INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DE FLORESTAS TROPICAIS

DINÂMICA DE RAÍZES FINAS EM FLORESTAS DE TERRA FIRME NA AMAZÔNIA CENTRAL

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Sinopse

Com o uso de minirhizotron, pôde-se avaliar a dinâmica das raízes finas em diferentes profundidades, classes de diâmetro, estações e em resposta à adição de fósforo. Também pôde-se comparar a dinâmica de raízes finas utilizando-se diferentes metodologias. Esses dados contribuem para um avanço na compreensão do ciclo do carbono, água e fósforo que são pouco estudados nos componentes abaixo do solo. Foi detectada que a dinâmica de raízes varia em profundidade, classe de diâmetro e entre estações, mas que não varia em resposta à adição de fósforo e que diferentes métodos podem chegar a estimativas similares de estoque e produtividade de raízes finas.

Palavras-chave: Produtividade, estoque, turnover, sazonalidade, fertilização, métodos

Dedico aos meus amados pais, irmão e avós.

RESUMO

Este estudo avaliou a dinâmica de raízes finas (estoque, produtividade e turnover) ao longo do perfil do solo e em diferentes classes de diâmetro ao longo do ano, em diferentes estações e em resposta à adição de fósforo (P) em dois sítios de floresta na Amazônia central. Analisou-se raízes finas <2mm de diâmetro utilizando minirizotrons até 90 cm de profundidade. Minirhizotrons foram calibrados com "Soil cores" e comparados com "Ingrowth cores". Modelos lineares mistos foram utilizados para controlar o desenho experimental hierárquico. Testes de Kruskal-Wallis foram realizados quando os dados não se ajustavam às hipóteses dos modelos lineares mistos. Para comparação de métodos, usamos testes-t. A maior parte do estoque (76,13%) e produtividade (82,25%) foram observadas em raízes mais finas (0 a 0,5mm). Além disso, aproximadamente metade do estoque (46.12%) e produtividade (40.61 %) estavam concentrados nas profundidades abaixo de 30cm. O turnover das raízes não variou entre classes de diâmetro e intervalos de profundidade. Considerando diferentes estações, a produtividade de raízes finas foi maior na estação chuvosa na camada mais superficial e o estoque de raízes foi maior na estação seca na camada mais profunda. Não houve divergência significativa nas estimativas de estoque quando minirhizotron foi comparado com "soil core", como também não houve diferença na produtividade de raízes quando se comparou "ingrowth core" e minirhizotron. No experimento de fertilização, a dinâmica das raízes finas não respondeu à adição de P. Em suma, a presença de uma alta proporção de dinâmica radicular abaixo de 30 cm nos mostra que raízes profundas não podem ser deixadas de medir. O fósforo pode não ser um nutriente limitante para as raízes na Amazônia Central no período de tempo avaliado.

Palavras-chave: Minirhizotron, dinâmica de raízes, raízes profundas, diâmetro, estação, fósforo, métodos.

ABSTRACT

This study evaluates fine root dynamics (stock, productivity and turnover) along the soil profile and in different diameter classes over the year, across seasons and in response to P addition in two sites in central Amazon rainforest. Roots <2mm were analyzed with minirhizotrons until 90 cm of depth. Minirhizotrons were calibrated with soil cores and compared with ingrowth cores. Linear mixed models were used to control for the hierarchical experimental design and Kruskal–Wallis tests were performed when the data did not fit the assumptions of linear mixed models. To compare methods, t tests were used. Fine root dynamics varied significantly between diameter classes and depth intervals. Most of the entire stock (76.13%) and productivity (82.25%) was observed in thinner roots (0 to 0.5mm). In addition, approximately half of fine root stock (46.12%) and productivity (40.61%) were concentrated in layers below 30cm. However, fine root turnover did not vary between diameter classes and depth intervals. Considering seasons, fine root productivity was higher in the wet season in the most superficial layer and root stock was higher in the dry season at the deepest layer. There was no significant divergence in stock estimates when minirhizotron was compared with soil core, nor was there any difference in root productivity when comparing ingrowth core and minirhizotron. Regarding the fertilization experiment, fine root dynamics did not respond to P addition. In summary, the presence of a high proportion of root dynamics below 30 cm presents that deep roots cannot be forgotten to measure. Phosphorus may not be a limiting nutrient to roots in Central Amazonia.

Key-words: Minirhizotron, root dynamics, deep roots, diameter, season, phosphorus, methods.

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INTRODUÇÃO

A floresta amazônica é uma das maiores reservas de carbono do mundo, armazenando aproximadamente 150-200Pg C em biomassa viva e nos solos (Feldpausch *et al.,* 2012; Brienen *et al.,* 2015). No entanto, ainda existem muitas incertezas sobre como essa floresta responderá ao crescente aumento das concentrações atmosféricas de CO_2, condições de seca e aumento de temperatura (Friedlingstein *et al.,* 2006; Meir *et al.,* 2008; Cox et al., 2013; Cleveland *et al.,* 2015; Doughty *et al.,* 2015; Lovejoy & Nobre, 2018). Assim, observações diretas da dinâmica das florestas tropicais frente às mudanças ambientais são cruciais para entender os atuais processos de fluxo de carbono e para prever, mais precisamente o que podemos esperar no futuro. Embora muitos estudos nos trópicos tenham se concentrado em estimativas de componentes acima do solo, há consideravelmente menos estudos com foco em processos abaixo do solo (Clark *et al.,* 2001).

Em particular, a dinâmica das raízes finas é um componente importante dos processos subterrâneos, porque estas raízes têm funções essenciais na ciclagem de água, nutrientes e carbono nos ecossistemas terrestres (Mccormack *et al.,* 2015). Elas são geralmente definidas como raízes com diâmetro menor ou igual a 2 mm, mas as raízes neste diâmetro podem diferir em forma e função. As raízes mais distais, de 1ª, 2ª e 3ª ordens, geralmente estão envolvidas na aquisição e captação de recursos do solo e têm menor tempo de residência nos solos, ou seja, têm um maior turnover, enquanto as raízes de maiores ordens, localizadas mais na parte interna do sistema radicular frequentemente têm funções relacionadas ao transporte, estrutura e armazenamento e têm maior longevidade (Mccormack *et al.,* 2015). Por ser um componente muito dinâmico, parte significativa (22% a 40%) de todo o carbono, fixado pela fotossíntese nos ecossistemas terrestres, é alocado para sua produtividade e turnover (Bloom, Chapin, Mooney, 1985; Jackson, Mooney, Schulze, 1997; Aragão *et al.,* 2009; Mccormack *et al.,* 2015).

Além disso, raízes finas mostraram algumas das respostas mais dinâmicas a mudança climática global em experimentos de manipulação de CO_2 de larga escala (Norby & Jackson, 2000; Iversen, 2010). Evidências experimentais de diferentes ecossistemas florestais indicam que o enriquecimento de CO_2 acarreta na distribuição das raízes finas em maiores profundidades o que é observado principalmente em ecossistemas de florestas limitadas por nutrientes. Essas mudanças na distribuição das raízes ao longo do perfil do solo podem afetar importantes processos do ecossistema, como a química das raízes, a função fisiológica, a infecção micorrízica, levando a

mudanças na taxa de absorção de nutrientes e taxas de turnover (Iversen, 2010). Portanto, quantificar os padrões de alocação de C para as raízes finas e identificar os possíveis fatores que influenciam nesse processo é essencial para que possamos entender melhor a estrutura e função do ecossistema, como também prever como tal ecossistema pode responder aos distúrbios e mudança climática (Vogt *et al.,* 1993; Norby & Jackson 2000).

Vários fatores ambientais podem influenciar na dinâmica das raízes finas, como por exemplo, a umidade do solo e disponibilidade de nutrientes (Cavelier *et al.,* 1999; Maycock & Congdon., 2000; McGroddy & Silver., 2000; Yavitt & Wright, 2001). A bacia amazônica tem anualmente períodos de pouca chuva ou seca, de modo que a água pode ser um recurso limitante durante os períodos mais secos. Por outro lado, a disponibilidade de P parece ser o recurso mais importante que impulsiona a dinâmica florestal em toda a bacia amazônica (Quesada *et al.,* 2012). Em particular, a baixa disponibilidade de fósforo (P) e cátions frequentemente limitam o crescimento de plantas tropicais (Vitousek & Sanford, 1986, Quesada *et al*., 2011), uma vez que os solos são altamente intemperizados e os nutrientes provenientes de rocha matriz tendem a ser lixiviados com o tempo (Walker & Syers, 1976; Crews *et al*., 1995). Nessas condições, foi proposto que as plantas deveriam alocar mais carbono para a produção de raízes finas quando / onde os recursos do solo (água / nutrientes) são limitantes, para que as plantas invistam em maior quantidade de biomassa de raízes finas para adquirir esses recursos (Thornley, 1972; Chapin, 1980; Bloom, Chapin, Mooney, 1985; Cannell & Dewar, 1994).

Em apoio à teoria de que a limitação de recursos que impulsionam os processos radiculares em paisagens tropicais, vários estudos mostram que a biomassa das raízes geralmente aumenta com a diminuição da disponibilidade de fósforo no solo em ecossistemas tropicais (Gower *et al.,* 1987; Maycock & Congdon, 2000; Ostertag, 2001; Powers et al., 2005; Espeleta & Clark, 2007). Por outro lado, alguns estudos mostram que a produtividade de raízes nas florestas tropicais é maior quando há mais recursos do solo, como água (Green *et al.,* 2005; Metcalfe *et al.,* 2008) e nutrientes (Cuevas & Medina, 1988; Raich *et al.,* 1994; Ostertag, 1998; McGrath *et al.,* 2001; Aragão *et al.,* 2009). Isso sugere que em solos com baixa disponibilidade de recursos, há maior biomassa de raízes finas, mas menor produtividade de raízes finas. Há algumas explicações possíveis para a relação entre a biomassa de raízes finas e a produtividade em resposta à disponibilidade de nutrientes e provavelmente está relacionada a mudanças no turnover de raízes finas (Nadelhoffer *et al.,* 2000).

Outra estratégia importante é produzir raízes onde os nutrientes estão disponíveis ao longo do perfil do solo (Prior *et al.,* 2003; Hodge, 2004). Em particular, na Amazônia Central, onde há baixa disponibilidade de fósforo e bases trocáveis, geralmente há um decréscimo no estoque de e na produtividade de raízes finas quando aumenta-se a profundidade do solo (Klinge, 1973; Silver *et al.,* 2000, Quesada *et al.,* 2011; Noguchi *et al.,* 2014); que é dominado pela proliferação de raízes finas perto da superfície do solo. Essas raízes são consideradas importantes para a aquisição de recursos, pois permitem a ciclagem direta de nutrientes da matéria orgânica, o que provavelmente é uma adaptação à baixa oferta de nutrientes nos solos inférteis (Went & Stark, 1968; Jordan, 1985; Richards, 1996; Sayer *et al.,* 2006). Portanto, a maioria dos estudos de raízes finas, particularmente em florestas tropicais, estão confinados aos 10 ou 30 cm superiores do perfil do solo. No entanto, experimentos manipulativos em florestas temperadas mostram que não apenas as raízes de superfície, mas as profundas são afetadas por mudanças ambientais (Norby *et al.,* 2004; Iversen *et al.,* 2010). Embora haja maior dinâmica de raízes finas nas camadas superficiais, as mais profundas podem ser relevantes em resposta a mudanças globais nas regiões tropicais.

Apesar da importância das raízes finas nos processos biogeoquímicos (Silver *et al.,* 2005), ainda existe conhecimento limitado da dinâmica de raízes finas em florestas tropicais. A razão é porque há poucos dados na região e por dificuldades metodológicas na avaliação dos compartimentos abaixo do solo (Judd *et al.,* 2015). Alguns pesquisadores sugeriram que há divergência entre os métodos usados para avaliar a dinâmica das raízes finas (Hendricks *et al.,* 1993; Nadelhoffer, 2000; Norby & Jackson, 2000). Na bacia amazônica, por exemplo, a maioria das medições foi baseada em "sequential coring" e "ingrowth cores" (Jiménez *et al.,* 2009). Embora esses métodos sejam bastante simples e possam fornecer insights significativos sobre a importância da dinâmica e das funções das raízes finas, eles requerem múltiplos distúrbios e não podem capturar padrões de produtividade de curto prazo e turnover de raízes efêmeras (Vogt *et al.,* 1998). O método mais adequado para acessar essa dinâmica em pesquisas de longo prazo é o minirhizotron, pois cada raiz pode ser rastreada ao longo do tempo (Hendrick & Pregitzer, 1996; Rewald & Ephrath, 2013). Uma desvantagem desse método é que as raízes analisadas não podem ser coletadas, portanto, as ordens das raízes não podem ser acessadas. No entanto, uma vez que o diâmetro da raiz está correlacionado com o turnover da raiz (Eissenstat *et al.,* 2000; Wells e Eissenstat 2001; King *et al.,* 2002; Matamala *et al.,* 2003; Baddeley & Watson, 2005; Majdi *et al.,* 2005), raízes de

minirhizotron ainda podem ser separadas em diferentes classes de diâmetro para avaliar a dinâmica das raízes de diferentes categorias: com maior ou menor tempo de residência no solo.

Em relação aos prós e contras de cada método, uma melhor compreensão do papel das raízes finas nos trópicos usando diferentes métodos e acessando múltiplos compartimentos do perfil do solo é fundamental para chegarmos a uma melhor compreensão sobre o ciclo C na região tropical. Portanto, o objetivo primário deste estudo foi avaliar a função das raízes finas em florestas tropicais amazônicas em uma alta resolução espacial e temporal usando minirhizotron pela primeira vez nesta floresta. Pretendemos quantificar os padrões e os fatores que controlam a produtividade, estoque e turnover de raízes finas, em uma área de floresta de terra firme na Amazônia Central, que está sob o tipo de solo mais abundante em toda a Bacia Amazônica. Os objetivos específicos deste estudo foram: avaliar a dinâmica das raízes finas (i) entre a estação chuvosa e a seca; (ii) através do perfil do solo; (iii) entre classes de diâmetros; (iv) em diferentes métodos no mesmo local, comparando-os para melhorar a robustez das estimativas de dinâmica de raízes finas (v) em resposta a um aumento na disponibilidade de P. Nossas hipóteses são de que: a maior parte da dinâmica das raízes finas será concentrada nas profundidades mais próximas da superfície e o turnover será maior nesta camada; a produtividade de raízes finas será maior na estação chuvosa, mas o estoque de raízes finas será o mesmo; produtividade de raiz fina e turnover serão maiores nas classes de menor diâmetro; diferentes métodos apresentarão diferentes estimativas de dinâmica de raiz fina e a produtividade de raízes finas aumentará em resposta a adição de P.

OBJETIVOS

1. Mensurar a mudança no estoque e produtividade de raízes finas ao longo do ano de 2017 e avaliar se existe variação entre estação seca e chuvosa;
2. Mensurar e comparar o estoque, produtividade e turnover, de raízes finas em diferentes profundidades do solo (0-30cm, 30-60cm e 60-90cm);
3. Mensurar e comparar o estoque, produtividade e turnover, de raízes finas em diferentes classes de diâmetro (0-0.5; 0.5-1; 1-1.5 e 1.5 a 2mm);
4. Comparar diferentes métodos (soil core x minirhizotron; ingrowth core x minirhizotron) que avaliam estoque e produtividade de raízes finas
5. Comparar o estoque, produtividade e turnover de raízes finas em parcelas fertilizadas com P e parcelas controle.

CAPITULO ÚNICO
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Fine root dynamics in *terra firme* forests in Central Amazon

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Abstract

- This study evaluates fine root dynamics (stock, productivity and turnover) along the soil profile and in different diameter classes over the year, across seasons and in response to P addition in two sites in central Amazon rainforest. It also compares different methods to access fine root dynamics.
- Fine roots (< 2 mm diameter) were measured using minirhizotrons to a soil depth of 90 cm. Minirhizotrons were calibrated with soil cores and compared with ingrowth cores using t-tests. Linear mixed models were used to control for the hierarchical experimental design and Kruskal–Wallis tests were performed when the data did not fit the assumptions of linear mixed models.
- Fine roots with a 0-0.5mm diameter comprised the majority of root standing stock biomass (76.13%) and productivity (82.25%). Approximately half of fine root stock biomass (46.12%) and productivity (40.61 %) were found in layers below 30 cm depth. Root turnover did not vary between diameter classes or depth intervals. Fine root productivity was higher in the wet season in the most superficial layer and root standing stock biomass was higher in the dry season at the deepest layer. Fine root dynamics were similar using different methods. Fine root dynamics did not respond to P addition.
- The presence of a high proportion of root dynamics below 30 cm suggests that deep roots need to be taken into account for accurate estimates of belowground plant processes. Fine root dynamics is related to water and nutrient availability. Further study is needed to determine whether soil phosphorus additions influence roots dynamics in Central Amazonia.

Key-words: Minirhizotron, root dynamics, root traits, rooting depth, seasonality, soil phosphorus, methods

Introduction

Amazon rainforest is one of the largest ecosystem carbon reserves in the world, storing approximately 150-200Pg C in living biomass and soils (Feldpausch *et al*., 2012; Brienen *et al.,* 2015). However, there is uncertainty on how this forest will behave under changing climate scenarios, such as increased atmospheric CO2 concentrations, drought conditions, and temperature (Friedlingstein *et al*.,2006; Meir *et al*.,2008; Cox *et al*., 2013; Cleveland *et al*.,2015; Doughty *et al*.,2015; Lovejoy & Nobre, 2018). Thus, direct observations of tropical forest dynamics in a changing world are crucial to understand current carbon flux processes and to better predict what we can expect in the future. Although many studies in the tropics have focused on estimates of above-ground components, there are considerably fewer studies focusing on belowground processes (Clark *et al.,* 2001).

In particular, fine root dynamics is an important component of belowground processes because they play an important role in the cycling of water, nutrients, and carbon in terrestrial ecosystems (Mccormack *et al*., 2015). They are usually defined as roots with ≤ 2mm in diameter, but roots in this diameter cutoff can differ in form and function. The most distal roots are usually involved in the acquisition and uptake of soil resources and have a higher turnover, whereas roots which occur higher in the branching hierarchy often have functions related to transport, structure and storage and are long lived (Mccormack *et al*., 2015). Also, because it is a very dynamic component, a significant part (22% to 40%) of all the carbon, fixed by photosynthesis in terrestrial ecosystems is allocated for its productivity and turnover (Bloom, Chapin, Mooney, 1985; Jackson, Mooney, Schulze, 1997; Aragão *et al*., 2009; Mccormack *et al*., 2015).

In addition, fine roots have shown some of the most dynamic responses to global climate change factors in large-scale CO_2 manipulation experiments (Norby & Jackson, 2000; Iversen, 2010). Experimental evidence from many different forested ecosystems indicates that CO_2 enrichment may lead to deeper rooting distributions and it is mainly observed in nutrient limited forest ecosystems. Those changes on root distribution along soil profile can affect important ecosystem processes such as root chemistry, physiological function, mycorrhizal infection leading to an altered nutrient uptake rate and turnover rates (Iversen, 2010). So, 1uantifying patterns of C allocation to fine roots and identifying drivers of this process is therefore paramount to the

understanding of ecosystem structure and function, and in predicting how these ecosystems may respond to disturbance and climate change (Vogt *et al.*, 1993; Norby & Jackson 2000).

Several environmental factors may contribute to changes in the dynamics of the fine roots, such as soil moisture and nutrient availability (Cavelier *et al*., 1999; Maycock & Congdon., 2000; McGroddy & Silver., 2000; Yavitt & Wright., 2001). The Amazon basin experiences periods of low rainfall or drought each year, so water can be a limiting resource during drier periods. On the other hand, nutrient availability seems to be the most important resource driving forest dynamics throughout the Amazon basin (Quesada *et al.,* 2012). In particular, the low availability of phosphorus (P) and cations often limit the growth of tropical plants (Vitousek & Sanford, 1986, Quesada *et al.,* 2011), since soils are highly weathered and nutrients from parent rock tend to be leached over time (Walker & Syers, 1976; Crews *et al.,* 1995).

It has been proposed that roots should allocate more carbon to produce fine roots when/where soil resources (water/nutrients) are limiting, so that plants should invest in a higher amount of fine root biomass (root standing stock) to acquire these resources (Thornley, 1972; Chapin, 1980; Bloom, Chapin, Mooney, 1985; Cannell & Dewar, 1994). In support of resource limitation driving root processes across tropical landscapes, several studies have found root biomass generally increases with decreasing soil phosphorous availability (Gower *et al.*, 1987; Maycock & Congdon, 2000; Ostertag, 2001, Powers *et al.,* 2005; Espeleta & Clark, 2007). On the other hand, some studies show that root productivity in tropical forests is greater when there are more soil resources such as water (Green *et al.*, 2005, Metcalfe *et al.,* 2008) and nutrients (Cuevas & Medina, 1988; Raich *et al.,* 1994; Ostertag, 1998; McGrath *et al*., 2001; Aragão *et al.,* 2009). It suggests that in soils with low resource availability, there is higher fine root biomass, but less fine root productivity. There are some possible explanations for the relationship between fine root biomass and productivity in response to nutrient availability and it is probably related to changes in fine root turnover (Nadelhoffer *et al.,* 2000).

Another important strategy is to produce roots where the nutrients are available along a soil profile (Prior *et al.*, 2003; Hodge, 2004). In particular, in the Central Amazon, where there is low phosphorus and base cation availability, there is generally a decrease of fine root standing stock and fine root productivity with soil depth (Klinge, 1973; Silver *et al*., 2000, Quesada *et al*., 2011; Noguchi *et al.,* 2014); which is dominated by the proliferation of fine roots near the soil surface. These roots are considered important for resource acquisition because they allow the direct cycling

of nutrients from organic matter, which probably is an adaptation to the low nutrient supply in infertile soils (Went & Stark,1968; Jordan, 1985; Richards, 1996; Sayer *et al.,* 2006). Therefore, most fine root studies, particularly in tropical forests, are confined to the top 10 or 30 cm of the soil profile. However, manipulative experiments in temperate forests show that not just surface roots, but deep ones are affected by environmental changes (Norby *et al*., 2004; Iversen *et al.,* 2010). Although there is higher fine root dynamics in surface layers, the deepest ones may be relevant in response to that global changes in tropical regions.

Despite the importance of fine roots on biogeochemical processes (Silver *et al.,* 2005), there is still limited knowledge of fine root dynamics in tropical forests. The reason why is because there are few data in the region and because of methodological difficulties in assessing belowground compartments (Judd *et al.*, 2015). Some investigators have suggested that there is some divergence between the methods used to assess fine root dynamics (Hendricks *et al.,* 1993; Nadelhoffer, 2000; Norby & Jackson, 2000). In the Amazon basin, for example, most of measurements have been based upon sequential soil coring and in-growth cores (Jiménez *et al.,* 2009). Although these methods are quite simple and can provide significant insights on the importance of fine root dynamics and functions, they require multiple disturbances and cannot capture short-term patterns of productivity and turnover of ephemeral roots (Vogt *et al*., 1998). The most suitable method to access this dynamic in long-term researches is the minirhizotron because each root can be tracked over time (Hendrick & Pregitzer, 1996; Rewald & Ephrath, 2013). One disadvantage of this method is that the roots analyzed cannot be collected, so root orders cannot be accessed. Nevertheless, since root diameter is correlated with root turnover (Eissenstat *et al.,* 2000; Wells & Eissenstat 2001; King *et al.,* 2002; Matamala *et al.,* 2003; Baddeley & Watson, 2005; Majdi *et al.,* 2005), roots from minirhizotron can still be separated in different diameter classes to evaluate root dynamics in long and short-term pools.

Regarding the pros and cons from each method, an improved understanding of the role of fine roots in the tropics using different methods and accessing multiple compartments of the soil profile is critical for a better comprehension about the C cycle in the tropical region. Therefore, the primary objective of this study was to evaluate fine root function in Amazonian tropical forests in a high spatial and temporal resolution by using minirhizotron for the first time in this forest. I wanted to quantify the patterns and controls of fine root productivity, stock and turnover with soil depth at a Central Amazonian Terra Firme forest site that is representative of the most soil type

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across the Amazon Basin. The specific objectives of this study were to evaluate fine root dynamics (i) between wet and dry season; (ii) across the soil profile; (iii) among different diameter classes; (iv) improve the robustness of fine root dynamics estimates by comparing different methods at the same site an also (v) in response to an increase in P availability. Our hypotheses are that: most of fine root dynamics will be concentrated at the depths closer to the surface and turnover will be higher at this layer; fine root productivity will be higher in the wet season, but fine root stock will be the same; fine root productivity and turnover will be higher at the smaller diameter classes; different methods will present different estimates of fine root dynamics and fine root productivity will increase in response to P addition.

Material and methods

Site description

Fine root dynamics were analyzed at two similar experimental sites located 70-90 km north of Manaus, Amazonas within the Cuieiras Reserve (02°35'33"S and 60°06'55"W), Brazil. Both forests are old-growth, closed canopy and located in a Terra Firme (non flooded) area. The type of forest (formally classified as Lowland Dense Ombrophylous and soil found on plateau forests along both sites are representative of a dominant fraction of the forests occurring in Central Amazon. Local variations in soil type, topography and drainage status have created distinct patterns in forest vegetation composition. On the plateaus where the experiments are located, well-drained clay favors high biomass forests, with tall canopy with ca. 35–40 m in height with emergent trees over 45 m tall, this being a typical Terra Firme forest for the region.

The soils at both sites have been described by Quesada *et al.,* (2010, 2011). It is classified in the World Reference Base (WRB) system as a Geric Ferralsol (Alumic, Hyperdystric, Clayic); which is equivalent to an Oxisol in the U.S. Soil Taxonomy (Norby *et al.,* 2016). In the FAO/UNESCO system are classified as xanthic ferralsols, and as yellow latosols (latossolo amarelo álico) in the Brazilian system (Laurance *et al*., 1999). It is a deep soil with good aggregation, friable, low density, and unsaturated (not anoxic), with 67.7 % clay, 19.9 % sand, 12.4% silt (Quesada *et al*., 2010). Soils there are highly weathered and nutrient depleted, with rock derived elements being particularly low in concentration. Soil surface total C ranges from 2.9 -

3.6%, total N from 0.2-0.3 %, and total P from 50-130 mg kg^{-1}, while the sum of exchangeable bases ranges from 0.12 to y 0.25 cmol$_c$ kg^{-1}. Both sites have the same climate regime (Fig.1) with an average annual temperature of 26°C (Araújo *et al.,* 2002) and the average annual precipitation between 1900 to 2400 mm, with a dry season from July to September (Lovejoy & Bierrgaard, 1990).

In order to understand about total C allocation to fine root productivity, its dynamics over time and seasons, its distribution along the soil profile and diameter classes and also to compare estimates from different methods, I conducted a study in the experimental area of the AmazonFACE program (Lapola & Norby 2014; Norby *et al*., 2015. It is located in the Biological Reserve of Cuieiras - ZF2 (2 ° 35'40 " S, 60 ° 12'28"W), approximately 70 km north of Manaus, managed by the National Institute of Amazonian Research (INPA). This study is part of the pretreatment research that has been conducted on the two AmazonFACE pilot plots. Forest structural parameters collected by other members of the AmazonFACE group are described on Table 1.

Table 1: Forest biomass, basal area, number of individuals, number of species, genus and families in 2 plots from AmazonFACE, Manaus. All trees with the diameter at breast height (dbh) ≥ 2cm were measured (Lapola *et al.,* unpublished).

Parameter	Plot	
	1	2
Tree live biomass aboveground (Mg.ha^{-1})	333.24	513.96
Basal area (m^2 ha^{-1})	30.12	40.05
# of individuals (per plot)	233	287
# of species (per plot)	88	85
# of genus (per plot)	70	71
# of families (per plot)	36	35

To better understand the effects of seasonality in fine root productivity and stock, the wet season was considered as the three wettest months in the year and dry season as the driest months in the year (Fig. 1). I chose five sample dates in the wet season, between February and April 2017 (06 Feb 2017, 23 Feb 2017, 17 Mar 2017, 04 Apr 2017, 20 Apr 2017), and five dry season sample dates between August and October 2017 (10 Aug 2017, 29 Aug 2017, 12 Sep 2017, 28 Sep 2017, 11 Oct 2017) to compare fine root dynamics between seasons. I also used precipitation data from the K34 tower which is approximately 2 km far from the plots to correlate fine root productivity with precipitation in the same period of time
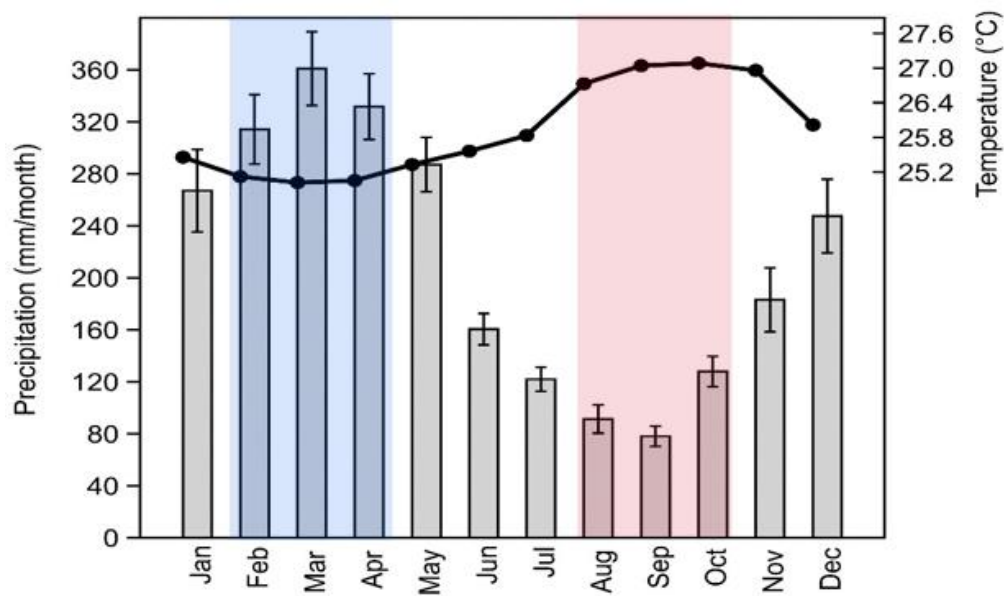
Fig. 1: Average ± standard error monthly rainfall and mean temperature from 1999 to 2016 in the K34 tower, 2 km far from AmazonFACE plots - Manaus, Brazil. Blue and red column represent the months considered as wet season and dry season respectively.

In order to understand the importance of soil P availability for fine root dynamics, I monitored root responses to P additions in comparison with control plots. This site is the experimental area of the Amazon Fertilization Experiment (AFEX), which is a large-scale fertilization experiment in a nested design. The experiment contains four blocks and each one contains a control plot and nutrient addition plots. The study area is located in the Forest Reserve of Km 41 (02° 24'S, 59°52'W), belonging to the Biological Dynamics of Forest Fragments Project (PDBFF). The reserve is located about 80 km from Manaus, at km 41 of the road ZF-3 of the BR-

174 highway. Forest structural parameters collected by other members of the AFEX group are described on Table 2.

This study was conducted in 8 plots from AFEX experiment in which half of them were used as control plots and another half were fertilized with P. All plots (50 x 50 meters each one) are separated by approximately 50 meters. In the P addition plots, 50 Kg P ha^{-1}year^{-1} was applied as triple superphosphate. The first fertilization was carried out in 1st May 2017 and it was repeated in 23th May 2017 and 13th June 2017, completing 50 Kg P ha^{-1} in this year. In 2018, 16.650 Kg P ha^{-1} was added in 2nd February and it was the last fertilization before the sample period for this study has finished. The high amount of P added was due to high adsorption rates of P in clay particles and in Fe and Al oxides. The fertilizers were spread by hand through a systematic walking within the plots.

Table 2: Basal area, number of individuals, number of species, genus and families in two treatments (Control and P addition) distributed in 4 blocks from AFEX, Manaus. All trees with the diameter at breast height (dbh) ≥ 10 cm were measured in each block. +P represents the plots with P addition and Control represents the control plots.

Parameter	Block 1		Block 2		Block 3		Block 4	
	+P	Control	+P	Control	+P	Control	+P	Control
Basal área (m^2 ha^{-1})	22,02	14,68	25,77	29,87	31,26	34,63	28,27	30,92
# of individuals (per plot)	169	144	119	161	134	150	162	170
# of species (per plot)	104	84	82	93	88	96	103	101
# of genera (per plot)	63	57	51	67	58	62	68	68
# of families (per plot)	30	29	22	32	29	32	33	31

Root standing stock, productivity and turnover

To study fine root dynamics from soil surface to deep layers (soil profile) throughout time, I used minirhizotrons (Fig. 2), which is a method originally proposed by Bates (1937). In December

2014, 10 acrylic minirhizotron tubes with five cm inner diameter and two m length were installed in two plots of AmazonFACE site (adapted from Norby *et al*, 2004). Each plot has 30 m diameter and are separated by approximately 90 meters. The holes for the tubes were made with an auger in a 45° angle. In each plot, five tubes were installed to a transversal depth of approximately 1.7 meters with a vertical depth of 1.2 meters. Five tubes were spatially distributed within each plot with at least 3 meters between tubes.
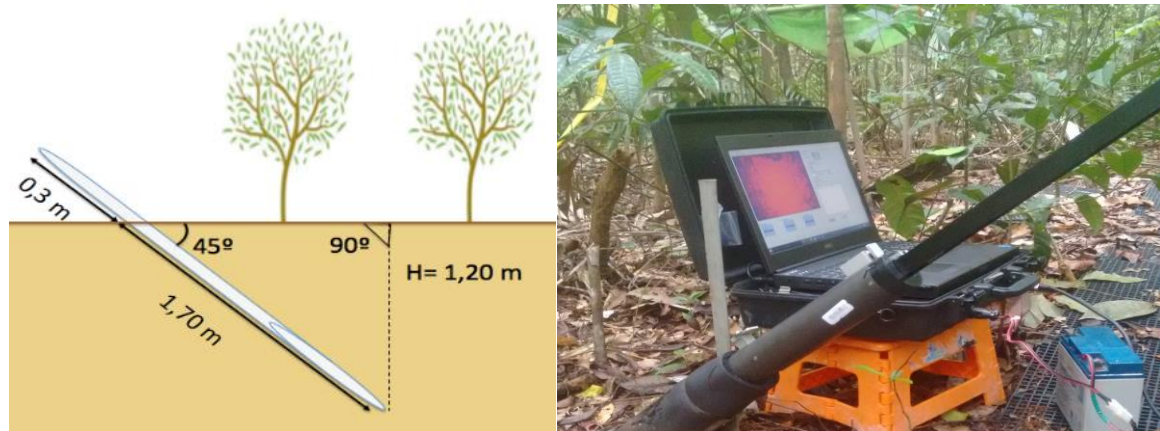
Fig. 2: Minirhizotron system. Left-Minirhizotron tube installed in the soil; Right: Minirhizotron camera, computer and battery at the field.

Images were recorded every month from November 2016 to December 2017 with a total period of 383 days. I started to collect the images two years after tubes were installed allowing the root system to stabilize after disturbance from the tube installation. Images were collected with a Minirhizotron BTC-2 camera (Bartz Technology, Santa Barbara, CA). Each soil tube had approximately 90 viewing windows (10.64 x 14.23 mm) with a total viewing area of 0.0136 m^2. Pictures were taken at the same position along each tube to observe root dynamics along the soil profile over time. One tube was removed (tube number 7) because most of the images there were completely covered with mud, so I could not see the roots. So, regarding that images from 9 tubes were collected 18 times in the period studied, the total number of images analyzed was approximately 14580 in total. All images were analyzed with the ROOTFLY software (http://www.plant-image-analysis.org/software/rootfly) (Fig. 3). Length and diameter of each root segment were measured. Root diameter had a range of 0.099 to 1.55 mm, so all roots from minirhizotron can be considered as fine roots.
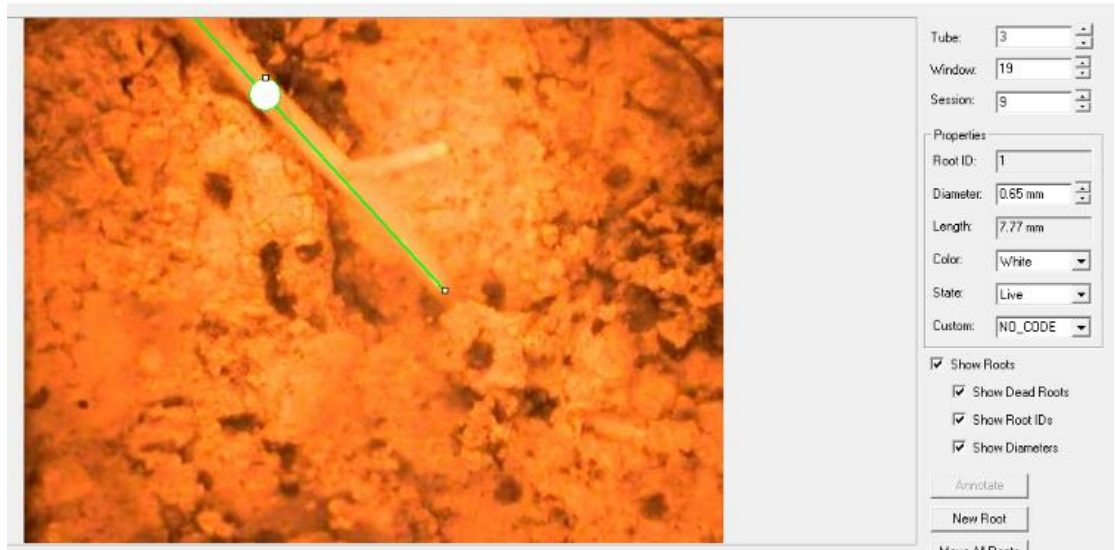
Fig.3: Minirhizotron image at ROOTFLY software. The green line is a root vectorized to obtain length and diameter data.

Fine root dynamics were calculated as described by Hendrick & Pregitzer (1993). Fine root standing stock was calculated as all the length (m) that was present at an image area (m^2) in a certain sampling date. Productivity was considered as all the length (m or mm) of roots that grew or appeared at an image area (m^2) in a period of time between sampling dates. Considering that this forest is in quasi-equilibrium (Malhi *et al*., 2009), fine root turnover ($year^{-1}$) was calculated as fine root productivity (m m^{-2} $year^{-1}$) divided by root standing stock (m m^{-2}) at the date of maximum standing stock, which can be called as peak standing stock date (Gill & Jackson, 2000). To compare each date, I calculated root productivity in a day basis as the period between sampling dates had slight differences in number of days.

Root standing stock, productivity and turnover were separated by depth intervals in two different ways. In both cases, the 90-100cm layer was removed as most of tubes did not reach 1-meter depth or have moved up over time. In the first approach, three equivalent depth levels of intervals were considered, which were 0-30, 30-60 and 60-90cm. This allowed direct comparison with root in-growth core methods (typically to 30cm depth) and improved visualization of variation through time. In the second approach, 9 depth intervals were considered, which were 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90cm. The second approach was used to calculate the percentage of root standing stock and productivity from each depth interval and diameter class in a more comprehensive way along soil profile. I also evaluated root depth distribution by using

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an equation ($Y=1- \beta^{D}$) where D is soil depth (cm), Y is the proportion of roots from the surface to depth D and β is a numerical index of rooting distribution (Gale & Grigal., 1987). When values of *β* are high, it indicates that there is a greater proportion of roots with soil depth (Jackson *et al.,* 1997). Root diameter classes were divided between 0 to 2.0 mm for all variables in four levels (0-0.5, 0.5-1, 1-1.5 and 1.5-2 mm) to calculate the amount of productivity and stock in the peak standing stock date in each diameter class. There was no root stock at the peak standing stock date for the 1.5-2mm diameter class, so the turnover for this class was not calculated.

As this was the first experiment with this type of equipment in the Amazon, I had a difficulty related to the change in the quality of some images over time. Probably due to intense rainfall and clay soil, some images become totally clay smeared and some roots could not be seen anymore. On the other hand, the number of unreliable images in the 0-90 cm layer of soil was only 12 images out of a total of 774 for all remaining tubes or only 1.55% of all images. These images were distributed in 4 tubes. In 3 tubes, these images could reach a depth of 2cm and in 1 tube up to 6cm. Because of the low number of totally dirty images in the tubes, the data is still reliable, but more attention should be given when discussing about the 0-6cm layer of the soil.

Furthermore, at the end of the study period, I cleaned all the tubes which had smudged images, so I could verify if the roots that were in the images that got smudged still continued in the image after cleaning. However, if in the period before cleaning, some roots appeared or disappeared, I could not count the productivity of that root or the stock added to a certain date. In this context, I did a conservative analysis, maintaining the stock of roots from the previous collection date in which I could observe all roots. Therefore, productivity and/or stock of fine roots in some windows may be underestimated. Cleaning tubes was also important because a new sampling collection with all tubes totally clean could initiate. The tubes were removed very carefully to not damage the roots which were being observed in the images. Then, the tubes were inserted back in the same hole and at the same position it was before.

To understand about fine root dynamics (productivity, stock and turnover) in response to P addition, I used the same methodology in the AFEX experiment as described for FACE experiment, but with some differences. This study was conducted in 8 plots and each of them had 1 acrylic minirhizotron tube. Therefore, there were 4 tubes in 4 plots fertilized with P and 4 tubes in 4 control plots. I installed that tubes in the middle of the plot to avoid border effect in June 2017. I started to collect images in the same date the tube was installed to quantify the time for root recovery from

disturbance. Images were recorded every month from June 2017 to February 2018 with a total period of 247 days and a total of 11 collection dates. I analyzed approximately 7920 images for this experiment. Root diameter had a range of 0.063 to 1.49 mm, so all roots from minirhizotron can be considered as fine roots.

Root standing stock, productivity and turnover were also separated by depth intervals and diameter classes in this experiment. Firstly, three levels of intervals were considered, which were 0-30, 30-60 and 60-90cm, so it could give a better visualization of variation trough time as well as simplifying statistical analysis. Secondly, the 0-10cm layer was analyzed separately as this is the layer with the highest probability of being affected by P addition. Diameter classes were separated in 0-0.5, 0.5-1 and 1-1.5mm.

The same problem related to the quality of the images was reported in AFEX tubes. In this experiment, the number of unreliable images in the 0-90 cm layer of soil was only 18 images out of a total of 792 or only 2.27% of all images. These images were distributed in 5 tubes. In 4 tubes, there were just 5 smudged images, which could reach a depth of 2cm, and in 1 tube there was 13 smudged images located in the 60-70cm layers. Because of the low number of totally dirty images in the tubes, the data from AFEX experiment is also reliable.

Estimation of root biomass and C allocation to roots (NPP)

All calculations for root dynamics over time, season, depth intervals and diameter classes from minirhizotron are initially reported in per unit length as directly measured by minirhizotron images. However, it is also important to consider mass basis to match belowground and aboveground productivity or to compare with other methods also measuring fine root dynamics (eg. Ingrowth core and soil core). I estimated biomass by finding a relationship between root mass, diameter and length from fine roots as described by Iversen $et\ al$ (2008). Roots were not collected inside the plots in order to not disturb them. Rather, roots were collected in areas along transects adjacent to the AmazonFACE plots. Root samples were collected from 18 soil cores with a diameter of 10.5 cm and a depth of 15 cm in February, April, August and November 2016. A subset of roots was randomly selected from each soil core, with roots being cleaned and scanned individually with WhinRHIZO^{TM}, to obtain length and diameter for each root (n=543). Roots were dried at 65°C for 72 hours or to constant weight and weighed for biomass. For each scanned root image, diameter, length and mass were used to find a relationship between RML (root mass/root

length ratio) and diameter for each root. Finally, I fitted an equation from the soil root samples to understand the relationship between root RML (mg cm^{-1}) and diameter (mm), with diameter as the independent variable.

The equation relating RML x Diameter was then used to estimate root biomass from minirhizotron images, taking root length and diameter from minirhizotron images to estimate root mass. Root mass (mg) was obtained by multiplying root length (cm) from minirhizotron by RML (mg cm^{-1}) obtained from the soil core roots. Root weight (mg per square meter viewing area) was converted to mg per unit land area by dividing by 0.0025 m effective depth of field (DOF) (better described by Johnson *et al*., 2001) and multiplying by 0.9 m^3 of soil per m^2 of land area to account for the soil volume observed to the maximum depth (cm) of the bottom of the tube which is 0.9 m depth in both of our experiments. The DOF (0.0025 m) was calculated for this research by comparing minirhizotron estimated mass with root mass harvested from 18 soil cores located around the plots in the same depth (0-15cm), same diameter cutoff (<1mm) and same date, also using cores that were very near by the plots where minirhizotron tubes were installed. The DOF was calculated when minirhizotron tubes were 100% clean (in the end of the period studied) in order to not underestimate fine root stock from minirhizotron. In other words, fine root standing stock estimates from minirhizotron (n=9) was calibrated with the real stock inside soil cores (n=18) for the same depth, volume and diameter cutoff.

To convert net primary productivity (NPP) data from Mg. ha^{-1} to Mg C. ha^{-1}, I used the percentage of C in roots which was 43.85% ($\pm$ 0.27 SE; n=66) for roots in the 0-15cm depth in the plots (Fuchslueger, 2016 – personal communication). In addition, fine root productivity estimates from minirhizotron were compared with estimates obtained from root ingrowth. To install the ingrowth cores, firstly, a soil core, which contains all roots from the soil in a known volume, was taken. In this case, 10 cores with 12cm of diameter and 15cm of depth were taken from the plots in December 2015. The soil cores removed were paired with each minirhizotron tube and located within 0.5 m from it. After that, all the roots that were inside that core were collected and the free-root soil was separated to use later. Inside the 10 holes in the soil, ingrowth cores were installed, which consist in mesh bags with the root-free soil that came from the cores. After approximately 3 months, ingrowth cores were sampled and all roots that grew inside them were collected and separated in diameter classes (<1mm, 1-5mm and >5mm). Then, fine root productivity in the 3 months period was calculated. This procedure was repeated quarterly over 2016 and 2017. I

compared fine root productivity from ingrowth cores (n=9) in 2017 with estimates from minirhizotron (n=9) in the same year (January to November 2017), the same depth (0-15cm) and the same diameter cutoff (<1mm).

<u>Statistical analyses</u>

Root dynamics from minirhizotron in the AmazonFACE experiment were analyzed using linear mixed models to control for the hierarchical experimental design where depth interval and diameter classes are nested within tube, and tubes are nested within plots. I tested whether and how different dates, seasons, depth intervals and diameter classes (fixed factors) influenced fine root dynamics (root productivity, root standing stock and turnover) for each dependent variable separately (R packages 'NLME', Pinheiro *et al.,* 2015; and 'LME4', Bates *et al*., 2013). Plot and tube were considered as random factors in the models, where tube is nested within plot. In those models, I tested the most parsimonious combinations of the fixed factors.

Root dynamics from minirhizotron in the AFEX experiment were analyzed in a similar way as in AmazonFACE experiment. I also analyzed using linear mixed models to control for the hierarchical experimental design, but in this case depth interval and diameter classes are nested within tube and tubes are nested within blocks. I tested whether P additions influenced fine root dynamics (root productivity, root standing stock and turnover) in comparison to control plots at each depth interval and for each diameter class. Block and tube were considered as random factors in the models, where tube is nested within block. Dates were considered as a random effect for some analysis.

All data was analyzed in software R (R Development Core Team, 2016). For both experiments, I used LME in the NLME as the main model and I tested model fits by using diagnostic plots (normality and residual plots). Weights were used to control for heteroscedasticity among factor levels when it was necessary (Pinheiro & Bates 2000). Post hoc Tukey tests were performed to check the significance of differences between least square means of each fixed factor. However, when the data did not fit the assumptions of linear mixed models, non-parametric tests were used. Kruskal–Wallis tests in the R package "agricolae" (de Mendiburu 2014) were used to examine fine root dynamics in response to different treatments within depth intervals and diameter classes.

To find RML (root mass divided by root length) equation, I tested different equations in software R. I chose the RML equation with the best Akaike information criterium (AIC) when compared with other equations including the linear one. To understand the relationship between fine root productivity and precipitation, I did a correlation test where fine root productivity at the 0-30cm layer was the dependent variable and precipitation in the same month was the independent variable.

In order to compare fine root dynamics using different methods, I used t-tests to compare the means of fine root standing stock from soil cores with standing stock from minirhizotrons; and to compare fine root productivity over the year from ingrowth cores with productivity from minirhizotron estimates. T-test were also used to compare means from the fine root standing stock amplitude for AmazonFACE and AFEX projects to compare the increase of stock over time from the different places where tubes were installed in different times.

Results

Total fine root productivity for the year was measured as 23.05 ± 3.27 m.m^{-2}. year^{-1}. The minimum root standing stock was 36.10 ± 4.84 m.m^{-2} and the maximum root standing stock was 46.58 ± 5.04 m.m^{-2} over the year. Total root turnover rate was calculated as 0.49 ± 0.18 year^{-1}. Using the RML equation (RML=2.879*Diameter^1.402) from Figure 4 and scaling up the total root standing stock and productivity from minirhizotron to ground area, I calculated total fine root biomass as 13.12 ± 1.98 Mg. ha^{-1} and NPP as 6.33 ± 0.83 Mg. ha^{-1} year^{-1}.
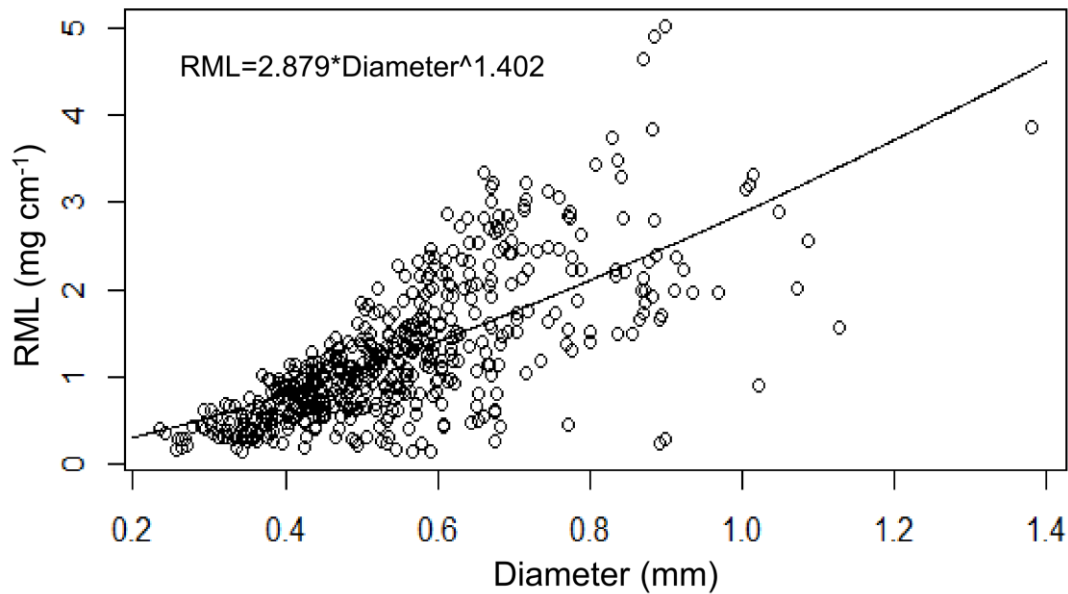
Fig. 4: Relationship between the RML ratio (root mass by root length) and root diameter from soil cores near AmazonFACE plots. n = 543. AIC = 938.04.

<u>Seasonal differences in root standing stock and productivity</u>

There was significant variation in fine-root standing stock ($F_{17, 136}$= 4.01, P <0.0001) during the year (Fig. 5A), with a slightly seasonal trend for higher standing stock in the dry season ($F_{1, 8}$= 4.00, P < 0.09). The minimum root standing stock of 36.10 ± 4.84 m.m^{-2} was observed in November 2016 and increased to the maximum root standing stock of 46.58 ± 5.04 m.m^{-2} which was observed in December 2017 (Figure 5A). Root standing stock also varied with depth ($F_{2, 16}$= 7.77, p < 0.005), both temporally and vertically (Fig. 5B) but with higher stocks consistently closer to surface. Soil layers varied significantly in root stocks between wet and dry season ($F_{1, 42}$= 7.61, P < 0.009). However, the only layer that varied between seasons ($F_{1, 8}$= 7.29, P < 0.003) was the deepest one (60-90 cm) clearly showing greater stocks during the dry season, suggesting that most of the seasonal variation comes from deeper soil layers.

Fine-root productivity also varied with time ($F_{16, 128}$= 5.26, P <0.0001) and season (Fig. 5C) with higher productivity on wet season ($F_{1, 8}$= 7.38, P < 0.003). Therefore, root productivity shows an opposite seasonal pattern when compared to standing stocks, for example being at its maximum in the wet season when standing stocks are low. Maximum and minimum productivity rates coincided with wet and dry season (January and October 2017), with 142.82 ± 5.51 and 15.12 ± 6.13 mm.m^{-2}.day^{-1}, respectively (Fig. 5C).

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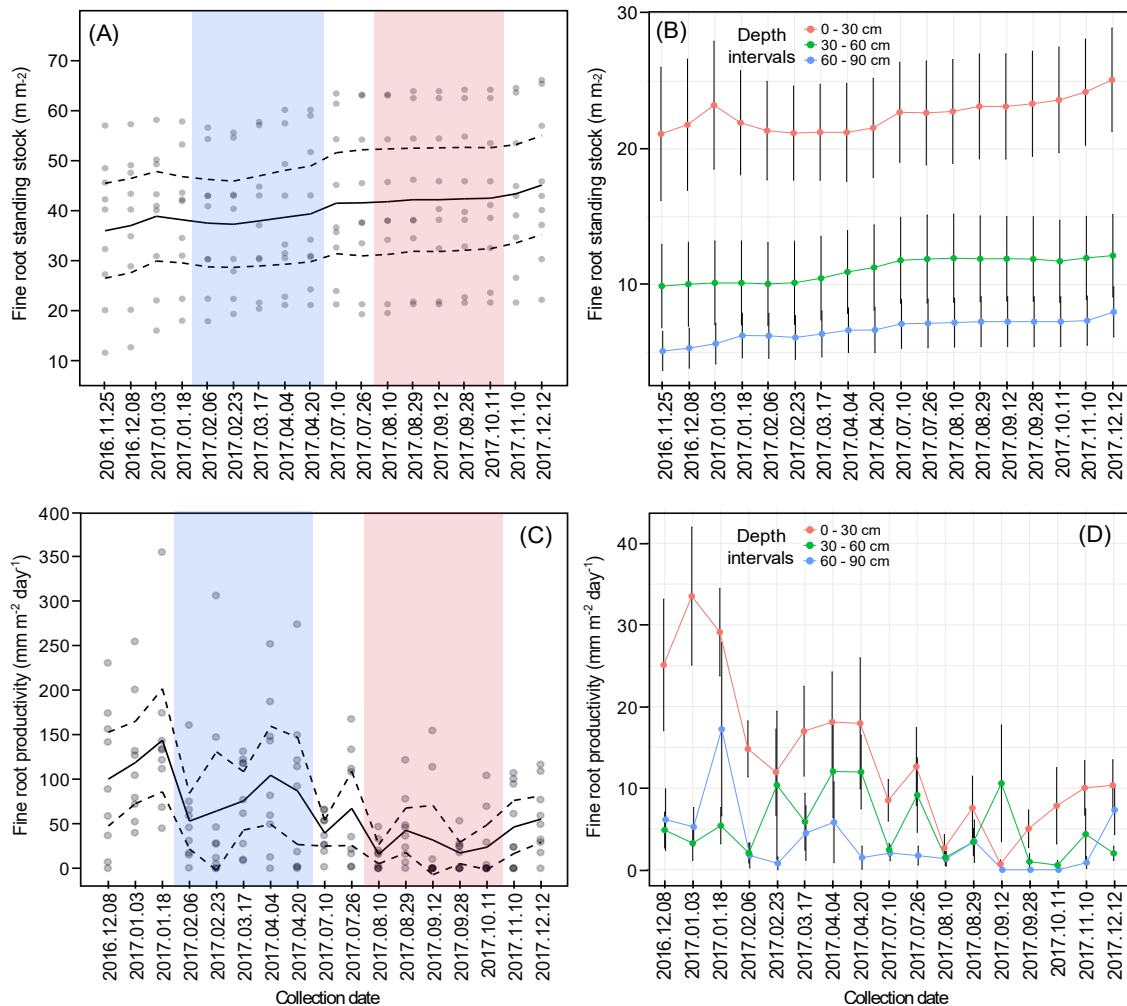
Fig. 5: Fine root dynamics in AmazonFACE experiment using minirhizotron; A: Seasonal pattern of total fine-root standing stock. Solid and dashed lines represent means ± 95% IC, respectively, from 9 tubes for each sample collection. Blue and red column represent wet season and dry season respectively; B: Seasonal pattern of fine-root standing stock in three depth intervals (0-30 cm, 30-60 cm and 60-90 cm) ± SE. Data are means ± SE. C: Seasonal pattern of total fine-root productivity. Solid and dashed lines represent means ± 95% IC, respectively; D: Seasonal pattern of fine-root productivity divided by depth intervals (0-30cm, 30-60cm and 60-90cm) ± SE.

Fine root productivity also differed among depths intervals ($F_{2, 16} = 8.14$, $P < 0.004$) with higher productivity at depths closer to surface (Fig. 5D) and a more stable productivity in deeper soil layers. The variation in productivity by depth intervals ($F_{2, 416} = 8.78$, $P < 0.003$) was significant throughout the year ($F_{16, 416} = 3.80$, $P < 0.0001$) and between wet and dry season ($F_{2, 42} = 6.05$, $P < 0.005$). However, the only depth interval that had a significant variation in productivity between dry and wet season was the most superficial one (0-30cm) with a higher productivity on wet season

($F_{1, 8} = 44.63$, $P < 0.0003$). There was also a positive correlation ($R^2 = 0.66$; $P < 0.003$) between fine root productivity (mm.m^{-2}.day^{-1}) at the 0-30 cm layer and precipitation (mm) over the year (Fig. 6).
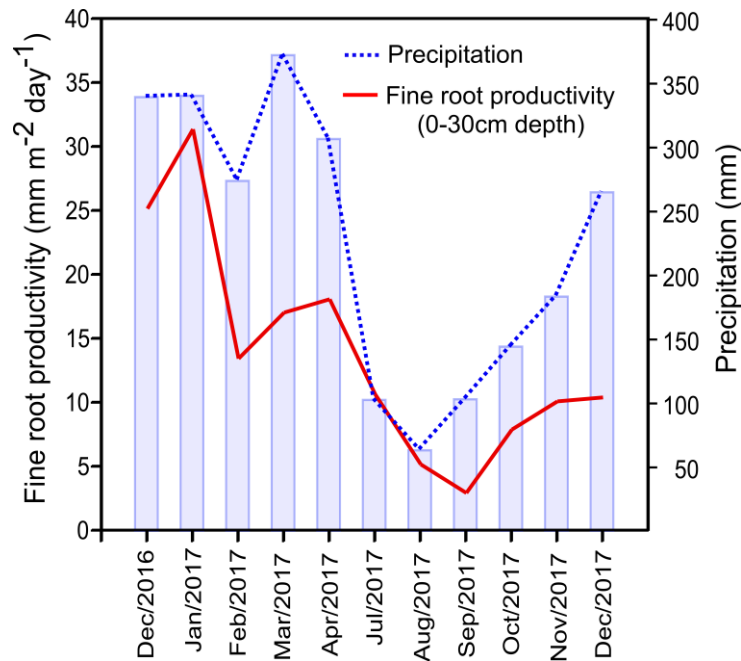
Fig. 6: Fine root productivity (mm.m^{-2}.day^{-1}) at the 0-30cm layer (red solid line) and precipitation (mm) over the year (blue bars and dashed line). Precipitation data is from the K-34 tower near AmazonFACE plots.

Diameter and depth distributions

All diameter classes (0-0.5 mm, 0.5-1 mm, 1-1.5 mm, 1.5-2 mm) showed different root standing stock ($F_{3, 11} = 20.97$, $P < 0.0001$). Smaller diameter roots (0-0.5 mm) had the greatest root standing stock when compared to larger diameters at the peak standing stock date (Fig. 7B). A similar pattern was observed for root productivity, with productivity varying significantly between diameter classes ($F_{3, 10} = 11.10$, $P < 0.002$). Considering the annual productivity across the whole profile, smaller diameter roots (0-0.5mm) also had the greatest productivity compared to larger diameter roots (Table 3; Fig. 7A).
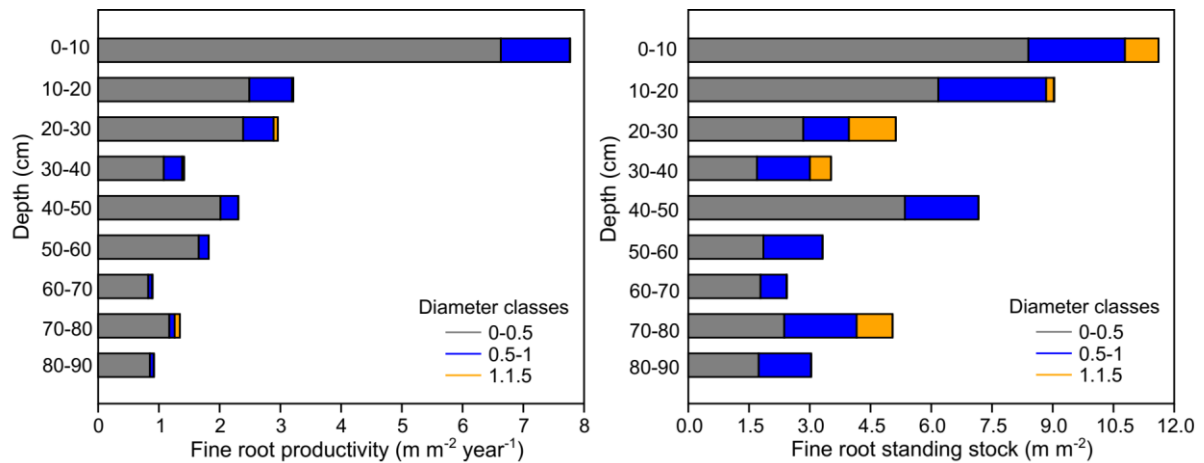
Fig. 7: Fine root dynamics by diameter classes and depth distribution at AmazonFACE site using minirhizotron; A: Fraction of fine root standing stock at its peak by depth and diameter class; B: Fraction of annual root productivity by depth and diameter class.

Most of the entire root stock was observed in roots from 0-0.5 mm diameter class (76.13%), with the remaining stock (23.87%) found in the 0.5-1, 1-1.5 mm diameter classes. The fractional distribution of root productivity by diameter class was 82.25% of productivity occurring on the smallest diameter class (0-0.5mm) and 17.75% in the other diameter classes until 2 mm. Root turnover rate also varied in diameter classes, being higher in smaller diameter classes (Table 3), even though this variation was not significant ($F_{2,11} = 0.15$, $P > 0.8$).

Table 3: Average values for root productivity, standing stock at the peak standing stock date and turnover rates and associated standard error (SE) for 95% confidence interval using minirhizotron in AmazonFACE experiment.

		Productivity (m.m^{-2}.year^{-1})		Standing stock (m.m^{-2})		Turnover (year^{-1})	
		Mean	SE	Mean	SE	Mean	SE
Total		23.05	3.27	46.58	5.04	0.49	0.18
Depth interval (cm)	0-30	13.69	2.2	25.1	4.02	0.54	0.11
	30-60	5.85	1.55	12.14	2.79	0.48	0.11
	60-90	3.51	1.03	9.34	1.83	0.37	0.1
Diameter classes (mm)	0-0.5	18.96	2.71	35.46	1.65	0.53	0.09
	0.5-1	3.87	0.71	10.57	3.5	0.36	0.12
	1-1.5	0.2	0.28	0.55	1.92	0.36	0.17
	1.5-2	0.02	0.15	-	-	-	-

Considering the whole soil profile, almost half of root length stock (53.88%) observed in the minirhizotrons was in roots from 0-30 cm layer, with the other half (46.12%) observed in the 30-90 cm layer. I also found a $\beta = 0.970$ which can be comparable with other studies evaluating the proportion of roots with soil depth. The fractional distribution of root productivity was similar to that of standing stock: 59.39% of length productivity occurring in 0-30 cm depth interval, and 40.61 % of root productivity occurring in the 30-90 cm depth (Fig. 7B). Root turnover rate also had a variation in depth intervals, decreasing with increasing depth (Table 3) but this difference was not significant ($F_{2,16} = 0,84$, $P > 0.4$).

Methods comparison

Fine root standing stock from minirhizotron (N=9) was calibrated with soil core (N=18) at the 15 cm depth with roots <1mm in diameter. Stock from minirhizotron and soil core at this depth was 2.67 ± 0.82 Mg. ha^{-1} and 2.66 ± 0.19 Mg. ha^{-1} respectively. There was no significant difference between these methods (t=0.02; $P > 0.9$). Fine root productivity from January 2017 to November 2017 at the 15 cm depth and roots <1mm in diameter also did not vary between ingrowth core and minirhizotron (t=0.3; $P > 0.7$). Total fine root productivity was 1.72 ± 0.46 Mg. ha^{-1}. year^{-1} for ingrowth core (N=9) and 1.90 ± 0.75 Mg. ha^{-1}. year^{-1} for minirhizotron (N=9). However, fine root productivity was not always equal for the two methods when the collection periods over the year were considered separately. For example, fine root productivity was significant different between ingrowth core and minirhizotron for two periods: January to April/May and August to November. On the other hand, in the period from April/May to August, there was no significant difference between the methods (Table 4). As annual fine root productivity and stock were the same for both methods, root turnover at the 0-15cm layer and for roots <1mm was also the same for both methods. However, when considering all the layers from minirhizotrons (0-90cm) and roots <2mm, total root turnover from minirhizotron was smaller (0.49 year^{-1}) than from ingrowth core (0.64 year^{-1}).

Table 4: Average values for fine root productivity (Mg. ha^{-1}. year[-1]) and associated standard error (SE) for 95% confidence interval by method (minirhizotron or ingrowth core) and in different periods from the year, for the same depth (15cm) and diameter cutoff (<1mm). N= number of samples; t=t-value; p=p-value reported in the t-test.

Method	Period (2017)	N	Mean ± SE (Mg.ha^{-1}.year^{-1})	t	p
Ingrowth core	25 Jan - 09 May	9	1.44 ± 0.28	2.11	0.05
Minirhizotron	18 Jan - 20 Apr	9	3.61 ± 0.98		
Ingrowth core	09 May - 20 Aug	9	1.09 ± 0.40	0.36	0.7
Minirhizotron	20 Apr - 29 Aug	9	1.32 ± 0.47		
Ingrowth core	20 Aug - 19 Nov	9	2.64 ± 0.54	3.02	0.008
Minirhizotron	29 Aug - 10 Nov	9	0.76 ± 0.28		
Ingrowth core	25 Jan - 19 Nov	9	1.72 ± 0.46	0.3	0.7
Minirhizotron	18 Jan - 10 Nov	9	1.90 ± 0.75		

Influence of P on root dynamics

The overall root standing stock in the peak standing stock date for Control plots was 23.74 ± 7.58 m.m^{-2} (8.90 ± 2.14 Mg. ha^{-1}) and 17.56 ± 3.87 m.m^{-2} (5.05 ± 0.95 Mg. ha^{-1}) for P plots. Root productivity for the whole period studied (June 2017 to February 2018) was 20.73 ± 4.38 m.m^{-2}. year^{-1} (5.72 ± 1.24 Mg.ha^{-1}.year^{-1}) for Control plots and 15.14 ± 2.66 m.m^{-2}.year^{-1} (5.50 ± 1.43 Mg.ha^{-1}.year^{-1}) for P plots. Total fine root turnover was 0.92 ± 0.22 year^{-1} for Control and 0.90 ± 0.19 year^{-1} for P treatment.

There was a significant variation of fine-root standing stock ($F_{10,70} = 7.84$, p <0.0001) during the year with stock increasing over time for both treatments (Fig 8A). There were no differences in fine root standing stock between treatments considering the whole period ($\chi2 = 2.08$, p = 0.15), in the beginning of the experiment (minimal standing stock) ($\chi2 = 2.08$, p = 0.15) or for the end of the experiment (peak standing stock) ($\chi2 = 0.33$, p = 0.56). I also decided to calculate the increment of stock between the first day of sampling and the last day. Then I could compare treatments regardless the amount of stock in the beginning of the period given. Nevertheless, there was no significant difference of stock increment between treatments ($F_{1,3} = 0.01$, p = 0.9106).

There were no differences in root productivity between treatments (P and Control) over the period sampled ($F_{1,3} = 0,41$, p = 0.5656) although there are different peaks of productivity in

Control and P over time (Fig 8B). Furthermore, total root turnover was not influenced by P addition ($F_{1,3} = 0.01$, p = 0.9283).
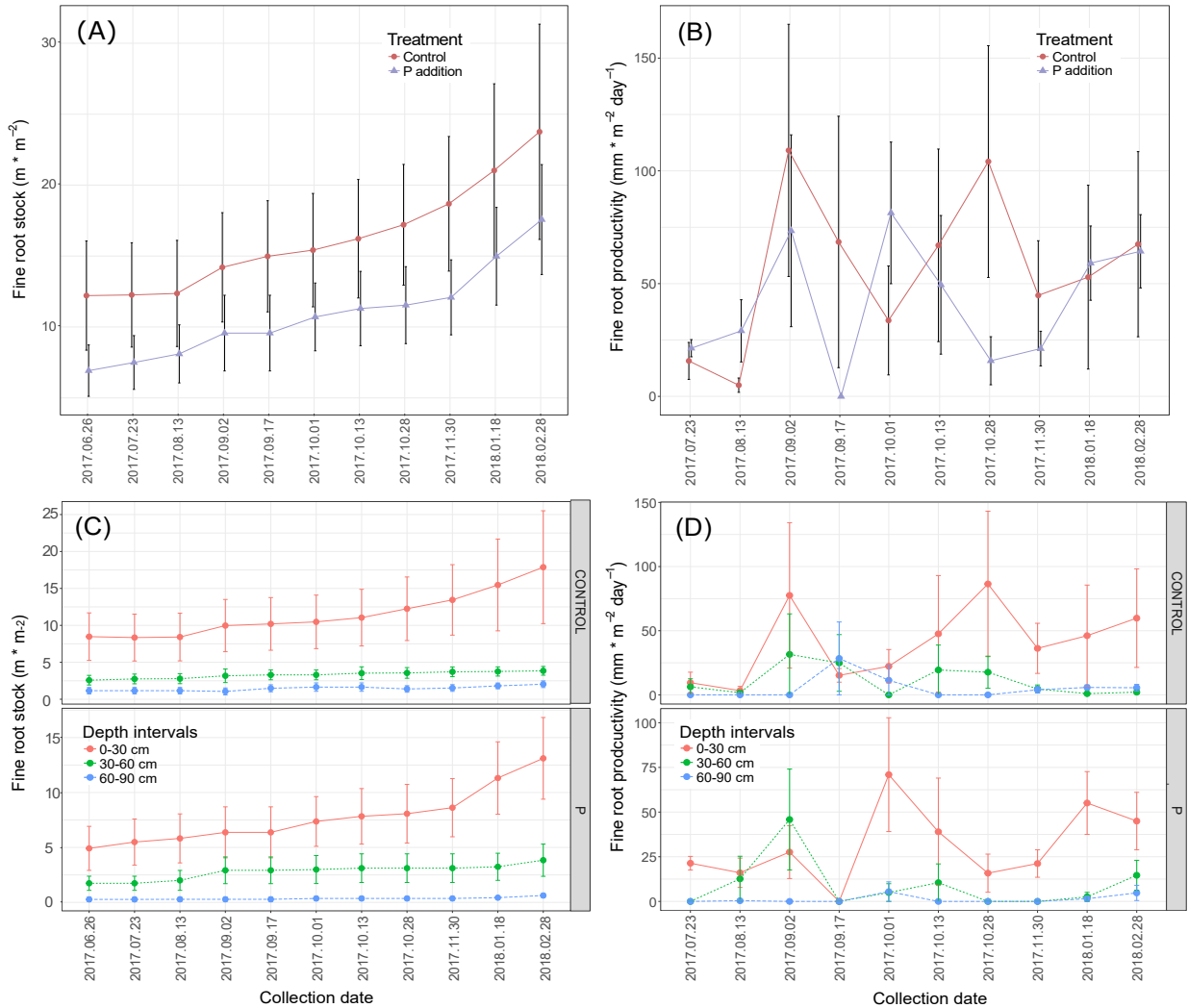
Fig. 8: Fine root dynamics in AFEX experiment using minirhizotron; A: Seasonal pattern of total fine-root standing stock by treatment (P and control). Data are means ± SE, from 8 tubes for each sample collection; B: Seasonal pattern of total fine-root productivity by treatment (P and control). Data are means ± SE, from 8 tubes for each sample collection; C: Seasonal pattern of fine-root standing stock in three depth intervals (0-30 cm, 30-60 cm and 60-90 cm) in different treatments. Data are means ± SE.; D: Seasonal pattern of fine-root productivity in three depth intervals (0-30 cm, 30-60 cm and 60-90 cm) in different treatments. Data are means ± SE.

Considering diameter classes and depth intervals separately (Table 5), there was no influence of treatment on the fine root stock at any depth intervals (0-10cm, 0-30cm, 30-60cm, 60-

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90cm) or diameter classes (0-0.5mm, 0.5-1mm, 1-1.5mm) in the peak standing stock date, which was the last collection date. Total root productivity over the whole period studied was also invariant between treatments in diameter classes and depth intervals separately. There was also no difference between treatments for root turnover in any depth interval and diameter class. I decided not to consider fine root turnover in in the 1-1.5 diameter class because there were 4 tubes without fine root stock, so that I could not calculate turnover by dividing productivity per stock as the number of repetitions was not enough.

Table 5: Average values for root productivity, standing stock at the peak standing stock date and turnover rates and associated standard error (SE) for 95% confidence interval in different treatments (P addition and Control) using minirhizotron at AFEX experiment.

		Productivity ($m.m^{-2}.year^{-1}$)				Standing stock ($m.m^{-2}$)				Turnover ($year^{-1}$)			
		P		Control		P		Control		P		Control	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Depth interval (cm)	0-10	7.31	2.58	10.12	5.62	7.15	3.2	13.08	5.04	1.26	0.39	0.89	0.25
	0-30	11.38	2.6	14.74	9.65	13.11	3.71	17.87	7.63	0.86	0.2	0.82	0.36
	30-60	3.32	1.61	3.98	1.8	3.83	1.47	3.84	0.59	0.86	0.25	1.03	0.39
	60-90	0.44	0.18	2.01	1.63	0.62	1.17	2.03	0.45	0.7	0.52	0.99	0.52
Diameter classes (mm)	0-0.5	9.45	3.16	17.51	10.11	12.69	4.5	16.89	4.1	0.74	0.07	1.03	0.21
	0.5-1	5.37	1.28	2.83	0.93	4.68	1.8	5.5	2.26	1.14	0.2	0.51	0.27
	1-1.5	0.32	0.33	0.39	0.39	0.19	0.18	1.35	0.61	-	-	-	-

Regarding the soil profile and diameter classes, it can be observed that fine root standing stock was influenced by depth intervals ($F_{2,14} = 10.72$, $p = 0.0015$) with most of stock concentrated in the 0-30cm layer (74.65% for the P treatment and 75.27% for the control plots; Fig 9A). In addition, fine root standing stock was also different between diameter classes ($F_{2,14} = 12.80$, $p = 0.0007$) with most of stock in the 0-0.5 diameter class (72.26% for the P treatment and 71.14% for the control plots; Fig 9C).

Fine root productivity was also influenced by depth intervals ($F_{2,14} = 12.86$, $p = 0.0007$) with a higher percentage in the 0-30cm layer (75.16% for the P treatment and 71.10% for the control plots; Fig 9B). It was also different between diameter classes ($F_{2,14} = 14.81$, $p = 0.0004$) with most of productivity in the 0-0.5 diameter class (62.41% for the P treatment and 84.46% for

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the control plots; Fig 9D). On the other hand, fine root turnover was not different between depth intervals ($F_{2,14} = 0.07$, $p = 0.9354$) and diameter classes ($F_{2,14} = 3.03$, $p = 0.0807$).
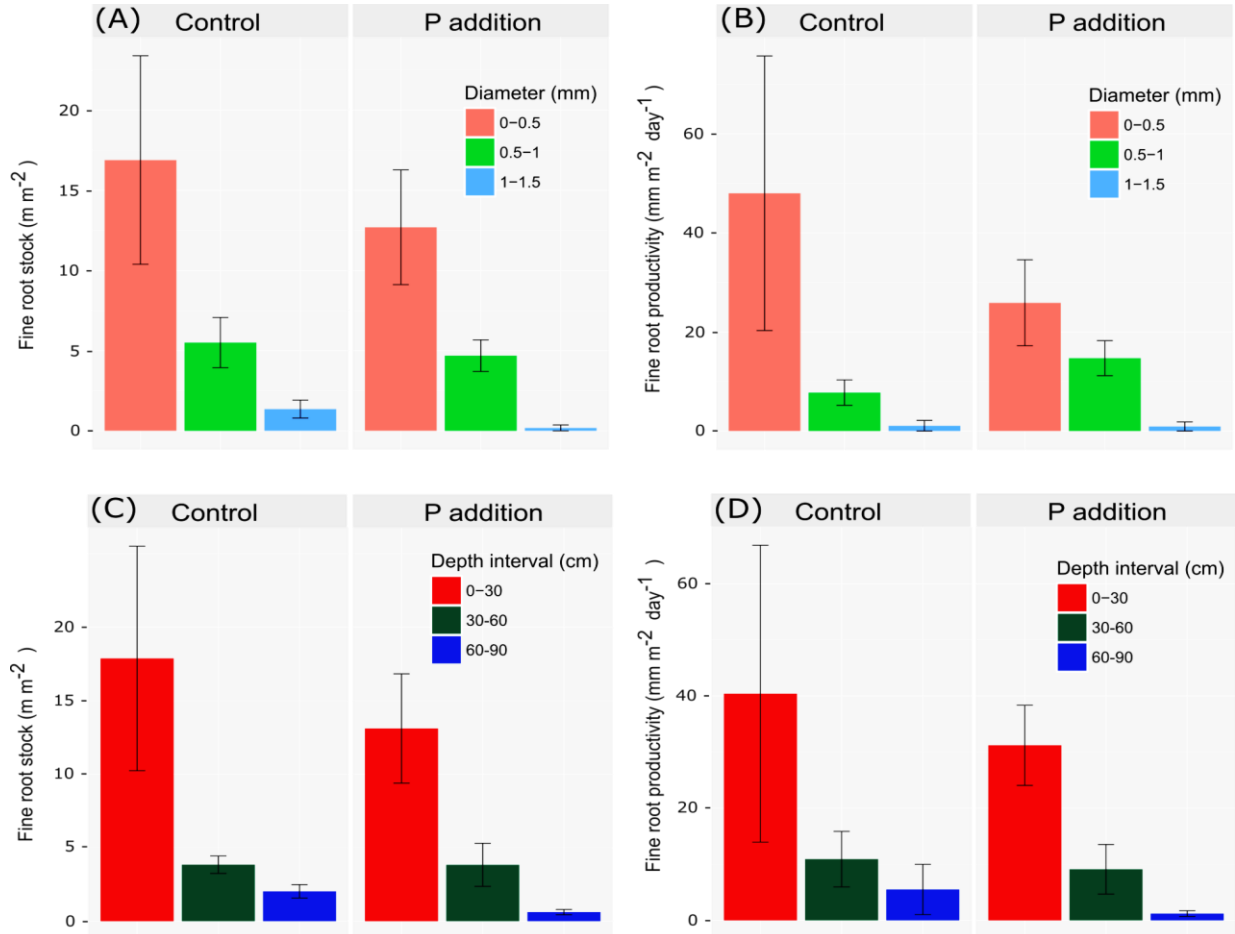
Fig. 9: Fine root dynamics by depth interval and diameter classes using minirhizotron in AFEX experiment; A: Fine root stock in the peak standing stock date by treatment and diameter classes (0-0.5, 0.5-1 and 1-1.5mm); B: Total fine-root productivity by treatment (P and control) and diameter classes; C: Fine root standing stock in the peak standing stock date by treatment and depth interval (0-30, 30-60 and 60-90cm); D: Total fine root productivity by treatment and depth intervals. Data are means ± SE.

Influence of installation disturbance on root dynamics

The fine root standing stock amplitude (Fig. 10) for the whole period of sampling was significant different between the two sites studied (p=0.02, n=8 for AFEX and n=9 for AmazonFACE). For the AFEX experiment, the tubes from P and Control plots were considered together as there was no significant difference between them. The increase in fine root standing

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stock in the AFEX experiment (151.44%) was more than the triple of the increase in AmazonFACE (39.03%) experiment over time.
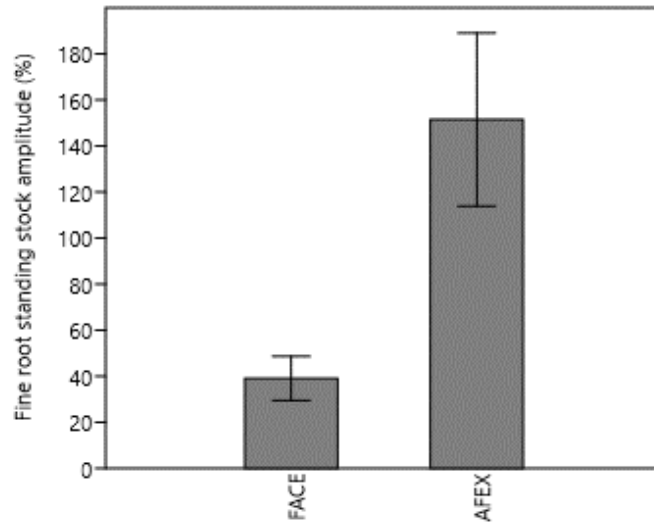
Fig. 10: Percentage of amplitude from the minimal to maximal stock which can be expressed as: Amplitude (%) = (Maximal stock – Minimal stock)*100/Minimal stock in each project (FACE and AFEX). N=8 minirhizotron tubes in AFEX and N=9 minirhizotron tubes in FACE experiments.

Discussion

Seasonal variation in root stocks and productivity

Our results show that fine root productivity occurs all year long, but fine root productivity is highly correlated with precipitation (R^2=0.66). Consequently, there is higher fine root productivity in the wet season, which is consistent with patterns observed in other tropical regions (Cavelier *et al,* 1999; Yavitt & Wright 2001; Green *et al*, 2005; Rodtassana & Tanner, 2018) and in the Amazonian Basin (Metcalfe *et al.,* 2008; Girardin *et al*., 2016). Variations on productivity patterns usually are related to resource availability and environmental changes. For example, studies in temperate forests show that changes in soil temperature are linked with patterns of root production (McCormack *et al.,* 2015). In tropical forests, however, root productivity is most likely related to water (Green *et al.*, 2005, Metcalfe *et al.,* 2008) and/or nutrients availability (Cuevas & Medina, 1988; Raich *et al.,* 1994; Ostertag, 1998; McGrath *et al*., 2001; Aragão *et al.,* 2009), being higher when water/nutrients are more available.

Soils from Central Amazonia have low nutrient content, being particularly low in rock derived nutrients such as P, which is an important nutrient limiting net primary productivity across the basin (Walker & Syers, 1976; Vitousek & Sandford 1986; Quesada *et a*l., 2010, 2012). Thus, one important strategy to overcome nutrient limitation is to increase fine roots where and when nutrients are available (Prior *et al*., 2003; Hodge, 2004). Therefore, in this region, plants acquire nutrients mostly coming from the litterfall. It happens because soils are strongly nutrient limited and most of the nutrient economy of such forests comes from the recycling of nutrients from organic matter (Vitousek, 1982; 1984). Plants can obtain nutrients as soon as they are mineralized (Jordan, 1985; Richards, 1996; Sayer *et al*., 2006) or even directly from the decaying material through interactions with mycorrhizas and/or exudation of extra-cellular compounds, in a process known as direct nutrient cycling (Went & Stark, 1968).

The peak of litterfall and new leaves productivity in Central Amazonia occurs on dry season (Wu *et al*., 2016), but the peak of litterfall decomposition occurs in early wet season, when there is enough moisture for microorganisms, with water availability playing an important role in mobilizing nutrients (Luizão & Schubart, 1987). Interestingly, in our study, the higher fine root productivity in the wet season just occurs at the 0-30 cm layer which seems reasonable as roots located closer to soil surface can have more access to nutrients coming from litterfall decomposition during the wet season. Greater fine root growth at surface layers on wet season has often been reported, while no growth occurs on dry season (Luizão, Luizão, Proctor, 2007; Rodtassana & Tanner, 2018).

Peak root growth comes at the expense of carbon used for aboveground growth, so that peak root growth may represent a trade-off between competing plant sinks (Comas *et al*., 2005). Because it is costly in terms of carbon, peak root growth may be timed to balance carbohydrate availability with periods of high nutrient and water availability, maximizing resource acquisition. It is exactly what can be observed in the present study. The peak of new leaves productivity occurring on dry season, when root production is in its lowest, suggests resource partitioning in time. This trade-off is corroborated by Girardin *et al* (2016) in the Amazonian basin who show that in humid plots there is evidence of constant allocation to wood and a seasonal trade-off between roots and canopy production. However, a more complete dataset may be needed to better understand this relationship.

Fine root standing stock in the present study also varied between dry and wet season, with a higher stock in the dry season, opposite from what was observed in productivity. This was more prominent at the 60-90 cm depth interval and it is probably related to water availability. The type of soil in this study presents a high concentration of macropores at the depth until 80 cm (Tomasella & Hodnett, 1996). As a consequence, it favors rapid water drainage and low water retention leading to a seasonal variation in water content with less soil moisture in the end of the dry season (Broedel *et al.,* 2017). On the other hand, from 80 to 480 cm of soil depth, there is a higher soil water content and a low variation of that between seasons when compared to the 0-80 cm layer. It is a consequence of an increase in total porosity because of the presence of microaggregates and microfissures among the microaggregates (Broedel *et al.,* 2017). These soil characteristics may have leaded to the higher stock of roots in deep layers in the dry season. It is probably a plant mechanism to absorb more water when it is limited (Bloom, Chapin & Mooney, 1985). In addition to water uptake, the higher stock at deep layers in the dry season may be related to P uptake as the release of water by roots into superficial dry soil layers, taken up from moist deeper layers (hydraulic lift), is expected to increase Pi acquisition (Prieto *et al.*, 2012).

Some studies have indicated that during the dry season, root growth increases in deeper soil layers to absorb water in these layers (Nepstad *et al.,* 1994; Dickmann *et al.,* 1996; Hendrick & Pregitzer, 1996; Joslin *et al.,* 2000; Sommer *et al.,* 2000; Vasconcelos *et al*., 2003). I did not observe a higher root growth in the deep layer during the dry season, but root growth over the year could have resulted in the higher stock of roots in deep layers in the dry season. On the other hand, the observed increase in fine root stock during the dry season may also be related to a decrease in fine root mortality. So, the trade-off between root standing stocks and productivity can also arise from variations in root turnover. Periods of increased production are not the same as higher stocks, suggesting an increased replacement of roots, perhaps to have them more active in periods of increased resource capture.

Furthermore, the studied forests have high biodiversity so probably the timing of root productivity can vary between species (McCormack *et al*., 2014). In competition, plants may advance the timing of peak root production in order to access resources and limit the fitness of neighbors (Eissenstat & Caldwell, 1988; Dybzinski *et al*., 2011). It may also explain variations in peaks of root productivity along the year in our results. In addition, shifts in root dynamics may be triggered by multiple factors such as local characteristics, recent environmental conditions, climatic

conditions and plant productivity (i.e. availability of carbohydrates) of the previous growing season(s) (McCormack *et al*., 2014). Therefore, a longer dataset of environmental effects on root dynamics and also experiments which consider endogenous factors may be necessary to a better understanding of what is influencing root dynamics.

Root stocks, productivity and turnover along soil depth profile

The decrease of fine root standing stock and fine root productivity with soil depth found here follows a general trend observed in tropical forests (Klinge 1973; Cavelier 1992; Duivenvoorden & Lips 1995; Pavlis & Jeník 2000; Silver *et al.,* 2000; Valverde-Barrantes *et al.,* 2006; Jiménez *et al.,* 2009) which is dominated by the proliferation of fine roots near the soil surface. In the present study, however, there is a lower percentage (~53%) of fine root standing stock (<2mm) until 30cm layer (for both length per area and mass per area) when compared to other study near Manaus which had ~62% of the fine root stock (both in length per area and mass per area) occurring in the 27cm layer (Klinge, 1973). Probably the difference between results is a consequence of different sites or methodologies as the present study was conducted with minirhizotron and in the other study roots were excavated from the soil. Anyway, in both experiments, there is a higher fine root stock at surface layers but there is also a high percentage of fine root biomass below 30cm depth which is not usually measured. In addition, I found a $\beta = 0.970$ in the date that fine root standing stock was in its peak, which is a slightly higher numerical index of rooting distribution when compared to other studies in tropical forests whose $\beta = 0.962$ for moisture tropical forests in general (Jackson *et al.*, 1996) and $\beta = 0.943$ in a tropical forest in Costa Rica (Valverde-Barrantes *et al*., 2006). It suggests a slightly greater proportion of roots with depth in our study.

It has been proposed that the high proportion of fine root standing stock closer to surface can be also related to soil texture. For example, in a site in Costa Rica, there was an increase in clay content with depth and it could impede fine root growth in deeper layers (Valverde-Barrantes *et al*., 2006). On the other hand, in the present study, the proportion of clay, silt and sand do not have a high variation along depths (Quesada *et al.,* 2011). Therefore, there was probably no soil impedance in depths. Although the proportion of fine root standing stock at the surface layers in the present study is lower than in others, there is still a considerably higher stock closer to surface which is a consequence of higher nutrient availability from decomposing litter as it was discussed

in the topic above. This root distribution through soil profile can be considered an important strategy for resource acquisition.

Although measurements of fine root turnover have rarely been conducted below the first few centimeters of the soil surface, the observation that fine root turnover decrease with soil depth in some studies (Joslin *et al.,* 2006; Germon *et al.*, 2015; this study) might indicate that the high construction costs of deep roots tends to be counter-balanced by lower maintenance costs through longer root longevity (Pierret *et al*., 2016). It may happen because as soil depth increases, microbial activity, nutrient availability and decomposition rates often decline (Gill & Burke, 2002). In the present study, there is a decrease in fine root turnover with depth, but it is not significant. The same result was found in other sites in the Amazon (Trumbore *et al.,* 2006). The observation that root turnover does not vary significantly between depths might have an important implication for future studies that need to estimate fine root productivity from fine root stock data along the soil profile. However, it needs to be considered cautiously and a longer dataset would be important to indeed prove it.

It is also important mentioning that I was measuring roots just until 90cm of depth, but roots or evidence of them were already found in higher depths at the Central Amazon such as below 6m and 4.8m (Chauvel *et al.,* 1992, Broedel *et al.*, 2017) and at the Eastern Amazon such as 8m, 10m and 11.5m (Nepstad *et al.*, 1994; Jipp *et al*., 1998; Bruno *et al.,* 2006; Markewitz *et al*., 2010).

<u>Root stocks, productivity and turnover among diameter classes</u>

In our study most of root length stock (93%) and length productivity (99%) occurs in the finest roots (<1mm). A high root length stock in very fine roots was also observed in another study in Central Amazonia in picked roots from soil (99% in roots <1mm) and in a temperate forest in Tennessee using minirhizotron (80% in roots <0.5mm) (Klinge, 1973; Norby *et al.,* 2004). Regarding fine root length productivity, the high percentage of smaller roots growth in our study is consistent with another study in an Amazonian site using ingrowth cores where roots <1 mm in diameter constituted a considerable proportion (69–75%) of total root length growth measuring roots from 0 to 5mm in diameter (Metcalfe *et al.,* 2008).

In addition, the positive relationship between RML (root mass/length) x diameter found in this study reveals that for smaller diameter roots there is a low RML which indicates that less C is being invested for the same length when compared to higher diameter roots. In other words, by

producing smaller diameter roots, plants can create roots with a higher length per C invested and then be more able to uptake and absorb nutrients (McCormack *et al*., 2015). Therefore, it indicates that plants are producing a higher quantity of finest roots as a morphological strategy to resources uptake and absorption. The relationship between RML x diameter is very similar in a temperate forest on Tennessee (Iversen *et al.,* 2008). However, despite of a similar result in our experiment, their samples fitted much better to the model in comparison to ours. This probably occurred because of the high variability of species in our experimental site compared with the one on Tennessee that has just one species.

Regarding fine root turnover, it is commonly assumed that it increases when root diameter decreases (Gill & Jackson 2000; Majdi *et al.,* 2005). Our result followed this pattern, but the differences in turnover in different diameter classes was not significative. This result may be a consequence of the fact that fine root diameter is not the only variable which can affect root turnover. For example, root longevity can be enhanced by mycorrhizal colonization and higher tissue density; and negatively correlated with nutrients concentration, root maintenance respiration and specific root length (Eissenstat *et al.,* 2000, Aragão *et al.,* 2009). In a highly diverse ecosystem is even more difficult to understand which factors are affecting root turnover as different species can be influenced in a different way to environmental constrains and changes.

Comparisons with other studies

Our study is within the reported range for tropical forests in the Amazon basin, but it is different when compared to temperate zones. For example, using the equation obtained from RML x diameter relationship to estimate biomass based on our length and diameter of roots from images obtained with minirhizotron, I obtained a root standing stock of 13.12 ± 1.98 Mg. ha^{-1} throughout the profile. This estimate is within the range of others also measured near Manaus. The first study in the region, measured fine root biomass (<2.5mm) to approximately 1 m depth as 8.4 Mg. ha^{-1} (Klinge, 1973). Another study measured root biomass (<2mm) until 40 cm depth from a similar site in Central Amazonia and found a value of 8.7 Mg. ha^{-1} (Noguchi *et al.,* 2014). Beyond the differences arising from different methodologies, the difference from that study can be caused by the measurement of root biomass until only 40 cm deep. Our results indicate that approximately 60% of root standing stock occurs until 40 cm (7.87 Mg. ha^{-1}), so if only this depth is considered, our values are very close to those reported by Noguchi *et al*., (2004). In a global meta-analysis of

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root distribution in the soil, Jackson et al., (1996) found that standing root biomass varied by over an order of magnitude across biomes, from approximately 2 to 50 Mg. ha^{-1}, with tropical evergreen forests presenting the highest root biomass. Our estimate is within the range, but far from the maximum value. It probably happened because in this study I am considering just roots <2mm in diameter.

In terms of fine root NPP, when considering just the 30 cm layer, I found that fine root NPP was 1.55 ± 0.7 Mg C.ha^{-1} year^{-1} for the top 30 cm which is almost equal to the value found in a similar site (1.58 ± 0.12 Mg C.ha^{-1} year^{-1} for the top 30 cm) near our site (Oblitas *et al.*, unpublished). Another study considering the 30 cm depth reported a mean annual value for NPP to fine roots of 3.81 ± 0.08 Mg C.ha^{-1} year^{-1} throughout humid forests (n=8) where forest composition is variable across sites (Girardin *et al.,* 2016). This estimate is higher than in the present study. Some good reasons may be that they did not account plots from Central Amazonia, considered roots between 2 and 5mm in diameter and measured NPP from 2009 to 2012, so their higher value for productivity may be a consequence of methodological, site or interannual differences.

When I considered NPP for the 90 cm depth, I found 2.77 ± 0.4 Mg C. ha^{-1} year^{-1}. At the same region, other estimates for fine root NPP up to 1-meter depth range from 2 to 7.6 Mg C. ha^{-1} year^{-1}, while forests more similar to Manaus had average fine root NPP of 3.3 Mg C. ha^{-1} year^{-1} (Aragão *et al.,* 2009). Another study reported an estimate of 2.1 ± 0.7 Mg C.ha^{-1} year^{-1} for Manaus forests, though this was only an average between other sites with measurement to 30 cm and 10 cm, but extrapolated to 100 cm depth (Malhi *et al.,* 2009). This extrapolation is calculated by assuming that fine root productivity per unit fine root biomass is invariant with depth. Then, root biomass profiles are used to estimate scaling factors to extrapolate results restricted to surface measurements into estimates for 100 cm depth. This approach somewhat matched our results since that in our study, fine root productivity divided by fine root biomass is invariant between depth intervals. However, our estimate of NPP is higher and it seems that such approach is a rough estimation at its best.

Total root turnover of this study (0.49 ± 0.18 year^{-1}) is within the range reported for other Amazonian forests (0.14 to 2.5 year^{-1}) (Jiménez *et al.*, 2009; Aragão *et al.*, 2009). The difference of fine root turnover throughout the Amazon basin can be related to soil nutrient content (Aragão *et al.*, 2009). There are some results from the literature which show that fine roots turnover is greater for plants adapted to soils with higher nutrient content than to nutrient-poor soils (Eissenstat

& Yanai, 1997). One reason for that is that roots maintenance costs can be higher than construction costs for new roots when soil nutrient resources are plentiful. The theory also predicts that plants adapted to nutrient-rich soils can increase root turnover in response to increased nutrient availability, in part, because nutrient pulses are more common in nutrient-rich than in nutrient-poor soils (Espeleta & Donovan, 2002). The relationship between turnover and nutrient-poor soils may be another explanation for the low turnover rate of the roots in our site as this site has low P and base cations content.

Direct comparison with other minirhizotron studies from temperate forests reveals significantly differences between these forest biomes. Root standing stock until 60cm depth in all dates from our site has higher values when compared to stocks from a FACE experiment in a sweetgum (*Liquidambar styraciflua L.*) plantation in eastern Tennessee for the same depth, both in control and under elevated CO_2 plots which were N limited (Norby *et al*., 2004). On the other hand, the peak in total root length growth from this study was much lower than the peaks from Tennessee in both treatments. The high difference of fine root productivity and stock between our study and that temperate forest lead to a huge difference in fine root turnover. Fine root turnover from our site (0.49 ± 0.18 year^{-1}) is smaller than the one from Tennessee FACE experiment (1.7 year^{-1}), which means that roots in the Central Amazon have a higher residence time when compared to roots from a temperate forest. This is the opposite of what was found by Gill & Jackson (2000) who evaluated root turnover in different terrestrial ecosystems. They found that root turnover decreased from tropical to high-latitude systems for all plant functional groups. In other words, it means that turnover in our site should be higher as it is in a tropical ecosystem If latitude was the only factor affecting root turnover. However, it can be influenced by many other factors such as microsite conditions, root development patterns, growing season length, plant mineral nutrient conservation, mycorrhizal colonization, root maintenance respiration and specific root length (Eissenstat *et al.,* 2000, Gill & Jackson, 2000; Majdi *et al.,* 2005; Aragão *et al.,* 2009).

Identifying patterns and variation in fine root dynamics across ecosystems represents a basic and necessary step to improve understanding of plant and ecosystem processes, especially in the context of climatic change and elevated CO_2.

<u>Methods comparison</u>

Direct comparison between studies is somewhat difficult since there are many methodological variations between them (Hendricks *et al.,* 2006). Different sampling methods lead to intrinsic variations in sampled root diameter, root morphologies (i.e. root orders), diameter cut-offs, depth and soil variations. The most commonly used method in root research is destructive soil coring (Rewald & Ephrath, 2013). The advantage of this method regarding fine root standing stock is that it reveals root biomass directly without the need for calibration (Ephrath *et al.,* 1999). Furthermore, roots from soil cores can often be reliably separated into live and dead roots or into root orders being an appropriate method to access root function (Rewald *et al.,* 2011; McCormack *et al.,* 2015).

In the opposite, roots from minirhizotron cannot be removed from soil, so separating roots into dead/alive or into root orders is not reliable. Also, fine root biomass need to be estimated by using the mass/length/diameter relationship (RML x diameter) from picked roots. In addition, assumptions regarding depth-of-field (DOF) must be made when root length is converted to biomass. Typically, the values used for DOF range from two to three millimeters (Sanders & Brown, 1978; Itoh, 1985; Steele *et al.,* 1997; Brown, 2009), so our DOF (2.5mm) is within this range. Although I had to calibrate the minirhizotron with data obtained from nearby soil cores to find DOF and estimate the minirhizotron soil volume occupied by roots, our minirhizotron biomass estimate seems reasonable when compared to other studies as discussed above.

Although minirhizotron in our site can present good estimates of fine root biomass and can even reach higher depths (90cm) than soil coring (15cm), it is still important having both methods to calibrate minirhizotrons for each site studied. Minirhizotrons are especially important in long-term studies because it has minimum site disturbance (Majdi, 1996). In addition, each root can be followed over time, so it is possible to follow peaks of root growth and mortality. Because of its high spatial and temporal resolution, it has been considered the most suitable method to access fine root productivity and turnover in long-term studies (Hendricks *et al.,* 2006; Addo-danso *et al.,* 2015). However, the main disadvantage of minirhizotron method is the substantial cost involved in acquiring equipment and processing the root data. Thus, the most common method to obtain fine root productivity throughout tropics is the ingrowth core, which is cheaper than minirhizotron (Addo-danso *et al.,* 2015).

Although its low cost, some studies, mainly in temperate forests, have generally found that ingrowth core could under-estimate fine root productivity when compared to minirhizotrons (see Appendix B at Addo-danso *et al.,* 2015). This may be related to the fast turnover of roots, which can be missed before the next sampling or to the mesh size of the ingrowth cores (Hertel & Leuschner, 2002; Adamek *et al.,* 2011; Milchunas, 2012). On the other hand, roots may also be stimulated to grow. The soil used for ingrowth cores are homogenized and replaced in each sample collection, so it can lead to changes in the physical and chemical properties of the soil becoming an artificial and less competitive soil environment (Majdi, 2006). Therefore, it can be colonized at different rates than other parts of the soil and may overestimate fine root productivity (Vogt *et al.,* 1998; Fahey *et al.,* 1999; Lauenroth, 2000).

Therefore, perhaps these limitations can be counterbalanced giving a good estimate of fine root productivity. Interestingly, in the present study, annual fine root productivity did not have a significant difference between methods (minirhizotron and ingrowth core). It is consistent with another study in the Amazon where fine root productivity from ingrowth cores and rhizotrons showed similar estimates (Girardin *et al.,* 2013). Nevertheless, in our study, fine root productivity was not always the same for the different methods. It can be a consequence of the differences between methodologies or between periods of sampling. Minirhizotrons and ingrowth cores were not measured exactly in the same dates, so peaks of growth may have happened in both methods, but the slight difference in sample dates could have leaded to different estimates in some periods. It seems that annual estimates are more trustable. However, further analysis is needed to evaluate if total fine root productivity over the year will keep equal between both methods.

Finally, a similar limitation of ingrowth core and minirhizotron is that both methods underestimate fine root productivity in the litterfall which is particularly important in the site studied. Therefore, other methodologies need to be used to access this component such as ingrowth cores and minirhizotron for surface.

Influence of P on root dynamics

P addition did not affect fine root dynamics in the site studied. It may be happening as a reason of the tubes stabilization time. It seems that roots are still recovering from disturbance as the amplitude of fine root stock over time is higher in AFEX experiment when compared the AmazonFACE site where tubes are probably stabilized. Even though, fine roots may be responding

in a different way from disturbance in the different treatments. Therefore, the fine root productivity in the treatments can still be compared, although more data collection is needed to find out if the results change after fine root stock near tubes stabilize.

Another possibility for the lack of community response to fertilization is that the treatment was not effective. Roots acquire P from the soil solution as soluble inorganic phosphate (Pi) as it is readily available for uptake by plants. Nevertheless, the Pi pool in the soil solution tends to be small (Pierre & Parker, 1927) because a large fraction of the Pi pool, can be strongly sorbed to oxides and hydroxides of iron and aluminium (Barrow, 1999; Borie & Rubio, 2003). Then, in the present study, the P added might have become unavailable to plants. However, I can`t affirm that as I do not have access to soil analysis yet. The soil analysis need to be undertaken to have a better comprehension about what has happened to the P added. On the other hand, I can still speculate about the reasons why that have happened and compare this study with other ones in the tropical region.

In that respect, to our knowledge, Aragão *et al*., (2009) has done the most comprehensive study about fine root productivity along the Amazon basin in relation to P availability. They reported that both above and below-ground production increased along a gradient of soil P availability. On the other hand, they presented that soil texture (proportion of clay in the soil), seemed to be more important than soil fertility in determining the proportion of NPP allocated below-ground. Sites with less clay content tended to allocate a higher proportion of their NPP to below-ground. A similar pattern was observed by Carvalheiro & Nepstad (1996) in eastern Amazon. They found that fine-roots proliferated in soils with low resistance to root penetration, even though levels of available nutrients in the soil were similar to, or lower than, levels in the adjacent soil offering higher resistance. This suggests that sandy soils may favor allocation to below-ground due to greater penetrability than clay-rich soils.

Because of that combining effect of soil P availability and texture, and other effects such as differences on climate or species distribution throughout a large area (i.e. Amazon basin), large-scale fertilization experiments are useful to maximize the effect of nutrients in our dependent variables and minimize the contribution of other effects, decreasing artifacts. For this reason, I decided to compare our results with results from other large-scale experiments in the tropics.

The large-scale experiments in the tropics are located in Costa Rica (2007-in progress), Borneo (1993-1998), Panama (1998 – in progress) and Cameroon (1995-1997). However, just two

of them have studied fine root dynamics. In Costa Rica, for instance, Alvarez-Clare et al., (2013) used ingrowth and soil cores to evaluate fine root dynamics and they did not detect any difference on root biomass or growth two years after initial P fertilization. In Panama, Yavitt *et al*., (2011) investigated root biomass and turnover using minirhizotron and found no difference after 2 years of P addition. In this case, fine root dynamics responded to added K rather than added P. Also, in Panama, Wurzburger & Wright (2015) used soil cores to evaluate fine root standing stock biomass, mycorrhizal association and root nutrients concentrations in response to P addition and they also found no change in fine root biomass after 14 years. In contrast, they found that P addition increased root mycorrhizal colonization and root P concentrations. Those authors also encountered that when P was combined with other nutrients such as N or K, total fine-root biomass declined by 50%.

It seems that multiple soil nutrients seem to regulate fine root dynamics in species-rich lowland tropical forests, rather than P alone. In addition, the insignificant effect of P addition on fine root dynamics does not mean that roots are not absorbing P. It has been demonstrated that P addition can affect other components of primary productivity such as litterfall and trees basal area (Wright *et al.*, 2011; Alvarez-Clare, Mack & Brooks, 2013). Therefore, it can be speculated that an increase on mycorrhizal colonization as P increases may help on P absorption. It can be supported by the fact that root P concentration increased after P addition in the Panama experiment (Wurzburger & Wright, 2015).

On the other hand, there are other possible explanations for this lack of response to fertilization in our study. Possibly, the plant community is co-limited by a nutrient other than P, potentially potassium or a combination of macronutrients (Herbert & Fownes 1995; Kaspari *et al*., 2008; Townsend *et al*., 2011; Wright *et al.,* 2011; Yavitt *et al.,* 2011; Baribault *et al*., 2012). In the opposite, some of the species may not be limited to low P availability. If the P added become available to plants, what may have happened was a species-specific response to an increase of P availability. Turner *et al.,* (2018) studying a steep natural P gradient in Panama forests, demonstrate that community-wide growth rates did not vary significantly across the P gradient. They argue that in species-rich plant communities, P limitation is species-specific, and this kind of environment contains species that can be productive on low-P soils. There are several mechanisms, which can be used to increase the ability of some species to acquire P.

For instance, mycorrhizal associations increase the affinity of infected roots for P in solution, lowering its threshold concentration to absorption (Mosse *et al*., 1973) and leading to

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more desorption of P from the labile pools. In addition, the P that is adsorbed by iron and aluminum oxides and otherwise unavailable to plant roots can also become available to plants as mycorrhizal associations can induce its release (Alexander, 1989). Furthermore, plants can secrete enzymes such as nucleases and acid phosphatases that mobilise inorganic-P (Pi) from the soil's organic-P pool (Plaxton & Tran, 2011), as well as release carboxylates that mobilise P from both organic-P and (Pi) sources (Lambers *et al.*, 2006; Lambers *et al*., 2008).

Therefore, even if those species are exposed to a higher amount of P available, they may already be adapted to low P conditions, not increasing their growth. In contrast, species that occur naturally in P-impoverished environments with a very low capacity to down-regulate their Pi-uptake system can be very sensitive to P toxicity (Shane *et al.,*2004; de Campos *et al.*, 2013). Interestingly, one such P-sensitive species, *Eucalyptus marginata* (jarrah), only shows severe P-toxicity symptoms following a toxic Pi pulse when it is not colonized by mycorrhizal fungi (Kariman *et al.*, 2014), which suggests that its Pi-uptake systems are downregulated only when the plants are mycorrhizal.

Conclusion

Our results highlight the important role of deep roots in fine root dynamics as almost half of fine root dynamics is found in the layers below 30cm. Therefore, depths below that should be taken into account because the deeper roots dynamics may respond in a different way to environmental changes when compared to roots closer to surface. Fine root stock and productivity were significant different between all depth intervals decreasing with depth, but turnover did not show variation and it is probably related to the higher nutrient availability at the surface layers. Fine root productivity and stock were different between diameter classes, being higher in roots with smaller diameter classes. It may be related to water and nutrients absorption as finest roots are the ones responsible for resources acquisition and absorption (McCormack *et al*., 2015). However, fine root turnover did not vary between diameter classes which can be a consequence of the many factors that can affect turnover. In addition, fine root productivity was totally related to precipitation, being higher when precipitation was also higher. Therefore, there was more productivity in early wet season, mainly in the 0-30cm layer interval and a fine root standing stock

was higher in the dry season, mainly in the 60-90cm layer. This difference was possibly also related to nutrient availability with the microorganisms playing an important role in this seasonality. Minirhizotron and ingrowth core gives similar values for fine root annual productivity, but it need to be re-evaluated for the next years as it has variations during the year. It seems that P availability may not have influence in root dynamics in a short-term period. There are some possible reasons for that, such as that the number of repetitions of our experiment was not enough; fine root dynamics may not respond in a short-term period; the minirhizotron tubes did not reach stability; the P added did not become available to plants; the forest is already adapted to low-P content in soil or roots could have responded to P addition by associating with mycorrhizas.

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References

Adamek M, Corre MD, Hölscher D. 2011. Responses of fine roots to experimental nitrogen addition in a tropical lower montane rain forest, Panama. J. *Trop. Ecol.* **27**, 73–81.

Addo-Danso SD, et al. 2015. Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: A review. *Forest Ecol. Manage*.

Alexander IJ .1989. Systematics and ecology of ectomycorrhizal legumes. In: Stirton CH, Zarucchi JL (eds) *Advances in Legume Biology*. Missouri Botanical Garden, Missouri, pp 607–624

Allen MF.1991. The ecology of mycorrhizae. *Cambridge University Press*.

Alvarez-Clare S, Mack MC, Brooks M. **2013**. A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. *Ecology* **94**: 1540–1551.

Aragão LEOC, Malhi Y, Metcalfe DB, Silva-Espejo JE, Jiménez E, Navarrete D, Almeida S, Costa ACL, Salinas N, Phillips OL, Anderson LO, Alvarez E, Baker TR, Gonçalvez PH, Huamán-Ovalle J, Mamani-Solórzano M, Meir P, Monteagudo A, Patiño S, Peñuela MC, Prieto A, Quesada CA, Rozas-Dávila Rudas A, Silva Jr JÁ, Vásquez R. 2009. Above-and below-ground net primary productivity across ten Amazonian forests on contrasting soils. *Biogeosciences* **6:** 2759–2778.

Araujo AC, Nobre AD, Kruijt B, Elbers JA, Dallarosa R, Stefani P, von Randow C, Manzi AO, Culf AD, Gash JHC, Valentini R, Kabat P. 2002. Comparative measurements of carbon dioxide fluxes from two nearby towers in a central amazonian rainforest: The manaus lba site. *Journal of Geophysical Research-Atmospheres***107**(D20): art. no.-8090.

Araujo AC., Nobre AD, Kruijt B, Elbers JA, Dallarosa R, Stefani P, von Randow C, Manzi AO, Culf AD, Gash JHC, Valentini R, Kabat P. 2002. Comparative measurements of carbon dioxide fluxes from two nearby towers in a central amazonian rainforest: The manaus lba site. *Journal of Geophysical Research-Atmospheres***107**(D20): art. no.-8090.

Baddeley JA, Watson CA. 2005. Influences of root diameter, tree age, soil depth and season on fine root survivorship in Prunus avium. *Plant and Soil* **276**: 15–22.

Baribault TW, Kobe RK, Finley AO. **2012**. Tropical tree growth is correlated with soil phosphorus, potassium, and calcium, though not for legumes. *Ecological Monographs* **82**: 189–203.

Barrow NJ. 1999. A simple equation to describe sorption of anions by goethite. *European Journal of Soil Science* **50**, 151–155.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**(1), 1-48.

Bates, G.H. 1937. A device for the observation of root growth in the soil. *Nature*, **139**, 966–967.

Blair BC, Perfecto I. 2001. Nutrient content and substrate effect on fine root density and size distribution in a Nicaraguan rain forest. *Biotropica* **33**: 697–701.

Bloom AJ, Chapin FS, Mooney HA. 1985. Plants-an Economic Analogy. *Annual Review of Ecology and Systematics* **16**: 363–392.

Booth BB. et al. 2012. High sensitivity of future global warming to land carbon cycle processes. *Environ. Res. Lett*. **7**, 024002.

Borie F and Rubio R. 2003. Total and organic phosphorus in Chilean volcanic soils. *Gayana Botanica* **60**, 69–73.

Brienen RJW, Phillips OL, Feldpausch TR, Gloor E, Baker TR, Lloyd J, Lopez-Gonzalez G, Monteagudo-Mendoza A, Malhi Y, Lewis SL, et al. 2015. Long-term decline of the Amazon carbon sink. *Nature* **519**: 344–8.

Broedel E, Tomasella J, Cândido LA, von Randow C. 2017. Deep soil water dynamics in an undisturbed primary forest in central Amazonia: Differences between normal years and the 2005 drought. *Hydrological Processes*. **31**:1749–1759.

Brown ALP, Day FP, Stover DB. 2009. Fine root biomass estimates from minirhizotron imagery in a shrub ecosystem exposed to elevated CO2. *Plant and Soil* **317**:145-153.

Bruno RD, Rocha HR, Freitas HC, Goulden ML, Miller SD. 2006. Soil moisture dynamics in an eastern Amazonian tropical forest. *Hydrological Processes*, **20**, 2477–2489.

Bugmann H, Bigler C. 2011. Will the CO2 fertilization effect in forests be offset by reduced tree longevity? *Oecologia* **165**, 533–544.

Cannell MGR, Dewar RC. 1994. Carbon allocation in trees: a review of concepts for modelling. *Adv Ecol Res* **25**:59–104.

Carvalheiro KO, Nepstad DC. **1996**. Deep soil heterogeneity and fine root distribution in forests and pastures of eastern Amazonia. *Plant and Soil* **182**: 279–285.

Cavelier J, Tanner EVJ, Santamaría J. 2000. Effect of water, temperature and fertilizers on soil nitrogen net transformations and tree growth in an elfin cloud forest of Colombia. J *Trop Ecol* **16**:83–99

Cavelier J, Wright S J and Santamaria J .1999. Effects of irrigation on litterfall, fine root biomass and production in a semidecidous lowland forest in Panama. *Plant Soil***211**, 207–213.

Cavelier J. **1992**. Fine-root biomass and soil properties in a semideciduous and a lower montane rain forest in Panama. *Plant and Soil* **142**: 187–201.

Cernusak LA, Winter K, Turner BL. 2010. Leaf nitrogen to phosphorus ratios of tropical trees: experimental assessment of physiological and environmental controls.*New Phytol* **185**:770–779.

Chapin FS. 1980. The mineral nutrition of wild plants. Annu. Rev. *Ecol. Syst.*, **11**, 233–260.

Chauvel A, Vital ART, Lucas Y, Desjardins T, Franken WK, Luizão FJ, Bedmar AP. 1992. O papel das raízes no ciclo hidrológico da floresta amazônica. Proceedings of the VII Brazilian Congress of Meteorology, São Paulo. September 1992.

Chen HYH, Brassard BW. **2013**. Intrinsic and Extrinsic Controls of Fine Root Life Span. *Critical Reviews in Plant Sciences* **32**: 151–161.

Clark DA, Brown S, Kicklighter DW, Chambers JQ, Thomlinson JR, Ni J. 2001. Measuring net primary production in forests: Concepts and field methods, *Ecol. Appl.*, **11**, 356–370.

Cleveland CC, Taylor P, Chadwick KD, Dahlin K, Christopher E. Doughty YM, Smith WK, Benjamin W. Sullivan WRW, Townse AR. 2015. A comparison of plot-based satellite and Earth system model estimates of tropical forest net primary production. *Global Biogeochemical Cycles*: 626–644.

Comas LH, Anderson LJ, Dunst RM, Lakso AN, Eissenstat DM. 2005. Canopy and environmental control of root dynamics in a long-term study of Concord grape.*New Phytol* **167**:829–840.

Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* **408**, 184–187.

Cox PM, Pearson D, Booth BB, Friedlingstein P, Huntingford C, Jones CD, Luke CM. 2013. Sensitivity of tropical carbon to climate change constrained by carbon dioxide variability. *Nature* **494**: 341–4.

Crews TE, et al. 1995. Change in soil phosphorus fractions and eco- system dynamics across a long chronosequence in Hawaii, *Ecology*, **76**, 1407–1424.

Cuevas E, Medina E. 1988. Nutrient dynamics within Amazonian forests. II. Fine root growth, nutrient availability and leaf litter decomposition. *Oecologia* **76**: 222–235.

de Campos MCR, Pearse SJ, Oliveira RS. *et al.* **2013.** Down-regulation of net phosphorus uptake capacity is inversely related to leaf phosphorus resorption in four species from a phosphorus-impoverished environment. *Annals of Botany* **111**, 445–454.

de Mendiburu F. 2014. agricolae: statistical procedures for agricultural research. R package version 1.2-1. http://CRAN. R-project.org/package=agricolae.

Dickmann DI, Nguyen PV, Pregitzer KS. 1996. Effects of irrigation and coppicing on above-ground growth, physiology, and fine-root dynamics of two field-growth hydric poplar clones. *Forest Ecology and Management* **80**.

Doughty CE, Metcalfe DB, Girardin CAJ, Amézquita FF, Cabrera DG, Huasco WH, Silva-Espejo JE, Araujo-Murakami A, da Costa MC, Rocha W, et al. 2015. Drought impact on forest carbon dynamics and fluxes in Amazonia. *Nature* **519**: 78–82.

Duivenvoorden JF and Lips JM. 1995. A land-ecological study of soils, vegetation, and plant diversity in Colombian Amazonia. The Tropenbos Foundation, Wageningen, Netherlands, **438** pp.

Dybzinski R, Farrior C, Wolf A, Reich PB, Pacala SW. 2011. Evolutionarily stable strategy carbon allocation to foliage, wood, and fine roots in trees competing for light and nitrogen: an analytically tractable, individual-based model and quantitative comparisons to data. *American Naturalist* **177**, 153–166.

Eissenstat DM, Caldwell MM. 1988. Competitive ability is linked to rates of water extraction— a field study of 2 arid land tussock grasses. *Oecologia* **75**, 1–7.

Eissenstat DM, Wells CE, Yanai RD. 2000. Building roots in a changing environment: implications for root longevity. New Phytologist. 33-42.

Eissenstat DM, Yanai RD. **1997**. The Ecology of Root Lifespan. *Advances in Ecological Research* **27**: 1–60.

Ephrath JE, Silberbush M, Berliner PR. 1999. Calibration of minirhizotron readings against root length density data obtained from soil cores. *Plant and Soil* **209**, 201–208.

Espeleta JF, Clark DA. 2007. Multi-scale variation in fine-root biomass in a tropical rain forest: A seven-year study, *Ecological monographs*, **77**(3), 377–404.

Espeleta JF, Donovan LA. **2002**. Fine root demography and morphology in response to soil resources availability among xeric and mesic sandhill tree species. *Functional Ecology* **16**: 113–121.

Fahey TJ, Bledsoe CS, Day FP, Ruess RW, Smucker AJM. 1999. Fine root production and demography. In: Robertson GP, Coleman DC, Bledsoe CS, Solins P, eds. Standard soil methods for long-term ecological research. New York, NY, USA: *Oxford University Press*, 437-455.

Feldpausch TR. et al. 2012. Tree height integrated into pantropical forest biomass estimates. *Biogeosciences* **9**, 3381–3403.

Freschet GT, Roumet C. **2017**. Sampling roots to capture plant and soil functions. *Functional Ecology* **31**: 1506–1518.

Friedlingstein P, Cox P, Betts R, Bopp L, von Bloh W, Brovkin V, Cadule P, Doney S, Eby M, Fung I, et al. 2006. Climate–Carbon Cycle Feedback Analysis: Results from the C 4 MIP Model Intercomparison. *Journal of Climate* **19**: 3337–3353.

Friend AD. et al. 2013. Carbon residence time dominates uncertainty in terrestrial vegetation responses to future climate and atmospheric CO2. *Proc. Natl Acad. Sci.* **111**, 3280–3285.

Gale MR, Grigal DF. 1987. Vertical root distribution of northern tree species in relation to successional status. *Can J For Res* **17**:829–834

Germon A, Cardinael R, Prieto I, et al.,. 2015. Unexpected phenology and life- span of shallow and deep fine roots of walnut trees grown in a silvoarable Mediterranean agroforestry system. *Plant and Soil* **401**: 409–426.

Gill RA, Burke IC. 2002. Influence of soil depth on the decomposition of Bouteloua gracilis roots in the shortgrass step. *Plant Soil* **241**, 233–242.

Gill RA, Jackson RB. 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* **147**: 13–31.

Girardin CAJ, Aragao LEO, Malhi Y, Huaranca Huasco W, Metcalfe DB, Durand L, Mamani M, Silva-Espejo JE, Whittaker RJ. 2013. Fine root dynamics along an elevational gradient in tropical Amazonian and Andean forests. Global *Biogeochemical Cycles*, v. **27**, n. 1, p. 252–264.

Girardin CAJ, Malhi Y, Doughty CE, Metcalfe DB, Meir P, Aguila-pasquel J, Araujo-murakami A, Costa ACL, Silva-espejo JE, Amézquita FF, et al. 2016. Seasonal trends of Amazonian rainforest phenology, net primary productivity, and carbon allocation. *Global Biogeochemical Cycles:* 700–715.

Gower ST. 1987. Relationships between mineral nutrient availability and fine root biomass in two Costa Rican wet forests: a hypothesis. *Biotropica* **19**:171–175.

Green JJ, Dawson LA, Proctor J, Duff EI, Elston DA. 2005. Fine root dynamics in a tropical rain forest is influenced by rainfall. *Plant and Soil*, **276**: 23-32.

Hendrick RL, Pregitzer KS. 1993. The dynamics of fine root length, biomass, and nitrogen content in two northern hardwood ecosystems. *Can. J. For. Res.* **23**, 2507–2520.

Hendrick RL, Pregitzer KS. 1996. Applications of minirhizotrons to understand root function in forests and other natural ecosystems. *Plant and Soil* **185**: 293–304.

Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecot SD, Guo D. 2006. Assessing the patterns and controls of fi ne root dynamics: an empirical test and methodological review. *J Ecol* **94**:40– 57.

Hendricks JJ, Nadelhoffer KJ, Aber JD. 1993. Assessing the role of fine roots in carbon and nutrient cycling. *Trends in Ecology and Evolution* **8**:174–178.

Hertel D, Leuschner C. 2002. A comparison of four different fine root production estimates with ecosystem carbon balance data in a Fagus-Quercus mixed forest. *Plant Soil* **239**, 237–251.

Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist* **162**: 9-24.

Huntingford C. et al. 2013. Simulated resilience of tropical rainforests to CO2-induced climate change. *Nature Geosci*. **6**, 268–273.

Itoh S. 1985. In situ measurement of rooting density by micro-rhizotron. *Soil Science and Plant Nutrition* **31**:653-656.

Iversen CM, Ledford J, Norby RJ. 2008. CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. *New Phytologist* **179**: 837–847.

Iversen CM. 2010. Digging deeper: Fine-root responses to rising atmospheric CO2 concentration in forested ecosystems. *New Phytologist* **186**: 346–357.

Jackson RB, Canadell J, Ehleringer JR, Mooney H a, Sala OE, Schulze ED. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* **108**: 389–411.

Jackson RB, Mooney H a, Schulze ED. 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 7362–7366.

Jiménez EM, Moreno FH, Nuela MCP, Pat No S, Lloyd J. 2009. Fine root dynamics for forests on contrasting soils in the Colombian Amazon. *Biogeosciences* **6**: 2809–2827.

Jiménez EM, Peñuela-Mora MC, Sierra CA, Lloyd J, Phillips OL, Moreno FH, Navarrete D, Prieto A, Rudas A, Álvarez E, Quesada CA, Grande-Ortíz MA, García-Abril A, Patiño S. 2014. Edaphic controls on ecosystem-level carbon allocation in two contrasting Amazon forests, J. *Geophys. Res. Biogeosci.,* **119**, doi:10.1002/2014JG002653.

Jipp P, Nepstad DC, Cassel DK, de Carvalho CR. 1998. Deep soil moisture storage and transpiration in forests and pastures of seasonally-dry Amazonia. *Climatic Change*, **39**, 395–412.

Johnson MG, Tingey DT, Phillips DL, Storm MJ. 2001. Advancing fine root research with minirhizotrons. *Environ Exp Bot* **45**:263–89.

Jordan CF. 1985. Nutrient Cycling in Tropical Forest Ecosystems. *John Wiley and Sons*, Ltd, pp 190.

Joslin JD, Gaudinski JB, Torn MS, Riley WJ, Hanson PJ. 2006. Fine-root turnover patterns and their relationship to root diameter and soil depth in a 14C-labeled hardwood forest. *New Phytologist* **172**: 523–535.

Joslin JD, Wolfe MH, Hanson PJ. 2000. Effects of altered water regimes on forest root systems. *New Phytologist* **147**: 117–129.

Judd LA, Jackson BE, Fonteno WC. **2015**. Advancements in Root Growth Measurement Technologies and Observation Capabilities for Container-Grown Plants. *Plants* **4**: 369–392.

Kariman K, Barker SJ, Finnegan PM. *et al.,* **2014**. Ecto- and arbuscular mycorrhizal symbiosis can induce tolerance to toxic pulses of phosphorus in jarrah (*Eucalyptus marginata*) seedlings. *Mycorrhiza* **24**, 501–509.

Kaspari M, Garcia MN, Harms KE, Santana M, Wright SJ, Yavitt JB. **2008**. Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecology Letters*.

King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherty P. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist* **154**: 389–398.

Klinge H. 1973. Root mass estimation in lowland tropical rain forests of central Amazonia, Brazil: I. Fine root masses of pale yellow latosol and a giant humus podzol. *Tropical Ecology* **14**: 29–38.

Lambers H, Raven JA, Shaver GR. *et al.* **2008.** Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution* **23**, 95–103.

Lambers H, Shane MW, Cramer MD. *et al.* **2006**. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* **98**, 693–713.

Lapola DM and Norby RJ. 2014. Amazon-FACE: Assessing the Effects of Increased Atmospheric CO2 on the Ecology and Resilience of the Amazon Forest - *Science Plan and Implementation Strategy*. Brasília: Ministério de Ciência, Tecnologia e Inovação.

Lauenroth WK. 2000. Methods of estimating belowground net primary production. In Sala OE, Jackson RB, Mooney HA and Howarth RW, eds. *Methods in Ecosystem Science*. New York: Springer. 58–71.

Lima TTS, Miranda IS, Vasconcelos, SS. 2010. Effects of water and nutrient availability on fine root growth in eastern Amazonian forest regrowth, Brazil. *New Phytol.*

Lovejoy T, Bierregaard R. 1990. Central Amazonian forests and the Minimum Critical Size of Ecosystem Project. In: Gentry A (eds) Central Amazonian forests and the Minimum Critical Size of Ecosystem Project. *Yale University Press*, New Haven, pp 60–71.

Lovejoy TE, Nobre C. 2018. Amazon Tipping Point. *Sci. Adv.4*, eaat2340.

Luizão FJ, Schubart HOR. 1987. Litter production and decomposition in a terra-firme forest of Central Amazonia. *Experientia* **43**: 259–265.

Luizão RCC, Luizão FJ, Proctor J. 2007. Fine root growth and nutrient release in decomposing litter in three contrasting vegetation types in central Amazonia. *Plant Ecology* **192**: 225–236.

Majdi H, Pregitzer K, Moren AS, Nylund JE, Agren GI. 2005. Measuring fine root turnover in forest ecosystems. *Plant and Soil* **276**: 1–8.

Majdi H. 1996. Root sampling methods – applications and limitations of the minirhizotron technique. *Plant and Soil* **185**: 255–258.

Malhi Y, Aragão LEOC, Metcalfe DB, Paiva R, Quesada CA, Almeida S, Anderson L, Brando P, Chambers JQ, Costa ACL, Hutyra L, Oliveira P, Patino S, Pyle EH, Robertson AL, Teixeira LM. 2009. Comprehensive assessment of carbon productivity, allocation and storage in three Amazonian forests, *Global Change Biol.*,**15**(5), 1255–1274.

Malhi Y, Doughty CE, Goldsmith GR., Metcalfe DB, Girardin CAJ, Marthews TR, Phillips OL. 2015. The linkages between photosynthesis, productivity, growth and biomass in lowland Amazonian forests. *Global Change Biology*, **21**(6), 2283–2295.

Malhi, Y, Doughty C, and Galbraith C. 2011. The allocation of ecosystem net primary productivity in tropical forests, *Philos.Trans.R. Soc.*, Ser. B, **366**(1582), 3225–3245, doi:10.1098/rstb.2011.0062.

Markewitz D, Devine S, Davidson EA, Brando P and Nepstad DC. 2010. Soil moisture depletion under simulated drought in the Amazon: Impacts on deep root uptake. *New Phytologist,* **187**, 592–607.

Matamala R, Gonzalez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH. 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science* **302**: 1385–1387.

Maycock CR, Congdon RA. 2000. Fine root biomass and soil N and P in north Queensland rain forests. **32**: 185–190.

McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM. 2014. Variability in root production, phenology, and turnover rate among 12 temperate tree species.*Ecology* **95**, 2224–2235.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, et al. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* **207**: 505–518.

McCormack ML, Gaines KP, Pastore M, Eissenstat DM. 2015. Early season root production in relation to leaf production among six diverse temperate tree species.*Plant and Soil* **389**, 121–129.

McGrath DA, Duryea ML, Cropper WP. 2001. Soil phosphorus availability and fine root proliferation in Amazonian agroforests 6 years following forest conversion.*Agriculture, Ecosystems and Environment* **83**: 271–284.

McGroddy M, Silver WL. 2000. Variations in belowground carbon storage and soil CO2 flux rates along a wet tropical climate gradient. *Biotropica* **32**: 614–624.

Meir P, Metcalfe D, Costa AC, Fisher R. 2008. The fate of assimilated carbon during drought: impacts on respiration in Amazon rainforests. Philosophical Transactions of the Royal Society B: *Biological Sciences* **363**: 1849–1855.

Metcalfe DB, Meir P, Aragão LEOC, Da Costa ACL, Braga AP, Gonçalves PHL, De Athaydes Silva Junior J, De Almeida SS, Dawson LA, Malhi Y, et al. 2008. The effects of water availability on root growth and morphology in an Amazon rainforest. *Plant and Soil.*

Milchunas DG. 2012. Root and errors associated with different root production methods and their effects on field estimates of belowground net primary production. In: *Mancuso (Ed.), Measuring Roots.* Springer, Berlin, pp. 303–339 (Chapter 16).

Mosse B, Hayman DS and Arnold DJ. 1973. Plant growth responses to VA mycorrhizas, *New Phytol.*, **72**, 809–815.

Nadelhoffer KJ, Aber JD, Melillo JM. **1985**. Fine Roots, Net Primary Production, and Soil Nitrogen Availability: A New Hypothesis. *Ecological Society of America* **66**: 1377–1390.

Nadelhoffer KJ. 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystems. *New Phytol.* **147**, 131–139.

Nepstad DC, Carvalho CJRD, Davidson EA, Jipp PH, Lefevbre PA, Negreiros GH, daSilva E, Stone TA, Trumbore SE, Viera S. 1994. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. *Nature* **372**: 666–669.

Noguchi H, Suwa R, Adélia C, Souza S D, Kajimoto T, Ishizuka M. 2014. Examination of vertical distribution of fine root biomass in a tropical moist forest of the central Amazon, Brazil. *Jarq* **48**: 231–235.

Norby R, Fitter A, Jackson R. **2000**. Root dynamics and global change: An ecosystem perspective. **147**: 3–12.

Norby RJ, De Kauwe, MG, Domingues TF, Duursma RA., Ellsworth DS, Goll DS, et al. 2016. Model–data synthesis for the next generation of forest free air CO2 enrichment (FACE) experiments. *New Phytol.* **209**, 17–28. doi: 10.1111/nph.13593.

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG. 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO2 enrichment. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 9689–93.

Ostertag R. 1998. Belowground effects of canopy gaps in a tropical wet forest. *Ecology* **79**: 1294–1304.

Ostertag R. 2001. Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montaine forests. *Ecology* **82**, 485–499.

Pavlis J, Jeník J. **2000**. Roots of pioneer trees in amazonian rain forest. *Trees - Structure and Function* **14**: 442–455.

Phillips OL, Gentry AH. 1994. Increasing turnover through time in tropical forests. *Science* **263**, 954–958.

Pierre WH and Parker FW. 1927. Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of the organic phosphorus to plants. *Soil Science* **24**, 119–128.

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. *Annals of Botany* **118**: 621–635.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models_. *R package version* **3**.1-131,

Pinheiro JC, Bates DM. 2000. Mixed-effects models in S and S-plus, *Springer*, New York.

Plaxton WC, Tran HT. 2011. Metabolic adaptations of phosphate-starved plants. *Plant Physiology* **156**, 1006–1015.

Powers JS, Treseder KK, Lerdau MT. 2005. Fine roots, arbuscular mycorrhizal hyphae and soil nutrients in four neotropical rain forests: Patterns across large geographic distances, *New Phytol.,* **165**, 913–921.

Prieto I, Armas C and Pugnaire FI. 2012. Water release through plant roots: new insights into its consequences at the plant and ecosystem level. *New Phytologist* **193**, 830–841.

Prior SA, Rogers HH, Mullins GL, Runion GB. 2003. The effects of elevated atmospheric C0$_2$ and soil placement on cotton root deployment. *Plant and Soil* **255**: 179-187.

Quesada CA, Lloyd J, Anderson LO, Fyllas NM, Schwarz M, Czimczik CI. **2011**. Soils of Amazonia with particular reference to the RAINFOR sites. *Biogeosciences* **8**: 1415–1440.

Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmerler J, *et al.* **2010**. Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* **7**: 1515–1541.

Quesada CA, Phillips OL, Schwarz M, Czimczik CI, Baker TR, Patiño S, Fyllas NM, Hodnett MG, Herrera R, Almeida S, *et al.* **2012**. Basin-wide variations in Amazon forest structure and function are mediated by both soils and climate. *Biogeosciences*.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raich JW, Riley RH, Vitousek PM. 1994. Use of root-ingrowth cores to assess nutrient limitations in forest ecosystems. *Canadian Journal of Forest Research* **24**: 2135–2138.

Rewald B, Ephrath JE, Rachmilevitch S. 2011. A root is a root is a root? Water uptake rates of citrus root orders. *Plant, Cell and Environment* **34**:33–42.

Rewald B, Ephrath JE. 2013. Minirhizotron techniques. Chapter 42. In: Eshel, A. & Beeckman, T. (Eds.) Plant roots: The hidden half. 4th edition. *CRC Press, New York, USA.* ISBN:978-1-4398-4648-3.

Richards PW. 1996. The Tropical Rain Forest: An Ecological Study, 2nd edn. by P.W. Richards Review by: Robert J. Whittaker Stable. *British ecological society* **84**: 791–792.

Rodtassana C, Tanner EVJ. 2018. Litter removal in a tropical rain forest reduces fine root biomass and production but litter addition has few effects. *Ecology.* **99**(3):735-742.

Sanders JL, Brown DA. 1978. A new fiber optic technique for measuring root growth of soybeans under field conditions. *Agronomy Journal* **70**:1073-1076.

Sayer EJ, Banin LF. **2016**. Tree Nutrient Status and Nutrient Cycling in Tropical Forest — Lessons from Fertilization Experiments.

Sayer EJ, Tanner EVJ, Cheesman AW. 2006. Increased litterfall changes fine root distribution in a moist tropical forest. *Plant and Soil* **281**: 5–13.

Shane MW, Szota C and Lambers H. 2004. A root trait accounting for the extreme phosphorus sensitivity of *Hakea prostrata* (Proteaceae). *Plant, Cell and Environment* **27**, 991–1004.

Silver WL, Neff J, McGroddy M, Veldkamp E, Keller M, Cosme R. **2000**. Effects of soil texture on belowground carbon and nutrient storage in a lowland Amazonian forest ecosystem. *Ecosystems* **3**: 193–209.

Silver WL, Thompson AW, McGroddy ME, Varner RK, Dias JD, Silva H, Crill PM, Keller M. 2005. Fine root dynamics and trace gas fluxes in two lowland tropical forests soils. Global Change *Biology*, **11**: 290-306.

Smeck, NE. 1985. Phosphorus dynamics in soils and landscapes. *Geoderma*, **36**(3–4), 185–199.

Sommer R, Denich M, Vlek PLG. 2000. Carbon storage and root penetration in deep soils under small-farmer land-use systems in the Eastern Amazon region, Brazil. *Plant and Soil* **219**: 231–241.

Steele SJ, Gower ST, Vogel JG, Norman JM. 1997. Root production, net primary production and turnover in aspen, Jack pine and Black spruce forests in Saskatchewan and Manitoba. *Tree Physiology* **17**:577-587.

Stewart CG. 2000. A test of nutrient limitation in two tropical montane forests using root ingrowth cores. *Biotropica* **32**: 369–373.

Thornley JHM. 1972. A balanced quantitative model for root: shoot ratios in vegetative plants. *Ann Bot (Lond)* **36**:431– 441.

Tomasella J, Borma LS, Marengo A, Rodriguez DA, Cuartas LA, Nobre CA, Prado MCR. 2010. The droughts of 1996-1997 and 2004-2005 in Amazonia: Hydrological response in the river main- stem. *Hydrological Processes*, **25**, 1228–1242. doi:10.1002/hyp.7889

Townsend AR, Cleveland CC, Houlton BZ, Alden CB, White JWC. **2011**. Multi-element regulation of the tropical forest carbon cycle. *Frontiers in Ecology and the Environment* **9**: 9–17.

Trumbore S, DaCosta ES, Nepstad DC, DeCamargo PB, Martinelli L, Ray D, Restom T, Silver W. 2006. Dynamics of fine root carbon in amazonian tropical ecosystems and the contribution of roots to soil respiration. *Global Change Biology* **12**: 217–229.

Turner BL, Brenes-arguedas T, Condit R. **2018**. Pervasive phosphorus limitation of tree species but not communities in tropical forests. *Nature Publishing Group* **0**.

Valverde-Barrantes OJ, Horning AL, Smemo KA, Blackwood CB. 2016. Phylogenetically structured traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms. *Plant and Soil* **404**:1–12.

Vasconcelos CM, Casagrande AA, Perecin D, Jorge LAC, Landell MGA. 2003. Avaliação do sistema radicular da cana-de-açúcar por diferentes métodos. *Sociedade Brasileira de Ciência do Solo.*

Vitousek PM, Sanford JR. 1986. Nutrient cycling in moist tropical forest. *Annual Review of Ecology and Systematics*, **17**(1):137-167.

Vitousek PM. 1982. Nutrient cycling and nutrient use efficiency. *American Naturalist* **119**:553-572.

Vitousek PM. **1984**. Litterfall, Nutrient Cycling, andNutrientLimitation in Tropical Forests. *Ecology* **65**: 285–298.

Vogt KA, Publicover DA, Bloomfield J, Perez JM, Vogt DA and Silver WL. 1993. Belowground responses as indicators of environmental change. *Environ. Exp. Bot.***33**, 189–205.

Vogt KA, Vogt DJ, Bloomfield J. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forest at in ecosystem level. *Plant and Soil.* V: **200**, p. 71-89.

Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H. 1996. Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant and Soil:* 159–219.

Walker AP, Zaehle S, Medlyn BE, De Kauwe MG, Asao S, Hickler T, Parton W, Ricciuto DM, Wang YP, Warlind D and Norby RJ. 2015. Predicting long-term carbon sequestration in response to CO2 enrichment: How and why do current ecosystem models differ? *Global Biogeochemical Cycles*. **29**(4): p. 476-495.

Walker TW, Syers JK. **1976**. The fate of phosphorus during pedogenesis. *Geoderma* **15**: 1–19.

Wells CE, Eissenstat DM. 2001. Marked differences in survivorship among apple roots of different diameters. *Ecology* **82**:882–893

Went FW, Stark N. 1968. Mycorrhiza, *BioScience*, 18, 1035–1039, doi:10.2307/1294552.

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE, *et al.* **2011**. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**: 1616–1625.

Wu J, Albert LP, Lopes AP, Restrepo-Coupe N, Hayek M, Wiedemann KT, Guan K, Stark SC, Christoffersen B, Prohaska N, et al. 2016. Leaf development and demography explain photosynthetic seasonality in Amazon evergreen forests. *Science* **351**: 972–976.

Wurzburger N and Wright J. 2015. Fine-root responses to fertilization reveal multiple nutrient limitation in a lowland tropical forest. *Ecology*, **96**(8), pp. 2137–2146.

Yavitt JB, Wright SJ. 2001. Drought and irrigation effects on fine root dynamics in a tropical moist forest, Panama. *Biotropica* **33**: 421–434.

Yavitt, J. B., K. E. Harms, M. N. Garcia, M. J. Mirabello, and S. J. Wright. 2011. Soil fertility and fine root dynamics in response to four years of nutrient (N, P, K) fertilization in a lowland tropical moist forest, Panama. *Austral Ecology* **36**: 433–445.

CONCLUSÃO

Nossos resultados destacam o importante papel das raízes profundas na dinâmica de raízes finas, pois quase metade da biomassa (45,51%) e produtividade líquida (43,45%) foram encontradas nas camadas abaixo de 30cm. Portanto, não devemos ignorar profundidades abaixo disso, porque a dinâmica das raízes mais profundas pode responder de maneira diferente às mudanças ambientais quando comparadas às raízes mais próximas da superfície. O estoque de raízes finas e a produtividade foram significativamente diferentes entre todos os intervalos de profundidade, diminuindo com a profundidade, mas o turnover não mostrou variação. Descobrimos também que a produtividade e o estoque de raízes finas foram diferentes entre as classes de diâmetro, sendo maiores nas raízes com menores classes de diâmetro. Isso pode estar relacionado à absorção de água e nutrientes, já que as raízes mais finas são normalmente as responsáveis pela aquisição e absorção de recursos. No entanto, o turnover dessas raízes não variou entre as classes de diâmetro. Além disso, foi possível observar que a produtividade de raízes finas foi maior no início da estação chuvosa, principalmente no intervalo de 0-30 cm enquanto o estoque foi maior na estação seca, principalmente na camada de 60-90cm. Essa diferença possivelmente também estava relacionada à disponibilidade de nutrientes e umidade. A disponibilidade de P pareceu não influenciar na dinâmica das raízes finas em um período de curto prazo. Existem algumas razões possíveis para isso, incluindo que o número de repetições do nosso experimento pode não ter sido suficiente; os tubos de minirhizotron podem não ter atingido a estabilidade; o P adicionado pode não ter se tornado disponível para as plantas; a floresta pode já está adaptada ao baixo teor de P no solo ou as raízes podem ter respondido à adição de P associando-se a micorrizas, porém o presente estudo não pode confirmar isso.

ANEXOS

ANEXO 1. Linear Mix Model parameters using LME (NLME package), random, fixed factors, degrees of freedom numerator (numDF), degrees of freedom denominator (denDF), F-value and p-value for the AmazonFACE experiment. Stock=Fine root standing stock; Productivity=Fine root productivity; Turnover = Fine root turnover; Depth intervals = 0-30, 30-60, 60-90cm; Diameter classes = 0.5,0.5-1,1-1.5mm.

Parameter	Random factors	Fixed factors	numDF	denDF	F-value	p-value
Stock	Plot|Tube	(Intercept)	1	136	71.22	<0.0001
		Date	17	136	4.01	<0.0001
Stock	Plot|Tube	(Intercept)	1	8	71.11	<0.0001
		Season	1	8	4.01	0.0802
Stock	Plot|Tube	(Intercept)	1	16	40.11	<0.0001
		Depth intervals	2	16	7.77	0.0044
Stock	Plot|Tube	(Intercept)	1	42	12.11	0.0012
		Depth intervals	2	42	17.56	<0.0001
		Season	1	42	7.62	0.0085
Stock (0-30cm)	Plot|Tube	(Intercept)	1	8	37.50	0.0003
		Season	1	8	1.04	0.3383
Stock (30-60cm)	Plot|Tube	(Intercept)	1	8	13.33	0.0065
		Season	1	8	3.59	0.0947
Stock (60-90cm)	Plot|Tube	(Intercept)	1	8	11.38	0.0097
		Season	1	8	7.29	0.0270
Stock	Plot|Tube	(Intercept)	1	11	66.00	<0.0001
		Diameter classes	3	11	20.97	0.0001
Productivity	Plot|Tube	(Intercept)	1	128	28.00	<0.0001
		Date	16	128	5.29	<0.0001
Productivity	Plot|Tube	(Intercept)	1	8	8.15	0.0213
		Season	1	8	7.39	0.0263
Productivity	Plot|Tube	(Intercept)	1	16	38.11	<0.0001
		Depth intervals	2	16	8.14	0.0036
Productivity	Plot|Tube	(Intercept)	1	432	41.10	<0.0001
		Depth intervals	2	432	29.23	<0.0001
		Date	16	432	3.43	<0.0001
Productivity	Plot|Tube	(Intercept)	1	42	32.67	<0.0001
		Depth intervals	2	42	6.06	0.0049
		Season	1	42	11.91	0.0013
Productivity (0-30cm)	Plot|Tube	(Intercept)	1	8	4.33	0.0711
		Season	1	8	44.63	0.0002
Productivity (30-60cm)	Plot|Tube	(Intercept)	1	8	8.68	0.0185
		Season	1	8	0.37	0.5599
Productivity (60-90cm)	Plot|Tube	(Intercept)	1	8	3.96	0.0816
		Season	1	8	2.46	0.1555
Productivity	Plot|Tube	(Intercept)	1	10	28.91	0.0003
		Diameter classes	3	10	11.10	0.0016

Parameter	Random factors	Fixed factors	numDF	denDF	F-value	p-value
Turnover	Plot|Tube	(Intercept)	1	16	12.31	0.0029
		Depth intervals	2	16	0.84	0.449
Turnover	Plot|Tube	(Intercept)	1	11	23.91	0.0005
		Diameter classes	2	11	0.15	0.8622

ANEXO 2. Linear Mix Model parameters using LME in the NLME package, random factors, fixed factors, degrees of freedom numerator (numDF), degrees of freedom denominator (denDF), F-value and p-value for the AFEX experiment. Stock = Fine root standing stock; Stock (increment) = Fine root stock in the last date – Fine root stock in the last date which is the increment over time; Stock (peak) = Fine root standing stock at the end of the experiment when the stock reaches its peak; Productivity = Fine root productivity; Turnover = Fine root turnover; Depth intervals = 0-30, 30-60, 60-90cm; Diameter classes = 0.5,0.5-1,1-1.5mm; Treatment = Control and P addition treatments.

Parameter	Random factors	Fixed factors	numDF	denDF	F-value	p-value
Stock	Block|Tube|Date	(Intercept)	1	70	31.35	<0.0001
		Date	10	70	7.84	<0.0001
Stock (increment)	Block|Tube	(Intercept)	1	3	9.14	0.0566
		Treatment	1	3	0.01	0.9106
Stock (peak)	Block|Tube	(Intercept)	1	14	44.76	<0.0001
		Treatment	1	3	8.78	0.0594
		Depth intervals	2	14	10.72	0.0015
Stock (peak)	Block|Tube	(Intercept)	1	14	26.15	0.0002
		Treatment	1	3	0.59	0.4998
		Diameter classes	2	14	12.80	0.0007
Productivity	Block|Tube|Date	(Intercept)	1	72	11.02	0.0014
		Treatment	1	3	0.41	0.5656
Productivity	Block|Tube	(Intercept)	1	14	31.45	0.0001
		Treatment	1	3	0.32	0.6132
		Depth intervals	2	14	12.86	0.0007
Productivity	Block|Tube	(Intercept)	1	14	2.21	0.1593
		Treatment	1	3	0.01	0.9176
		Diameter classes	2	14	14.81	0.0004
Turnover	Block|Tube	(Intercept)	1	3	39.54	0.0081
		Treatment	1	3	0.01	0.9283
Turnover	Block|Tube	(Intercept)	1	14	23.56	0.0003
		Treatment	1	3	0.01	0.9334
		Depth intervals	2	14	0.07	0.9354
Turnover	Block|Tube	(Intercept)	1	14	33.02	0.0001
		Treatment	1	3	0.51	0.5260
		Diameter classes	2	14	3.03	0.0807

ANEXO 3. Kruskal-wallis statistics presenting parameters, fixed factors, Chisq, Df and p.chisq for the AFEX experiment. Stock (average) = Average of fine root standing stock for the whole period collected; Stock (beginning) = Fine root standing stock at the first date of the experiment; Stock (peak) = Fine root standing stock at the end of the experiment when the stock reaches its peak; Productivity = Fine root productivity; Turnover = Fine root turnover; Fine root stock, productivity and turnover were analyzed separately by each depth interval (0-10, 0-30, 30-60, 60-90cm) and diameter class (0-0.5, 0.5-1, 1-1.5mm); Treatment = Control and P addition treatments.

Parameter	Fixed factors	Chisq	Df	p.chisq
Stock (average)	Treatment	2.08	1	0.15
Stock (beggining)	Treatment	2.08	1	0.15
Stock (peak)	Treatment	0.33	1	0.56
Stock (peak) 0-10cm	Treatment	0.33	1	0.56
Stock (peak) 0-30cm	Treatment	0.33	1	0.56
Stock (peak) 30-60cm	Treatment	0.03	1	0.87
Stock (peak) 60-90cm	Treatment	1.89	1	0.17
Stock (peak) 0-0.5mm	Treatment	0.00	1	1.00
Stock (peak) 0.5-1mm	Treatment	0.08	1	0.77
Stock (peak) 1-1.5mm	Treatment	2.86	1	0.09
Productivity 0-10cm	Treatment	0.08	1	0.77
Productivity 0-30cm	Treatment	0.13	1	0.72
Productivity 30-60cm	Treatment	1.00	1	0.32
Productivity 60-90cm	Treatment	2.29	1	0.13
Productivity 0-0.5mm	Treatment	0.08	1	0.77
Productivity 0.5-1mm	Treatment	2.08	1	0.15
Productivity 1-1.5mm	Treatment	0.04	1	0.85
Turnover 0-10cm	Treatment	0.75	1	0.39
Turnover 0-30cm	Treatment	0.08	1	0.77
Turnover 30-60cm	Treatment	0.33	1	0.56
Turnover 60-90cm	Treatment	0.75	1	0.39
Turnover 0-0.5mm	Treatment	0.75	1	0.39
Turnover 0.5-1mm	Treatment	0.75	1	0.39