text{N}$ nas folhas coletadas aproximadamente 1,5 mês após a aplicação de um marcador com ^{15}N permite avaliar se as raízes profundas estão ou não absorvendo água e nitrogênio. Bordron et al. (2019) detectaram que há uma especialização funcional de raízes finas

para árvores jovens de *Eucalyptus grandis*, dependendo da profundidade do solo, como observado anteriormente em árvores de *E. grandis* de 6 anos de idade na mesma região (DA SILVA et al., 2011). A absorção de NO_3^- - ^{15}N por árvores de *E. grandis* até os 3 metros de profundidade aos 20 meses de idade (BORDRON et al., 2019) é consistente com a exploração rápida do solo de raízes finas que podem atingir uma profundidade de 13 metros aos 18 meses de idade em Latossolos profundos (PINHEIRO et al., 2016). Isso mostra que o ^{15}N pode ser um marcador eficiente para explorar a variabilidade espacial de absorção das raízes nas florestas.

A análise dos isótopos estáveis de abundância natural da água, deutério e ^{18}O são ferramentas úteis para estimar a profundidade de absorção de água nas soluções do solo quando seguem um gradiente desde a superfície até as camadas profundas do solo (ROUPSARD et al., 1999; DAWSON et al., 2002). Estudos de absorção de água usualmente baseados em marcação de isótopo duplo do oxigênio (^{18}O) e/ou do hidrogênio (deutério) são inovadores, mas a complexidade e o custo da amostragem podem limitar consideravelmente o uso desses métodos (STAHL et al., 2013).

Através de pulsos de deutério, pequenas árvores em uma floresta tropical amazônica mostraram diferentes padrões de enraizamento; enquanto a maioria das espécies absorveu o traçador de deutério apenas perto do tronco, algumas espécies apresentaram altas concentrações de deutério até 10 m da distância horizontal da área de injeção (STERNBERG et al., 2002).

Brum et al. (2018) avaliaram a profundidade de enraizamento de 12 espécies de árvores dominantes em uma floresta amazônica sazonal analisando os isótopos estáveis de água ($\delta^{18}\text{O}$ e $\delta^2\text{H}$) coletada do xilema de árvores e solos a partir de uma gama de profundidades. Como encontraram um desajustamento entre as faixas abrangidas pelo $\delta^2\text{H}$ da planta e do solo e faixas similares entre o $\delta^{18}\text{O}$ da planta e do solo, utilizaram apenas o $\delta^{18}\text{O}$ do xilema como um indicador da profundidade de absorção da água.

As plantas com raízes profundas são mais propensas a sobreviver em períodos extensos de seca, por meio do acesso a camadas inferiores do solo, as quais contêm maiores níveis de umidade (CHAVES et al., 2003). Em geral, assume-se que as plantas respondem à seca nas camadas superficiais, deslocando a absorção de água e nutrientes para camadas mais profundas do solo (SHARP e DAVIES, 1985), o que recentemente foi confirmado num estudo em pastagens mistas temperadas usando a variação natural ^{18}O (HOEKSTRA et al., 2014). Entretanto, os efeitos da seca na

absorção de recursos pela planta parecem ser inconsistentes ainda (KULMATISKI e BEARD, 2013).

Complementaridade de nicho

A hipótese de complementaridade de nicho implica que espécies de plantas ou grupos funcionais ocupam nichos funcionalmente distintos em um ecossistema e usam recursos de forma complementar. Consequentemente, quando espécies ou grupos se extinguem, seus nichos permanecem desocupados, levando à redução da exploração de recursos e ao declínio das funções dos ecossistemas (KAHMEN et al., 2006). Evidências funcionais e ecofisiológicas diretas para a complementaridade de nichos são escassas na literatura científica.

A complementaridade de nicho abaixo do solo entre espécies de raízes profundas e espécies de raízes superficiais misturadas pode resultar numa exploração mais completa dos recursos disponíveis no solo do que pode ser atingido por algumas espécies que crescem em monocultura (HOEKSTRA et al., 2015). Essa complementaridade seria ainda melhor se a ocupação do nicho abaixo do solo de uma determinada espécie se afastasse das zonas de intensa competição por recursos, quando estas espécies crescerem juntas (MOMMER et al., 2010).

Essa complementaridade de nicho pode ser mais importante quando os recursos são limitados, isto é, sob condições secas, quando comparada a condições controladas ou em consórcios de espécies não leguminosas comparadas apenas a consórcios incluindo leguminosas (PAQUETTE e MESSIER, 2011). Esse aumento total na utilização de água e nutrientes pode levar a uma maior produção de biomassa aérea do que o esperado de uma combinação de desempenhos de monoculturas (HOEKSTRA et al., 2015).

Se, e como os diferentes nichos hidrológicos impulsionam as diferenças nas características de resistência à seca e nos processos ecossistêmicos não são totalmente compreendidos (CHRISTOFFERSEN et al., 2016; IVANOV et al., 2012). Hoekstra et al. (2015) mostraram que espécies com raízes mais profundas tiveram uma maior absorção proporcional de ambos os traçadores (^{15}N e Rb) na profundidade de solo de 35 cm do que as espécies com raízes superficiais. Sob condições secas, todas as espécies aumentaram a absorção proporcional do traçador na camada de

solo mais profunda, e as espécies com maior absorção proporcional de ^{15}N na camada de solo mais profunda foram mais resistentes à seca.

As árvores coexistentes em uma área da Floresta Amazônica sazonal foram em grande parte segregadas ao longo de um único eixo de nicho hidrológico definido por diferenças de profundidade das raízes, acesso à luz e tolerância ao baixo potencial hídrico. As diferenças na profundidade do enraizamento foram fortemente relacionadas ao tamanho da árvore (BRUM et al., 2018).

Justificativas e objetivos

Este trabalho propôs estudar o papel das raízes profundas na absorção de água e de nutrientes em dois tipos de formações florestais de grande importância para o Brasil: (1) uma área de cerradão, representando formações florestais nativas de alta diversidade, a fim de se conhecer o comportamento de diferentes espécies em relação à dinâmica de absorção de água e nutrientes ao longo do perfil de solos profundos em um contexto de um clima mais seco; e (2) em plantios monoclonais de eucalipto de alta produtividade de biomassa e grande valor econômico, a fim de estudar a variabilidade entre árvores de status sociais diferentes (mesmas condições de crescimento e genótipo) em diferentes idades. A distância máxima do tronco e a profundidade máxima no solo onde as árvores possuem a capacidade de absorver água e nutrientes é muito pouco conhecida, seja em floresta boreal, temperada ou tropical.

Levando em consideração as aplicações práticas desse estudo para uma empresa do setor florestal com plantios comerciais de eucalipto, conhecer a distância, tanto horizontal como vertical, que as raízes das árvores dominantes e dominadas de eucalipto podem absorver água e nutrientes em função da idade, é uma informação muito importante para a adubação, pois pode reduzir os custos com fertilizantes. Por exemplo, se a aplicação do adubo for concentrada uma única vez no início da rotação e em algumas entrelinhas, uma parte do adubo será lixiviada para as camadas profundas do solo. Assim, é necessário mostrar se todas as árvores ou somente as árvores dominantes têm acesso aos nutrientes nas camadas profundas do solo, uma informação essencial para garantir a homogeneidade dos plantios.

Além disso, a capacidade de absorção de água e nutrientes entre árvores dominantes e árvores dominadas pode ser diferente, tanto em relação à distância da

base da árvore como também em profundidade. No primeiro caso, uma heterogeneidade de adubação poderia afetar a homogeneidade do plantio. Já no segundo caso, haveria uma informação muito importante para saber se todas as árvores de um povoamento têm a mesma capacidade de sobrevivência quando há ocorrência de um período de seca severa.

Já em relação ao Cerrado, o segundo maior bioma da América Latina, os estudos sobre absorção de água e nutrientes podem ser de grande importância para se ter um conhecimento inovador do comportamento de algumas espécies deste bioma dentro dos pressupostos efeitos que podem ser causados pelas mudanças climáticas globais, visto que ajudarão a se ter uma melhor compreensão sobre a complementaridade entre essas espécies em relação ao acesso aos recursos naturais do solo (água e nutrientes). Este assunto ainda não foi estudado em camadas muito profundas do solo e utilizando ^{15}N enriquecido, e começou a ser alvo de interesse apenas nos últimos anos. Assim, tem o potencial de ser um precursor para estudos complementares similares envolvendo outras espécies do Cerrado e até mesmo espécies de um outro bioma nativo.

Considerando que, num futuro próximo, o estresse hídrico tende a aumentar cada vez mais, inclusive em muitas áreas de produção, é fundamental conhecer com mais detalhes as estratégias usadas pelas plantas, em escala de árvore, por exemplo, para tentar encontrar soluções mais eficazes e viáveis ambiental e economicamente.

Dentro de tal contexto, o principal objetivo desse trabalho foi melhorar a compreensão sobre a absorção de água e nutrientes pelas raízes das árvores em camadas muito profundas do solo e também a grandes distâncias horizontais do tronco em função da estação do ano, das espécies, da idade e do status social das árvores. Foram objetivos mais específicos:

- Avaliar a distância máxima do tronco onde as raízes finas de *Eucalyptus* absorvem água e nutrientes móveis nas soluções do solo em Neossolos Quartzarênicos muito profundos, em plantios de idades diferentes;
- Avaliar a distância máxima (horizontal e vertical) onde raízes finas de espécies comuns do Cerrado absorvem nutrientes móveis em Neossolos Quartzarênicos profundos, nas estações seca e chuvosa;
- Avaliar se existe uma complementaridade de nicho no solo entre essas espécies do Cerrado com uma absorção do ^{15}N em camadas diferentes, dependendo da espécie, nas estações seca e chuvosa.

Uma primeira hipótese deste estudo foi de que os nutrientes móveis são absorvidos a distâncias maiores do tronco e em maiores profundidades do solo por árvores dominantes do que por árvores dominadas, independentemente da idade do plantio.

A outra hipótese foi de que árvores de espécies diferentes do bioma Cerrado absorvem água e nutrientes móveis em camadas diferentes do solo, o que permite uma complementaridade de nicho entre as espécies.

CAPÍTULO 1

DISTANCE AND DEPTH OF UPTAKE OF LABELLED NITRATE FOR DOMINANT AND SUPPRESSED TREES THROUGHOUT THE ROTATION IN BRAZILIAN *EUCALYPTUS* PLANTATIONS

Rafael Costa Pinheiro^a, Jean-Pierre Bouillet^{b,c}, Bruno Bordron^d, Lívia Lanzi Aló^a, Vladimir Eliodoro Costa^e, Clayton Alcarde Alvares^f, Karel Van den Meersche^b, José Luiz Stape^f, Iraê Amaral Guerrini^a, Jean-Paul Laclau^{a, b, c}

^a Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu, SP, Brazil

^b CIRAD, UMR Eco&Sols, F-34398 Montpellier, France

^c Eco&Sols, CIRAD, INRA, IRD, Montpellier, SupAgro, University of Montpellier, Montpellier, France

^d ESALQ, Universidade de São Paulo, Piracicaba, SP CEP13418-900, Brazil

^e Universidade Estadual Paulista (Unesp), Centro de Isótopos Estáveis, Botucatu, SP, Brazil

^f Suzano SA Company, Brazil

ABSTRACT

Climate changes will increase the probability of drought, which is likely to dramatically increase tree mortality. The capacity of trees to withdraw water in deep soil layers is an important trait likely to account for tree survival over prolonged droughts. Our study aimed to gain insight into the maximum distance from the trunk where *Eucalyptus* fine roots take up water and mobile nutrients in deep sandy soils during dry periods. NO₃⁻-¹⁵N was injected in the soil at the end of the rainy season in commercial *Eucalyptus* stands planted with the same *E. urophylla* x *E. grandis* clone. The ¹⁵N tracer was applied in the middle of the inter-row (3 replications): at 5 depths (from 0.1 to 6 m) at age 0.6 year, at 4 depths (from 0.1 to 9 m) at age 1.2 years, at 5 depths (from 0.1 to 12 m) at age 2.2 years, and at 6 depths (from 0.1 to 15 m) at age 6.4 years. δ¹⁵N was determined in leaves sampled in dominant and suppressed trees at different distances from each injection area, 4-5 months after NO₃⁻-¹⁵N injection (after the dry season).

While dominant trees took up NO_3^- - ^{15}N down to a depth of 6 m between 7 and 12 months after planting, the maximum depth of NO_3^- - ^{15}N uptake for suppressed trees was between 3 and 4.5 m. From 1.5 to 6 years after planting, a foliar enrichment in ^{15}N was mainly detected when the NO_3^- - ^{15}N tracer was injected in the upper soil layers and only for a few trees at a depth of 6 m. Most of the uptake of ^{15}N occurred within 2 m of horizontal distance from the injection point, whatever tree age and tree social status. Low amounts of NO_3^- - ^{15}N were taken up for injection points located between 2 m and 5 m from the trunk, and ^{15}N uptake was never detected at horizontal distances greater than 6 m from the trunk. *Eucalyptus* fine roots can take up nitrates at depths between 6 and 8 m the first year after planting. However, the velocity of exploration of deep soil layers depends on the social status of the trees. Fertilizers must be applied within 2 m from the trunks in *Eucalyptus* plantations to be taken up by all trees, regardless of their social status. When large amounts of fertilizers are applied the first months after planting in sandy soils, nutrient leaching in deep layers might increase the heterogeneity of the stands since mobile nutrients could only be taken up by dominant trees.

Keywords: nutrient uptake; water uptake; fine roots; ^{15}N ; eucalypt plantations; Brazil

1.1 INTRODUCTION

Climate changes will increase the probability of exceptional droughts, which may dramatically increase tree mortality worldwide (Wu et al., 2011). Improving our understanding of the structural and physiological mechanisms by which trees maintain tissue hydration and photosynthesis in response to water deficit is therefore essential to predict the effects of climate change on tree survival, carbon sequestration, and use of water in forest ecosystems (McDowell et al., 2013; Klein et al., 2014). A better understanding of the effects of tree nutrition on the mechanisms involved in tree response to drought is essential to adapt the silviculture to a probable future drier climate (Battie-Laclau et al., 2014). Both an increase of atmospheric carbon dioxide (Iversen et al., 2010; Nie et al., 2013) and prolonged droughts (Germon et al., 2019) should increase the exploration of deep soil layers by plant roots in the future. Deep rooting can enhance the tolerance of some plant species to drought making it possible

to reach water stored in the subsoil (Chaves et al., 2003; Hoekstra et al., 2015). In a context of climate change, the maximum depth reached by plant roots is therefore an important trait to select genotypes tolerant to prolonged droughts (Comas et al., 2013; Pinheiro et al., 2016).

Roots have been little studied at depths of more than 5 m (Jackson et al., 1997; Pierret et al., 2016), and the maximum rooting depth has probably been underestimated in many tropical forests (Freycon et al., 2015). Most of the studies dealt with the description of fine root distributions and fine root dynamics throughout very deep soil profiles (Maeght et al., 2013; Pinheiro et al., 2016; Lambais et al., 2017). Even though labelling techniques have been used to locate the areas of nutrient uptake in the soil (Lehman and Muraoka, 2001; Lehman, 2003), the contribution of deep roots to supply tree nutrient requirements is still poorly documented in highly weathered tropical soils (Poszwa et al., 2002; Pradier et al., 2017). A functional specialization of fine roots to take up cations depending on the depth has been shown for tree species (Göransson et al., 2008; da Silva et al., 2011) but the uptake of nitrate was little influenced by soil depth for *Eucalyptus grandis* trees (Bordron et al., 2019).

The depth of water uptake by tree roots is commonly estimated monitoring soil water contents throughout soil profiles (Guderle and Hildebrandt, 2015), through modelling approaches (e.g. Kumar et al., 2014; Christina et al., 2017), or using stable isotopes of hydrogen (deuterium, ^2H) and oxygen (^{18}O) (Beyer et al., 2016, 2018; Koeniger et al., 2016; Trogisch et al., 2016). Deuterium and ^{18}O techniques can be used to estimate the depth of water uptake when their natural abundances in soil solutions follow a clear gradient from the soil surface to deep soil layers (Roupsard et al., 1999). Injecting ^2H and ^{18}O at different depths makes it possible to detect the areas of water uptake in the soil at the date of sampling. For example, a dual-isotope labelling technique in a tropical rainforest (^2H applied at soil surface and ^{18}O injected at 120-cm depth) showed that some tree species had a high plasticity in their depth of water uptake, exhibiting an efficient strategy for water resource acquisition below 100-cm depth during dry periods (Stahl et al., 2013). However, the depth of water uptake in tropical forests can be highly variable from one week to another depending on the occurrence of rainfall events (Christina et al., 2017). It would be therefore necessary to repeat deuterium and ^{18}O sampling along the year to estimate the maximum distance of water uptake from trees. Moreover, isotopic techniques based on deuterium and ^{18}O measurements are expensive and time-consuming. Repeated

measurements of ^2H and ^{18}O uptake at different depths in the soil throughout the year are therefore rare in tropical forests.

Da Silva et al. (2011) showed that ^{15}N from labelled nitrates injected at a depth of 3 m was only detected in young leaves of 6-year-old *Eucalyptus* trees when the gravitational soil solutions reached 3 m depth, which suggests that NO_3^- - ^{15}N uptake could be a tracer of water uptake in these plantations. Nitrate is highly mobile in soil solutions and the uptake of large amounts of nitrates is dependent on mass flow transport up to fine roots (Hinsinger et al., 2011; White et al., 2013; McMurtrie and Näsholm, 2018). Nitrate labelling could therefore be a proxy of the cumulative uptake of water between the date of NO_3^- - ^{15}N injection in the soil and leaf sampling a few months later, since a clear enrichment in foliar ^{15}N content can only occur if large amounts of NO_3^- - ^{15}N are transported through mass flow up to fine roots at the vicinity of the injection area.

Eucalyptus planted forests cover more than 20 million hectares worldwide and are expanding rapidly in tropical and subtropical regions (Booth, 2013; Gonçalves et al., 2013). In Brazil, these fast-growing plantations are intensively managed over 5.7 million hectares and account for 73 % of the total area of planted forests (Ibá and Pöyry, 2016). However, highly productive *Eucalyptus* plantations are sensitive to prolonged drought periods. The cumulative area affected by tree mortality as a result of drought over the last decade in the state of Minas Gerais (the largest producer of *Eucalyptus* in Brazil) was about 200,000 hectares (Gonçalves et al., 2017). The variability among trees within the same stand to reach water and nutrients stored far from the trunk (horizontally and in depth) might be an important factor likely to help explain why only some trees survive during drought periods. While the influence of deep roots on drought-adaptative mechanisms of tree species is well documented (McDowell et al., 2008; Fang and Xiong, 2015), the relationship between the social status of trees and their ability to withdraw water in very deep soil layers has been little investigated in monospecific forests.

Our study aimed to assess the maximum distance from the trunk where *Eucalyptus* fine roots take up water and mobile nutrients in soil solutions in very deep Oxisols. We hypothesized that mobile nutrients are taken up farther from the trunk and more deeply for dominant trees than for suppressed trees.

1.2. MATERIAL AND METHODS

Study site

Our study was carried out in one *Eucalyptus* stand at Avaré (23°00'41"S, 48°55'10"W) and in two neighboring *Eucalyptus* stands at Itatinga (23°14'54"S, 48°35'28"W), in southeast Brazil (São Paulo State). The distance between the stands sampled at Itatinga and Avaré was 40 km. The same clone of the *Eucalyptus urophylla* (S.T. Blake) x *Eucalyptus grandis* (Hill ex Maid) hybrid was managed by the Suzano SA Company at both sites with the same silvicultural practices. The annual rainfall and the mean temperature over the study period were 1578 mm and 20.2 °C at Itatinga, and 1980 mm and 20.6 °C at Avaré, respectively (Figure 1). The soils at both sites were very deep Oxisols (USDA Soil Taxonomy) developed on Cretaceous sandstone of the Marília formation, Bauru group. The topography was a plateau at an elevation of 700-900 m at both sites and the water table was very deep (more than 15 m, which was the deepest hole made to inject the tracer in our study). Soil properties show that the soil type was common for commercial *Eucalyptus* plantations in Brazil (Table 1).

Experimental design

In the youngest stand at Avaré, 15 circular plots (20 m in radius) were randomly located with a minimum distance of 50 meters between the centre of each plot: 5 depths of tracer injection (0.1, 1.5, 3.0, 4.5 or 6.0 m) and 3 replications per depth. At 3 months after planting, 50% of the trees were totally defoliated manually in each plot to induce a variability of growth between trees making it possible to study dominant and suppressed trees. Fully defoliations of young trees due to ants are common in young *Eucalyptus* stands. The $\text{NO}_3^-^{15}\text{N}$ tracer was applied in the middle of the inter-row at the center of each plot, at age 0.6 years (7 months after planting) (Figure 2).

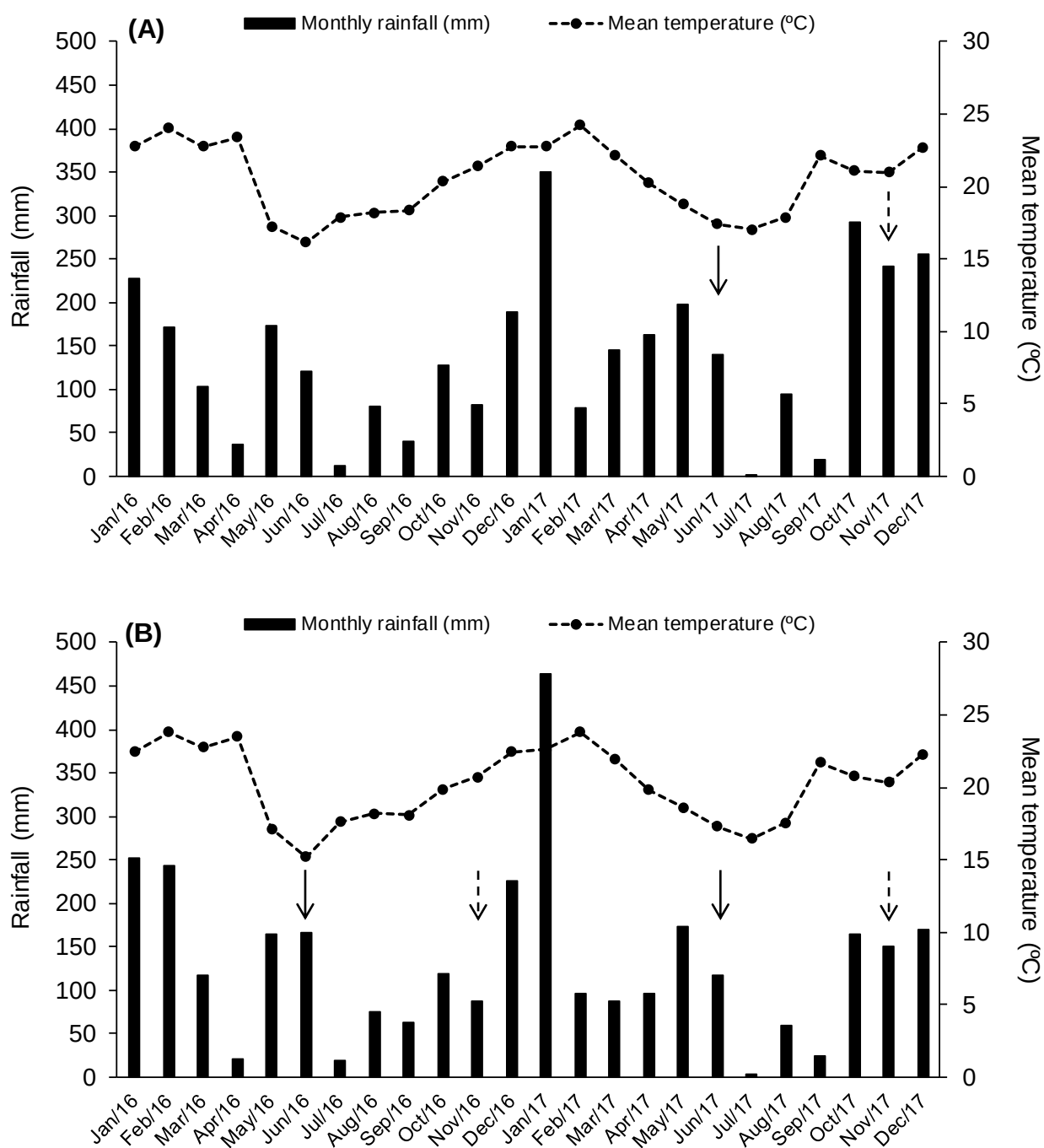


Fig. 1. Time course of monthly rainfall and mean temperature over the study period at Avaré (A) and at Itatinga (B). Continuous arrows show the ^{15}N tracer injection periods in 1.2- and 6.4-year-old stands in June 2016, and in 0.6- and 2.2-year-old stands in June 2017. Dashed arrows indicate the leaf sampling periods, five months after tracer injection.

Table 1. Main physical and chemical soil properties at Itatinga and Avaré. Mean values and standard errors are indicated ($n = 3$).

Soil layer	Sand	Silt	Clay	pH	O. M.	P _{resin}	H+Al	K	Ca	Mg	SB	CEC
(m)	Particle size distribution (%)			CaCl ₂	g.kg ⁻¹	mg.kg ⁻¹	mmolc.kg-1					
Itatinga												
0-0.25	92.8 ± 0.3	1.4 ± 0.5	5.8 ± 0.4	4.20 ± 0.18	14.13 ± 3.50	12.59 ± 3.47	27.94 ± 4.96	0.36 ± 0.04	22.16 ± 4.95	4.54 ± 0.33	27.05 ± 4.79	55.00 ± 6.20
0.25-0.5	91.0 ± 0.4	2.1 ± 0.4	6.9 ± 0.5	4.04 ± 0.06	6.48 ± 0.35	3.87 ± 0.91	24.26 ± 2.59	0.26 ± 0.11	15.48 ± 0.55	2.68 ± 0.58	18.42 ± 0.68	42.68 ± 3.22
0.5 - 1	89.3 ± 0.4	1.9 ± 0.4	8.8 ± 0.1	4.03 ± 0.06	5.52 ± 0.36	2.48 ± 0.41	18.87 ± 2.41	0.22 ± 0.01	14.36 ± 1.01	2.05 ± 0.35	16.63 ± 0.76	35.49 ± 2.94
1 - 2	88.7 ± 0.4	2.0 ± 0.9	9.3 ± 0.6	4.07 ± 0.11	5.63 ± 0.25	2.28 ± 0.27	17.92 ± 2.71	0.35 ± 0.22	12.58 ± 3.20	2.29 ± 0.33	15.21 ± 3.35	33.14 ± 4.01
2 - 4	88.8 ± 0.7	1.8 ± 0.8	9.4 ± 0.2	4.23 ± 0.20	3.28 ± 0.88	1.87 ± 0.37	11.59 ± 1.74	0.35 ± 0.24	12.54 ± 1.52	2.57 ± 0.34	15.46 ± 1.86	27.04 ± 1.87
4 - 6	86.9 ± 1.3	2.6 ± 0.5	10.5 ± 0.8	4.17 ± 0.10	2.66 ± 0.67	1.59 ± 0.21	10.38 ± 0.80	0.16 ± 0.04	12.27 ± 2.05	2.47 ± 0.20	14.89 ± 2.15	25.27 ± 2.53
6 - 8	87.1 ± 0.5	2.3 ± 1.0	10.7 ± 0.6	4.20 ± 0.03	1.99 ± 0.23	2.08 ± 0.61	10.29 ± 0.52	0.14 ± 0.00	12.44 ± 0.49	2.12 ± 0.13	14.70 ± 0.44	24.99 ± 0.14
8 - 10	86.3 ± 0.2	3.8 ± 1.5	9.9 ± 1.5	4.19 ± 0.07	1.93 ± 0.14	1.73 ± 0.09	9.81 ± 0.42	0.14 ± 0.00	15.32 ± 3.39	1.87 ± 0.24	17.34 ± 3.16	27.15 ± 3.42
10 - 12	87.0 ± 0.4	2.4 ± 0.3	10.6 ± 0.3	4.12 ± 0.08	2.27 ± 0.21	1.77 ± 0.18	10.17 ± 0.44	0.14 ± 0.00	13.63 ± 3.48	2.11 ± 0.27	15.88 ± 3.66	26.05 ± 3.96
Avaré												
0-0.25	84.7 ± 2.4	3.4 ± 1.6	11.9 ± 1.6	4.13 ± 0.39	22.5 ± 4.84	8.24 ± 2.51	43.36 ± 10.73	0.81 ± 0.47	8.82 ± 6.27	3.16 ± 2.13	12.79 ± 8.38	56.15 ± 5.92
0.25-0.5	81.8 ± 2.3	4.8 ± 1.9	13.4 ± 1.1	3.94 ± 0.10	9.82 ± 0.46	4.27 ± 1.13	37.78 ± 4.66	0.38 ± 0.17	6.59 ± 8.96	1.41 ± 1.52	8.38 ± 10.64	46.16 ± 8.05
0.5 - 1	81.5 ± 1.6	4.7 ± 0.9	13.8 ± 1.2	3.87 ± 0.06	8.81 ± 1.54	2.49 ± 0.61	30.34 ± 2.07	0.26 ± 0.18	4.88 ± 6.85	1.11 ± 1.03	6.25 ± 8.07	36.59 ± 7.65
1 - 2	77.3 ± 3.7	6.3 ± 3.8	16.4 ± 0.6	3.97 ± 0.05	6.43 ± 1.24	1.89 ± 0.13	23.83 ± 3.64	0.24 ± 0.08	7.63 ± 11.58	1.51 ± 1.70	9.39 ± 13.35	33.23 ± 9.98
2 - 4	77.6 ± 2.8	5.6 ± 2.8	16.7 ± 0.3	4.21 ± 0.05	4.12 ± 0.79	1.93 ± 0.14	14.75 ± 0.72	0.13 ± 0.05	6.38 ± 8.63	1.34 ± 1.41	7.86 ± 10.07	22.61 ± 10.03
4 - 6	75.9 ± 1.5	5.6 ± 1.0	18.5 ± 0.9	4.33 ± 0.04	2.55 ± 0.58	1.96 ± 0.12	11.80 ± 0.40	0.16 ± 0.00	4.96 ± 6.17	1.22 ± 1.20	6.33 ± 7.37	18.13 ± 7.59

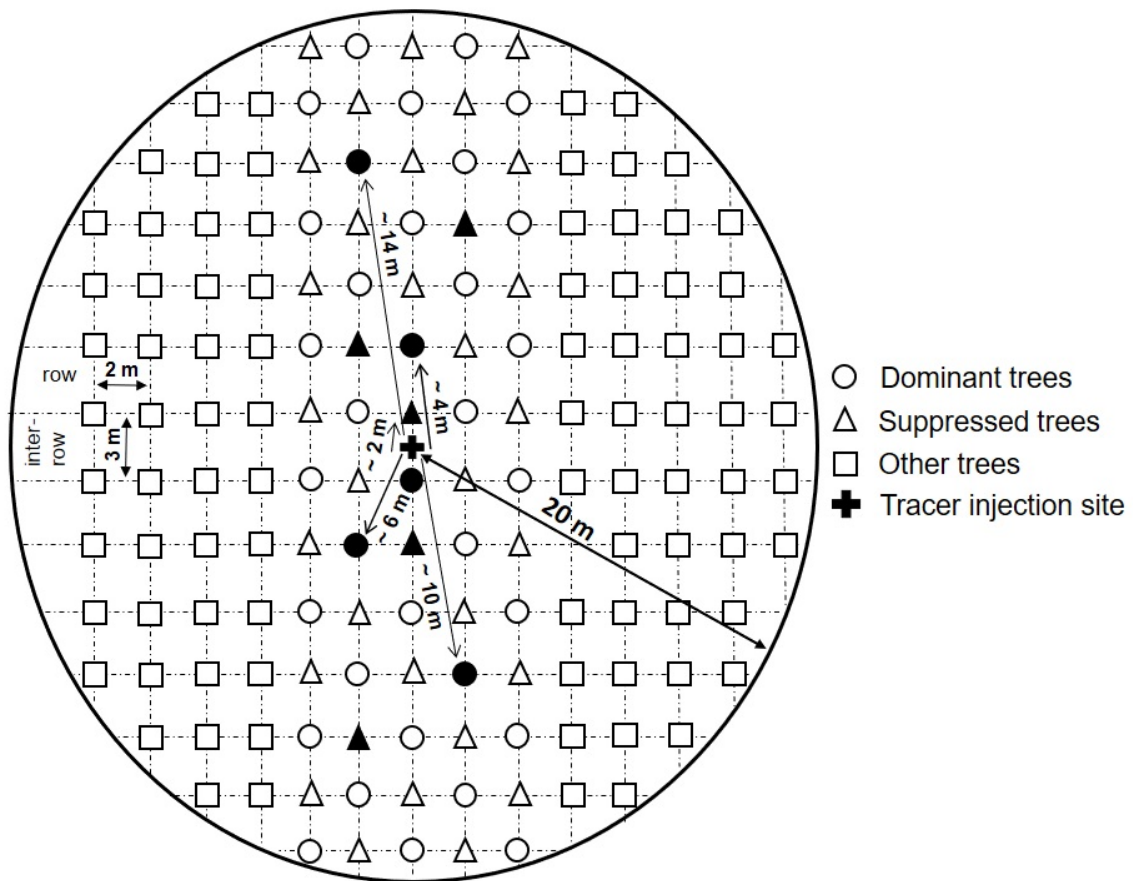


Fig. 2. Layout of a sampled plot. The ^{15}N tracer was injected at the center of the plot. Solid circles and triangles indicate the location of 4 dominant and 4 suppressed sampled trees, respectively. Undisturbed trees (square symbol) in the plot are indicated. The horizontal distances between the sampled trees and the injection point ranged between 1 m and 14 m.

At Itatinga, 27 circular plots (20 m in radius) were randomly located in a 9-month-old stand, with a minimum distance of 50 meters between the center of each plot: (i) 12 plots to apply the NO_3^- - ^{15}N tracer at 4 depths (0.1, 3.0, 6.0 or 9.0 m) and 3 replications per depth at 1.2 years after planting, and (ii) 15 plots to apply the NO_3^- - ^{15}N tracer at 5 depths (0.1, 3.0, 6.0, 9.0 or 12.0 m) and 3 replications per depth at 2.2 years after planting. In order to induce a growth heterogeneity among trees in this stand, 50% of the trees were partially defoliated (about 80% of leaf area removed) at age 9 months.

In a 6-year-old stand at Itatinga, 18 circular plots (20 m in radius) were randomly located with a minimum distance of 50 meters between the center of each plot. The NO_3^- - ^{15}N tracer was applied at age 6.4 years at the center of each plot. The 18 plots in this stand corresponded to 6 depths of tracer injection (0.1, 3.0, 6.0, 9.0, 12.0 or 15.0 m) and 3 replications per depth.

An inventory in each stand at the date of NO_3^- - ^{15}N tracer injection showed large differences in the basal area between dominant and suppressed trees (Appendix 1).

In each plot, 4 dominant and 4 suppressed trees were selected at distances of approx. 2 m, 6 m, 10 m and 14 m from the injection point to sample recently expanded leaves in the upper part of the crown (Figure 2).

Tracer applications

Our study aiming to assess the maximum distance from the trunk where labelled nitrate can be taken up was carried out during the dry season because a recent study in Brazilian *Eucalyptus* plantations showed that trees withdraw preferentially water in the topsoil during rainy periods (Christina et al., 2017). One day before application in the field, a labelled solution was prepared in the laboratory and maintained at 4 °C. 16.6 g of $\text{NH}_4^{15}\text{NO}_3$ commercial compound (10 atom% or 29,221.98 ‰ $\text{NO}_3^{-15}\text{N}$) dissolved in 20 ml of distilled water were injected at a single depth at the center of each plot. Holes were drilled down to the target application depth using a cylindrical auger with an inner diameter of 9 cm and soil water content was measured every meter in all the samples collected during drilling (Appendix 2). Soil blocks from the inner part of the auger were collected for the deepest meter in each hole to assess fine root densities (< 2 mm in diameter) close to the area of $\text{NO}_3^{-15}\text{N}$ injection.

A PVC tube (2.5 cm in diameter) was inserted into each hole to avoid any contamination of the soil with $\text{NO}_3^{-15}\text{N}$ during tracer application. A 0.4-cm polyethylene tube, attached to an iron rod, was inserted into the PVC tube, with a length depending on the application depth. A plastic sheet was placed around each hole to avoid any contamination with $\text{NO}_3^{-15}\text{N}$ at the soil surface. 20 ml of the labelled solution was applied at the selected depth using a syringe. Thereafter, 280 ml of distilled water were injected to rinse the polyethylene tube and to promote nitrate uptake in the soil area where the tracer was applied. Lastly, the polyethylene and PVC tubes were carefully withdrawn from the holes, which were filled with the soil removed during drilling, respecting the original order of the soil layers. Similar methodologies were used by da Silva et al. (2011) and Bordron et al. (2019) to study fine root specialization to take up nutrients in *Eucalyptus* plantations. Previous studies have shown that nitrogen fluxes (nitrate form) dissolved in gravitational solutions in *Eucalyptus* plantations are very low in sandy tropical soils below a depth of 2 m (Binkley et al., 2018). The $\text{NO}_3^{-15}\text{N}$ tracer remained therefore close to the area of injection during the dry season between tracer injection and leaf sampling in our study.

Leaf sampling

Thirty young leaves (approx. from 2 weeks to 3 months after bud emergence) were collected in the upper half of the crown of each sampled tree, about 5 months after tracer injection. In similar leaves sampled in the upper crown of *Eucalyptus grandis* trees, da Silva et al. (2011) showed that $\delta^{15}\text{N}$ remained roughly constant between 2 and 7 months after NO_3^- - ^{15}N injection in the soil.

In each *Eucalyptus* stand, young leaves were also sampled in 12 control trees (6 dominant and 6 suppressed trees) located far from the injection point (> 50 m) in order to estimate the prediction interval of foliar $\delta^{15}\text{N}$ values in natural abundance. Control trees were sampled away from each other throughout the stand to cover the variability of the ^{15}N natural abundance. In the young stands (1 year and 1.6 years after planting), the leaves were collected from the ground using a long pruner. In the 2.7- and in the 6.8-year-old stands, a professional team of tree climbers used a 5-m pruner to cut the branches from the selected trees. In total, 30 young leaves (from 2-4 branches per tree) were collected on 132, 108, 132 and 156 trees at 1, 1.6, 2.7 and 6.8 years after planting, respectively.

Isotopic analyses of ^{15}N

In the laboratory, the leaves were washed in deionized water and oven-dried at 65 °C for 72 hours. The dried samples were ground (< 60 μm) in a cryogenic mill (2010 Geno Grinder, SPEX Sample Prep, Metuchen, USA) at -196 °C for homogenization and stored in sealed acrylic pots until isotopic analysis. An aliquot of 4500-5000 μg of each dry and milled sample was weighed into a 6 mm height, 4 mm diameter cylindrical tin capsule (D1106 - Elemental Microanalysis, Okehampton, UK) with a 1 μg resolution scale (XP6 - Mettler Toledo, Greifensee, Switzerland). The ^{15}N analyses were performed at the Center for Stable Isotopes at São Paulo State University using a continuous flow system in a CF-IRMS isotope ratio mass spectrometer (Delta V Advantage - Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (Flash 2000 OEA - Thermo Scientific, Bremen, Germany) with an interface (ConFlo IV Universal - Thermo Scientific, Bremen, Germany) that determines the isotope ratio of the sample ($R_{\text{sample}} = ^{15}\text{N}/^{14}\text{N}$).

The ^{15}N values in isotopic delta ($\delta^{15}\text{N}$) in ‰ with regard to R_{sample} and the isotopic ratio of atmospheric air ($R_{\text{air}} = 0.0036765$) as international standard according to equation 1 (Coplen, 2011):

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{air}}} - 1 \right) \cdot 1000\text{‰}$$

The standard uncertainty in $\delta^{15}\text{N}$ was ± 0.3 ‰ for samples that are slightly enriched with ^{15}N .

Data analysis

For each *Eucalyptus* stand, a prediction interval with a threshold of 99% for $\delta^{15}\text{N}$ natural abundance was calculated from the $\delta^{15}\text{N}$ values of the 12 control trees using the equation $\bar{X}_n \pm T_\alpha s_n \sqrt{1 + 1/n}$, where $\bar{X}_n$ and s_n are the average and standard deviation of the control values, n the number of observations, and T_α the 99.5 percentile of a Student's t-distribution with $(n-1)$ degrees of freedom (Geisser, 1993). Calculations were done with the statistical software R (R Core Team, 2019). Foliar $\delta^{15}\text{N}$ values higher than the upper boundary of the prediction interval can be considered significantly different from the control population ($p < 0.01$) and suggest that the sampled trees took up the NO_3^- - ^{15}N tracer injected in the same plot. The $\delta^{15}\text{N}$ values of each sampled tree (both dominant and suppressed) were plotted relative to the horizontal distance up to the tracer application point.

1.3. RESULTS

NO_3^- - ^{15}N uptake from 0.6 to 1 year after planting

Foliar $\delta^{15}\text{N}$ values showed that the trees within 2 m from the injection point (both dominant and suppressed) took up the largest amounts of the NO_3^- - ^{15}N tracer injected at a depth of 0.1 m (Fig. 3A). The highest $\delta^{15}\text{N}$ value was measured in a suppressed tree (273‰). The foliar $\delta^{15}\text{N}$ value was slightly above the upper boundary of the prediction interval of natural abundance for one dominant tree at a distance of 4 m from the injection point. The behavior was similar for the injection depth of 1.5 m, but with

lower foliar $\delta^{15}\text{N}$ peaks in dominant and suppressed trees than for the injection depth of 0.1 m (data not shown).

When NO_3^- - ^{15}N was injected at a depth of 3 m, both dominant and suppressed trees took up the tracer within 2 m from the injection point (Figure 3B). Only dominant trees took up the NO_3^- - ^{15}N tracer applied at a depth of 4.5 m and only within 2 m from the injection point. High foliar $\delta^{15}\text{N}$ values ($> 100\text{‰}$) showed that large amounts of tracer were taken up by those trees (Figure 3C). At an injection depth of 6 m, only dominant trees took up the NO_3^- - ^{15}N tracer (foliar $\delta^{15}\text{N}$ values reached 94.4‰ in one tree) and one dominant tree took up the tracer at a distance of 3.7 m from the injection point (Figure 3D).

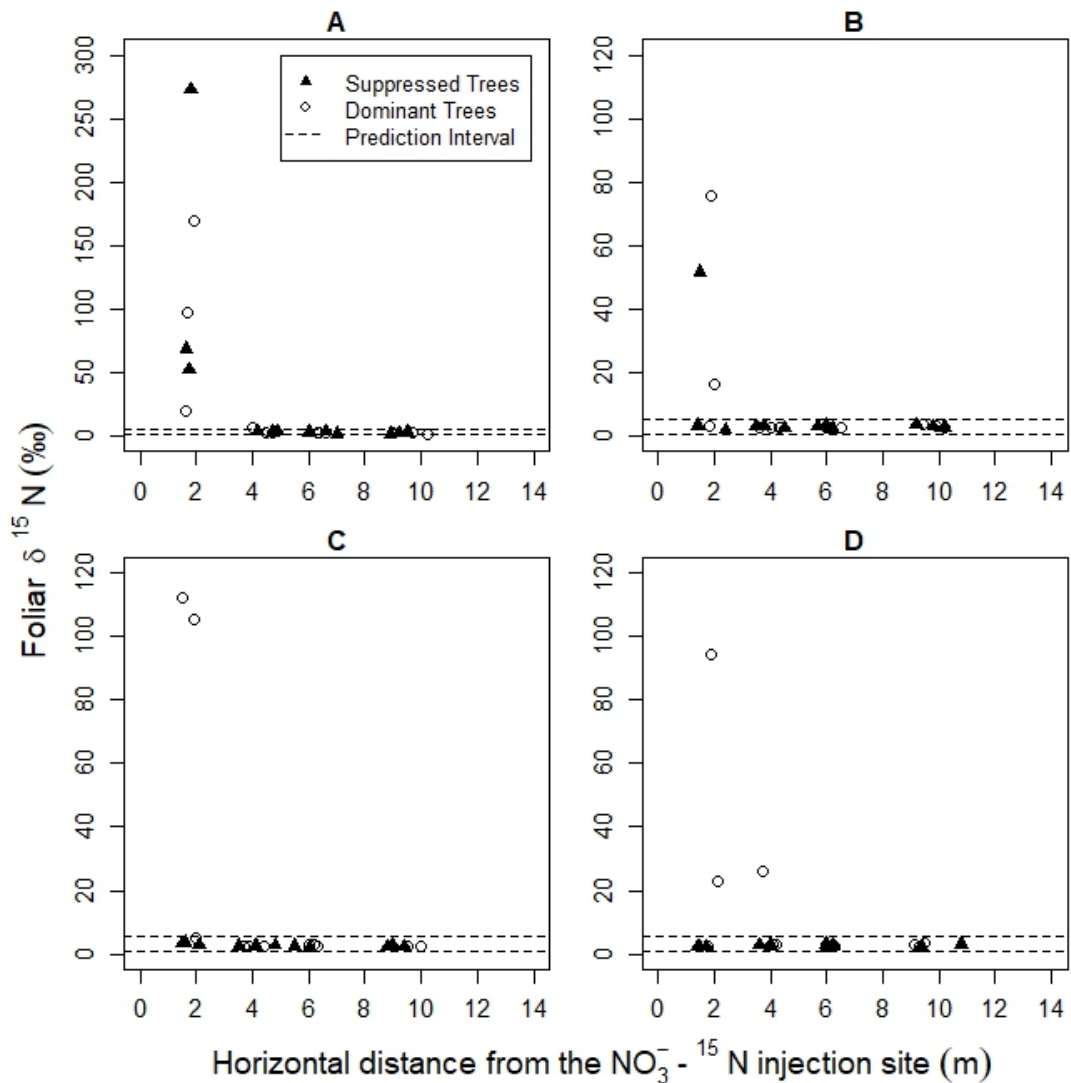


Fig. 3. Foliar $\delta^{15}\text{N}$ values in 1-year-old *E. urophylla* $\times$ *E. grandis* trees, 5 months after application of NO_3^- - ^{15}N tracer A) at a depth of 0.1 m, B) at a depth of 3 m, C) at a depth of 4.5 m, and D) at a depth of 6 m. Dominant trees (empty circle) and suppressed trees (solid triangle) were sampled. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 12 trees (6 dominant and 6 suppressed trees) sampled at more than 50 m from all the injection points.

Soil coring to inject NO_3^- - ^{15}N showed that fine roots were present in all the areas of tracer injection down to a depth of 4.5 m in the 0.6-year-old stand (Appendix 3). However, it cannot be excluded that fine roots belonging to the previous *Eucalyptus* stand (before clearcutting and replanting) were not distinguishable from the fine roots of young trees. While soil texture was roughly constant throughout the soil profile (Table 1), soil water contents sharply increased below the depth of 4 m at age 0.6 years (Appendix 2), which suggests that water was mainly withdrawn by tree roots in the upper 4 m before the first dry season.

NO_3^- - ^{15}N uptake from 1.2 to 1.6 years after planting

Dominant and suppressed trees took up large amounts of the NO_3^- - ^{15}N tracer applied at a depth of 0.1 m, within 2 m from the injection point with a peak of foliar $\delta^{15}\text{N}$ at 420 ‰ for a suppressed tree. Foliar $\delta^{15}\text{N}$ in one suppressed tree at a distance of 7.5 m from the injection point was slightly above the upper boundary of the prediction interval of natural abundance (Figure 4A). When the NO_3^- - ^{15}N tracer was applied at a depth of 3 m, foliar $\delta^{15}\text{N}$ values were higher than the upper boundary of the prediction interval of natural abundance for all the dominant trees located within 2 m from the injection point and only for one suppressed tree. No tracer uptake was detected at greater distances from the injection point (Figure 4B). Only dominant trees within a distance of 2 m from the injection point took up large amounts of NO_3^- - ^{15}N applied at a depth of 6 m. Foliar $\delta^{15}\text{N}$ values were slightly above the upper boundary of the prediction interval of natural abundance for two trees at a distance of 6 m from the injection point (Figure 4C).

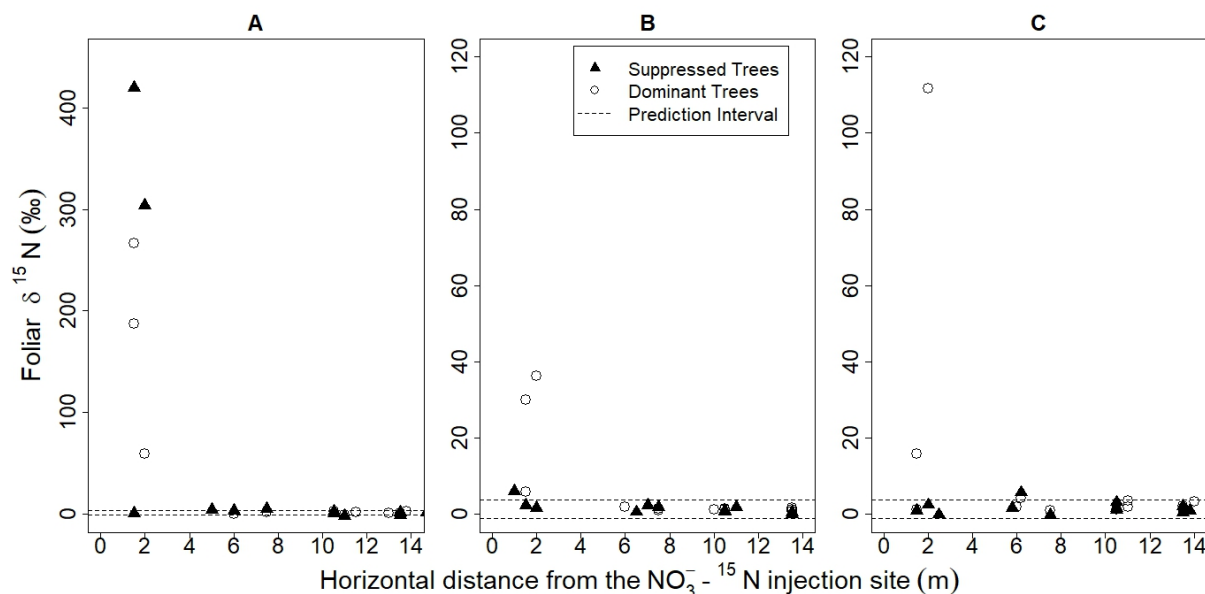


Fig. 4. Foliar $\delta^{15}\text{N}$ values in 1.6-year-old *E. urophylla* $\times$ *E. grandis* trees, 5 months after application of the NO_3^- - ^{15}N tracer A) at a depth of 0.1 m, B) at a depth of 3 m, and C) at a depth of 6 m. Dominant trees (empty circle) and suppressed trees (solid triangle) were sampled. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 12 trees sampled at more than 50 m from all the injection points.

Although some fine roots were sampled at a depth of 9 m when the NO_3^- - ^{15}N tracer was injected (Appendix 3), the foliar $\delta^{15}\text{N}$ values for all the sampled trees were within the interval of prediction of natural abundance, which showed that large amounts of the tracer were not taken up by the sampled trees at this depth.

NO_3^- - ^{15}N uptake from 2.2 to 2.7 years after planting

Large amounts of the NO_3^- - ^{15}N tracer injected at a depth of 0.1 m were taken up both by dominant and suppressed trees within 2 m from the injection point (Figure 5A). The same pattern was observed when the NO_3^- - ^{15}N tracer was applied at a depth of 3 m (Figure 5B). Only one dominant tree took up a detectable amount of NO_3^- - ^{15}N at a depth of 6 m and it was located within 2 m from the injection point (Figure 5C).

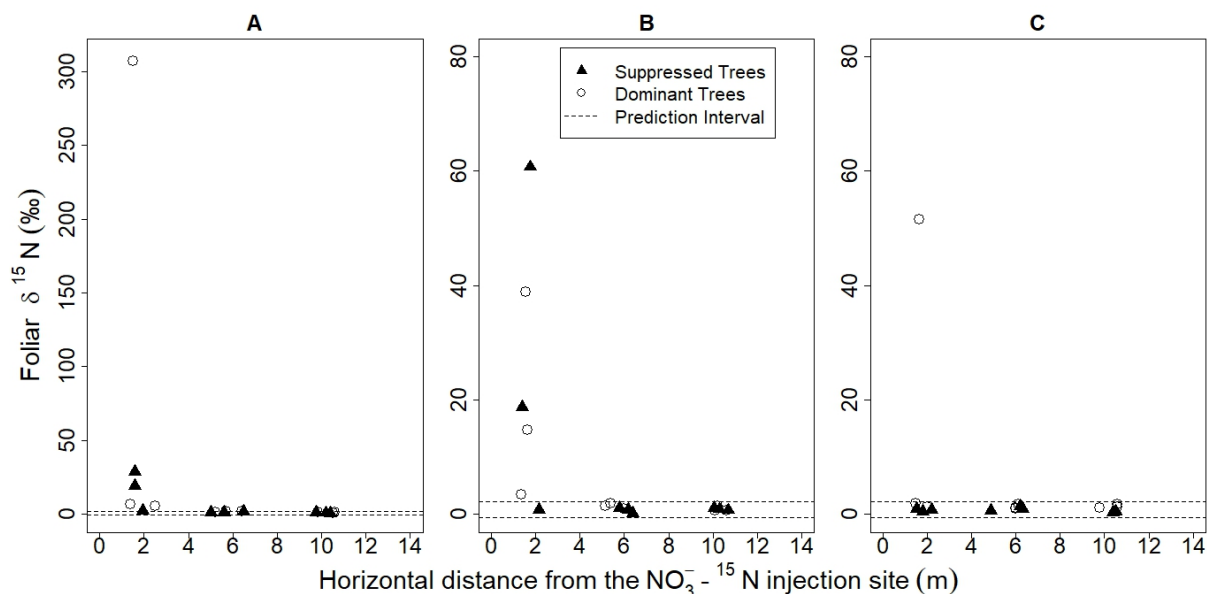


Fig. 5. Foliar $\delta^{15}\text{N}$ values in 2.7-year-old *E. urophylla* x *E. grandis* trees, 5 months after application of NO_3^- - ^{15}N tracer A) at a depth of 0.1 m, B) at a depth of 3 m, and C) at a depth of 6 m. Dominant trees (empty circle) and suppressed trees (solid triangle) were sampled. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 12 trees sampled at more than 50 m from all the injection points.

Foliar $\delta^{15}\text{N}$ values for all the sampled trees were within the prediction interval of natural abundance in the plots where the NO_3^- - ^{15}N tracer was injected at depths of 9 m and 12 m (data not shown). Soil coring to inject NO_3^- - ^{15}N showed that fine roots were present in all the areas of tracer injection down to a depth of 12 m in the 2.2-year-old stand (Appendix 3). Changes in soil water contents between 1.2 and 2.2 years after planting, in the same stand and at the same period (during drilling), suggest that tree roots withdrew water between the depths of 4 m and 9 m (Appendix 2).

NO₃⁻-¹⁵N uptake from 6.4 to 6.8 years after planting

A foliar enrichment in NO_3^- - ^{15}N was only detected when the NO_3^- - ^{15}N tracer was injected in the upper soil layer at a depth of 0.1 m. Both dominant and suppressed trees took up NO_3^- - ^{15}N within 3 m from the injection point. The suppressed trees had higher $\delta^{15}\text{N}$ values within leaves than the dominant trees, with a peak at 349 ‰ (Figure 6A). At all the other depths of injection of the NO_3^- - ^{15}N tracer (i.e. 3, 6, 9, 12 and 15 m), foliar $\delta^{15}\text{N}$ values were within the prediction interval of natural abundance (data not shown below 6 m of depth). Soil coring to inject NO_3^- - ^{15}N in the 6.4-year-old stand

showed that fine roots were present in all the areas of tracer injection down to a depth of 15 m (Appendix 3).

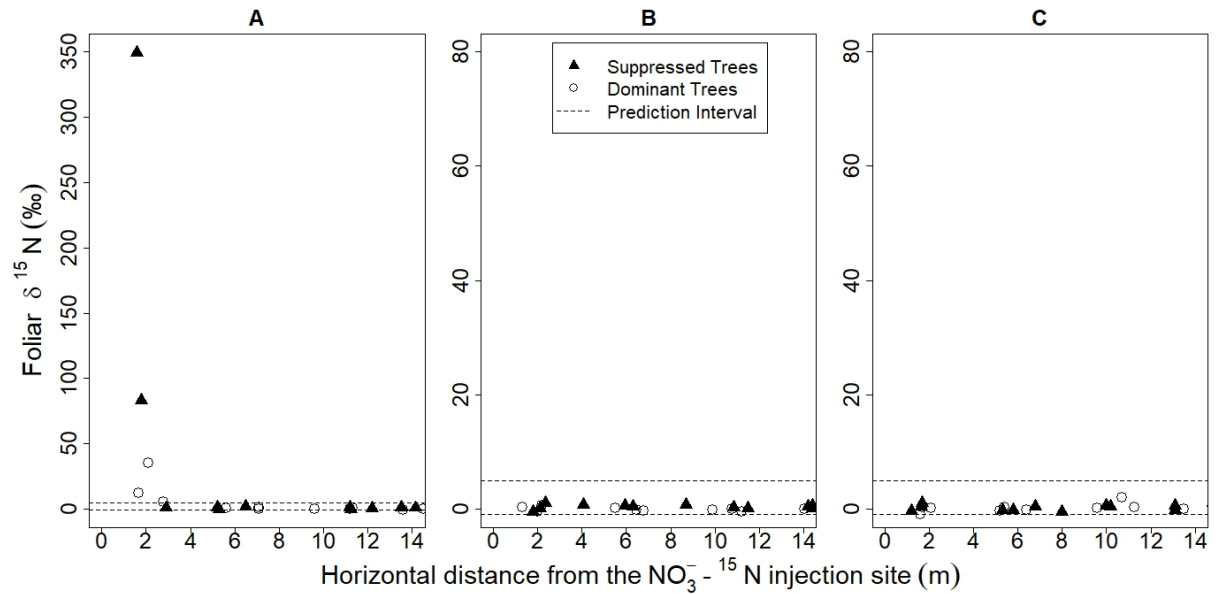


Fig. 6. Foliar $\delta^{15}\text{N}$ values in 6.8-year-old *E. urophylla* x *E. grandis* trees, 5 months after application of NO_3^- - ^{15}N tracer A) at a depth of 0.1 m, B) at a depth of 3 m, and C) at a depth of 6 m. Dominant and suppressed trees were sampled. The prediction interval was calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 12 trees sampled at more than 50 m from all the injection points.

1.4. DISCUSSION

Reliability of our method to detect the maximum distance of water uptake from trees

The depths of NO_3^- - ^{15}N uptake in our study are consistent with other studies dealing with water use in tropical *Eucalyptus* plantations, which suggests that the uptake of labelled nitrate could be a simple proxy of cumulative water uptake in specific soil areas. In particular, a modelling approach based on soil water content monitoring and eddy-covariance measurements over 4.5 years in a commercial *Eucalyptus* plantation at about 20 km from our study sites (and in the same soil type) showed that water was withdrawn from the soil surface to a depth of 6-7 m the first year after planting, from the soil surface to 10 m depth the second dry season, then only in the upper soil layers and close to the water table at a depth of more than 12 m until harvesting (Christina et al., 2017). The depth of uptake of labelled nitrate by *Eucalyptus* trees in our study is consistent with the dynamics of water uptake throughout deep soil

profiles over long periods in other *Eucalyptus* plantations in the same region (Laclau et al., 2013; Marsden et al., 2013; Christina et al., 2017). However, a strong demonstration of the validity of our method (enrichment in foliar ^{15}N after injection of NO_3^- - ^{15}N in the soil) as a proxy of water uptake by tree roots would require to monitor foliar ^{15}N as well as ^2H and ^{18}O in the trees after the injection in the same soil areas of NO_3^- - ^{15}N , ^2H and ^{18}O tracers.

Although we injected NO_3^- - ^{15}N down to a depth of 15 m, we did not detect any significant uptake of labelled nitrate at depths of more than 9 m. This pattern does not demonstrate that water was not taken up at these depths but suggests that large amounts of labelled nitrate were not taken up by neighboring trees. Fine root densities are very low at depths of more than 6 m in *Eucalyptus* plantations (Christina et al., 2011; Laclau et al., 2013), and it is possible that the trees sampled to determine foliar ^{15}N contents in our study did not explore the small soil areas where the tracer was injected. The simple method used here to detect water and nitrate uptake is also probably less sensitive than isotopic methods based on ^2H and ^{18}O measurements to detect small amounts of water taken up throughout the study period.

Influence of tree social status on the distance of uptake of labelled nitrate

In agreement with our hypothesis, our study clearly shows for the same genotype in monoclonal stands that dominant trees have a higher capacity to use soil resources in the very deep soil layers than suppressed trees. However, the horizontal distance of NO_3^- - ^{15}N uptake from the trunk was not clearly influenced by the social status of trees. From 0.6 to 1 year after planting, most of the trees close to the injection points took up NO_3^- - ^{15}N in the upper 3 m of the soil profile and only dominant trees took up the tracer at depths of 4.5 m and 6 m. While some dominant trees took up large amounts of ^{15}N at a depth of 6 m the second and third years after planting, the lack of clear enrichment in foliar ^{15}N for all the suppressed trees suggests that their ability to access to soil resources in very deep soil layers was low relative to dominant trees.

This pattern seems contradictory with the observation that suppressed trees can survive better to drought events than dominant trees (Bennett et al., 2015; McDowell and Allen, 2015). However, many factors other than the maximum depth reached by fine roots can account for tree survival during drought periods. A low water demand of

suppressed trees relative to dominant trees might help avoid hydraulic failure during drought (Grote et al., 2016). While drought periods impacted less the growth of suppressed trees relative to dominant trees in some forests (Martín-Benito et al., 2008; Martínez-Vilalta et al., 2012; Zang et al., 2012), the pattern was less clear in other forests and a more pronounced impact of drought in the understory than for dominant trees has also been reported (Castagneri et al., 2012; Trouvé et al., 2014; Merlin et al., 2015). Tall trees preferentially extracted water from layers below a depth of 1 m in a tropical rainforest, while smaller trees exhibited large variations in the mean depth of water uptake, which prevented the use of tree dimensions to parameterize functional models in this multi-specific forest (Stahl et al., 2013). Our study suggests that the patterns of water uptake can be different in the upper soil layers and in very deep soil layers, and the relationship between tree size and water uptake in the subsoil would deserve further studies in tropical forests.

Consequences for the management of *Eucalyptus* stands in deep tropical soils

Our study was also designed to answer a question from forest managers in Brazil: which proportion of the inter-rows in *Eucalyptus* plantations must be fertilized (after weeding) to make it possible for all the trees to take up the applied nutrients? Fertilization and weeding are seasonal activities with a peak at the mid-rainy season, and an extension of these activities throughout the year could be useful to reduce the cost of the silviculture. The staff and the equipment needed to cope with the peak of activity over the rainy season could indeed be reduced if the intervention could be postponed of a few months in a significant proportion of the inter-rows within each stand.

Whatever stand age and tree social status, most of the labelled nitrate injected at different soil depths was taken up by trees located within 2-3 m from the injection point. Our results show that N fertilizers must be applied close to the trunks to be taken up by all the trees, whatever stand age. Spacing in commercial *Eucalyptus* plantations managed in short rotations is commonly 2 m x 3 m or 3 m x 3 m. Our results suggest that fertilization (and weeding) in half of the inter-rows during the rainy season could be sufficient to make it possible for all the trees to take up the applied nutrients. However, the proportion of fertilized inter-rows cannot be lower than 50%. Applying fertilizers in 1/3 of the inter-rows would prevent most trees that are not adjacent to the

fertilized inter-row to take up the nutrients and would probably lead to a high heterogeneity of growth within the stand.

The risk of nutrient losses by deep drainage will be higher if only 50% of the inter-rows are fertilized because only about half of the root system would have access to the applied nutrients. Laclau et al. (2010) showed that nutrient requirements are high the first two years after planting *Eucalyptus* trees to produce leaves and fine roots. A combination of high nutrient requirements and high transpiration rates of *Eucalyptus* trees in highly weathered tropical soils account for very low nutrient losses by deep drainage (Mareschal et al., 2013; Versini et al., 2014; Caldeira et al., 2019). We show in our study that nitrate ions leached in deep Oxisols could be taken up by tree roots at a depth of 6 m between 0.6 and 2.5 years after planting in commercial *Eucalyptus* plantations. However, the velocity of exploration of deep soil layers depends on the social status of the trees. When N fertilization is concentrated in the first months after planting (and even more if fertilizers are only applied in 50% of the inter-rows), nitrate leaching in very deep soil layers might increase the heterogeneity of the stands since deep nitrates could only be available for dominant trees. Field trials are needed to assess if weeding and fertilizing only 50% of the inter-rows during the rainy season (and the other 50% a few months later) would affect stand productivity. Field trials will show whether weeds in half of the inter-rows during the rainy season would decrease significantly (or not) stand productivity. In drought-prone areas, an increase in stand evapotranspiration due to weeds during the rainy season might reduce the availability of water for trees during the next dry season and therefore might increase the risk of tree mortality during prolonged drought periods (Christina et al., 2018). The suitability of an extension of the period of weeding and fertilization throughout the year is therefore probably dependent on climate and soil properties. Further studies dealing with the relationship between the social status of the trees and the distance of water and nutrient uptake are also needed to improve tree-based modeling approaches that intend to predict the response of tropical forests to climate change.

Acknowledgements

We thank Raul Chaves and forest engineers in the Eucflux project (<http://www.ipef.br/eucflux/en/>) for interesting questions relative to the necessity or not to apply fertilizers in 100% of the inter-rows within *Eucalyptus* stands. This work was

supported by the São Paulo Research Foundation (FAPESP, project 2015/25946-0). Additional and essential funding came from Suzano SA Company (<http://www.suzano.com.br/en/>) that also provided the study areas and assistance in the field activities supported by forest technician Dileto Benedito Bau. We are grateful to the staff at the Itatinga Experimental Station (in particular Rildo Moreira e Moreira - ESALQ/USP), Eder Araujo da Silva (<http://www.floragroapoio.com.br>) and tree climbers of the Forestry Science and Research Institute (<http://www.ipef.br/english/seeds/>) for their technical support. We also acknowledge the Stable Isotopes Center from the Institute of Biosciences (UNESP).

REFERENCES

- Battie-Laclau, P., Laclau, J.-P., Domec, J.-C., Christina, M., Bouillet, J.-P., Piccolo, M. C., Gonçalves, J. L. M., Moreira, R. M., Krusche, A. V., Bouvet, J.-M., Nouvellon, Y., 2014. Effects of potassium and sodium supply on drought-adaptive mechanisms in *Eucalyptus grandis* plantations. *New Phytol.* 203, 401-413. <https://doi.org/10.1111/nph.12810>
- Bennett, A. C., McDowell, N. G., Allen, C. D., Anderson-Teixeira, K. J., 2015. Larger trees suffer most during droughts in forests worldwide. *Nature Plants* 1 (15139). <https://doi.org/10.1038/nplants.2015.139>
- Beyer, M., Koeniger, P., Gaj, M., Hamutoko, J. T., Wanke, H., Himmelsbach, T., 2016. A deuterium-based labelling technique for the investigation of rooting depths, water uptake dynamics and unsaturated zone water transport in semiarid environments. *J. Hydrol.* 533, 627-643. <https://doi.org/10.1016/j.jhydrol.2015.12.037>
- Beyer, M., Hamutoko, J. T., Wanke, H., Gaj, M., Koeniger, P., 2018. Examination of deep root water uptake using anomalies of soil water stable isotopes, depth-controlled isotopic labeling and mixing models. *J. Hydrol.* 566, 122-136. <https://doi.org/10.1016/j.jhydrol.2018.08.060>
- Binkley, D., Adams, M., Fredericksen, T., Laclau, J.-P., Mäkinen, H., Prescott, C., 2018. Connecting ecological science and management in forests for scientists, managers and pocket scientists. *For. Ecol. Manage.* 410, 157–163. <https://doi.org/10.1016/j.foreco.2017.11.022>
- Booth, T. H., 2013. Eucalypt plantations and climate change. *For. Ecol. Manage.* 301, 28–34. <http://dx.doi.org/10.1016/j.foreco.2012.04.004>
- Bordron, B., Robin, A., Oliveira, I. R., Guillemot, J., Laclau, J.-P., Jourdan, C., Nouvellon, Y., Abreu-Junior, C. H., Trivelin, P. C. O., Gonçalves, J. L. M., Plassard, C., Bouillet, J.-P., 2019. Fertilization increases the functional specialization of fine roots in deep soil layers for young *Eucalyptus grandis* trees. *For. Ecol. Manage.* 431, 6-16. <https://doi.org/10.1016/j.foreco.2018.03.018>
- Caldeira, A., et al., 2019. Impact of clear-cutting and replanting Eucalyptus stands on soil solution chemistry and nutrient leaching in fine and coarse textured tropical soils. *For. Ecol. Manage.*, submitted.
- Castagneri, D., Nola, P., Cherubini, P., Motta, R., 2012. Temporal variability of size–growth relationships in a Norway spruce forest: the influences of stand structure, logging, and climate. *Can. J. For. Res.* 42(3), 550-560. <https://doi.org/10.1139/x2012-007>
- Chaves, M. M., Maroco, J. P., Pereira, J. S., 2003. Understanding plant responses to drought - from genes to the whole plant. *Funct. Plant Biol.* 30, 239-264. <https://doi.org/10.1071/FP02076>

Christina, M., Laclau, J.-P., Gonçalves, J. L. M., Jourdan, C., Nouvellon, Y., Bouillet, J.-P., 2011. Almost symmetrical vertical growth rates above and below ground in one of the world's most productive forests. *Ecosphere* 2(3). <https://doi.org/10.1890/ES10-00158.1>

Christina, M., Nouvellon, Y., Laclau, J.-P., Stape, J. L., Bouillet, J.-P., Lambais, G. R., le Maire, G., 2017. Importance of deep water uptake in tropical eucalypt forest. *Funct. Ecol.* 31 (2), 509-519. <https://doi.org/10.1111/1365-2435.12727>

Christina, M., le Maire, G., Nouvellon, Y., Vezy, R., Bordron, B., Battie-Laclau, P., Gonçalves, J. L. M., Delgado-Rojas, J. S., Bouillet, J.-P., Laclau, J.-P., 2018. Simulating the effects of different potassium and water supply regimes on soil water content and water table depth over a rotation of a tropical *Eucalyptus grandis* plantation. *For. Ecol. Manage.* 418, 4-14. <https://doi.org/10.1016/j.foreco.2017.12.048>

Comas, L. H., Becker, S. R., Cruz, V. M. V., Byrne, P. F., Dierig, D. A., 2013. Root traits contributing to plant productivity under drought. *Front. Plant Sci.* 4 (442). <https://doi.org/10.3389/fpls.2013.00442>

Coplen, T. B., 2011. Guidelines and recommended terms for the expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Commun. Mass Spectrom.* 25 (17), 2538-2560. <http://doi.org/10.1002/rcm.5129>

da Silva, E.V., Bouillet, J.-P., Gonçalves, J. L. M., Abreu-Junior, C. H., Trivelin, P.C.O., Hinsinger, P., Jourdan, C., Nouvellon, Y., Stape, J.L., Laclau, J.-P., 2011. Functional specialization of Eucalyptus fine roots: contrasting potential uptake rates for nitrogen, potassium and calcium tracers at varying soil depths. *Funct. Ecol.* 25, 996-1006. <http://dx.doi.org/10.1111/j.1365-2435.2011.01867.x>

Fang, Y., Xiong, L., 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. *Cell. Mol. Life Sci.* 72, 673-689. <https://doi.org/10.1007/s00018-014-1767-0>

Freycon, V., Wonkam, C., Fayolle, A., Laclau, J.-P., Lucot, E., Jourdan, C., Cornu, G., Gourlet-Fleury, S., 2015. Tree roots can penetrate deeply in African semi-deciduous rain forests: evidence from two common soil types. *Journal Tropical Ecol.* 31 (01), 13-23. <https://doi.org/10.1017/S0266467414000595>

Geisser, S., 1993. Predictive Inference: An Introduction, Monographs on Statistics and Applied Probability. CRC Press.

Germon, A., Jourdan, C., Bordron, B., Robin, A., Nouvellon, Y., Gonçalves, J.L.M., Chapuis-Lardy, L., Pradier, C., Guerrini, I.A., Laclau, J.-P., 2019. Consequences of clear-cutting and drought on fine roots dynamics down to 17 m in coppice-managed eucalypt plantations. *For. Ecol. Manage.* 445, 48-59. <https://doi.org/10.1016/j.foreco.2019.05.010>

Gonçalves, J.L. de M., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., Ferraz, S.F. de B., Lima, W. de P., Brancalion, P.H.S., Hubner, A., Bouillet, J.P.D., Nouvellon, Y., Epron, D., 2013. Integrating genetic and silvicultural strategies to

minimize abiotic and biotic constraints in Brazilian eucalypt plantations. *For. Ecol. Manage.* 301, 6–27. <https://doi.org/10.1016/J.FORECO.2012.12.030>

Gonçalves, J.L., Alvares, C.A., Rocha, J.H., Brandani, C.B., Hakamada, R., 2017. Eucalypt plantation management in regions with water stress. *South. For. a J. For. Sci.* 1–15. <https://doi.org/10.2989/20702620.2016.1255415>

Göransson, H., Ingerslev, M., Wallander, H., 2008. The vertical distribution of N and K uptake in relation to root distribution and root uptake capacity in mature *Quercus robur*, *Fagus sylvatica* and *Picea abies* stands. *Plant Soil* 306, 129–137. <http://dx.doi.org/10.1007/s11104-007-9524-x>

Grote, R., Gessler, A., Hommel, R., Poschenrieder, W., Priesack, E., 2016. Importance of tree height and social position for drought-related stress on tree growth and mortality. *Trees* 30, 1467–1482. <https://doi.org/10.1007/s00468-016-1446-x>

Guderle, M., Hildebrandt, A., 2015. Using measured soil water contents to estimate evapotranspiration and root water uptake profiles – a comparative study. *Hydrol. Earth Syst. Sci.* 19, 409–425. <https://doi.org/10.5194/hess-19-409-2015>

Hinsinger, P., Brauman, A., Devau, N., Gérard, F., Jourdan, C., Laclau, J.-P., Le Cadre, E., Jaillard, B., Plassard, C., 2011. Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? *Plant Soil* 348, 29–61. <http://dx.doi.org/10.1007/s11104-011-0903-y>

Hoekstra, N. J., Suter, M., Finn, J. A., Husse, S., Lüscher, A., 2015. Do belowground vertical niche differences between deep-and shallow-rooted species enhance resource uptake and drought resistance in grassland mixtures? *Plant Soil* 394, 21–34. <https://doi.org/10.1007/s11104-014-2352-x>

IBÁ (Indústria Brasileira de Árvores) and PÖYRY, 2016. Report 2017. https://iba.org/images/shared/Biblioteca/IBA_RelatorioAnual2017.pdf.

Iversen, C. M., 2010. Digging deeper: fine-root responses to rising atmospheric CO₂ in forested ecosystems. *New Phytol.* 186, 346–357. <https://doi.org/10.1111/j.1469-8137.2009.03122.x>

Jackson, R. B., Mooney, H. A., Schulze, E.-D., 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proc. Natl. Acad. Sci.* 94, 7362–7366. <https://doi.org/10.1073/pnas.94.14.7362>

Klein, T., Yakir, D., Buchmann, N., Grünzweig, J. M., 2014. Towards an advanced assessment of the hydrological vulnerability of forests to climate change-induced drought. *New Phytol.* 201, 712–716. <https://doi.org/10.1111/nph.12548>

Koeniger, P., Gaj, M., Beyer, M., Himmelsbach, T., 2016. Review on soil water isotope based groundwater recharge estimations. *Hydrol. Process.* 30, 2817–2834. <https://doi.org/10.1002/hyp.10775>

Kulmatiski, A., Adler, P.B., Stark, J.M., Tredennick, A.T., 2017. Water and nitrogen uptake are better associated with resource availability than root biomass. *Ecosphere* 8, 1–10. <http://dx.doi.org/10.1002/ecs2.1738>

Kumar, R., Shankar, V., Jat, M. K., 2014. Evaluation of root water uptake models – a review. *ISH J. Hydraul. Eng.* 21, 115-124. <http://dx.doi.org/10.1080/09715010.2014.981955>

Laclau, J.-P., Ranger, J., Gonçalves, J. L. M., Maquère, V., Krusche, A. V., M'Bou, A. T., Nouvellon, Y., Saint-André, L., Bouillet, J.-P., Piccolo, M. C., Deleporte, P., 2010. Biogeochemical cycles of nutrients in tropical Eucalyptus plantations. Main features shown by intensive monitoring in Congo and Brazil. *For. Ecol. Manage.* 259, 1771-1785. <https://doi.org/10.1016/j.foreco.2009.06.010>

Laclau, J.-P., da Silva, E. A., Lambais, G. R., Bernoux, M., le Maire, G., Stape, J. L., Bouillet, J.-P., Gonçalves, J. L. M., Jourdan, C., Nouvellon, Y., 2013. Dynamics of soil exploration by fine roots down to a depth of 10 m throughout the entire rotation in *Eucalyptus grandis* plantations. *Front. Plant Sci.* 4 (243). <https://doi.org/10.3389/fpls.2013.00243>

Lambais, G.L., Jourdan, C., Piccolo, M.C., Germon, A., Pinheiro, R.C., Nouvellon, Y., Stape, J.L., Campoe, O.C., Robin, A., Bouillet, J.-P., le Maire, G., Laclau, J.-P., 2017. Contrasting phenology of *Eucalyptus grandis* fine roots in upper and very deeper soil layers in Brazil. *Plant Soil* 421, 301-318. <https://doi.org/10.1007/s11104-017-3460-1>

Lehmann, J., Muraoka, T., 2001. Tracer methods to assess nutrient uptake distribution in multistrata agroforestry systems. *Agro. Systems* 53, 133-140. <https://doi.org/10.1023/A:1013368302450>

Lehmann, J., 2003. Subsoil root activity in tree-based cropping systems. *Plant Soil* 255, 319-331. <https://doi.org/10.1023/A:1026195527076>

Maeght, J.-L., Rewald, B., Pierret, A., 2013. How to study deep roots – and why it matters. *Front. Plant Sci.* 4 (299). <http://dx.doi.org/10.3389/fpls.2013.00299>

Mareschal, L., Laclau, J.-P., Nzila, J.-D.-D., Versini, A., Koutika, L.-S., Mazoumbou, J.-C., Deleporte, P., Bouillet, J.-P., Ranger, J., 2013. Nutrient leaching and deep drainage under Eucalyptus plantations managed in short rotations after afforestation of an African savanna: Two 7-year time series. *For. Ecol. Manage.* 307, 242-254. <https://doi.org/10.1016/j.foreco.2013.06.038>

Marsden, C., Nouvellon, Y., Laclau, J.-P., Corbeels, M., McMurtrie, R. E., Stape, J. L., Epron, D., le Maire, G., 2013. Modifying the G'DAY process-based model to simulate the spatial variability of Eucalyptus plantation growth on deep tropical soils. *For. Ecol. Manage.* 301, 112-128. <https://doi.org/10.1016/j.foreco.2012.10.039>

Martín-Benito, D., Cherubini, P., del Río, M., Cañellas, I., 2008. Growth response to climate and drought in *Pinus nigra* Arn. trees of different crown classes. *Trees* 22, 363-373. <https://doi.org/10.1007/s00468-007-0191-6>

Martínez-Vilalta, J., López, B. C., Loepfe, L., Lloret, F., 2012. Stand- and tree-level determinants of the drought response of Scots pine radial growth. *Oecologia* 168, 877-888. <https://doi.org/10.1007/s00442-011-2132-8>

McDowell, N. G., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G., Yepez, E. A., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* 178, 719-739. <https://doi.org/10.1111/j.1469-8137.2008.02436.x>

McDowell, N. G. et al, 2013. Evaluating theories of drought-induced vegetation mortality using a multimodel-experiment framework. *New Phytol.* 200, 304-321. <https://doi.org/10.1111/nph.12465>

McDowell, N. G., Allen, C. D., 2015. Darcy's law predicts widespread forest mortality under climate warming. *Nat. Clim. Change* 5, 669-672. <https://doi.org/10.1038/nclimate2641>

McMurtrie, R.E., Näsholm, T., 2018. Quantifying the contribution of mass flow to nitrogen acquisition by an individual plant root. *New Phytologist* 218, 119–130. <https://doi.org/10.1111/nph.14927>

Merlin, M., Perot, T., Perret, S., Korboulewsky, N., Vallet, P., 2015. Effects of stand composition and tree size on resistance and resilience to drought in sessile oak and Scots pine. *For. Ecol. Manage.* 339, 22-33. <https://doi.org/10.1016/j.foreco.2014.11.032>

Nie, M., Lu, M., Bell, J., Raut, S., Pendall, E., 2013. Altered root traits due to elevated CO₂: a meta-analysis. *Glob. Ecol. Biogeogr.* 22, 1095-1105. <https://doi.org/10.1111/geb.12062>

Pierret, A., Maeght, J.-L., Clément, C., Montoroi, J. P., Hartmann, C., Gonkhamdee, S., 2016. Understanding deep roots and their functions in ecosystems: an advocacy for more unconventional research. *Ann. Bot.* 118, 621–635. <https://doi.org/10.1093/aob/mcw130>

Pinheiro, R. C., de Deus Júnior, J. C., Nouvellon, Y., Campoe, O. C., Stape, J. L., Aló, L. L., Guerrini, I. A., Jourdan, C., Laclau, J.-P., 2016. A fast exploration of very deep soil layers by *Eucalyptus* seedlings and clones in Brazil. *For. Ecol. Manage.* 366, 143-152. <https://doi.org/10.1016/j.foreco.2016.02.012>

Poszwa, A., Dambrine, E., Ferry, B., Pollier, B., Loubet, M., 2002. Do deep tree roots provide nutrients to the tropical rainforest? *Biogeochemistry* 60, 97-118. <https://doi.org/10.1023/A:1016548113624>

Pradier, C., Hinsinger, P., Laclau, J.-P., Bouillet, J.-P., Guerrini, I. A., Gonçalves, J.L.M., Asensio, V., Abreu-Junior, C.H., Jourdan, C., 2017. Rainfall reduction impacts rhizosphere biogeochemistry in eucalypts grown in a deep Ferralsol in Brazil. *Plant Soil* 414, 339-354. <https://doi.org/10.1007/s11104-016-3107-7>

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>

Roupsard, O., Ferhi, A., Granier, A., Pallo, F., Depommier, D., Mallet, B., Joly, H.I., Dreyer, E., 1999. Reverse phenology and dry-season water uptake by *Faidherbia albida* (Del.) A. Chev. in an agroforestry parkland of Sudanese West Africa. Functional Ecology 13, 460–472. <https://doi.org/10.1046/j.1365-2435.1999.00345.x>

Stahl, C., Hérault, B., Rossi, V., Burban, B., Bréchet, C., Bonal, D., 2013. Depth of soil water uptake by tropical rainforest trees during dry periods: does tree dimension matter? Oecologia 173, 1191-1201. <https://doi.org/10.1007/s00442-013-2724-6>

Trogisch, S., Salmon, Y., He, J.-S., Hector, A., Scherer-Lorenzen, M., 2016. Spatio-temporal water uptake patterns of tree saplings are not altered by interspecific interaction in the early stage of a subtropical forest. For. Ecol. Manage. 367, 52-61. <https://doi.org/10.1016/j.foreco.2016.02.018>

Trouvé, R., Bontemps, J.-D., Collet, C., Seynave, I., Lebourgeois, F., 2014. Growth partitioning in forest stands is affected by stand density and summer drought in sessile oak and Douglas-fir. For. Ecol. Manage. 334, 358-368. <https://doi.org/10.1016/j.foreco.2014.09.020>

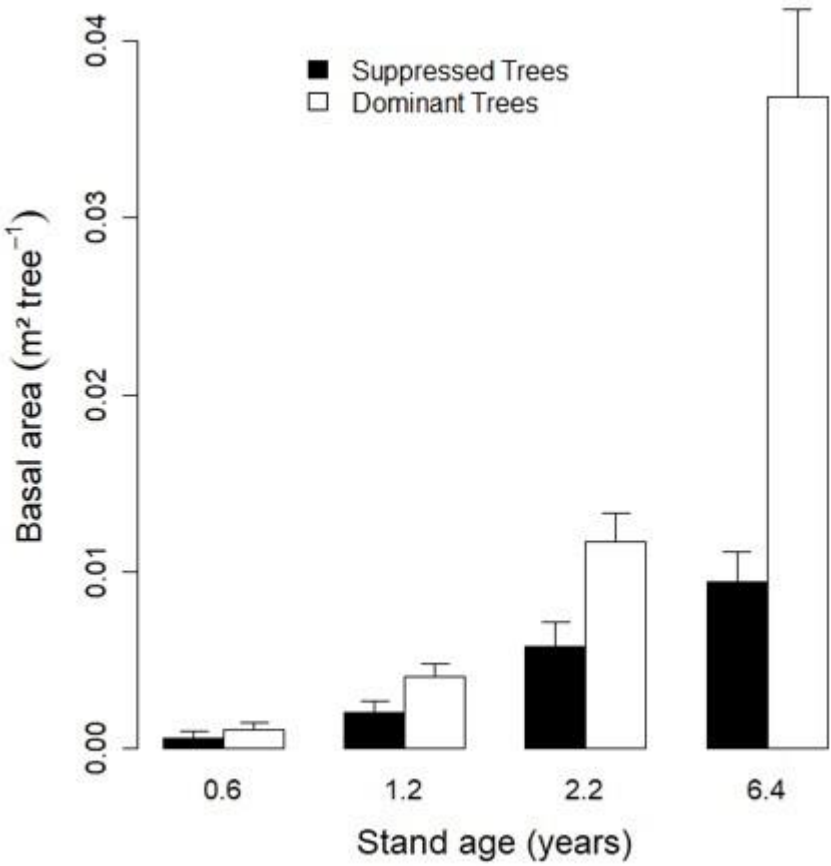
Versini, A., Mareschal, L., Matsoumbou, T., Zeller, B., Ranger, J., Laclau, J.-P., 2014. Effects of litter manipulation in a tropical *Eucalyptus* plantation on leaching of mineral nutrients, dissolved organic nitrogen and dissolved organic carbon. Geoderma 232-234, 426-436. <https://doi.org/10.1016/j.geoderma.2014.05.018>

White, P. J., George, T. S., Gregory, P. J., Bengough, A. G., Hallett, P. D., McKenzie, B. M., 2013. Matching roots to their environment. Ann. Bot. 112 (2), 207-222. <https://doi.org/10.1093/aob/mct123>

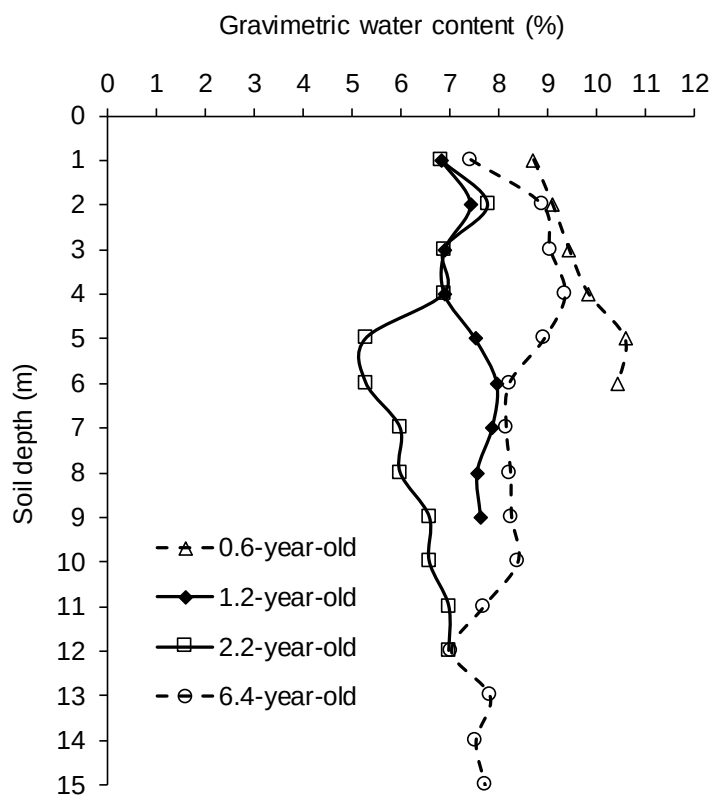
Wu, Z., Dijkstra, P., Koch, G. W., Peñuelas, J., Hungate, B. A., 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Glob. Change Biol. 17, 927-942. <https://doi.org/10.1111/j.1365-2486.2010.02302.x>

Zang, C., Pretzsch, H., Rothe, A., 2012. Size-dependent responses to summer drought in Scots pine, Norway spruce and common oak. Trees 26, 557-569. <https://doi.org/10.1007/s00468-011-0617-z>

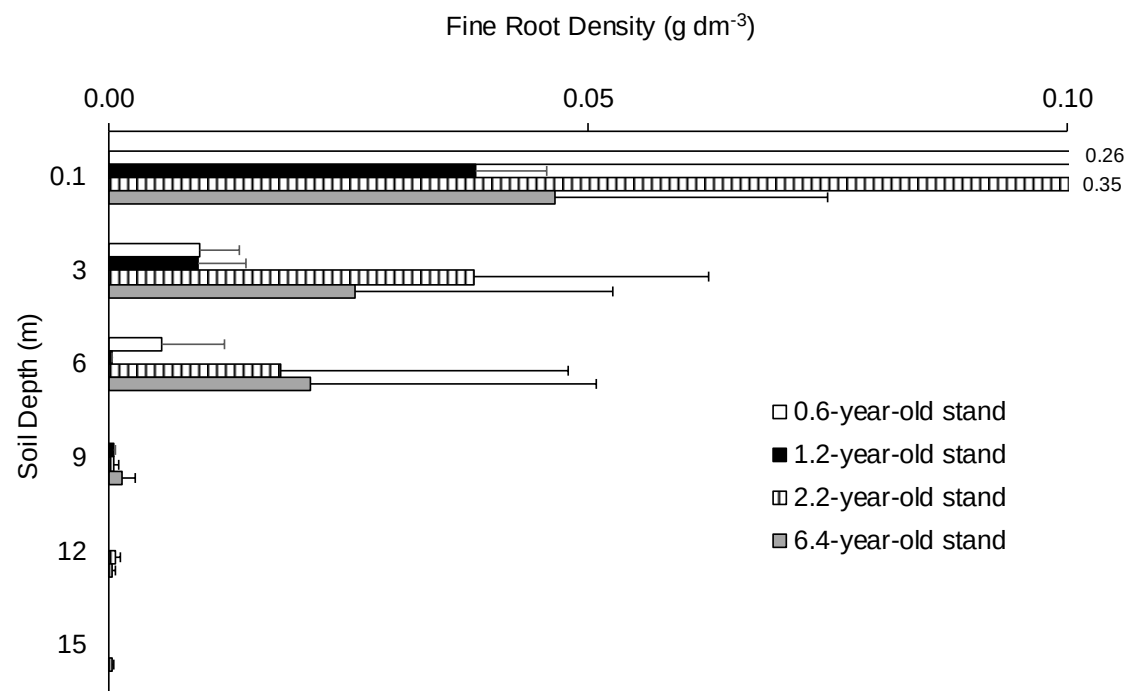
APPENDIXES



Appendix 1. Mean basal area of dominant and suppressed eucalypt trees at the date of ¹⁵N tracer injection. Standard deviations are indicated (*n* = 60 in the 0.6- and 2.2-year-old stands, *n* = 48 in the 1.2-year-old stand, and *n* = 72 in the 6.4-year-old stand).



Appendix 2. Mean gravimetric soil water contents down to the maximum depth where the tracer was injected at each sampling age ($n = 3$ in each soil layer at each age).



Appendix 3. Fine root density down to the maximum depth where the tracer was injected at each sampling age. Mean values and standard deviations in each stand are indicated ($n = 3$).

CAPÍTULO 2

NICHE COMPLEMENTARITY BETWEEN PLANT SPECIES TO TAKE UP LABELLED-NITRATE DOWN TO A DEPTH OF 12 M IN A BRAZILIAN SAVANNA

Rafael Costa Pinheiro ^a, Lívia Lanzi Aló ^a, Iraê Amaral Guerrini ^a, Jean-Pierre Bouillet ^{b, c}, Vladimir Eliodoro Costa ^d, Vânia Pivello ^e, Karel Van den Meersche ^b, Jean-Paul Laclau ^{a, b, c},

^a São Paulo State University (Unesp), School of Agriculture, Botucatu, SP, Brazil

^b CIRAD, UMR Eco&Sols, F-34398 Montpellier, France

^c Eco&Sols, Univ Montpellier, CIRAD, INRA, IRD, Montpellier SupAgro, Montpellier, France

^d São Paulo State University (Unesp), Institute of Biosciences, Botucatu, SP, Brazil

^e Department of Ecology, University of São Paulo, São Paulo, SP, Brazil

ABSTRACT

Cerrado savannas are the second largest vegetation formation in Brazil after the Amazon rainforest, with about 2 million km². Roots have been shown in very deep soil layers in Cerrado ecosystems, which suggests that deep rooting might have a crucial role to supply water and nutrients over dry periods. Our study aimed to gain insight into the distance from the trunk (horizontal and vertical) where fine roots of common Cerrado species take up mobile nutrients in very deep soils and to assess whether there is a niche complementarity in nitrate uptake between these species. The NO₃⁻-¹⁵N tracer was injected in a closed-canopy savanna with dense woody understory (cerradão) at six depths (0.1, 1.5, 3.0, 6.0, 9.0 and 12.0 m) with three plots per depth, at two dates (in rainy season and in dry season). Five months after tracer injection, young leaves were sampled in three plant species (*Coussarea hydrangeifolia*, *Miconia albicans* and *Xylopia aromatica*) in each plot and foliar δ¹⁵N was determined. Large differences in depth of NO₃⁻-¹⁵N uptake between the three species showed a niche complementarity in this savanna. *Xylopia aromatica* trees took up NO₃⁻-¹⁵N at a depth

of 6 m during both seasons and exploited a much larger soil volume than the two other species, with an uptake of ^{15}N by trees located at up to 5 m from the injection site. The behaviour of *M. albicans* and *C. hydrangeifolia* plants was similar, with a strong uptake of NO_3^- - ^{15}N only in the 0 - 1.5 m soil layer, within 2 m of horizontal distance between the trunk and the injection site. The depth of nitrate uptake over the dry season was unrelated with the size of the plants. Further studies are needed to improve our understanding of the role of very deep roots in the biogeochemical cycles of tropical savannas.

Keywords: deep roots; ^{15}N ; water; nutrient; subsoil; Cerrado; Brazil

2.1. INTRODUCTION

The Cerrado biome covers about 2 million km^2 and is, after the Amazon rainforest, the largest Brazilian plant formation, which represents about a quarter of Brazil's territory (BRASIL, 2019). The Cerrado is classified as one of the global 35 biodiversity hotspots (MYERS et al., 2000), because of its exceptional concentration of endemic biodiversity and high levels of damaging economic exploitation (LAHSEN et al., 2016). Nevertheless, its importance is often neglected in Brazil, and studies dealing with the functioning of Cerrado ecosystems remain scarce, with most of the available information focusing on nitrogen and carbon cycles (COLETTA et al., 2009). The increasing replacement of the Cerrado by grasses, crops and forest plantations is likely to influence the regional climate (RODRIGUES et al., 2014).

Deep rooting can help survive over extended drought periods by shifting the uptake of water to deeper soil layers (SHARP AND DAVIES, 1985; HOEKSTRA et al., 2014; CHRISTINA et al., 2017). Deep rooting is common in seasonally dry environments such as neotropical savannas, providing access to water stored in deep soil layers, which makes it possible to maintain plant transpiration and CO_2 assimilation over dry periods (OLIVEIRA et al., 2005). Canadell et al. (1996) pointed out that tropical savannas are the biome with the highest average rooting depth (15 ± 5.4 m), and with the deepest rooting ever recorded (68 m). Even though the deep rooting habit of some Cerrado plants is recognized (JACKSON et al., 1999; FRANCO, 2002), their functional significance and their contribution to water and nutrient acquisition have been little explored (OLIVEIRA et al., 2005). In the Cerrado biome, the shrub-tree vegetation

stands out for its richness of phenological and morphological types, which suggests contrasting distribution patterns of root systems as well as a broad range of water use strategies (FRANCO, 2005). The structure of the Cerrado formations can be variable, ranging from natural landscapes dominated by grasses and dwarf shrubs (“campo sujo”) to forest formations (“cerradão”), with differences in floristic and phytosociological compositions (GOODLAND AND FERRI, 1979).

While niche partitioning of soil resources is considered to fulfil a crucial role for the coexistence of plants in native ecosystems, methodological difficulties have considerably limited the measurements of the timing and location of nutrient uptake in deep soil layers (KULMATISKI AND BEARD, 2013; MAEGHT et al., 2013). In agreement with the stress-gradient hypothesis, which predicts that competitive interactions decrease and facilitation increases under stressful environmental conditions (FORRESTER, 2014), higher niche complementarity for soil resources between plant species under harsh conditions than under favorable conditions might contribute to explaining a positive effect of diversity on net primary productivity (PAQUETTE AND MESSIER, 2011; LÜSCHER et al., 2014). Indeed, belowground niche complementarity between deep-rooted and shallow-rooted species may result in a more complete use of available soil resources than in monospecific stands (HOEKSTRA et al., 2015).

Isotopic approaches are commonly used to trace water and nutrient dynamics in forest soils (POSZWA et al., 2014). The depth of water uptake can be estimated using natural-abundance isotopic techniques. However, the isotopic natural abundance of deuterium and ^{18}O can only be used when there is a clear gradient in water isotope ratios with depth. Moreover, the depth of water uptake is highly dependent on climatic conditions on the days prior to sampling (BERRY et al., 2017). Studies in eucalypt plantations suggest that ^{15}N determinations within leaves after injection of ^{15}N -labelled nitrates in a specific soil area could be useful to gain insight into the localization of water uptake by tree roots. Indeed, ^{15}N -labelled nitrates injected at a depth of 3 m were only detected in young leaves of 6-year-old Eucalyptus trees when the gravitational soil solutions reached the depth of labelled nitrate injection (DA SILVA et al., 2011), which suggested that NO_3^- - ^{15}N uptake could be a tracer of water uptake in tropical plantations (PINHEIRO et al., 2019). Nitrate labelling could be a proxy of the cumulative water uptake between the date of NO_3^- - ^{15}N injection in the soil

and leaf sampling a few months later since a clear enrichment in foliar ^{15}N content can only occur if large amounts of NO_3^- - ^{15}N are transported through mass flow up to fine roots close to the injection area. A recent study in monoclonal eucalypt plantations showed that the tallest trees took up labelled nitrate more deeply than small trees in the same stand the first years after planting (PINHEIRO et al., 2019). Hydrological niche segregation was recently demonstrated in a seasonal Amazon forest, with differences in depth of water uptake strongly related to the diameter at breast height of the trees and a trade-off between access to deep water and tolerance of very low water potentials (BRUM et al., 2018).

Our study aimed to gain insight into the distance from the trunk (horizontal and vertical) where fine roots of common Cerrado species take up mobile nutrients in very deep soils. We hypothesized: i) a niche complementarity between tree species in Cerrado savannas, some species taking up labelled nitrates in deeper soil layers than other species, ii) a deeper uptake of labelled nitrates during the dry season than during the rainy season as a result of deep-water uptake in the subsoil over dry periods, and iii) an uptake of labelled nitrates more deeply for big trees than for small trees and shrubs.

2.2 MATERIAL AND METHODS

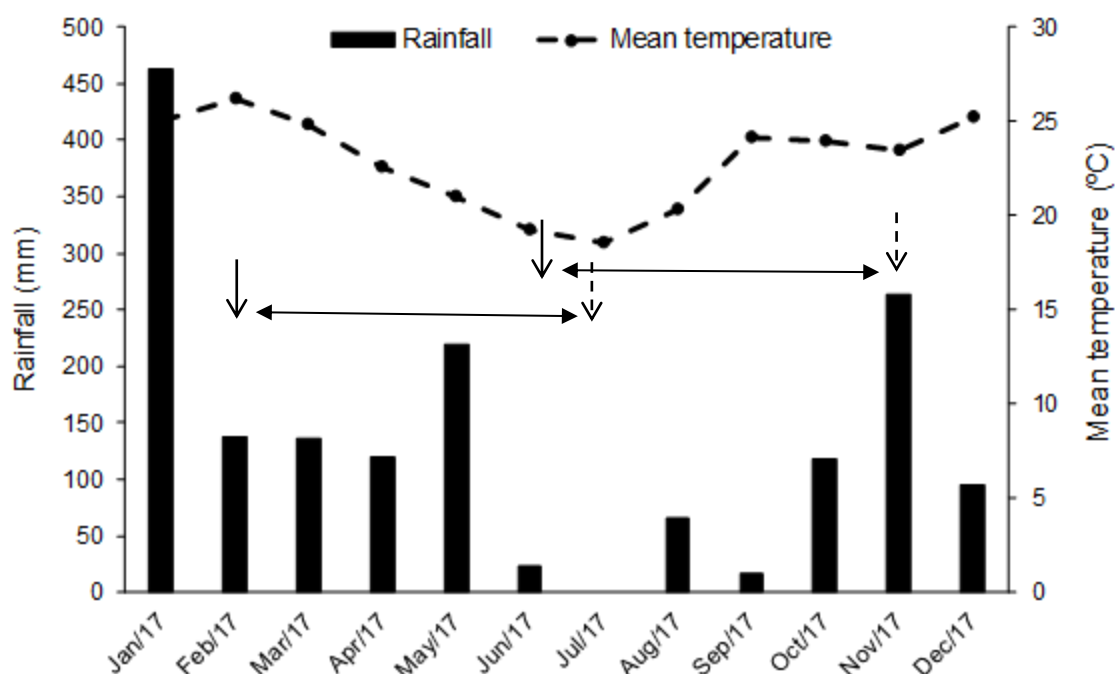
Study site

The study was carried out in a large and flat savanna area at an altitude of 530 meters in Brazil (22°20'30" S, 40°00'30" W). The vegetation ranged from grassland to sclerophyllous forest (cerradão). This area at Jardim Botânico Municipal de Bauru has conserved the last fragments of native vegetation in the centre-west region of the state of São Paulo, with a total area of more than 200 hectares, and is considered as a priority for conservation (DURIGAN et al., 2004). From a total area of 321.7 hectares, 277 hectares are composed of cerradão (WEISER, 2007). The physiognomy of dense cerradão was chosen because it is the Cerrado formation with the largest area in the state of São Paulo nowadays.

The regional climate of Bauru is humid subtropical (Cwa, Köppen's classification) with dry winter and hot summer (ALVARES et al., 2013). The annual rainfall and the mean temperature were 1655 mm and 22.9 °C, respectively, over our study period in 2017, and 1394 mm and 22.7 °C over the last 10 years. The wettest

month in 2017 was January (462 mm) while no rainfall was recorded in July. The hottest month was February (31.8 °C), while the minimum temperature was in July (12.6 °C) (Fig. 1).

Figure 1 - Monthly precipitation and mean temperature over the study period at Bauru. Full vertical arrows show the ^{15}N tracer injection dates in rainy (early February) and dry (end of June) seasons in 2017. Dashed vertical arrows show the leaf sampling dates, five months after tracer injection (early July and early November)



The soil was sandy (Arenosols, FAO classification) with high acidity ($\text{pH} \leq 4.3$) and very low nutrient contents (Table 1). The water table was very deep (more than 12 m, which was the deepest hole made in our study).

Experimental design

Based on a preliminary botanical survey at the same site (WEISER, 2007), we selected the three most common species for our study: i) *Xylopia aromatica* (Lam.) Mart.; ii) *Coussarea hydrangeifolia* (Benth.) Müll. Arg.; and iii) *Miconia albicans* (Sw.) Steud. While *Xylopia aromatica* and *Coussarea hydrangeifolia* are tree species, *Miconia albicans* is a shrub (Appendix A). *Xylopia aromatica* is an evergreen species with a widespread but irregular and discontinuous distribution, occurring typically at low frequency. Despite its pioneering characteristics, its growth is rather slow

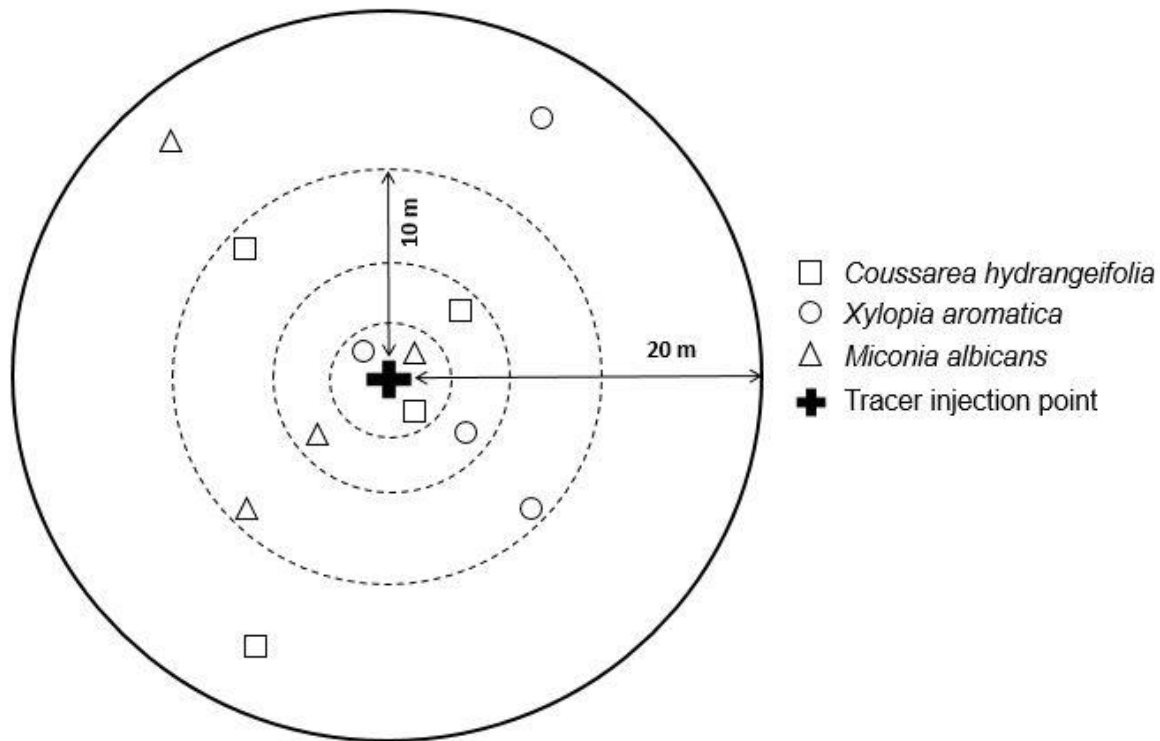
(LORENZI, 2008). *Coussarea hydrangeifolia* is another evergreen pioneer species common in Cerrado formations found preferentially on sandy soils (LORENZI, 2008). *Miconia albicans* is a shrub species characteristic of Cerrado and savanna formations (MARTINS et al., 1996), which loses part of its leaf area in the dry season (MATTOS et al., 1997).

Thirty-six circular plots (20 m in radius) were randomly attributed in the cerradão to sample two seasons (rainy and dry), six depths of tracer injection (0.1, 1.5, 3.0, 6.0, 9.0 or 12.0 m) with three replications for each depth and season. The distance between the centres of the plots was at least 50 meters. The $\text{NO}_3^-^{15}\text{N}$ tracer was applied at the centre of each plot, at a single soil depth. In each plot, four intervals of horizontal distance between the injection site and the trunk of the sampled plant were predefined: 0 – 3 m, 3 – 6 m, 6 – 10 m, and ≥ 10 m (Fig. 2). An inventory of plants with a diameter at breast height (DBH) of more than 1.5 cm (in a radius of 10 m from the tracer injection site in each plot) showed that the basal area of *Miconia albicans* was much lower than the basal areas of *Xylopia aromatica* and *Coussarea hydrangeifolia*. The basal areas of the three selected species were low compared to the total basal area but these three species were the only ones found in all the plots (Appendix B).

Table 1 - Main physical and chemical soil properties of the Cerrado area at Bauru. Mean values and standard deviations are indicated ($n = 3$)

Soil layer	Sand	Silt	Clay	pH	O. M.	P _{resin}	H+Al	K	Ca	Mg	SB	CEC
(m)	Particle size distribution (%)			CaCl ₂	g kg ⁻¹	mg kg ⁻¹	mmol _c kg ⁻¹					
0 - 0.25	89.6 ± 1.1	2.2 ± 0.1	8.2 ± 1.0	3.9 ± 0.2	13.9 ± 5.2	3.6 ± 0.9	33.7 ± 7.6	0.6 ± 0.3	2.0 ± 1.4	1.4 ± 1.0	4.0 ± 2.5	37.7 ± 6.3
0.25 - 0.5	87.6 ± 1.0	2.4 ± 0.5	10.0 ± 1.3	3.8 ± 0.1	10.0 ± 1.0	2.3 ± 0.7	32.0 ± 5.3	0.5 ± 0.4	1.7 ± 0.1	1.0 ± 0.7	3.1 ± 1.0	35.1 ± 4.3
0.5 - 1	85.7 ± 0.9	2.9 ± 0.7	11.4 ± 0.3	3.7 ± 0.0	5.3 ± 0.9	1.8 ± 0.1	26.7 ± 2.5	0.2 ± 0.1	1.1 ± 0.5	0.5 ± 0.0	1.8 ± 0.5	28.6 ± 2.9
1 - 2	83.6 ± 1.1	3.3 ± 0.9	13.1 ± 0.3	3.9 ± 0.1	4.3 ± 0.5	1.8 ± 0.1	20.4 ± 3.7	0.3 ± 0.2	1.2 ± 0.5	0.5 ± 0.0	1.9 ± 0.5	22.3 ± 4.2
2 - 4	83.2 ± 1.5	2.9 ± 0.6	13.9 ± 1.2	4.1 ± 0.0	2.7 ± 0.4	1.8 ± 0.0	15.6 ± 1.1	0.2 ± 0.0	1.5 ± 0.5	0.5 ± 0.0	2.1 ± 0.5	17.8 ± 1.4
4 - 6	80.7 ± 0.7	3.4 ± 0.9	15.9 ± 1.6	4.0 ± 0.1	2.2 ± 0.2	2.1 ± 0.5	16.5 ± 1.5	0.1 ± 0.0	1.5 ± 0.5	0.5 ± 0.0	2.2 ± 0.6	18.7 ± 1.1
6 - 8	80.1 ± 1.7	3.6 ± 0.8	16.3 ± 1.1	4.0 ± 0.0	2.1 ± 0.2	1.9 ± 0.1	18.3 ± 2.0	0.2 ± 0.0	1.2 ± 0.5	0.5 ± 0.0	1.9 ± 0.5	20.2 ± 1.5
8 - 10	80.2 ± 2.3	4.3 ± 1.3	15.5 ± 2.7	4.0 ± 0.1	3.4 ± 1.6	1.8 ± 0.0	21.0 ± 3.6	0.3 ± 0.2	1.2 ± 0.5	0.6 ± 0.1	2.1 ± 0.5	23.1 ± 3.2
10 - 12	78.7 ± 0.7	6.1 ± 1.8	15.2 ± 1.6	3.9 ± 0.2	3.4 ± 1.3	2.2 ± 0.6	26.5 ± 10.8	0.7 ± 0.6	1.2 ± 0.6	2.5 ± 1.8	4.5 ± 2.5	30.0 ± 13.6

Figure 2 - Layout of one sampled plot. The ^{15}N tracer was injected at the centre of the plot. Plants were sampled at 4 intervals of distance from the injection site: i) < 3 m; ii) 3 – 6 m; iii) 6 – 10 m; iv) > 10 m.



Among the three studied species at our site, *Coussarea hydrangeifolia* had the largest basal area and the highest density with on average 1103 trees per hectare (Table 2). The height of *Coussarea hydrangeifolia* trees ranged from 2.8 to 8.1 m with a diameter at breast height (DBH) ranging from 3.0 to 10.4 cm. The mean density of *Xylopia aromatica* trees was 756 trees per hectare, with heights ranging from 4.3 to 10.5 m and DBHs from 2.5 to 10.8 cm. *Miconia albicans* plants had a lower density than the two other species with 337 trees per hectare on average, heights ranging from 1.2 to 6.4 m and DBHs from 0.1 to 7.0 cm.

Table 2 - Height, diameter at breast height (DBH), basal area and absolute density of the three species studied in the Cerrado area at Bauru from an inventory made within a 10 m radius of the tracer injection site in each one of the 36 allocated plots. Height values were only measured for the plants whose leaves were sampled for isotopic analysis but DBH was measured for all plants with a DBH of more than 1.5 cm. Mean standard deviations are indicated.

Species	Height (m)	DBH (cm)	Basal area (m ² ha ⁻¹)	Absolute density (# plants ha ⁻¹)
<i>Coussarea hydrangeifolia</i>	5.3 (1.1)	4.3 (2.0)	2.3 (0.8)	1103 (560.2)
<i>Miconia albicans</i>	3.1 (1.0)	2.1 (1.2)	0.2 (0.2)	337 (174.6)
<i>Xylopia aromatica</i>	7.3 (1.0)	4.5 (2.1)	1.8 (0.9)	756 (335.0)
Large trees sampled	nd	23.0 (9.5)	5.2 (2.2)	106 (15.4)
Other shrubs and trees	nd	6.4 (6.0)	20.8 (7.3)	3185 (1100.1)

Tracer applications

One day before application in the field, a labelled solution was prepared in the laboratory and maintained at 4 °C. 16.6 g of NH₄¹⁵NO₃ commercial compound (10 atom% or 29,222 ‰ NO₃⁻¹⁵N) dissolved in 20 ml of distilled water were injected at a single depth at the centre of each plot (indicated as 'injection site' thereafter). Holes were drilled down to the target application depth using a cylindrical auger with an inner diameter of 9 cm and soil water contents (SWCs) were measured every meter in all the samples collected during the drilling (Appendix C). Soil blocks from the inner part of the auger were collected for the deepest meter in each hole to assess fine root density (< 2 mm in diameter) close to the area of NO₃⁻¹⁵N injection. Gravitational SWCs from the soil surface to a depth of 11 m ranged from 17 to 25% in February 2017 (mid of the rainy season), and from 5 to 10% in June 2017, at the onset of the dry season (Appendix C). SWCs sharply increased below a depth of 10 m, which might reflect a decrease in water withdrawal by tree roots relative to upper soil layers since the clay and silt contents did not increase below 10 m (Table 1).

A PVC tube (2.5 cm in diameter) was inserted into each hole to avoid any contamination of the soil in ¹⁵N during tracer injection. A 0.4-cm polyethylene tube, attached to an iron rod, was inserted into the PVC tube, with a length depending on the application depth. A plastic sheet was placed around each hole to avoid any contamination with ¹⁵N at the soil surface. 20 ml of the labelled solution was injected at the selected depth using a syringe. Thereafter, 280 ml of distilled water were injected to rinse the polyethylene tube and to increase soil moisture in the area where the tracer was applied in order to promote nitrate uptake. Lastly, the polyethylene and PVC tubes

were carefully withdrawn from the holes, which were filled with the soil removed during drilling, respecting the original order of the soil layers. Similar methodologies were used by da Silva et al. (2011) and Pinheiro et al. (2019).

Leaf sampling

Young leaves were sampled in the upper part of the crown of one plant of each selected species, for each interval of distance from the injection site in each plot. All the leaves were collected from the ground using a pruning shear. Leaves were sampled in July 2017 in the plots with tracer injection in February to study nitrate uptake in rainy season, and in November 2017 in the plots with tracer injection in June to study nitrate uptake in dry season (Fig. 1). The number of young leaves sampled for isotopic analyses was adapted to the size of the leaves for each species. Twenty leaves of *Coussarea hydrangeifolia* (large leaf), 30 leaves of *Miconia albicans* (medium-size leaf) and 40 leaves of *Xylopia aromatica* (small leaf) were sampled on several branches for each plant.

Another sampling was carried out in November 2017 to assess whether the biggest trees had the ability to take up the tracer at a larger distance from the injection site than the three studied species (*Miconia albicans*, *Xylopia aromatica* and *Coussarea hydrangeifolia*). Thirty young leaves were sampled in the upper part of the crown of the tree with the largest diameter at breast height (whatever the species) at each predefined interval of distance of the tracer injection site in each plot (1 tree x 3 distance intervals from the centre of the plot x 18 plots = 54 trees).

In addition, young leaves were sampled in 8 (rainy season) and 12 (dry season) control trees of each species, far from the injection sites (> 10 m), to determine foliar $\delta^{15}\text{N}$ values in natural abundance. Control trees were sampled far from each other throughout the study area to cover the spatial variability of ^{15}N natural abundance. Leaves were sampled about 5 months after tracer injection in both seasons (Fig. 1).

Isotopic analyses of ^{15}N

In the laboratory, the leaves were washed in deionized water and oven-dried at 65 °C for 72 hours. The dried samples were ground (< 60 μm) in a cryogenic mill (2010 Geno Grinder, SPEX Sample Prep, Metuchen, USA) at -196 °C for homogenization and stored in sealed acrylic pots until isotopic analysis. An aliquot of 4500-5000 μg of each dry and milled sample was weighed into a cylindrical tin capsule (D1106 -

Elemental Microanalysis, Okehampton, UK) with a 1 µg resolution scale (XP6 - Mettler Toledo, Greifensee, Switzerland). The ^{15}N analyses were performed at the Centre for Stable Isotopes at São Paulo State University using a continuous flow system in a CF-IRMS isotope ratio mass spectrometer (Delta V Advantage - Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (Flash 2000 OEA - Thermo Scientific, Bremen, Germany) with an interface (ConFlo IV Universal - Thermo Scientific, Bremen, Germany) that determines the isotope ratio of sample ($R_{\text{sample}} = ^{15}\text{N}/^{14}\text{N}$).

The ^{15}N values in isotopic delta ($\delta^{15}\text{N}$, expressed in ‰) were calculated from Coplen (2011):

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{air}}} - 1 \right) \cdot 1000\text{‰} \quad (1)$$

Where R_{sample} is the $^{15}\text{N}/^{14}\text{N}$ ratio in the sample and R_{air} is the isotopic ratio of atmospheric air ($R_{\text{air}} = 0.0036765$) as an international standard. The standard uncertainty in $\delta^{15}\text{N}$ was ± 0.3 ‰ for samples slightly enriched in ^{15}N .

Data analyses

For the three selected species, a prediction interval (PI) of $\delta^{15}\text{N}$ in natural abundance (with a threshold of 99%) was calculated from the $\delta^{15}\text{N}$ values of control trees using equation 2:

$$\text{PI} = \bar{X}_n \pm T_\alpha s_n \sqrt{1 + 1/n} \quad (2)$$

Where $\bar{X}_n$ and s_n were the average and the standard deviation of $\delta^{15}\text{N}$ values in control trees, respectively, n the number of observations, and T_α the 99.5 percentile of a Student's t-distribution with $(n-1)$ degrees of freedom (GEISSER, 1993). The statistical software R was used (R CORE TEAM, 2019).

Foliar $\delta^{15}\text{N}$ values higher than the upper boundary of the prediction interval were considered significantly different from the control population ($P < 0.01$), which indicated that the sampled trees took up the NO_3^- - ^{15}N tracer injected in the same plot. The $\delta^{15}\text{N}$ values of each sampled tree were plotted relative to the horizontal distance between the trunk and the tracer application site and the prediction interval of $\delta^{15}\text{N}$ in natural abundance was shown in each plot.

A covariance analysis (ANCOVA) was made to identify the differences of the effects of species, season, injection depth and horizontal distance from the injection point (up to 6 m), and the interactions between these variables on foliar $\delta^{15}\text{N}$ values, for the three studied species (*Coussarea hydrangeifolia*, *Miconia albicans* and *Xylopia aromatica*).

2.3. RESULTS

Uptake of NO_3^- - ^{15}N by *Xylopia aromatica*, *Coussarea hydrangeifolia* and *Miconia albicans* plants

The analysis of covariance revealed a highly significant ($P < 0.01$) effect of the depth of injection of the NO_3^- - ^{15}N tracer on foliar $\delta^{15}\text{N}$ values (Table 3). The interaction between the depth of tracer injection and the plant species shows that the three species did not take up the NO_3^- - ^{15}N tracer at the same depths.

Table 3 - ANCOVA statistics assessing the effects of species, season, injection depth and horizontal distance from the injection point (up to 6 m) and the interactions on foliar $\delta^{15}\text{N}$ values in the Cerrado area at Bauru for the three studied species (*Coussarea hydrangeifolia*, *Miconia albicans* and *Xylopia aromatica*)

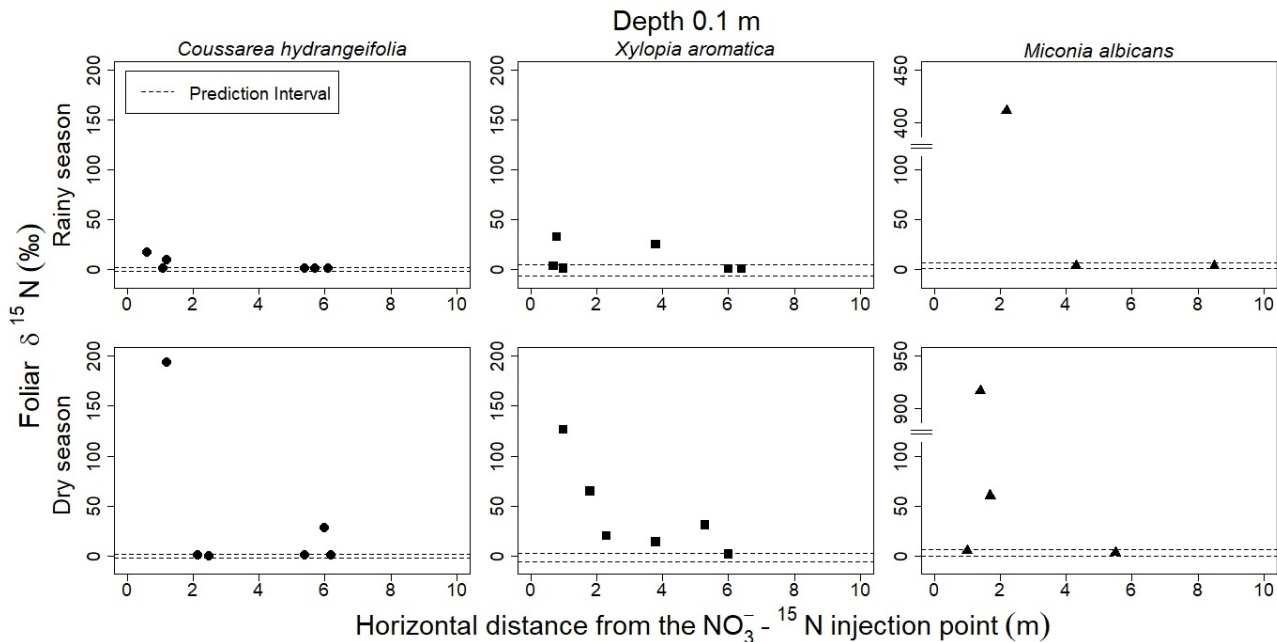
ANCOVA	df	Sum. Sq.	Mean Sq.	F value	Pr (> F)
Species	2	28999	14499	2.556	0.082
Season	1	5212	5212	0.919	0.340
Depth	5	118182	23636	4.167	0.002 **
Distance	1	16696	16696	2.943	0.089
Species x Season	2	1321	661	0.116	0.890
Species x Depth	10	155274	15527	2.737	0.005 **
Species x Distance	2	9402	4701	0.829	0.439
Season x Depth	5	7385	1477	0.260	0.934
Season x Distance	1	4102	4102	0.723	0.397
Depth x Distance	5	38078	7616	1.343	0.252
Species x Season x Depth	10	1725	173	0.030	1.000
Species x Season x Distance	2	152	76	0.013	0.987
Species x Depth x Distance	10	74550	7455	1.314	0.233
Season x Depth x Distance	5	838	168	0.030	1.000
Residuals	104	589951	5673		

** denote a 1% of significance ($p < 0.01$) tested by ANCOVA

Foliar $\delta^{15}\text{N}$ values were not significantly influenced by the season of tracer application. The non-significant species x season interaction shows a similar behavior of the studied species in rainy and in dry seasons. The effects of the species and the distance from the injection site on foliar $\delta^{15}\text{N}$ values were only marginally significant ($P < 0.10$).

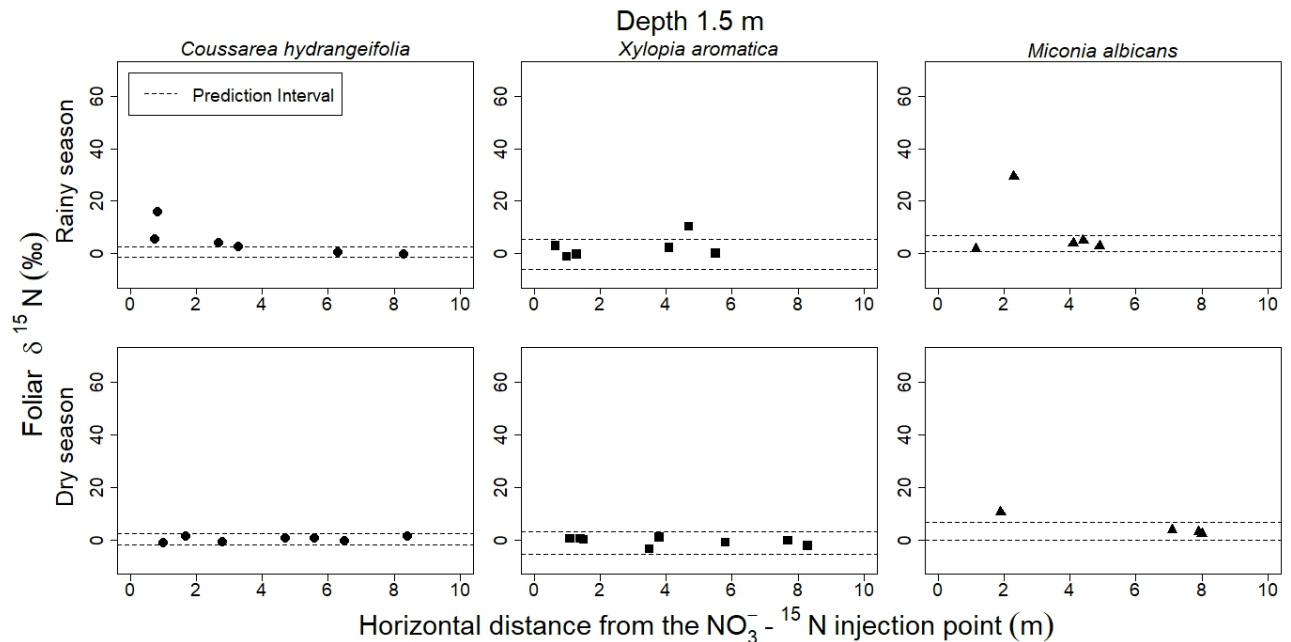
Foliar $\delta^{15}\text{N}$ values showed that the patterns of uptake of the NO_3^- - ^{15}N tracer applied at a depth of 0.1 m were highly variable between individuals of the same species (Fig. 3). At a horizontal distance from the trunk of less than 3 m, the highest foliar $\delta^{15}\text{N}$ value in the rainy season was measured in one *M. albicans* individual (411 ‰, at 2.2 m from the trunk). The two other species also took up the tracer in the rainy season, but foliar $\delta^{15}\text{N}$ values were considerably lower. At distances between 3 m and 6 m from the injection site, the highest foliar $\delta^{15}\text{N}$ value was 25.2 ‰ for one *Xylopia aromatica* tree. Over the dry season, the three studied species took up large amounts of the NO_3^- - ^{15}N tracer applied at a depth of 0.1 m within 3 m from the injection site, with a peak of foliar $\delta^{15}\text{N}$ at 917 ‰ for one *M. albicans* individual (at 1.4 m from the trunk). There was no significant tracer uptake for only two *C. hydrangeifolia* trees at 2.1 and 2.5 m from the injection site and one *M. albicans* plant at 1 m from the injection site. The foliar $\delta^{15}\text{N}$ value of the other *C. hydrangeifolia* tree was 194 ‰ at a distance of 1.2 m from the injection site. Between 3 m and 6 m from the injection site, the highest foliar $\delta^{15}\text{N}$ value was found in one *X. aromatica* individual (30.9 ‰ at a distance of 5.3 m). Large amounts of NO_3^- - ^{15}N were also taken up by another *X. aromatica* tree ($\delta^{15}\text{N} = 13.9$ ‰) and one *C. hydrangeifolia* individual ($\delta^{15}\text{N} = 28.4$ ‰) at 3.8 and 6 m from the injection site, respectively.

Figure 3 - Foliar $\delta^{15}\text{N}$ values in the three studied Cerrado species, 5 months after application of NO_3^- - ^{15}N tracer, at the depth of 0.1 m during rainy and dry seasons. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 8 trees (rainy season) and 12 trees (dry season) of each species sampled at more than 10 m from all the injection sites. The lower and the upper boundaries of the prediction interval calculated were, respectively: -1.3 ‰ and 2.7 ‰ for *C. hydrangeifolia*, -6.0 ‰ and 5.5 ‰ for *X. aromatica*, 0.9 ‰ and 6.8 ‰ for *M. albicans* in the rainy season; and -1.7 ‰ and 2.2 ‰ for *C. hydrangeifolia*, -5.4 ‰ and 3.0 ‰ for *X. aromatica*, -0.1 ‰ and 6.6 ‰ for *M. albicans* in the dry season



At the injection depth of 1.5 m, the uptake of the NO_3^- - ^{15}N tracer led to much lower $\delta^{15}\text{N}$ values than for tracer injection in the topsoil (Fig. 4). As for the injection depth of 0.1 m, the highest $\delta^{15}\text{N}$ value was again in one *M. albicans* individual (29.4 ‰) in the rainy season, at 2.3 m from the injection site. Three *C. hydrangeifolia* trees also took up ^{15}N in lower amounts, two of them with foliar $\delta^{15}\text{N}$ values only 1.5-3 ‰ above the upper boundary of the prediction interval of natural abundance. Once again, at more than 3 m from the injection site (at 4.7 m), the highest foliar $\delta^{15}\text{N}$ value (10.3 ‰) was measured in one *X. aromatica* individual. During the dry season, the highest $\delta^{15}\text{N}$ value was measured in one *M. albicans* individual (10.4 ‰) placed at 1.9 m from the injection site (Fig. 4).

Figure 4 - Foliar $\delta^{15}\text{N}$ values in the three studied Cerrado species, 5 months after application of NO_3^- - ^{15}N tracer, at the depth of 1.5 m during rainy and dry seasons. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 8 trees (rainy season) and 12 trees (dry season) of each species sampled at more than 10 m from all the injection sites. The lower and the upper boundaries of the prediction interval calculated were, respectively: -1.3 ‰ and 2.7 ‰ for *C. hydrangeifolia*, -6.0 ‰ and 5.5 ‰ for *X. aromatica*, 0.9 ‰ and 6.8 ‰ for *M. albicans* in the rainy season; and -1.7 ‰ and 2.2 ‰ for *C. hydrangeifolia*, -5.4 ‰ and 3.0 ‰ for *X. aromatica*, -0.1 ‰ and 6.6 ‰ for *M. albicans* in the dry season

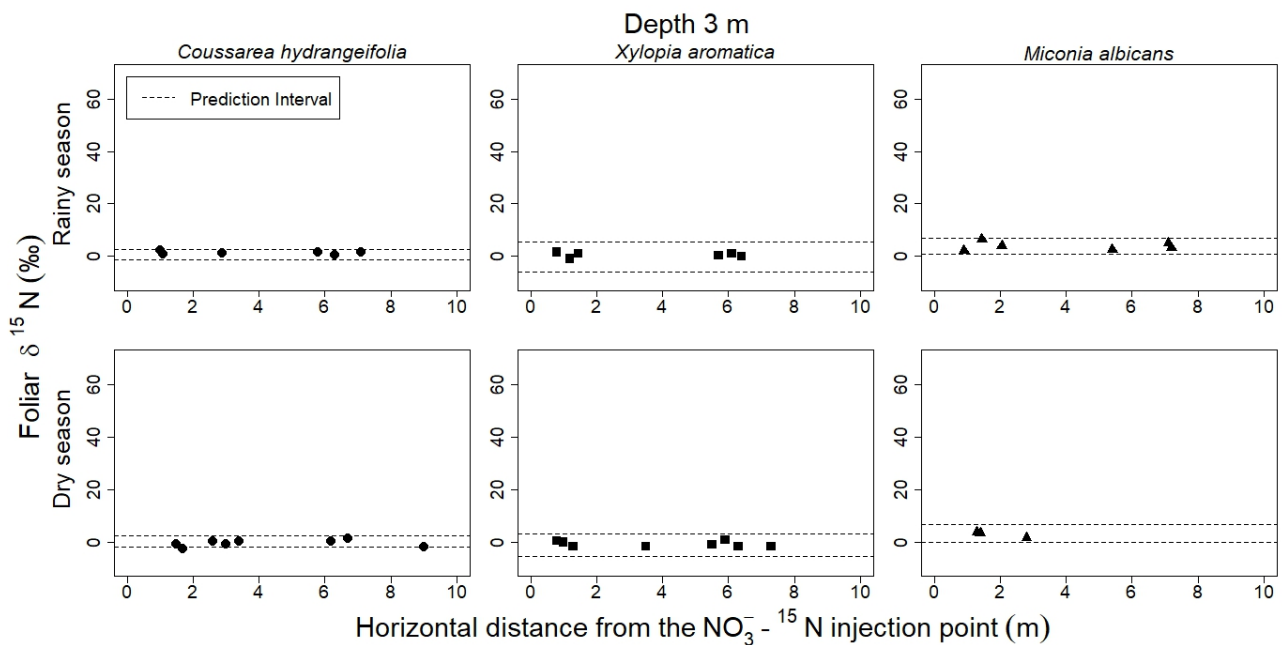


When the NO_3^- - ^{15}N tracer was injected at a depth of 3 m, all the foliar $\delta^{15}\text{N}$ values were within the prediction interval of natural abundance, whatever the species and the season (Fig. 5). The highest foliar $\delta^{15}\text{N}$ value in the rainy season was only 6.4 ‰ for a *M. albicans* individual sampled at 1.5 m from the injection site.

Contrary to the injection depth of 3 m, large amounts of the NO_3^- - ^{15}N tracer injected at a depth of 6 m were taken up by some plants during both seasons (Fig. 6). In the rainy season, a high foliar $\delta^{15}\text{N}$ value (17.1 ‰) was measured in one *X. aromatica* individual at 0.8 m from the injection site, more than 10 ‰ above the upper boundary of the prediction interval of natural abundance. During the dry season, two *X. aromatica* individuals took up large amounts of the NO_3^- - ^{15}N tracer applied at a depth of 6 m, within 2 m from the injection site, with a peak of foliar $\delta^{15}\text{N}$ at 66.7 ‰ (Fig. 6).

Significant enrichment in ^{15}N was not detected in leaves sampled at more than 2 m from the tracer injection site.

Figure 5 - Foliar $\delta^{15}\text{N}$ values in the three studied Cerrado species, 5 months after application of NO_3^- - ^{15}N tracer, at the depth of 3 m during rainy and dry seasons. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 8 trees (rainy season) and 12 trees (dry season) of each species sampled at more than 10 m from all the injection sites. The lower and the upper boundaries of the prediction interval calculated were, respectively: -1.3 ‰ and 2.7 ‰ for *C. hydrangeifolia*, -6.0 ‰ and 5.5 ‰ for *X. aromatica*, 0.9 ‰ and 6.8 ‰ for *M. albicans* in the rainy season; and -1.7 ‰ and 2.2 ‰ for *C. hydrangeifolia*, -5.4 ‰ and 3.0 ‰ for *X. aromatica*, -0.1 ‰ and 6.6 ‰ for *M. albicans* in the dry season



Whatever the species and the season, foliar $\delta^{15}\text{N}$ values were not highly enriched in ^{15}N when the NO_3^- - ^{15}N tracer was applied at the depths of 9 m and 12 m. Nevertheless, the foliar $\delta^{15}\text{N}$ value (6.2 ‰) in one *X. aromatica* individual at 2.4 m from the injection site in the rainy season was 0.7 ‰ above the upper boundary of prediction interval at a depth of 9 m (Fig. 7). This suggests that a small amount of labelled-nitrate was taken up, consistently with the strong foliar enrichment in ^{15}N for the same species when the tracer was injected at a depth of 6 m. Foliar $\delta^{15}\text{N}$ values for all the sampled trees were within the prediction interval of natural abundance in the plots where the NO_3^- - ^{15}N tracer was injected at a depth of 12 m.

Figure 6 - Foliar $\delta^{15}\text{N}$ values in the three studied Cerrado species, 5 months after application of $\text{NO}_3^- -^{15}\text{N}$ tracer, at the depth of 6 m during rainy and dry seasons. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 8 trees (rainy season) and 12 trees (dry season) of each species sampled at more than 10 m from all the injection sites. The lower and the upper boundaries of the prediction interval calculated were, respectively: -1.3 ‰ and 2.7 ‰ for *C. hydrangeifolia*, -6.0 ‰ and 5.5 ‰ for *X. aromatica*, 0.9 ‰ and 6.8 ‰ for *M. albicans* in the rainy season; and -1.7 ‰ and 2.2 ‰ for *C. hydrangeifolia*, -5.4 ‰ and 3.0 ‰ for *X. aromatica*, -0.1 ‰ and 6.6 ‰ for *M. albicans* in the dry season

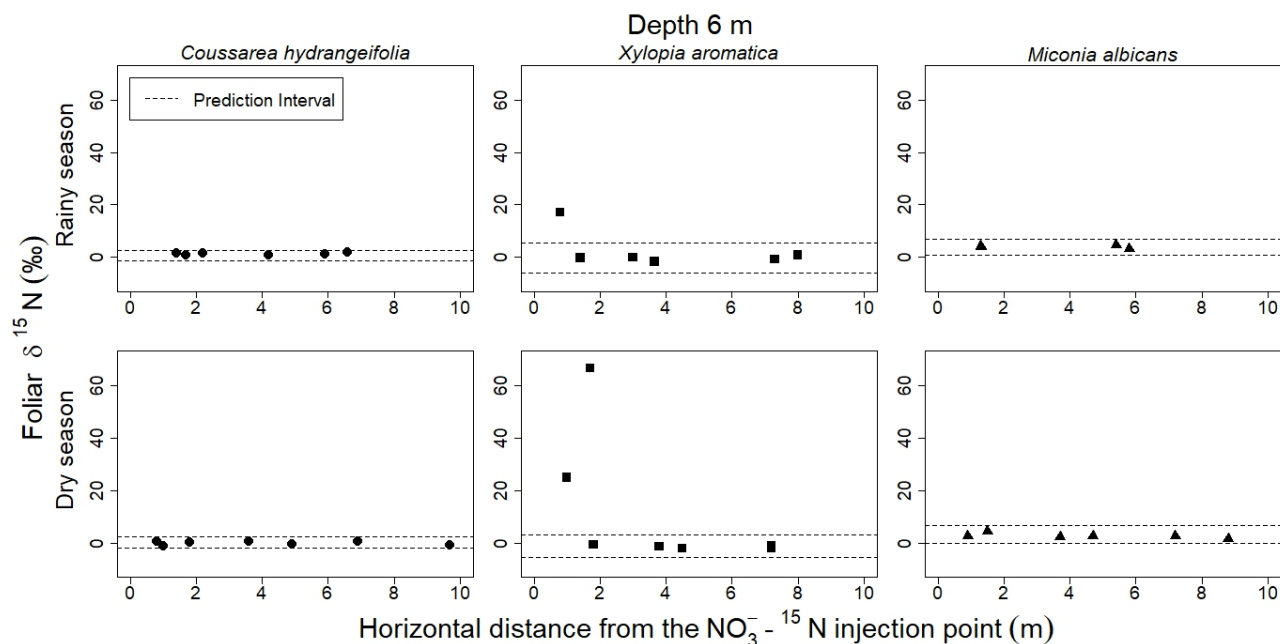
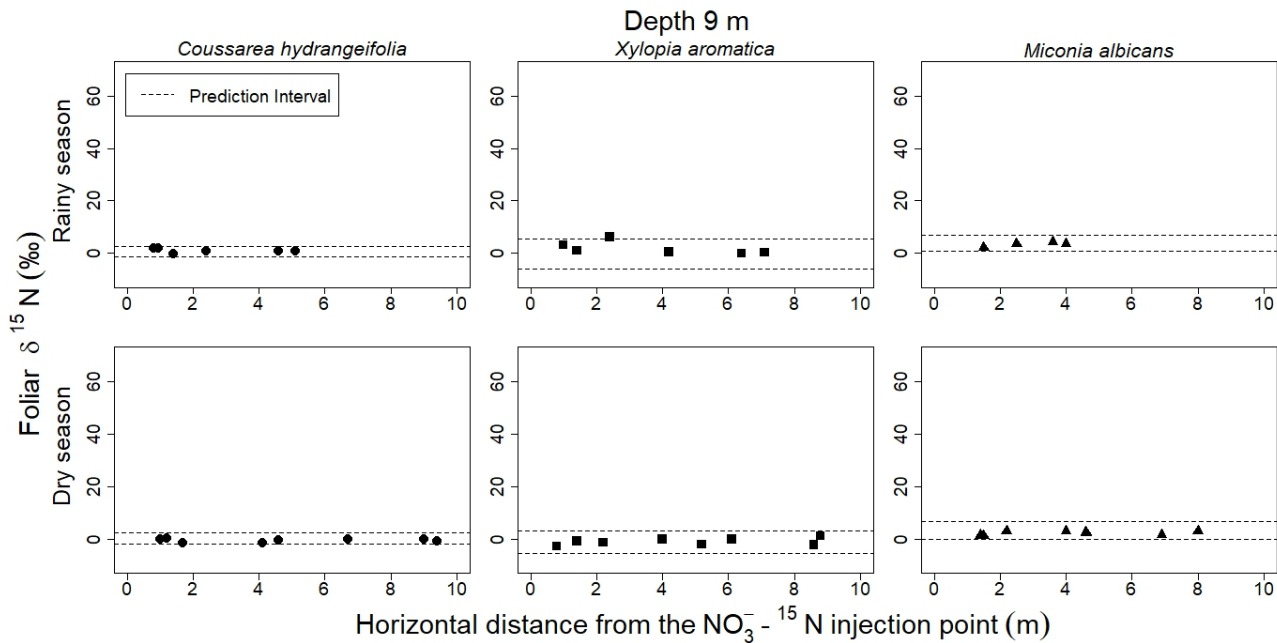


Figure 7 - Foliar $\delta^{15}\text{N}$ values in the three studied Cerrado species, 5 months after application of NO_3^- - ^{15}N tracer, at the depth of 9 m during rainy and dry seasons. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 8 trees (rainy season) and 12 trees (dry season) of each species sampled at more than 10 m from all the injection sites. The lower and the upper boundaries of the prediction interval calculated were, respectively: -1.3 ‰ and 2.7 ‰ for *C. hydrangeifolia*, -6.0 ‰ and 5.5 ‰ for *X. aromatica*, 0.9 ‰ and 6.8 ‰ for *M. albicans* in the rainy season; and -1.7 ‰ and 2.2 ‰ for *C. hydrangeifolia*, -5.4 ‰ and 3.0 ‰ for *X. aromatica*, -0.1 ‰ and 6.6 ‰ for *M. albicans* in the dry season

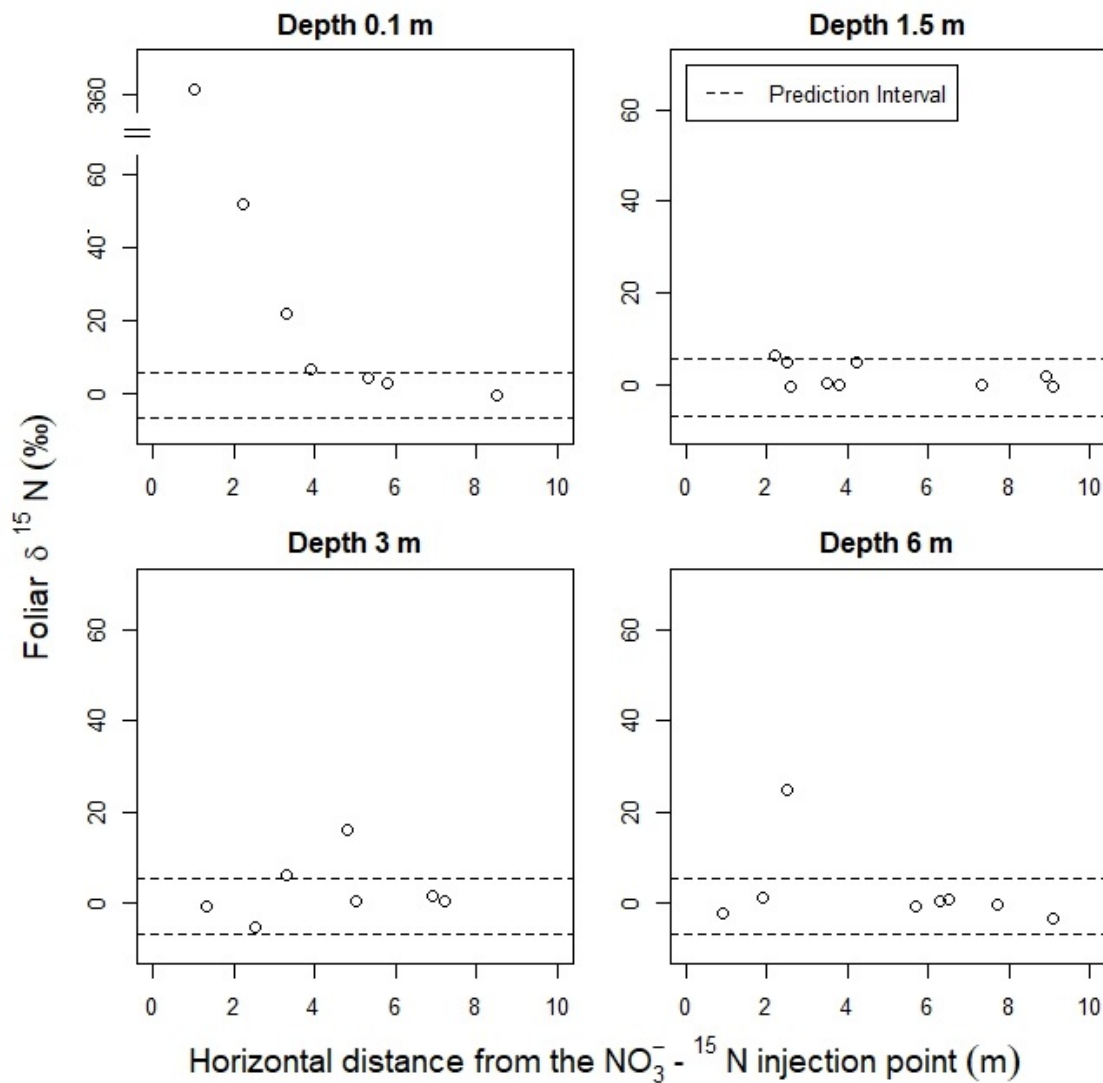


NO_3^- - ^{15}N uptake of large trees during the dry season

Whatever the depth of injection of the NO_3^- - ^{15}N tracer, we did not observe a clear relationship between the DBH of the trees (whatever the species) close to the injection site (within 4 m) and the uptake of ^{15}N at the end of the dry season (Fig. 8). When the NO_3^- - ^{15}N tracer was applied in the topsoil, the largest trees sampled at more than 3.5 m from the injection site did not exhibit high foliar $\delta^{15}\text{N}$ values (maximum of 6.4 ‰ for one tree at 4 m from the injection site). The peak of foliar $\delta^{15}\text{N}$ was 362 ‰ in one big tree at 1 m from the injection site (Fig. 9A).

NO_3^- - ^{15}N tracer (i.e. 9 and 12 m), foliar $\delta^{15}\text{N}$ values were within the prediction interval of natural abundance.

Figure 9 - Foliar $\delta^{15}\text{N}$ values in large trees 5 months after application of the NO_3^- - ^{15}N tracer at the depths of 0.1 m (A), 1.5 m (B), 3 m (C) and 6 m (D), during the dry season. The prediction intervals ($P < 0.01$) were calculated from $\delta^{15}\text{N}$ values in natural abundance measured for 12 large trees of several species (unidentified) sampled at more than 10 m from all the injection sites, irrespective of the depth of tracer injection. The lower and the upper boundaries of the prediction interval were - 6.9 ‰ and 5.4 ‰, respectively



2.4. DISCUSSION

Natural abundance of ^{15}N and methodological issues

Foliar $\delta^{15}\text{N}$ values in natural abundance in our study are within the range reported in other Brazilian Cerrado ecosystems. Foliar $\delta^{15}\text{N}$ values in natural abundance ranged from -5 to 7.9 ‰ in 45 different plant species in a Cerrado ecosystem in central Brazil (BUSTAMANTE et al., 2004), and from -2.5 to 4.5 ‰ in 22 species of another Cerrado ecosystem in the São Paulo state (COLETTA et al., 2009). In our study, the highest foliar $\delta^{15}\text{N}$ values in natural abundance were determined in the shrub species in both seasons (*Miconia albicans*), with values ranging from 1.8 to 5.5 ‰, whilst foliar $\delta^{15}\text{N}$ values were between -3.1 and 1.6 ‰ in both tree species. Similarly, foliar $\delta^{15}\text{N}$ values in natural abundance were significantly higher for shrub leaves than for tree leaves in a Cerrado ecosystem in central Brazil (BUSTAMANTE et al., 2004). However, foliar $\delta^{15}\text{N}$ values in natural abundance were uncorrelated with plant height in another Cerrado (COLETTA et al., 2009). In general, the $\delta^{15}\text{N}$ values of plants in native ecosystems range from -5 to +8 ‰ depending on soil conditions (EHLERINGER, 2017). While some studies compiling global data suggest that tropical forest ecosystems are enriched in ^{15}N relative to temperate forest ecosystems (MARTINELLI et al., 1999), the contrary has also been reported (SHENG et al., 2014).

Tracers have been injected in specific soil zones for several decades to gain insight into the areas of water and nutrient uptake by plant roots (LEHMANN, 2003). However, studies using tracers are scarce in tropical ecosystems and, as far as we are aware, tracers have never been injected as deeply as in our study. Although plants in Cerrado ecosystems are known to explore very deep soil layers, fine root densities are very low below a depth of 1 m (ABDALA et al., 1998; CASTRO AND KAUFFMAN, 1998; LILIENFEIN et al., 2001; OLIVEIRA et al., 2005). The probability that the plants sampled in our plots had roots exploring the soil zone exactly at the injection site was low, which probably explains why many plants near the centre of the plots did not show a marked uptake of labelled nitrate.

In our study, the ability to detect the uptake of the tracer by plant roots was dependent on the accuracy of the determination of foliar $\delta^{15}\text{N}$ values in natural abundance. The consistency of the determinations of foliar $\delta^{15}\text{N}$ values between individuals of each species made it possible to establish narrow prediction intervals

and therefore to detect small amounts of nitrate taken up by plant roots. Although fine roots were sampled at a depth of 12 m, no significant ^{15}N foliar enrichment was observed in our study for any plant sampled during wet and dry seasons. A comprehensive study combining eddy covariance measurements and ecophysiological modeling in eucalypt plantations in a neighboring area showed that even though fine roots were found at depths of more than 10 m, they took up water only when the soil was dry in the upper soil layers (CHRISTINA et al., 2017). The lack of detection of tracer uptake at the depths of 9 and 12 m in our study could result from numerous factors, in particular: exclusion in our sampling design of the plant species with the deepest roots in this ecosystem, and/or amounts of labelled nitrate absorbed too low to be detected in the foliage, and/or a period not dry enough to require the absorption of very deep water by plant roots. Another explanation might be that soil water contents in deep soil layers were too low when the tracer was injected at the beginning of the dry season to make possible the diffusion of nitrate ions to the roots. Indeed, large changes in soil water contents between the two dates of tracer injection suggest that tree roots had already withdrawn large amounts of water between the depths of 4 m and 10 m when the tracer was injected in June 2017 (Appendix C). Nitrates are mobile ions in soil solution and a previous study close to our study site showed that labelled nitrates were not taken up by trees in deep soil layers when soil water contents were low. An enrichment in ^{15}N in tree leaves occurred only when gravitational water reached deep soil layers at the beginning of the rainy season (DA SILVA et al., 2011).

Niche complementarity between the three studied species to take up soil resources

In agreement with our first hypothesis, foliar ^{15}N values indicate a niche complementarity for the uptake of labelled nitrate between the three studied species. *Xylopia aromatica* trees exploit a considerably larger soil volume than the other species, with high foliar $\delta^{15}\text{N}$ values showing uptake of labelled nitrate injected at the depths of 0.1 and 6 m in both seasons, as well as at a depth of 1.5 m in the rainy season. The behaviour of the other two species is different, with a clear uptake of the ^{15}N tracer only in the upper soil layers, down to a depth of 1.5 m.

Partitioning of resource use among plant species has been shown in other native forest ecosystems. A review on hydrological niches in plant communities

ranging from dry to wet environments suggests that niche segregation is widespread, occurring in 43 of the 48 studies considered in the review (SILVERTOWN et al., 2015). Kulmatiski and Beard (2013) showed that niche partitioning increased when the availability of resources decreased and highlighted that niche partitioning can occur on short time and spatial scales. Handley and Scrimgeour (1997) showed that the strategies of N acquisition of plant taxa in natural mixed stands can differ chemically (with the ability to take up different N forms, with or without microbial associations), spatially (depending on root architecture, exploitation of spatially heterogeneous sources, storage and resorption of N, and activity of assimilative enzymes) and temporally (depending on plant demand for both external and internal N). In an African semiarid environment, deuterium measurements showed a sharing of water resources between shrubs and trees in the upper meter of the soil, while the roots of the trees had access to deeper soil layers (BEYER et al., 2016). Other studies in forest ecosystems have shown that different tree species can take up water and/or nutrients in different soil layers (JACKSON et al., 1999; OLIVEIRA et al., 2005; SILVERTOWN et al., 2015).

While *X. aromatica* trees take up nitrate within 5 m of horizontal distance from the injection site, the uptake was only detected within 2 m for the two other species. A single *C. hydrangeifolia* tree showed a specific behaviour with the uptake of NO_3^- - ^{15}N at a distance of 6 m from the injection site in the dry season when the tracer was injected at a depth of 0.1 m. The horizontal distance of tracer uptake in our Cerrado ecosystem is consistent with studies in other environments. Göttlicher et al. (2008) showed that the lateral root spread of trees in monospecific boreal forests was 4–5 m on average. However, the ^{15}N tracer was not taken up by all trees close to the injection site, suggesting a high asymmetry of the root system as in our study. In an Amazonian forest, while high deuterium concentrations were measured in the leaves for some species at about 10 m of horizontal distance from the injection site, most of the small trees sampled (DBH from 2.5 to 22.0 cm) took up the deuterium tracer close to the trunk (STERNBERG et al., 2002). In a semiarid environment, Beyer et al. (2016) showed that the *Combretum collinum* tree species was able to take up the deuterium tracer at a distance of 6 m from the application site.

Contrary to our second hypothesis, the uptake of labelled nitrate was not significantly influenced by the season for the three studied species. While we expected an uptake of large amounts of labelled nitrate at the depths of 9 m and 12 m during dry

periods, we did not detect a clear enrichment in foliar ^{15}N in the three studied species as well as in large trees sampled close to the injection zones. However, the simple method used here to detect water and nutrient uptake through the injection of nitrates in the soil (that need the uptake of water to reach roots) is probably less sensitive than isotopic methods based on ^2H and ^{18}O measurements to detect small amounts of water taken up by plants between the dates of tracer injection and leaf sampling.

Relationship between the size of the plants and the depth of nitrate uptake

Contrary to our third hypothesis, we did not observe a clear relationship between the size of the trees and the depth of NO_3^- - ^{15}N uptake. The largest trees sampled in dry season (irrespective of the species) did not take up detectable amounts of the NO_3^- - ^{15}N tracer at the depths of 9 and 12 m, although some fine roots (with a very low density) were sampled (Appendix D). This pattern does not demonstrate that water was not taken up at these depths but suggests that large amounts of labelled nitrate were not taken up by neighboring large trees. Two decades ago, by comparing the stable hydrogen isotope composition (δD) of xylem sap and soil water at different depths down to 4 m over two consecutive dry seasons in a savanna (cerrado) in central Brazil, Jackson et al. (1999) pointed out a specific division of water resources between 10 representative woody species. In this study, deciduous species had more negative xylem sap δD values than evergreen species, which showed that deciduous species preferentially tapped deeper sources of soil water than evergreen species. As in our study for the uptake of labelled nitrate, the size of the plant did not have a marked influence on the partitioning of water in the soil among the woody species in this Cerrado.

An interesting dual-isotope labelling approach (applying deuterium in surface soil and oxygen at 120-cm depth) in an Amazon forest, did not find a strong relationship between tree dimensions and depth of water uptake (STAHL et al., 2013). While tall trees preferentially extract water from layers below 100-cm depth, shorter trees show broad variations in mean depth of water uptake.

Although we did not find a significant relationship between plant size and depth of ^{15}N uptake, only trees with a DBH greater than 5 cm in our study were able to take up the tracer at more than 3 m from the bottom of the trunk, horizontally and vertically (except for a single *C. hydrangeifolia* individual when the NO_3^- - ^{15}N tracer was injected in the topsoil). A large tree took up the NO_3^- - ^{15}N tracer applied at a depth of 3 m, at a

horizontal distance of 5 m from the injection site. However, our methodology was probably not well suited to study the relationship between the depth of nitrate uptake and the size of the trees because the probability to sample leaves of trees with roots exploring exactly the area of tracer injection was low. Studies based on the natural abundance of stable isotopes are better suited to test this hypothesis. A comprehensive study sampling stable isotopes of water ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) in tree xylem and down to a depth of 10 m in the soil in a seasonal Amazon forest showed that coexisting trees were widely segregated along a single axis of a hydrological niche defined by differences in root depth, light access and tolerance to low water potential. In this forest, the differences in rooting depth of 12 dominant tree species were strongly related to tree size (BRUM et al., 2018), and it would be interesting to study whether the same behaviour exists in Brazilian Cerrado.

2.5. CONCLUSION

The labelled nitrate was taken up at different depths and distances from the trunk depending on the species in the Brazilian savanna studied, which showed a niche complementarity throughout the soil profile down to a depth of at least 6 m. Even though we did not observe a clear relationship between the size of the plants and the depth of uptake of labelled nitrate, only trees with a DBH of more than 5 cm were able to take up the NO_3^- - ^{15}N tracer injected at depths of more than 1.5 m. Unexpectedly, trees did not take up the labelled nitrates more deeply in the dry season than in the rainy season.

Further studies dealing with nutrient and water uptake in very deep soil layers are needed to improve modeling approaches that intend to predict the response of tropical savannas to climate change. Improving our understanding of the functional ecology of tropical savannas such as the Brazilian Cerrado is essential for their preservation in the context of global change.

Acknowledgements

This work was supported by the São Paulo Research Foundation (FAPESP, project 2015/25946-0). We are grateful to the staff at the Jardim Botânico Municipal de Bauru

(http://www.bauru.sp.gov.br/semma/unidades_ambientais/jardim_botanico.aspx),

which provides the study area and field aid, and also to Eder Araujo da Silva (<http://www.floragroapoio.com.br>) for their technical support. We also acknowledge the Stable Isotopes Centre from the Institute of Biosciences of Botucatu (Unesp).

REFERENCES

- ABDALA, G. C. et al. Above and belowground organic matter and root:shoot ratio in a cerrado in central Brazil. **Brazilian Journal of Ecology**, v. 2, p. 11-23, 1998.
- ALVARES, C. A. et al. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, n. 6, p. 711-728, 2013. <https://doi.org/10.1127/0941-2948/2013/0507>
- BERRY, Z. C. et al. The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward. **Ecohydrology**, v. 11, n. 3, e1843, 2017. <https://doi.org/10.1002/eco.1843>
- BEYER, M. et al. A deuterium-based labelling technique for the investigation of rooting depths, water uptake dynamics and unsaturated zone water transport in semiarid environments. **Journal of Hydrology**, v. 533, p. 627–643, 2016. <https://doi.org/10.1016/j.jhydrol.2015.12.037>
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Serviço Florestal Brasileiro. **Florestas do Brasil em resumo**. Brasília, DF: MAPA/SFB, 2019. 207 p.
- BRUM, M. et al. Hydrological niche segregation defines forest structure and drought tolerance strategies in a seasonal Amazon forest. **Journal of Ecology**, v. 107, p. 318–333, 2018. <https://doi.org/10.1111/1365-2745.13022>
- BUSTAMANTE, M. M. C. et al. ^{15}N in natural abundance in woody plants and soils of central Brazilian savannas (Cerrado). **Ecological Applications**, v. 14, n. 4, p. 200–213, 2004. <https://doi.org/10.1890/01-6013>
- CANADELL, J. et al. Maximum rooting depth of vegetation types at the global scale. **Oecologia**, v. 108, p. 583–595, 1996. <https://doi.org/10.1007/BF00329030>
- CASTRO, E. A.; KAUFFMAN, J. B. Ecosystem structure in the Brazilian Cerrado: a vegetation gradient of aboveground biomass, root mass and consumption by fire. **Journal of Tropical Ecology**, v. 14, p. 263-283, 1998. <https://doi.org/10.1017/S0266467498000212>
- CHRISTINA, M. et al. Importance of deep water uptake in tropical eucalypt forest. **Functional Ecology**, v. 31, p. 509–519, 2017. <https://doi.org/10.1111/1365-2435.12727>
- COLETTA, L. D. et al. Isotopic view of vegetation and carbon and nitrogen cycles in a Cerrado ecosystem, Southeastern Brazil. **Scientia Agricola**, v. 66, p. 467-475, 2009. <https://doi.org/10.1590/S0103-90162009000400006>
- COPLEN, T. B. Guidelines and recommended terms for the expression of stable-isotope-ratio and gas-ratio measurement results. **Rapid Communications in Mass Spectrometry**, v. 25, n. 17, p. 2538-2560, 2011. <http://doi.org/10.1002/rcm.5129>
- DA SILVA, E. V. et al. Functional specialization of *Eucalyptus* fine roots: contrasting potential uptake rates for nitrogen, potassium and calcium tracers at varying soil

depths. **Functional Ecology**, v. 25, p. 996-1006, 2011.

<http://dx.doi.org/10.1111/j.1365-2435.2011.01867.x>

DURIGAN, G. et al. A vegetação dos remanescentes de cerrado no Estado de São Paulo. In: BITTENCOURT, M. D.; MENDONÇA, R. R. (Orgs.). **Viabilidade de conservação dos remanescentes de Cerrado no Estado de São Paulo**. São Paulo: Annablume e FAPESP, 2004. p. 29-56.

EHLERINGER, J.R. Interpreting Stable Isotopes Ratios in Plants and Plant-based Foods. In: CARTER, J. F.; CHESSON, L. A. (Eds.). **Food Forensics – Stable Isotopes as a Guide to Authenticity and Origin**. 1. ed. Boca Raton, FL: CRC Press, 2017. p. 46-62.

FORRESTER, D. I. The spatial and temporal dynamics of species interactions in mixed-species forests: From pattern to process. **Forest Ecology and Management**, v. 312, p. 282–292, 2014. <https://doi:10.1016/j.foreco.2013.10.003>

FRANCO, A. C. Ecophysiology of woody plants. In: OLIVEIRA, P. S.; MARQUIS, R. J. (Eds.). **The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna**. New York, USA: Columbia University Press, 2002. p. 178–197.

FRANCO, A. C. Biodiversidade de forma e função: implicações ecofisiológicas das estratégias de utilização de água e luz em plantas lenhosas do Cerrado. In: SCARIOT, A.; SOUSA-SILVA, J. C.; FELFILI, J. M. (Orgs.). **Cerrado: Ecologia, Biodiversidade e Conservação**. Brasília: Ministério do Meio Ambiente, 2005. p. 179-196.

GEISSER, S. **Predictive Inference: An Introduction, Monographs on Statistics and Applied Probability**. CRC Press, 1993.

GOODLAND, R.; FERRI, M. G. **Ecologia do Cerrado**. São Paulo: Editora da Universidade de São Paulo – Edusp, 1979. 193 p.

GÖTTLICHER, S. G. et al. The lateral spread of tree root systems in boreal forests: estimates based on ^{15}N uptake and distribution of sporocarps of ectomycorrhizal fungi. **Forest Ecology and Management**, v. 255, p. 75–81, 2008.

<https://doi.org/10.1016/j.foreco.2007.08.032>

HANDLEY, L. L.; SCRIMGEOUR, C. M. Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish Old Field. **Advances in Ecological Research**, v. 27, p. 133–212, 1997. [https://doi.org/10.1016/S0065-2504\(08\)60008-2](https://doi.org/10.1016/S0065-2504(08)60008-2)

HOEKSTRA, N. J.; FINN, J. A.; LÜSCHER, A. The effect of drought and interspecific interactions on the depth of water uptake in deep-and shallow-rooting grassland species as determined by $\delta^{18}\text{O}$ natural abundance. **Biogeosciences**, v.11, p. 4493-4506, 2014. <https://doi.org/10.5194/bg-11-4493-2014>

HOEKSTRA, N. J. et al. Do belowground vertical niche differences between deep- and shallow-rooted species enhance resource uptake and drought resistance in

grassland mixtures? **Plant Soil**, v. 394, p. 21-34, 2015.

<https://doi.org/10.1007/s11104-014-2352-x>

JACKSON, P. C. et al. Partitioning of soil water among tree species in a Brazilian Cerrado ecosystem. **Tree Physiology**, v. 19, p. 717-724, 1999.

<https://doi.org/10.1093/treephys/19.11.717>

KULMATISKI, A.; BEARD, K. H. Root niche partitioning among grasses, saplings, and trees measured using a tracer technique. **Oecologia**, v. 171, n. 1, p. 25-37, 2013. <https://doi.org/10.1007/s00442-012-2390-0>

LAHSEN, M.; BUSTAMANTE, M. M. C.; DALLA-NORA, E. L. Undervaluing and Overexploiting the Brazilian Cerrado at Our Peril. **Environment: Science and Policy for Sustainable Development**, v. 58, n. 6, p. 4-15, 2016.

<https://doi.org/10.1080/00139157.2016.1229537>

LEHMANN, J. Subsoil root activity in tree-based cropping systems. **Plant and Soil**, v. 255, p. 319–331, 2003. https://doi.org/10.1007/978-94-017-2923-9_31

LILIENFEIN, J. et al. Nutrient storage in soil and biomass of native Brazilian Cerrado. **Journal of Plant Nutrition and Soil Science**, v. 164, p. 487–495, 2001.

[https://doi.org/10.1002/1522-2624\(200110\)164:5<487::AID-JPLN487>3.0.CO;2-I](https://doi.org/10.1002/1522-2624(200110)164:5<487::AID-JPLN487>3.0.CO;2-I)

LORENZI, H. **Árvores brasileiras**: manual de identificação e cultivo de plantas arbóreas do Brasil v. 1, 5 ed., Nova Odessa, SP: Instituto Plantarum, 2008.

LÜSCHER, A. et al. Potential of legume-based grassland-livestock systems in Europe: a review. **Grass and Forage Science**, v. 69, p. 206-228, 2014.

<https://doi.org/10.1111/gfs.12124>

MAEGHT, J. L.; REWALD, B.; PIERRET, A. How to study deep roots – and why it matters. **Frontiers in Plant Science**, v. 4, n. 299,

2013. <https://doi.org/10.3389/fpls.2013.00299>

MARTINELLI, L. A. et al. Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. **Biogeochemistry**, v. 46, p. 45–65, 1999.

<https://doi.org/10.1007/BF01007573>

MARTINS, A. B. et al. O gênero *Miconia* Ruiz & Pav. (Melastomataceae) no Estado de São Paulo. **Acta Botanica Brasilica**, v. 10 n. 2, p. 267-316, 1996.

<http://dx.doi.org/10.1590/S0102-33061996000200005>

MATTOS, E. A.; REINERT, F.; MORAES, J. A. P. V. Comparison of carbon isotope discrimination and CO₂ and H₂O gas exchange between the dry and wet season in leaves of several wood species. **Revista Brasileira de Fisiologia Vegetal**, v. 9, n. 2, p. 77-82, 1997.

MYERS, N. et al. Biodiversity Hotspots for Conservation Priorities. **Nature**, v. 403, n. 6772, p. 853–58, 2000. <https://doi.org/10.1038/35002501>

OLIVEIRA, R. S. et al. Deep root function in soil water dynamics in cerrado savannas of central Brazil. **Functional Ecology**, v. 19, p. 574-581, 2005.

<https://doi.org/10.1111/j.1365-2435.2005.01003.x>

PAQUETTE, A.; MESSIER, C. The effect of biodiversity on tree productivity: from temperate to boreal forests. **Global Ecology and Biogeography**, v. 20, n. 1, p. 170-180, 2011. <https://doi.org/10.1111/j.1466-8238.2010.00592.x>

PINHEIRO, R. C. et al. Distance from the trunk and depth of uptake of labelled nitrate for dominant and suppressed trees in Brazilian *Eucalyptus* plantations:

Consequences for fertilization practices. **Forest Ecology and Management**, v. 447, p. 95-104, 2019. <https://doi.org/10.1016/j.foreco.2019.05.011>

POSZWA, A. et al. Isotopic approach to trace water and nutrient dynamics in forest soils. **Revue Forestière Française**, v. 66, 2014. <https://doi.org/10.4267/2042/56265>

R CORE TEAM. **R: A language and environment for statistical computing**. R

Foundation for Statistical Computing, Vienna, Austria, 2019. URL <http://www.R-project.org/>

RODRIGUES, T. R. et al. Seasonal variation in energy balance and canopy conductance for a tropical savanna ecosystem of south central Mato Grosso, Brazil.

Journal of Geophysical Research: Biogeosciences, v. 119, p. 1-13, 2014.

<http://dx.doi.org/10.1002/2013JG002472>

SHARP, R.; DAVIES, W. Root growth and water uptake by maize plants in drying soil. **Journal of Experimental Botany**, v. 36, p. 1441-1456, 1985.

<https://doi.org/10.1093/jxb/36.9.1441>

SHENG, W. et al. Regional patterns of ¹⁵N natural abundance in forest ecosystems along a large transect in eastern China. **Nature Scientific Reports**, v. 4, p. 1-6, 2014.

<http://dx.doi.org/10.1038/srep04249>

SILVERTOWN, J.; ARAYA, Y.; GOWING, D. Hydrological niches in terrestrial plant communities: a review. **Journal of Ecology**, v. 103, p. 93-108, 2015.

<http://dx.doi.org/10.1111/1365-2745.12332>

STAHL, C., et al. Depth of soil water uptake by tropical rainforest trees during dry periods: does tree dimension matter? **Oecologia**, v. 173, p. 1191-1201, 2013.

<https://doi.org/10.1007/s00442-013-2724-6>

STERNBERG, L. S. L.; MOREIRA, M. Z.; NEPSTAD, D. C. Uptake of water by lateral roots of small trees in an Amazonian Tropical Forest. **Plant and Soil**, v. 238, n. 1, p. 151-158, 2002.

<https://doi.org/10.1023/A:1014214404699>

WEISER, V. L. **Trees, shrubs and climbers of the cerrado of the Bauru**

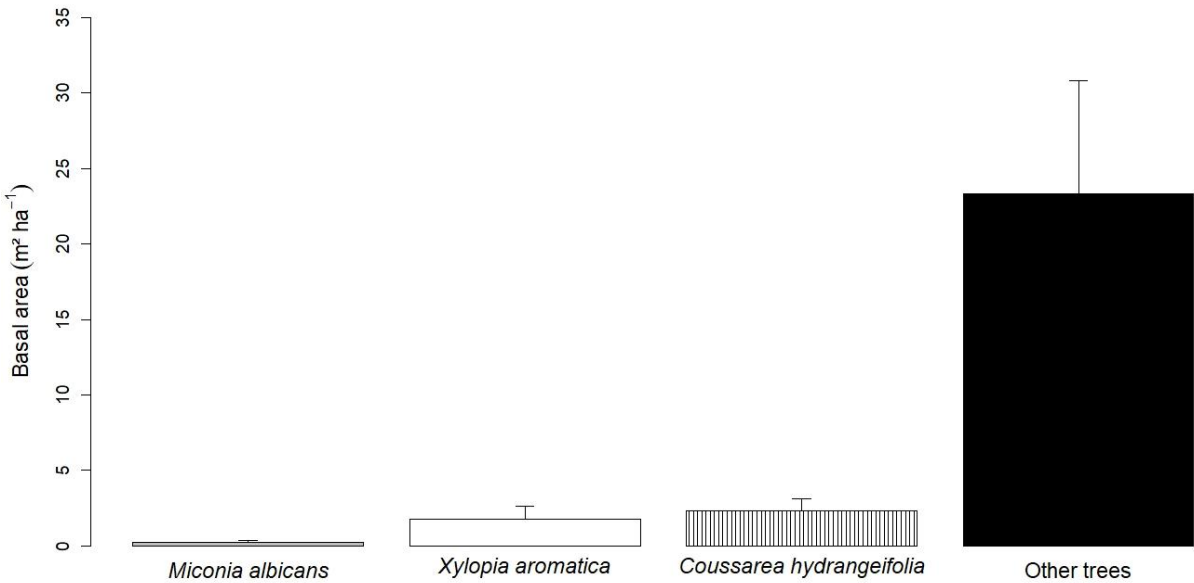
Botanical Garden, SP. 2007. 111 p. Thesis (PhD in Ecology) - Institute of Biology, University of Campinas (Unicamp), Campinas, 2007.

APPENDIXES

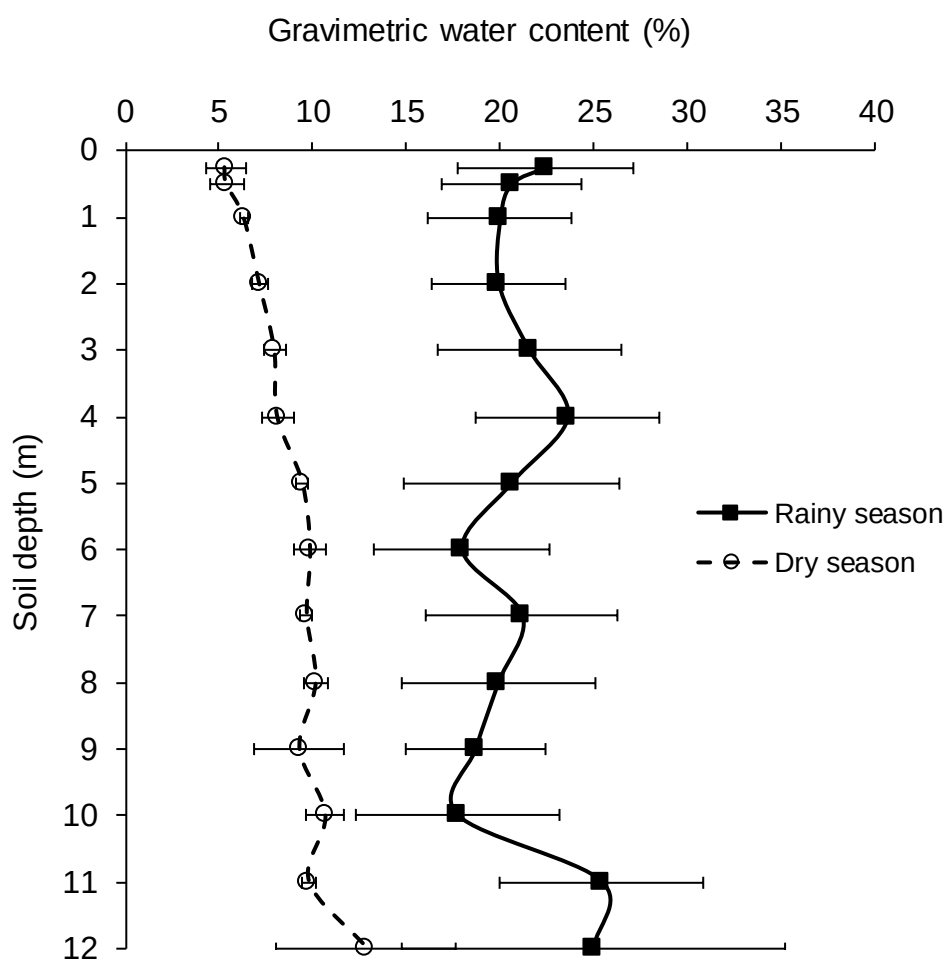
Appendix A - Overall view of the three-plant species sampled: (A) *Xylopia aromatica* (Lam.) Mart.; (B) *Coussarea hydrangeifolia* (Benth.) Müll. Arg.; and (C) *Miconia albicans* (Sw.) Steud.



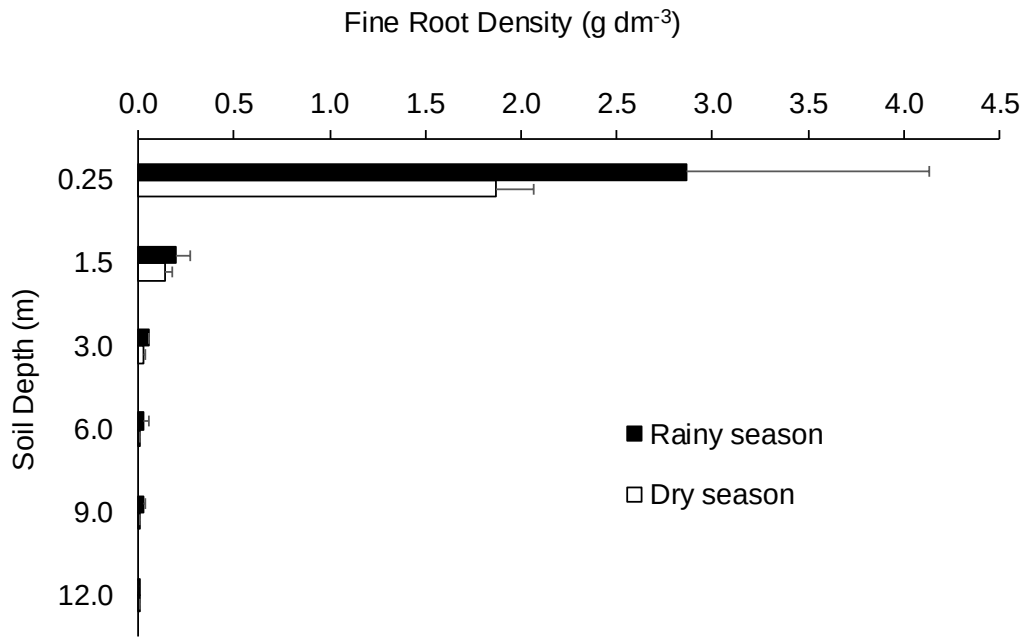
Appendix B - Mean basal area of the three studied species and the other trees and shrubs of the area with a Diameter at Breast Height (DBH) higher than 1.5 cm. A forest inventory was carried out within a 10 m radius from the tracer injection point in each plot (36 in total). Standard deviations are indicated.



Appendix C - Gravimetric soil water contents (SWCs) down to the maximum depth where the tracer was injected at each season. Standard deviations are indicated ($n = 6$ in soil layers 0.25, 0.50, 1.0, 2.0, 3.0, 6.0 and 9.0 m at each season and $n = 3$ in the other soil layers at each season).



Appendix D - Mean fine root densities (g dm^{-3}) down to a depth of 12 m when the NO_3^- - ^{15}N tracer was injected. Standard deviations are indicated ($n = 3$).



CONSIDERAÇÕES FINAIS

Os resultados desse estudo mostram que os fertilizantes em plantios comerciais de eucalipto devem ser aplicados próximos aos troncos para serem absorvidos por todas as árvores, independentemente da idade do povoamento e do status social das árvores. Isso porque a maior parte do nitrato marcado injetado em diferentes profundidades do solo foi absorvida por árvores localizadas de 2 a 3 metros do ponto de aplicação do marcador isotópico.

Nossos resultados também sugerem que a fertilização em metade das entrelinhas de plantios de eucalipto durante a estação chuvosa pode ser suficiente para permitir que todas as árvores absorvam os nutrientes aplicados. Isso pode reduzir o custo de uma adubação comercial convencional amplamente utilizada pelas empresas do setor florestal atualmente, além de possibilitar uma melhor divisão das atividades ao longo do ano. Ou seja, realizar o controle de plantas daninhas e a adubação em 50% das entrelinhas de plantio na estação chuvosa e o controle de plantas daninhas nos outros 50% das entrelinhas durante os períodos de menor atividade no campo. No entanto, a proporção de entrelinhas fertilizadas não pode ser inferior a 50%. A aplicação de fertilizantes em um terço ($1/3$) da entrelinha evitaria que a maioria das árvores não adjacentes à entrelinha fertilizada absorvesse os nutrientes e provavelmente levaria a uma alta heterogeneidade de crescimento dentro do povoamento.

Testes de campo são necessários para avaliar se o controle de plantas daninhas e a adubação de apenas 50% das entrelinhas de plantio durante a estação chuvosa (e os outros 50% alguns meses depois) afetaria a produtividade do povoamento. Esses testes de campo mostrarão se as ervas daninhas na metade das entrelinhas durante a estação chuvosa diminuiriam significativamente, ou não, a produtividade do povoamento.

Além disso, o estudo mostra que os íons de nitrato lixiviados em Latossolos profundos podem ser absorvidos pelas raízes das árvores a uma profundidade de 6 metros entre 0,6 e 2,5 anos após o plantio, em plantios comerciais de eucalipto. Entretanto, a velocidade de exploração das camadas profundas do solo depende do status social das árvores. Quando a fertilização nitrogenada é concentrada nos primeiros meses após o plantio (e ainda mais se os fertilizantes forem aplicados apenas em 50% das entrelinhas), a lixiviação de nitrato em camadas muito profundas

do solo pode aumentar a heterogeneidade dos povoamentos, uma vez que os nitratos profundos só poderiam estar disponíveis para árvores dominantes (as árvores dominantes têm maior capacidade de usar os recursos do solo nas camadas mais profundas do que as árvores dominadas).

As profundidades de absorção de NO_3^- - ^{15}N nesse estudo são consistentes com outros estudos que tratam do uso de água em plantações tropicais de eucalipto, o que sugere que a absorção de nitrato marcado poderia ser um simples indicador da absorção cumulativa de água em áreas específicas do solo.

Na área de Cerrado, o nitrato marcado foi absorvido em diferentes profundidades e distâncias do tronco, dependendo da espécie estudada, o que sugere uma complementaridade de nicho em todo o perfil do solo até uma profundidade de pelo menos 6 metros, o que é extremamente importante em ambientes onde os recursos são mais escassos. Embora não tenhamos observado uma relação clara entre o tamanho das plantas e a profundidade de absorção do NO_3^- - ^{15}N , apenas as árvores com um diâmetro à altura do peito (DAP) maior que 5 cm foram capazes de absorver o marcador injetado em profundidades de mais de 1,5 metros. Inesperadamente, as árvores não absorveram os nitratos marcados mais profundamente na estação seca do que na estação chuvosa.

Os estudos realizados com a mesma metodologia mostram um comportamento semelhante em ecossistemas florestais de diversidade extrema (povoamentos clonais de eucalipto e fragmentos de cerradão), mostrando uma complementaridade de nicho entre as árvores, com a absorção do nitrato em camadas diferentes do solo, dependendo do status social das árvores e/ou da espécie.

A aplicação da metodologia utilizada nesse estudo pode ser muito interessante em futuros estudos, sendo mais fácil que estudos que utilizam os isótopos estáveis da água deutério (^2H) e oxigênio-18 (^{18}O). O nitrato marcado serviu como um indicador da absorção de água em áreas específicas do solo, pois o nitrato consiste em um íon que é móvel no solo, necessitando de um fluxo de difusão de água do solo até as raízes para que possa ser absorvido e detectado nas folhas.

Enfim, esse estudo sugere que os padrões de absorção de água podem ser diferentes nas camadas superiores do solo e em camadas muito profundas do mesmo e a relação entre o tamanho da árvore, a estação do ano e a absorção de água no subsolo merece mais estudos em florestas tropicais. Outros estudos que tratam da relação entre o status social das árvores e a distância e profundidade de absorção de

água e nutrientes também são necessários para melhorar as abordagens de modelagem baseadas em árvores que pretendem prever a resposta de formações florestais tropicais às mudanças climáticas.

REFERÊNCIAS

- ADAMS, H.D. et al. Empirical and process-based approaches to climate-induced forest mortality models. **Frontiers in Plant Science**, v. 4, nov. 2013. <https://doi.org/10.3389/fpls.2013.00438>
- ALMEIDA, A. C. et al. Mapping the effect of spatial and temporal variation in climate and soils on *Eucalyptus* plantation production with 3-PG, a process based growth model. **Forest Ecology and Management**, v. 259, p. 1730-1740, 2010. <https://doi.org/10.1016/j.foreco.2009.10.008>
- BATTIE-LACLAU, P. et al. Effects of potassium and sodium supply on drought-adaptive mechanisms in *Eucalyptus grandis* plantations. **New Phytologist**, v. 203, p. 401-413, 2014. <https://doi.org/10.1111/nph.12810>
- BENNETT, A. C. et al. Larger trees suffer most during droughts in forests worldwide. **Nature Plants**, v. 1, n. 15139, 2015. <https://doi.org/10.1038/nplants.2015.139>
- BERDANIER, A. B.; CLARK, J. S. Multiyear drought-induced morbidity preceding tree death in southeastern US forests. **Ecological Applications**, v. 26, p. 17-23, 2016. <https://doi.org/10.1890/15-0274>
- BINKLEY, D. et al. Explaining growth of individual trees: light interception and efficiency of light use by *Eucalyptus* at four sites in Brazil. **Forest Ecology and Management**, v. 259, p. 1704–1713, 2010. <https://doi.org/10.1016/j.foreco.2009.05.037>
- BINKLEY, D. et al. Light absorption and use efficiency in forests: why patterns differ for trees and stands. **Forest Ecology and Management**, v. 288, p. 5–13, jan. 2013. <https://doi.org/10.1016/j.foreco.2011.11.002>
- BIRCHER, N.; CAILLERET, M.; BUGMANN, H. The agony of choice: different empirical mortality models lead to sharply different future forest dynamics. **Ecological Applications**, v. 25, p. 1303–1318, 2015. <https://doi.org/10.1890/14-1462.1>
- BLAIS, L. et al. Inventaire forestier. In: BÉRARD, J. A.; CÔTÉ, M. (Ed.). **Manuel de Foresterie**. Québec: Les Presses de l'Université Laval, 1996. p. 389-473.
- BOLTE, A.; KAMPF, F.; HILBRIG, L. Space sequestration below ground in old-growth spruce-beech forests—signs for facilitation? **Frontiers in Plant Science**, v. 4, n. 322, p. 1-11, 2013. <https://doi.org/10.3389/fpls.2013.00322>
- BORCHERT, R. Water storage in soil or tree stems determines phenology and distribution of tropical dry forest trees. **Ecology**, v. 75, p. 1437-1449, 1994. <https://doi.org/10.2307/1937467>
- BORDRON, B. et al. Fertilization increases the functional specialization of fine roots in deep soil layers for young *Eucalyptus grandis* trees. **Forest Ecology and Management**, v. 431, p. 6-16, 2019. <https://doi.org/10.1016/j.foreco.2018.03.018>

BRANG, P. et al. Suitability of close-to-nature silviculture for adapting temperate European forests to climate change. **Forestry**, v. 87, p. 492–503, 2014. <https://doi.org/10.1093/forestry/cpu018>

BRUM, M. et al. Hydrological niche segregation defines forest structure and drought tolerance strategies in a seasonal Amazon forest. **Journal of Ecology**, v. 107, n. 1, p. 318-333, 2018. <https://doi.org/10.1111/1365-2745.13022>

BRUNNER, I. et al. How tree roots respond to drought. **Frontiers in Plant Science**, v. 6, n. 547, jul. 2015. <https://doi.org/10.3389/fpls.2015.00547>

BRUNO, R. D. et al. Soil moisture dynamics in an eastern Amazonian tropical forest. **Hydrological Processes**, v. 20, p. 2477-2489, ago. 2006. <https://doi.org/10.1002/hyp.6211>

BUCCI, S. J. et al. Water relations and hydraulic architecture in Cerrado trees: adjustments to seasonal changes in water availability and evaporative demand. **Brazilian Journal of Plant Physiology**, v. 20, n. 3, p. 233-245, 2008. <http://dx.doi.org/10.1590/S167704202008000300007>

CAMPOE, O. C. et al. Stem production, light absorption and light use efficiency between dominant and non-dominant trees of *Eucalyptus grandis* across a productivity gradient in Brazil. **Forest Ecology and Management**, v. 288, p. 14-20, jan. 2013. <https://doi.org/10.1016/j.foreco.2012.07.035>

CASTRO, L. H. R.; KAUFFMAN, J. Ecosystem structure in the Brazilian Cerrado: a vegetation gradient of aboveground biomass, root mass and consumption by fire. **Journal of Tropical Ecology**, v. 14, p. 263-283, 1998. <https://doi.org/10.1017/S0266467498000212>

CHAVES, M. M.; MAROCO, J. P.; PEREIRA, J. S. Understanding plant responses to drought - from genes to the whole plant. **Functional Plant Biology**, v. 30, p. 239-264, 2003. <https://doi.org/10.1071/FP02076>

CHRISTINA, M. et al. Almost symmetrical vertical growth rates above and below ground in one of the world's most productive forests. **Ecosphere**, v. 2(3), n. 27, mar. 2011. <https://doi.org/10.1890/ES10-00158.1>

CHRISTINA, M. et al. Measured and modeled interactive effects of potassium deficiency and water deficit on gross primary productivity and light-use efficiency in *Eucalyptus grandis* plantations. **Global Change Biology**, v.21, n. 5, p. 2022-2039, mai. 2015. <https://doi.org/10.1111/gcb.12817>

CHRISTOFFERSEN, B. O. et al. Linking hydraulic traits to tropical forest function in a size-structured and trait-driven model (TFS v.1-Hydro). **Geoscientific Model Development**, v. 9, n. 11, p. 4227–4255, 2016. <https://doi.org/10.5194/gmd-9-4227-2016>

COMAS, L. H. et al. Root traits contributing to plant productivity under drought. **Frontiers in Plant Science**, v. 4, n. 442, nov. 2013.
<https://doi.org/10.3389/fpls.2013.00442>

CRAINE, J. M. et al. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. **New Phytologist**, v. 183, p. 980–992, 2009.
<https://doi.org/10.1111/j.1469-8137.2009.02917.x>

CRAINE, J. M. et al. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. **Plant and Soil**, v. 396, p. 1–26, 2015.
<https://doi.org/10.1007/s11104-015-2542-1>

DA SILVA, E. V. et al. Functional specialization of *Eucalyptus* fine roots: contrasting potential uptake rates for nitrogen, potassium and calcium tracer at varying soil depths. **Functional Ecology**, v. 25, n. 5, p. 996–1006, 2011.
<https://doi.org/10.1111/j.1365-2435.2011.01867.x>

DAWSON, T. D. et al. Stable isotopes in plant ecology. **Annual Review of Ecology and Systematics**, v. 33, n.1, p. 507–559, 2002.
<https://doi.org/10.1146/annurev.ecolsys.33.020602.095451>

DUURSMA, R. A.; MEDLYN, B. E. MAESPA: a model to study interactions between water limitation, environmental drivers and vegetation function at tree and stand levels, with an example application to [CO₂] × drought interactions. **Geoscientific Model Development Discussions**, n. 5, p. 459–513, 2012.
<https://doi.org/10.5194/gmd-5-919-2012>

EHLERINGER, J. R. Interpreting Stable Isotopes Ratios in Plants and Plant-based Foods. In: CARTER, J. F.; CHESSON, L. A. (Ed.). **Food Forensics – Stable Isotopes as a Guide to Authenticity and Origin**. Boca Raton, FL: CRC Press, 2017. v. 1, cap. 3, p. 46–62.

EVANS, R. D. Physiological mechanisms influencing plant nitrogen isotope composition. **Trends in Plant Science**, v. 6, n. 3, p. 121–126, 2001.
[https://doi.org/10.1016/S1360-1385\(01\)01889-1](https://doi.org/10.1016/S1360-1385(01)01889-1)

FLOYD, M. L. et al. Structural and regenerative changes in old-growth piñon–juniper woodlands following drought-induced mortality. **Forest Ecology and Management**, v. 341, p. 18–29, 2015. <https://doi.org/10.1016/j.foreco.2014.12.033>

FRANCO, A. C. Ecophysiology of woody plants. In: OLIVEIRA, P. S.; MARQUIS, R. J. (Ed.). **The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna**. New York (USA): Columbia University Press, 2002. p. 178–197.

FRANCO, A. C. Biodiversidade de forma e função: implicações ecofisiológicas das estratégias de utilização de água e luz em plantas lenhosas do Cerrado. In: SCARIOT, A.; SOUSA-SILVA, J. C.; FELFILI, J. M. (Org.). **Cerrado: Ecologia**,

Biodiversidade e Conservação. Brasília: Ministério do Meio Ambiente, 2005. p. 179-196.

FREYCON, V. et al. Tree roots can penetrate deeply in African semi-deciduous rain forests: evidence from two common soil types. **Journal of Tropical Ecology**, v. 31, n. 01, p. 13-23, jan. 2015. <https://doi.org/10.1017/S0266467414000595>

GERMON, A. et al. Consequences of clear-cutting and drought on fine roots dynamics down to a depth of 17 m in coppice-managed eucalypt plantations. **Forest Ecology and Management**, v. 445, p. 48-59, 2019. <https://doi.org/10.1016/j.foreco.2019.05.010>

GONÇALVES, J. L. M. et al. Eucalypt plantation management in regions with water stress. **Southern Forests: a Journal of Forest Science**, v. 79, n. 3, p. 169–183, 2017. <https://doi.org/10.2989/20702620.2016.1255415>

GOODLAND, R; FERRI, M. G. **Ecologia do Cerrado**. Editora da Universidade de São Paulo – Edusp/Livraria Itatiaia Editora Ltda., Belo Horizonte, 1979.

GROSSIORD, C. et al. Impact of interspecific interactions on the soil water uptake depth in a young temperate mixed species plantation. **Journal of Hydrology**, v. 519, p. 3511-3519, 2014. <https://doi.org/10.1016/j.jhydrol.2014.11.011>

GROTE, R. et al. Importance of tree height and social position for drought-related stress on tree growth and mortality. **Trees**, v. 30, n. 5, p. 1467-1482, 2016. <https://doi.org/10.1007/s00468-016-1446-x>

HOBBIE, S. E. Plant species effects on nutrient cycling: Revisiting litter feedbacks. **Trends in Ecology and Evolution**, v. 30, p. 357–363, 2015. <https://doi.org/10.1016/j.tree.2015.03.015>

HOEKSTRA, N. J. et al. The effect of drought and interspecific interactions on the depth of water uptake in deep-and shallow-rooting grassland species as determined by $\delta^{18}\text{O}$ natural abundance. **Biogeosciences**, v. 11, p. 4493-4506, 2014. <https://doi.org/10.5194/bg-11-4493-2014>

HOEKSTRA, N. J. et al. Do belowground vertical niche differences between deep- and shallow-rooted species enhance resource uptake and drought resistance in grassland mixtures? **Plant Soil**, v. 394, p. 21-34, 2015. <https://doi.org/10.1007/s11104-014-2352-x>

HOFFMAN, W. A.; FRANCO, A. C. Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically-independent contrasts. **Journal of Ecology**, v. 91, p. 475-484, 2003. <https://doi.org/10.1046/j.1365-2745.2003.00777.x>

IPCC, 2014: Summary for Policymakers. In: **Climate Change 2014: Mitigation of Climate Change**. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel

and J.C. Minx (Eds.)). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

IVANOV, V. Y. et al. Root niche separation can explain avoidance of seasonal drought stress and vulnerability of overstory trees to extended drought in a mature Amazonian forest. **Water Resources Research**, v. 48, n. 12, p. 1–21, 2012. <https://doi.org/10.1029/2012WR011972>

IVERSEN, C. M. Digging deeper: fine-root responses to rising atmospheric CO₂ in forested ecosystems. **New Phytologist**, v. 186, p. 346–357, 2010. <https://doi.org/10.1111/j.1469-8137.2009.03122.x>

JACKSON, R. B.; MOONEY, H. A.; SCHULZE, E.-D. A global budget for fine root biomass, surface area, and nutrient contents. **Proceedings of the National Academy of Sciences**, v. 94, p. 7362–7366, jul. 1997.

JENSEN, A.; LÖF, M.; WITZELL, J. Effects of competition and indirect facilitation by shrubs on *Quercus robur* saplings. **Plant Ecology**, v. 213, n. 4, p. 535–543, 2012. <https://doi.org/10.1007/s11258-012-0019-3>

KAHMEN, A. et al. Niche complementarity for nitrogen: an explanation for the biodiversity and ecosystem functioning relationship? **Ecology**, v. 87, n. 5, p. 1244–1255, 2006. [https://doi.org/10.1890/00129658\(2006\)87\[1244:NCFNAE\]2.0.CO;2](https://doi.org/10.1890/00129658(2006)87[1244:NCFNAE]2.0.CO;2)

KLEIN, T. et al. Towards an advanced assessment of the hydrological vulnerability of forests to climate change-induced drought. **New Phytologist**, v. 201, p. 712–716, 2014. <https://doi.org/10.1111/nph.12548>

KOBA, K. et al. Natural abundance of nitrogen-15 in a forest soil. **Soil Science Society of America Journal**, v. 62, p. 778–781, 1998.

KULMATISKI, A.; BEARD, K. H. Root niche partitioning among grasses, saplings, and trees measured using a tracer technique. **Oecologia**, v. 171, n. 1, p. 25–37, 2013. <https://doi.org/10.1007/s00442-012-2390-0>

LACLAU, J.-P. et al. Dynamics of soil exploration by fine roots down to a depth of 10 m throughout the entire rotation in *Eucalyptus grandis* plantations. **Frontiers in Plant Science**, v. 4, n. 243, jul. 2013. <https://doi.org/10.3389/fpls.2013.00243>

LAMBAIS, G. R. et al. Contrasting phenology of *Eucalyptus grandis* fine roots in upper and very deeper soil layers in Brazil. **Plant and Soil**, v. 421, p. 301–318, 2017. <https://doi.org/10.1007/s11104-017-3460-1>

LENZA, E.; KLINK, C. A. Comportamento fenológico de espécies lenhosas em um cerrado sentido restrito de Brasília, DF. **Brazilian Journal of Botany**, v. 29, p. 627–638, 2006. <http://dx.doi.org/10.1590/S0100-84042006000400013>

LIMA, W. P. et al. Assessing the hydrological effects of forest plantations in Brazil. In: BOON, P. J., RAVEN, P. J. (Ed.), **River conservation and management**. Oxford: Wiley-Blackwell, 2012. p. 57–66.

LUO, Y.; CHEN, H. Y. H. Competition, species interaction and ageing control tree mortality in boreal forests. **Journal of Ecology**, v. 99, n. 6, p. 1470-1480, nov. 2011. <https://doi.org/10.1111/j.1365-2745.2011.01882.x>

MAEGHT, J.-L.; REWALD, B.; PIERRET, A. How to study deep roots – and why it matters. **Frontiers in Plant Science**, v. 4, n. 299, ago. 2013. <https://doi.org/10.3389/fpls.2013.00299>

MAEGHT, J.-L. et al. Seasonal patterns of fine root production and turnover in a mature rubber tree (*Hevea brasiliensis* Müll. Arg.) stand-differentiation with soil depth and implications for soil carbon stocks. **Frontiers in Plant Science**, v. 6, n. 1022, nov. 2015. <https://doi.org/10.3389/fpls.2015.01022>

MARSDEN, C. et al. Modifying the G'DAY process-based model to simulate the spatial variability of *Eucalyptus* plantation growth on deep tropical soils. **Forest Ecology and Management**, v. 301, p. 112-128, 2013. <https://doi.org/10.1016/j.foreco.2012.10.039>

MARTÍNEZ-VILALTA, J. et al. Stand- and tree-level determinants of the drought response of Scots pine radial growth. **Oecologia**, v. 168, p. 877-888, 2012. <https://doi.org/10.1007/s00442-011-2132-8>

MCDOWELL, N. G. et al. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? **New Phytologist**, v. 178, p. 719-739, 2008. <https://doi.org/10.1111/j.1469-8137.2008.02>

MCDOWELL, N. G. et al. Evaluating theories of drought-induced vegetation mortality using a multimodel-experiment framework. **New Phytologist**, v. 200, p. 304-321, 2013. <https://doi.org/10.1111/nph.12465>

MERLIN, et al. Effects of stand composition and tree size on resistance and resilience to drought in sessile oak and Scots pine. **Forest Ecology and Management**, v. 339, p. 22-33, 2015. <https://doi.org/10.1016/j.foreco.2014.11.032>

METZ, J. et al. Site-adapted admixed tree species reduce drought susceptibility of mature European beech. **Global Change Biology**, v. 22, n. 2, p. 903–920, 2016. <https://doi.org/10.1111/gcb.13113>

MOMMER, L. et al. Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. **Journal of Ecology**, v. 98, n. 5, p. 1117-1127, set. 2010. <https://doi.org/10.1111/j.1365-2745.2010.01702.x>

MOREIRA, A. G.; KLINK, C. A. Biomass allocation and growth of tree seedlings from two contrasting Brazilian savannas. **Ecotropics**, v. 13, p. 43-51, 2000.

NEPSTAD, D. C. et al. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. **Nature**, v. 372, 1994. <https://doi.org/10.1038/372666a0>

NIE, M. et al. Altered root traits due to elevated CO₂: a meta-analysis. **Global Ecology and Biogeography**, v. 22, p. 1095-1105, 2013.
<https://doi.org/10.1111/geb.12062>

PAQUETTE, A.; MESSIER, C. The effect of biodiversity on tree productivity: from temperate to boreal forests. **Global Ecology and Biogeography**, v. 20, n. 1, p. 170-180, 2011. <https://doi.org/10.1111/j.1466-8238.2010.00592.x>

PARDO, L. et al. Natural abundance ¹⁵N in soil and litter across a nitrate-output gradient in New Hampshire. **Forest Ecology and Management**, v. 251, p. 217–230, 2007. <https://doi.org/10.1016/j.foreco.2007.06.047>

PAULILO, M. T. S.; FELIPPE, G. M. Growth of the shrub-tree flora of the Brazilian cerrados: a review. **Tropical Ecology**, v. 39, p. 165-174, 1998.

PICHLER, P.; OBERHUBER, W. Radial growth response of coniferous forest trees in an inner Alpine environment to heat-wave in 2003. **Forest Ecology and Management**, v. 242, p. 688-699, 2007. <https://doi.org/10.1007/s10342-013-0777-z>

PINHEIRO, R. C. et al. A fast exploration of very deep soil layers by *Eucalyptus* seedlings and clones in Brazil. **Forest Ecology and Management**, v. 366, p. 143-152, 2016. <https://doi.org/10.1016/j.foreco.2016.02.012>

RIGNOT, E. et al. **The Copenhagen Diagnosis, 2009**: Updating the World on the Latest Climate Science. The University of New South Wales Climate Change Research Centre (CCRC), Sydney, Australia, 2009.

RIVERA, G. et al. Increasing day-length induces spring flushing of tropical dry forest trees in the absence of rain. **Trees**, v. 16, p. 445-456, 2002.
<https://doi.org/10.1007/s00468-002-0185-3>

ROBINSON, D. ¹⁵N as an integrator of the nitrogen cycle. **Trends in Ecology & Evolution**, v. 16, p. 153–162, 2001.

ROUPSARD, O. et al. Reverse phenology and dry-season water uptake by *Faidherbia albida* (Del.) A. Chev. in an agroforestry parkland of Sudanese West Africa. **Functional Ecology**, v. 13, p. 460–472, 1999. <https://doi.org/10.1046/j.1365-2435.1999.00345.x>

ROWLAND, L. et al. Death from drought in tropical forests is triggered by hydraulics not carbon starvation. **Nature**, v. 528, p. 119-122, 2015.
<https://doi.org/10.1038/nature15539>

SARACENO, M. I. **Efeitos da fertilização a longo prazo no metabolismo fotossintético, nas características foliares e no crescimento em árvores do Cerrado**. 2006. 66 f. Dissertação (Mestrado em Ecologia) - Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, 2006.

SARMIENTO, G.; GOLDSTEIN, G.; MEINZER, F. Adaptive strategies of woody species in neotropical savannas. *Biological Reviews*, v. 60, p. 315-355, 1985. <https://doi.org/10.1111/j.1469-185X.1985.tb00420.x>

SCHOLZ, F. G. et al. Biophysical and life history determinants of hydraulic lift in Neotropical savanna trees. **Functional Ecology**, v. 22, p. 773-786, 2008. <https://doi.org/10.1111/j.1365-2435.2008.01452.x>

SHARP, R.; DAVIES, W. Root growth and water uptake by maize plants in drying soil. **Journal of Experimental Botany**, v. 36, p. 1441-1456, 1985. <https://doi.org/10.1093/jxb/36.9.1441>

SHENG, et al. Regional patterns of ¹⁵N natural abundance in forest ecosystems along a large transect in eastern China. **Nature – Scientific Reports**, v. 4, n. 4249, 2014. <https://doi.org/10.1038/srep04249>

SPRACKLEN, D. V., ARNOLD, S. R., TAYLOR, C. M. Observations of increased tropical rainfall preceded by air passage over forests. **Nature**, v. 489, p. 282-286, 2012. <https://doi.org/10.1038/nature11390>

STAHL, C. et al. Depth of soil water uptake by tropical rainforest trees during dry periods: does tree dimension matter? **Oecologia**, v. 173, p. 1191-1201, 2013. <https://doi.org/10.1007/s00442-013-2724-6>

STERNBERG, L. S. L., MOREIRA, M. Z., NEPSTAD, D. C. Uptake of water by lateral roots of small trees in an Amazonian Tropical Forest. **Plant and Soil**, v. 238, n. 1, p. 151–158, 2002. <https://doi.org/10.1023/A:1014214404699>

TROUVÉ, R. et al. Stand density, tree social status and water stress influence allocation in height and diameter growth of *Quercus petraea* (Liebl.). **Tree Physiology**, v. 35, n. 10, p. 1035-1043, 2015. <https://doi.org/10.1093/treephys/tpv067>

VAN DER MOLEN, M. K. et al. Drought and ecosystem carbon cycling. **Agricultural and Forest Meteorology**, v. 151, p. 765–773, 2011. <https://doi.org/10.1016/j.agrformet.2011.01.018>

VENEGAS-GONZÁLEZ, A. et al. Sensitivity of tree ring growth to local and large-scale climate variability in a region of southeastern Brazil. **Theoretical and Applied Climatology**, v. 123, p. 233–245, 2016. <https://doi.org/10.1007/s00704-014-1351-4>

WU, Z. et al. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. **Global Change Biology**, v. 17, p. 927-942, 2011. <https://doi.org/10.1111/j.1365-2486.2010.02302.x>

ZANG, C.; PRETZSCH, H.; ROTHE, A. Size-dependent responses to summer drought in Scots pine, Norway spruce and common oak. **Trees**, v. 26, p. 557-569, 2012. <https://doi.org/10.1007/s00468-011-0617-z>