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Ecophysiology and growth of *Bertholletia excelsa* Bonpl. in response to thinning, liming and phosphorus addition

By
KAREN CRISTINA PIRES DA COSTA

Manaus, Amazonas
2019

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ADVISOR: Dr. José Francisco de Carvalho Gonçalves

Thesis presented to the graduate program in Tropical Forest Sciences of the National Institute of Amazonian Research (INPA) in partial fulfillment of the requirements for the degree of doctor in Tropical Forest Sciences, with a focus on Tropical silviculture.

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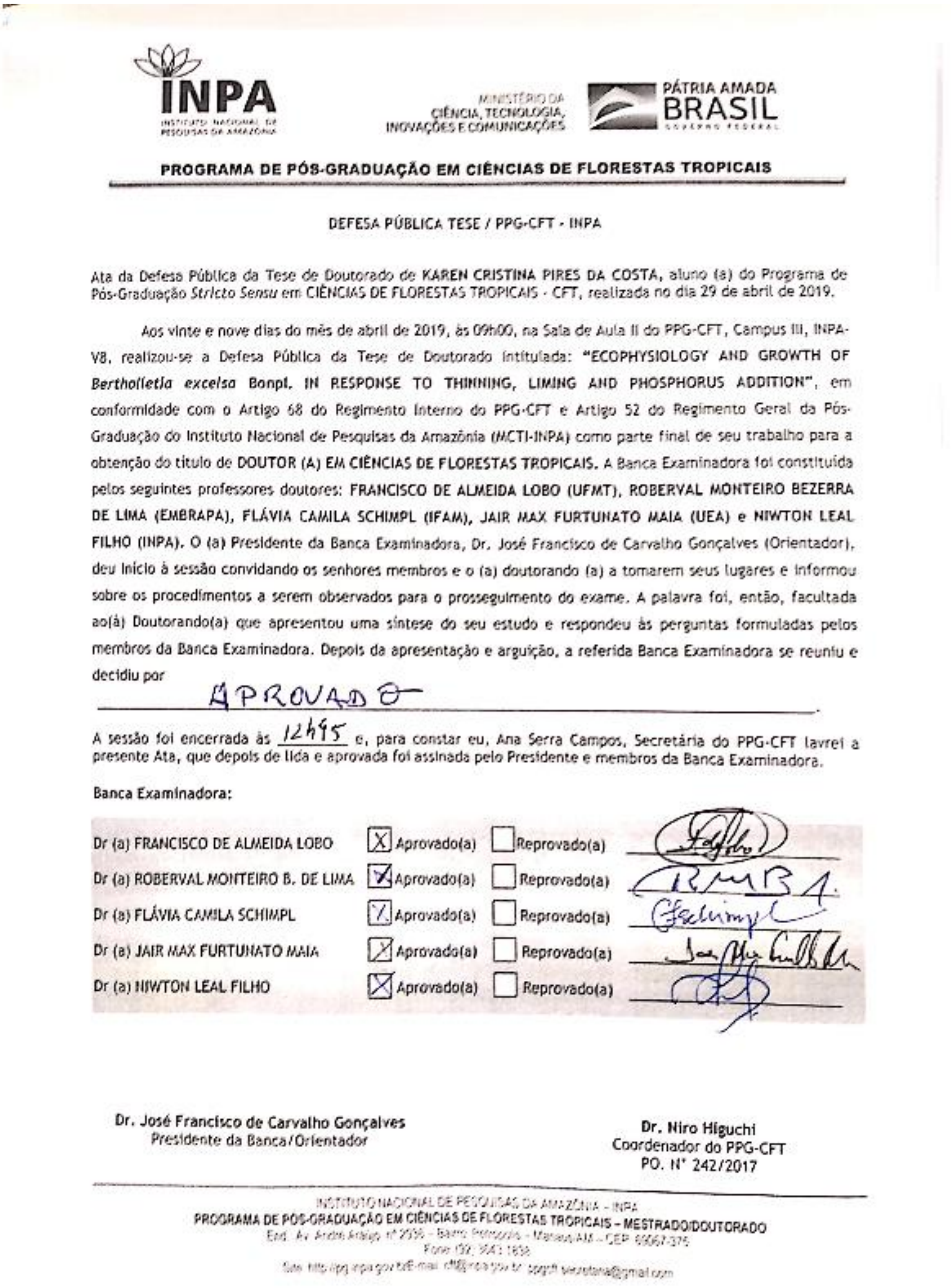

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Ata da Defesa Pública da Tese de Doutorado de **KAREN CRISTINA PIRES DA COSTA**, aluno (a) do Programa de Pós-Graduação *Stricto Sensu* em CIÊNCIAS DE FLORESTAS TROPICAIS - CFT, realizada no dia 29 de abril de 2019.

Aos vinte e nove dias do mês de abril de 2019, às 09h00, na Sala de Aula II do PPG-CFT, Campus III, INPA-V8, realizou-se a Defesa Pública da Tese de Doutorado intitulada: **"ECOPHYSIOLOGY AND GROWTH OF *Bertholletia excelsa* Bonpl. IN RESPONSE TO THINNING, LIMING AND PHOSPHORUS ADDITION"**, em conformidade com o Artigo 68 do Regimento Interno do PPG-CFT e Artigo 52 do Regimento Geral da Pós-Graduação do Instituto Nacional de Pesquisas da Amazônia (MCTI-INPA) como parte final de seu trabalho para a obtenção do título de **DOUTOR (A) EM CIÊNCIAS DE FLORESTAS TROPICAIS**. A Banca Examinadora foi constituída pelos seguintes professores doutores: **FRANCISCO DE ALMEIDA LOBO (UFMT), ROBERVAL MONTEIRO BEZERRA DE LIMA (EMBRAPA), FLÁVIA CAMILA SCHIMPL (IFAM), JAIR MAX FURTUNATO MAIA (UEA) e NIWTON LEAL FILHO (INPA)**. O (a) Presidente da Banca Examinadora, Dr. José Francisco de Carvalho Gonçalves (Orientador), deu início à sessão convidando os senhores membros e o (a) doutorando (a) a tomarem seus lugares e informou sobre os procedimentos a serem observados para o prosseguimento do exame. A palavra foi, então, facultada ao(à) Doutorando(a) que apresentou uma síntese do seu estudo e respondeu às perguntas formuladas pelos membros da Banca Examinadora. Depois da apresentação e arguição, a referida Banca Examinadora se reuniu e decidiu por

APROVADO.

A sessão foi encerrada às 12h45 e, para constar eu, Ana Serra Campos, Secretária do PPG-CFT lavrei a presente Ata, que depois de lida e aprovada foi assinada pelo Presidente e membros da Banca Examinadora.

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Sinopse:

Os efeitos do desbaste, calagem e fertilização fosfatada sobre a ecofisiologia e o crescimento de um plantio de *Bertholletia excelsa* Bonpl. foram estudados. Aspectos de crescimento, morfologia, trocas gasosas, *status* nutricional, *status* hídrico e a fluorescência da clorofila *a* foram analisados.

Palavras-chave: Amazônia, Castanheira-da-amazônia, Fotossíntese, Fluorescência da clorofila *a*, Tratamentos silviculturais.

DEDICATÓRIA

Dedico esta tese a Deus que tem preenchido
a minha alma com amor e esperança.

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ABSTRACT

Bertholletia excelsa has a high potential for timber production, and although it is widely planted in the Amazon, information on management practices of this species in planting condition is scarce. Understanding of the effects of thinning, liming and phosphate fertilization on the ecophysiology and growth of native species is fundamental for the definition of management strategies. Thus, the objective of this research was to investigate how the thinning, liming and phosphate fertilization, as well as the combination between these treatments in the dry and rainy season influence the ecophysiology and growth of *B. excelsa*. The study was carried out in a planting, located in the area of the Agronomic Company Fazenda Aruanã, Itacoatiara, AM. The planting was submitted simultaneously to two levels of thinning (removal of 0 and 50% of the basal area of the stand), liming (application of 0 and 2.0 Mg ha^{-1} of dolomitic limestone) and phosphate fertilization (application of 0 and 150 kg ha^{-1} of super triple phosphate). The experimental design was in randomized blocks with six treatments (1-Control; 2-Liming; 3-Liming + Phosphorus; 4-Thinning; 5-Thinning + Liming; 6-Thinning + Liming + Phosphorus) and eight replications. The treatments were compared in relation to light, water and nutrients availability, leaf and fine-root morphology, elementary stoichiometry at the soil-plant interface, photosynthesis and resource use efficiency for two years (2016-2018). Thinning increased light availability from 51 to 423 μmol m^{-2} s^{-1}. Liming boosted soil pH from 4.2 to 5.3 and phosphorus fertilization increased soil P from 2.1 to 88.2 mg kg^{-1}. Trees under thinning showed the highest growth rates (9.0 mm year^{-1}) independent of liming or phosphate fertilization application. Light was the site factor that exerted the greatest effect on the ecophysiology and growth of *B. excelsa*, but when light availability was low the pH and P availability in soil exerted the greatest effects on growth. The higher growth rates of trees under thinning were influenced by changes in leaf morphology, N and P concentrations, increase in photosynthetic rates, and efficiency in the use of light, N and P. While the higher growth rates of trees under liming treatment and phosphorus fertilization were influenced by changes in fine-root morphology, Ca, Mg and P concentrations, increase of photosynthetic rates, and efficiency in the use of light, N and P. The highest growth rates were observed in the rainy season, while the highest photosynthetic rates were observed in the dry season. Thus, it is concluded that although soil conditions (pH and P availability) have an effect on the growth of *B. excelsa*, light is the most limiting factor, and thinning is indicated to increase the light availability and, consequently, yield of the plantations formed by this species.

Key-words: Brazil nuts; Ecophysiology of trees; Site quality; Seasonality of precipitation; Silvicultural treatments.

RESUMO

Bertholletia excelsa é uma espécie de elevado potencial para a produção de madeira e, embora seja amplamente plantada na Amazônia, informações sobre práticas de manejo dessa espécie em condição de plantio são escassas. O entendimento dos efeitos do desbaste, calagem e fertilização fosfatada sobre a ecofisiologia e o crescimento de espécies nativas é fundamental para definição de estratégias de manejo. Assim, o objetivo desta pesquisa foi investigar como o desbaste, a calagem e a fertilização fosfatada, bem como a combinação entre esses tratamentos em períodos de baixa e alta precipitação influenciam a ecofisiologia e o crescimento de *B. excelsa*. O estudo foi realizado em plantio, localizado na área da Empresa Agropecuária Fazenda Aruanã, Itacoatiara, AM. O plantio foi submetido simultaneamente a dois níveis de desbaste (retirada de 0 e 50% da área basal do povoamento), calagem (aplicação de 0 e 2,0 Mg ha^{-1} de calcário dolomítico) e fertilização fosfatada (aplicação de 0 e 150 kg ha^{-1} de fosfato super triplo). O delineamento experimental foi em blocos casualizados com seis tratamentos (1-Controle, 2-Calagem, 3-Desbaste, 4-Calagem + Fósforo, 5-Calagem + Desbaste e 6-Calagem + Fósforo + Desbaste) e oito repetições. Os tratamentos foram comparados quanto à disponibilidade de recursos, morfologia de folhas e raízes finas, estequiometria elementar na interface solo-planta, fotossíntese e eficiência no uso de recursos durante dois anos (2016-2018). O desbaste aumentou a disponibilidade de luz de 51 para 423 µmol m^{-2} s^{-1}. A calagem aumentou o pH do solo de 4,2 para 5,3 e a fertilização fosfatada aumentou o P no solo de 2,1 para 88,2 mg kg^{-1}. As árvores sob tratamento de desbaste apresentaram as maiores taxas de crescimento (9.0 mm ano^{-1}) independente da aplicação da calagem ou da fertilização fosfatada. A luz foi o fator de sítio que exerceu maior efeito sobre a ecofisiologia e crescimento de *B. excelsa*, mas quando a disponibilidade de luz foi baixa o pH e a disponibilidade de P no solo exerceram os maiores efeitos sobre o crescimento. As maiores taxas de crescimento das árvores sob tratamento de desbaste foram influenciadas por mudanças na morfologia de folhas, concentrações de N e P, aumento das taxas fotossintéticas e eficiência no uso de luz, N e P. Ao passo que as maiores taxas de crescimento das árvores sob tratamento de calagem e fertilização fosfatada foram influenciadas por mudanças na morfologia de raízes finas, concentrações de Ca, Mg e P, aumento das taxas fotossintéticas e eficiência no uso de luz, N e P. As maiores taxas de crescimento foram observadas no período de maior precipitação, enquanto que as maiores taxas fotossintéticas foram observadas no período de menor precipitação. Desta forma, conclui-se que embora as condições edáficas (pH e disponibilidade de P) exerçam efeito sobre o crescimento de *B. excelsa*, a luz é o fator mais limitante, sendo indicada a realização do desbaste para aumentar a disponibilidade de luz e, consequentemente, o crescimento das plantações formadas por esta espécie.

Palavras-chave: Castanheira da Amazônia; Ecofisiologia de árvores; Qualidade de sítio; Sazonalidade de precipitação; Tratamentos silviculturais.

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GENERAL INTRODUCTION

Tropical forests will not be able to sustain increasing domestic and international demands for wood due a low growth rates, environmental concerns and rapidly diminishing resource (FAO 2018). Therefore, plantations are necessary to meet the demand for wood and reduce pressure deforestation on natural forests (Knoke et al. 2009). Although Brazil is prominent in the silviculture of *Eucalyptus* species, little is known about the silviculture of native species, especially of the Amazon forest. Among Amazon species, *Bertholletia excelsa* is one of the most promising tree species used for the formation of forest plantations, due to its high economic value, high growth rates, and excellent silvicultural traits (Scoles and Gribel 2012*a*, Costa et al. 2015).

Forest plantations management necessarily contemplates the adoption of silvicultural treatments such as thinning, pruning and fertilization. In this case, thinning and fertilization have greater effects on growth rates of trees (Forrester 2013). Thinning has, as main objective, to reduce the density of trees in a stand, while fertilization has, as main objective, to improve soil chemical conditions (Forrester 2013, Mo et al. 2015*a*). In case of Amazon Latosols, pH values vary from 3.78 to 4.29 and only 2% of the total phosphorus (P_{total}) is in the form readily available to plants (Quesada et al. 2010). Thus, liming and phosphate fertilization are indicated as important practices that improve the chemical conditions of the amazon soil (Quesada et al. 2010).

The pattern observed in several studies is that thinning, liming and phosphate fertilization influence changes in the availability of resources that result in increased yield of forest plantations (Forrester 2013, Forey et al. 2015*a*, Zavišić and Polle 2018). Increasing of growth rates of the plantations under thinning, liming and phosphorus fertilization are attributed to changes in the ecophysiology traits of the trees, that are normally related to the increase on the resources availability (light, water and nutrients) and also to the efficient use of these resources (Lamber et al. 2006, Wu et al. 2018*b*).

Eucalyptus plantations, for instance, showed an increasing of 34% in the growth rates of trees (Forrester 2013). The higher growth rates of plantations under thinning treatment have been attributed mainly to the high availability of light, which can favor the improvement of the nutritional and water status of the trees and result in increased photosynthetic capacity (Gauthier and Jacobs 2009). In addition, it has also been observed that thinning can too cause changes in the anatomy and morphology of leaves that favor the capture and utilization of resources (Gauthier and Jacobs 2009).

Liming, in turn, increased the growth rate of *Pinus* plantations by 20%, which may be related to the greater photosynthetic capacity and the improvement of the nutritional status of the trees (Røsberg et al. 2015). In the case of phosphate fertilization, a 50% boost in growth rates was observed in *Eucalyptus* plantations, which was related to the increase in photosynthetic rates, higher photochemical efficiency (F_V/F_M and PI_{ABS}) and improvement of the nutritional status (Graciano et al. 2006). Furthermore, changes in fine root morphology (diameter and specific root area) have also been associated with plant responses to P availability (Steidinger et al. 2006; Ushio et al. 2015).

Effects of thinning, liming and phosphate fertilization on the growth of forest plantations can be intensified through the interaction between these treatments. *Eucalyptus* plantations, for instance, grew more under the effect of the interaction between thinning and fertilization when compared to the growth rates of the trees when submitted to each of these treatments alone (Forrester 2013). The reason is that, in places where nutrients are not limiting, reducing the competition for light usually results in higher tree growth rates. On the other hand, in sites where soil resources are relatively more limiting than light, it is observed that thinning may have little effect on tree growth rates of trees (Forrester 2013).

Effects of thinning, liming and phosphate fertilization on the growth of forest plantations also can be influenced by variation in water and light seasonality, as it occurs naturally along periods of low and high precipitation (Forrester 2013). In periods of low rainfall the ecophysiological performance of trees in forest plantations can be compromised by the reduction of water availability associated with high irradiance and temperature. Thinning, liming and phosphate fertilization, in this case, may reduce the effects of water restriction on trees, favoring increased availability of water in soil, liming increasing root surface, and phosphorus contributing to the maintenance of cellular turgidity (Forrester 2013). Nevertheless, periods of high precipitation, despite the most favorable conditions, are accompanied by lower irradiance, implying reduction of energy for photosynthetic processes (Mulkey et al. 1996), in this case, thinning, liming and phosphate fertilization can increase the capture of resources such as light and carbon dioxide through the adoption of mechanisms associated with efficient interception, absorption and utilization of resources (Valadares and Niinemets 2008).

Researches showed that *B. excelsa* responds positively to the practices of thinning, liming and phosphate fertilization. In a greenhouse experiment, for instance, was found a positive linear relationship between P availability and *B. excelsa* growth (Corrêa et al. 2013). Additionally, recent results showed that liming resulted in increased growth rates of *B. excelsa* planted in an agroforestry system (Schroth et al. 2015). As for thinning, *B. excelsa* trees submitted to this treatment grew about three times more than the control trees (Costa 2015). Despite these studies, information on the effects of these treatments in the field and how they influence the ecophysiology and growth of *B. excelsa* in dry and rainy seasons is still absent in the literature.

Thus, in order to better understand the behavior of *B. excelsa* silvicultural interventions of thinning, liming and phosphate fertilization, as well as the combination between these treatments considering dry and rainy seasons, this thesis intends to investigate how environmental changes imposed by the application of these silvicultural treatments influenced the availability of primary resources (water, light and nutrients) and how changes in the availability of these resources influenced ecophysiology and growth of *B. excelsa*. For this purpose, this thesis was divided into following chapters:

1) *Bertholletia excelsa*: a review on the plasticity in resource availability;

2) *Bertholletia excelsa* growth under thinning, liming and P fertilization: a linkage between traits below- and above-ground and resource use efficiency;

3) Soil-trees elemental stoichiometry and their effects on nutrients use efficiency and biomass of *Bertholletia excelsa* under thinning, liming and phosphorus addition;

4) Photosynthesis traits of *Bertholletia excelsa* plantations following thinning, liming and phosphorus fertilization in Central Amazon; and

5) Seasonal responses of physiological traits and growth of *Bertholletia excelsa* subjected thinning, liming and phosphorus fertilization.

This research contributes with information on the silviculture of *B. excelsa*, and it is also one more step to break with 3-4 decades of silence about this topic in the

Amazon region. This thesis involves the use of basic and applied research, and is expected to answer, with the results of this work, not only "how" the factors evaluated influence the productivity of *B. excelsa* plantations, but also "why" these factors are influencing the productivity of these plantations. We accurately pinpoint the site factors and physiological mechanisms that are associated with growth, helping to define which site and tree characteristics should be managed in order to increase crop productivity. In addition, we provide a database that can be used to make inferences about forest conservation practices and possible impacts of climate change on Amazonian vegetation.

Bertholletia excelsa: a review on the plasticity to resource availability

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Abstract

Bertholletia excelsa Bonpl. is an amazon native species that belongs to the family Lecythidaceae. It is a multi-product supplier and historically played a key role in the development of complex societies in the region. Besides that, *B. excelsa* plays a fundamental role in the contribution of environmental services, being considered one of the species that contributes most to carbon sequestration in the Amazon forests and is still considered one of the most important species in the silvicultural scenario of this region. The importance of the *B. excelsa* in social, economic and environmental scenarios of the Amazon can be attributed, in part, to its plasticity. Thus, in order to infer about the plasticity of *B. excelsa*, in this study we review the effect of resources availability in populations traits of *B. excelsa*. We found that *B. excelsa* show phenotypical plasticity to light, water and nutrients. Both in forests and in plantations the availability of these resources influences the population structure, growth, and fruit production. Light is the major factor that determine the structure of populations and growth in both forests and plantations. In addition, we found evidence indicating that increase in irradiance can also boost fruit production; however, fruit production seems to be more influenced by edaphic factors, especially phosphorus availability. Regarding to water, the species shows tolerance to the reduction of water availability in soils. These results contribute with information that helps, not only the determination of management techniques for *B. excelsa*, which would induce Brazil nuts forest plantations, but also allow us to make inferences about sustainability and possible impacts of climate change on the amazon forest.

Key-words: Amazonian plants ecology, Brazil nuts, primary plant resources.

1 INTRODUCTION

Historically, *Bertholletia excelsa* Bonpl. contributed to the development of complex societies inside the Amazon region, with its earliest cultivation dating 4,500 years ago (Maezumi et al. 2018). Currently, *B. excelsa* is one of the most important native species in the silvicultural scenery in the Amazon region, and plays fundamental role in carbon sequestration, coming in third place, among 3,458 species, in the accumulation of biomass, and in first place, among species economically important, in the carbon sequestration. Besides that, *B. excelsa* supports human livelihoods through the production of brazil nut seeds that sustain the multimillion dollar extractive economies in Bolivia, Brazil and Peru (Vieira et al. 2005, Kainer et al. 2007, Ferreira et al. 2012, Selaya et al. 2017, Thomas et al. 2018).

B. excelsa is an Amazon native species that belongs to the family Lecythidaceae (Poiteau 1825) and was described in 1808 by Aimé de Bonpland (Mori 2018). The genus terminology is dedicated to the chemist Claude Louis Berthollet, in reference to his scientific stature. While the epithet refers to its grandiosity (excellently = eminent) (Mori 2018). The importance of *B. excelsa* in the social, economic and ecological scenarios of Amazonia is due, in part, to the capacity of this species to show high rates of survival and growth, even in sites with adverse conditions, which includes acid soils, with low availability of nutrients and water, and a high irradiance incident (Ferreira et al. 2015, 2016, Schroth et al. 2015, Costa et al. 2017, Shimpl et al. 2019). This performance of *B. excelsa* has been attributed, in part, to the ability of this species to tolerate the low availability of soil resources and also due to its plasticity to light, water and nutrients.

Plasticity, in this case, is defined as the ability of an organism to adjust its performance by changing its phenotype, which are induced by changes the availability of resources at the sites (Bradshaw 1965, 2006, Huang et al. 2011*a*, Gratani 2014). The plant species have plasticity to resource acclimation due to the ability to adjust key characteristics that favor the maintenance or increase of carbon acquisition and growth according to the demands for water, light and nutrients (dosAnjos et al. 2015). The plasticity in response to a range of resource conditions denote a further advantage for plant species establishment, carbon gain, and light, water and nutrients stress tolerance (Valladares et al. 2006, dosAnjos et al. 2015).

The natural history of *B. excelsa* already indicates that this species has plasticity of resources availability. The main hypotheses that explain the current and wide distribution of *B. excelsa* are the hypothesis of anthropogenic distribution (Shepard and Ramirez 2011) and the hypothesis of the natural refuges (Thomas et al. 2014), both consider that the acclimatization capacity of this species to different environmental conditions was determinant for its distribution throughout the Amazon region, either by traditional populations that cultivated the species in different regions or that the species was able to survive periods of cooling/heating and drying/moistening.

In natural populations has been observed recurrent reports highlighting, mainly, the effect of light availability on regeneration, density of individuals and growth in diameter of the species (Peres et al. 2003, Wadt et al. 2005, Kainer et al. 2014, Tonini et al. 2014, de Souza Neves et al. 2016). Light seems to be one of the most determinant factors for *B. excelsa* performance (Myers et al. 2000, Scoles et al. 2014). The availability of light still seems to influence fruit production and also the pattern of distribution of populations (Kainer et al. 2006, Cotta et al. 2008, Tonini and Pedrozo 2014, Rockwell et al. 2015). Less information has been directed to the other primary resources, but evidence suggests that water availability may influence seedling survival rates and that the availability of nutrients, especially phosphorus, seems to be determinant for increased fruit yield (Zuidema and Boot 2002, Kainer et al. 2007). Still with respect to seedlings, recent study point to strong resilience of *B. excelsa* to drought (Schimpl et al. 2019).

In forest plantations, the light has also showed to be one of the most limiting factors for the growth of *B. excelsa* and evidence also suggests that the light availability although favors the growth rates, does not influence the establishment and the survival in the initial phase and this has been attributed to their physiological plasticity (Scoles et al. 2014). *B. excelsa* has also been shown to be tolerant to nutrient availability since it has high growth rates when compared to other native species of the region, even in acid soils with low nutrient availability (Ferreira et al. 2009*a*).

Research with seedlings of *B. excelsa* under controlled conditions confirms what has been verified in the natural populations and in forest plantations for its plasticity to resources. Studies evaluating the ecophysiological responses of the species, when submitted to the variation in the availability of light, water and nutrients, allowed to verify that *B. excelsa* is benefited by the increase in availability of light, is tolerant to the reduction of water availability, and it also has high efficiency in the use of nutrients (Corrêa 2013, Ferreira et al. 2015, Schroth et al. 2015, Shimpl et al. 2019). These responses are associated with morpho-physiological strategies that the species develops, that favor the capture and use of light, water and nutrients, confering plasticity to *B. excelsa* (Corrêa 2013, Ferreira et al. 2015, Schroth et al. 2015, Shimpl et al. 2019).

Considering the role of *B. excelsa* in the social, economic and environmental scenario of the Amazon, in this first chapter we performed a review on the effects of light, water and nutrients availability on the traits of the *B. excelsa* populations with the objective of inferring on its plasticity to resource availability, because the understanding of factors associated with ecophysiology and growth of *B. excelsa* contributes not only to information that helps the definition of management techniques, but also allow make inferences about conservation practices and possible impacts of climate change on the Amazon forest.

1.1 Natural history of *B. excelsa* indicate plasticity to resources

The origin and distribution of *B. excelsa* throughout the Amazon basin still intrigues researchers. The most widespread hypothesis for the place of origin of the species is based on linguistic and phylogenetic evidence and suggest that *B. excelsa* originated in Central Amazonia, with *Lecythis* as ancestral (Huang et al. 2011*b*, Shepard and Ramirez 2011), but Thomas et al. (2014) pointed that the hypothesis of the origins of *B. excelsa* is very difficult to prove, considering that the species very likely occurred in the region for at least several hundreds of thousands, if not millions of years ago.

Regarding *B. excelsa* distribution along the Amazon basin, several theories have been proposed throughout history. Ducker in 1946 proposed the hypothesis of anthropogenic distribution, Peres and Baider in 1976 rejected this hypothesis and suggested that predators, such as short-range agouti and long-distance monkeys, are involved in dispersal events. Shepard and Ramirez (2011) also suggest anthropogenic distribution. Anthropogenic distribution hypothesis has been strengthened by research that showed that *B. excelsa* were found in association with geoglyphs dated between 1,000 and 2,500 years ago (Pärssinen et al. 2009; Ranzi et al. 2007). In addition, genetic studies also reinforce the hypothesis of anthropogenic distribution due to the low genetic variability of the populations, at the same time with a wide geographic distribution of the species.

Thomas et al. (2014) suggest that the *B. excelsa* distribution may have been restricted to potential refuges across southern Amazon. Conditions for establishment in the species date from the Last Inter-glacial Period (LIG), period in which areas more prone to the establishment of this species were located in the West to the Central Amazon. In the Last Glacial Maximum (LGM), due to the low temperatures and the strong decrease of precipitation, the conditions of suitable habitats for the species were reduced considerably, and the distribution of Brazil nuts during LGM, and the distribution of suitable habitat may have been restricted primarily to several potential refuges across southern Amazon (Thomas et al. 2014). During the mid-Holocene, evidence suggests an expansion of favorable habitats for the establishment of *B. excelsa*, which are already considered comparable to current distribution conditions. During the Holocene, populations of Brazil nut trees probably expanded again from the different areas of refuge where they survived during the glaciations (Thomas et al. 2014).

The fact is that, taking into account the different theories that seek to explain the origin and distribution of *B. excelsa*, they considered that the acclimatization capacity of this species to different environmental conditions was determinant for its distribution throughout the Amazon, either by traditional populations that cultivated the species in different regions or that the species was able to survive periods of cooling/heating and drying/moistening (Shepard and Ramirez 2011; Thomas et al. 2014).

1.2 *Bertholletia excelsa* phenotypical variation

B. excelsa trees have shown some morphological differences in the color and quality of wood, fruit production and shape of the trunk, crown, fruit and considering these different characteristics, *B. excelsa* trees are classified by the local population of the States of Acre, Amazonas, Roraima, Rondônia, and Mato Grosso into types: red and white in Acre, Rondônia and Amazonas; pink and white in Roraima; and pink, rajada (striped), and mirim (small) in Mato Grosso (Sujii et al. 2013). These differences led John Miers in 1874 to describe a species called *B. nobilis*, but the characters were not considered valid and consequently, *B. nobilis* is synonymous with the name *B. excelsa* described by Bonpland.

In the State of Mato Grosso, the phenotypical classification is based on wood color, size of fruit and quantity of seeds per fruit. The Brazil nuts trees called “pink” has greater fruits and wood of most quality (Camargo et al. 2010). In the State of Acre, the phenotypical classification is based on the color and quality of wood, as well as the size of the tree. The red Brazil nut trees have red wood and are believed to have a better quality for construction, with thicker trunk and branches; they also have a larger crown and greater fruit production when compared to the white Brazil nut trees (Sujii et al. 2013).

Phenotypical variation of *B. excelsa* around amazonian regions has been commonly pointed as a consequence of human selection, because fruit size, wood quality and shape of the crown are considered typical traits selected by humans in incipient domestication of managed species (Shepard and Ramirez 2011). Despite this, the effect of resource availability on the phenotypic variation of *B. excelsa* cannot be ruled out and evidence suggests that the availability of resources, especially light and nutrients, can directly affect fruit and seed production, as well as shape and size of crown, and fruits of *B. excelsa* trees.

1.3 Populations traits of *Bertholletia excelsa* and resource availability

Bertholletia excelsa is found in “terra firme” forests throughout a large geographic range, from 5° N latitude to 14° S, that comprises throughout the Brazilian Amazon and adjacent areas in Bolivia, Peru, Colombia, Venezuela, and the Guianas (Shepard and Ramirez 2011). The occurrence of *B. excelsa* is associated with average temperatures ranging from 24.3ºC to 27.2ºC, a total annual rainfall between 1,400 mm and 2,800 mm, and a mean annual relative humidity between 79 and 86% (Diniz and Basto 1974). This species presents better growth performance in soils with a clayey to very clayey texture and its growth is impaired in sandy soils (Müller 1995; Lima et al. 2018). The main edaphic factors related to the growth of *B. excelsa*: P, Zn, Na , Al, Mg, total sand, silt and water available (Lima et al. 2018).

Bertholletia excelsa is characterized by a long life span: large trees with >1.6 m of diameter at breast height measured at 1.3 m above ground level (DBH) may age over three centuries and the age at entering the first reproductive category (0.6-0.8 m DBH) is more than 120 years (Zuidema 2000). In eastern Amazon, Pires (1976) reported *B. excelsa* trees (4.46 m DBH) with age estimated to be approximately 800 and 1000 years-old. Tree-ring analyses estimate individuals of up to 400 years-old (Brienen & Zuidema, 2006; Schöngart et al. 2015) and radiocarbon dating suggests a maximum lifespan of over 1000 years (Vieira et al. 2005). Camargo et al. (1994), through radiocarbon dating, estimated an age of 440 ± 60 years for tree of 2.33 m of DBH, that is, mean annual increment of the diameter of 5.3 mm year^{-1}. Salomão (2009) extrapolated this result to nine trees with diameters between 3.05 and 3.87 m, recorded in the National Forest of Saracá-Taquera (Pará), estimating ages between 576 and 731 years-old. Schöngart et al. (2015) estimated that the maximum age of Brazil nut trees was 361 years-old in the Purus and 401 years-old in the Trombetas.

Brazil nut trees, in general, are found in groves of 50-100 individuals known as castanhais (Portuguese), with groves separated by considerable distances of compatible habitat, where the species is completely absent (Peres and Baider 1997). However, a random distribution pattern has also been reported in the literature (Zuidema 2000, Tonini et al. 2008). Grove versus more scattered distribution patterns may also be partially due to differences in forest types, in which Brazil nut naturally occurs (Wadt et al. 2005). Perhaps, the open forest provides more consistent favorable regeneration conditions, such as higher levels of light, resulting in a more scattered spatial distribution pattern and more constant seedling establishment over time (Wadt et al. 2005).

The largest populations are concentrated in Brazil (Acre, Amazonas, Rondônia and Pará), with a density varying from 1.3 to 23 trees ha^{-1} and in Bolivia (Beni, Pando and La Paz) with around 1.3 to 5.0 trees ha^{-1} (Table 1). The structure of the *B. excelsa* population has a reverse-J size class distribution, having greater representation in smaller size classes, and slightly declining proportional representation with increasing size and with highest densities occurring in the intermediate classes (0.8 m < DBH < 1.60 cm) (Viana et al. 1998; Zuidema 2000; Zuidema and Boot 2002; Zuidema 2003; Peres et al. 2003, Scoles and Grible 2012).

Variations in density of trees in populations of *B. excelsa* in different studies has been attributed, especially, to the lack of standardization in inventories, such as the use of sample units of different sizes and shapes, differences in minimum sampling diameter, and selection criteria for the location of the unit samples (Wadt et al. 2005). This makes it difficult to generate more precise inferences about the factors that determine the population structure of *B. excelsa*. Despite this, some evidence suggests that many traits of the *B. excelsa* population may result from anthropogenic factors (Scoles and Gribel 2011), mainly, from current tendencies of occupation and use of the Brazil nuts, and also with respect to its historical occupation that influenced especially the light availability.

Table 1. *Bertholletia excelsa* population's traits in natural forest in different Amazon regions.

Amazon region	**Density (tree ha^{-1})**	**Juveniles (%)**	**DBH (m)**	**Authors**
Trombetas River, Pará, Brazil	6.8	7.4	1.28	Scoles and Gribel 2011
Capanã Grande, Amazonas, Brazil	12.5	18.0	0.73	Scoles and Gribel 2011
Cajari, Amapá, Brazil	12.2	1.5		Baider 2000
Saracá-Taquera Forest, Pará, Brazil	5.6	0.7		Salomão 2009
Forest Reserve El Tigre, Beni, Bolívia	1.7	5.9		Zuidema and Boot (2002)
Indigenous Area Pinkaiti, Pará, Brazil	4.8	43.3		Peres and Baider (1997)
Marabá, Pará, Brazil	1.3	23.7	1.31	Salomão 1991
Carajás, Pará, Brazil	4.2		1.35	Salomão 1992
Bolíva			2.67	Zuidema and Boot (2002)
Bolívia			1.90	Zuidema and Boot (2002)
São João Baliza, Roraima, Brazil	3.7	35.3	0.66	Tonini et al. 2008
Caracaraí, Roraima, Brazil	12.9	26.7	0.75	Tonini et al. 2008
Bolívia				Peña-Claros et al. 2002
Nova Esperança, Acre, Brazil		40		Viana et al. 1998
Caxiuanã, Pará, Brazil	25	54.5	0.65	Sousa et al. 2014
Fallow, Acre, Brazil	12.7			Cotta et al. 2008
Mature Forest, Acre, Brazil	5.3			Cotta et al. 2008
RESEX Chico Mendes, Acre, Brazil	1.35	25.5	0.86	Wadt et al. 2005
Cachoeira, Acre, Brazil	2.5		0.93	Wadt et al. 2008
Pindamonhangaba, Acre, Brazil	2.2		0.72	Wadt et al. 2008
Filipinas, Acre, Brazil	1.5		0.71	Wadt et al. 2008
Oriximiná, Pará, Brazil	2.0	4.6	1.59	Scoles et al. 2016
Água Branca, Amapá, Brazil	6.8		0.93	Neves et al. 2016
Sororoca, Amapá, Brazil	11.2		1.09	Neves et al. 2016
Plato Almeida, Pará, Brazil	1.5	1.21		Salomão 2009
Plato Aviso, Pará, Brazil	0.005	71.4		Salomão 2009
RESEX Cajari, Amapá, Brazil – Flores	7	21	1.12	Guedes 2014
RESEX Cajari, Amapá, Brazil – Capo	11	63	0.57	Guedes 2014

* DBH = Diameter at breast height measured at 1.3 m above ground level

Availability of light has also been commonly considered to explain the population traits of *B. excelsa*. Although, some relations show that the availability of light does not seem to influence the establishment of seedlings (Zuidema 2000), many other studies highlight the effect of light on the population structure. For instance, *B. excelsa* grows best in large gaps during the initial stages of its life cycle (Mori and Prance 1990; Salomão, 1991). Pereira (1994) observed that the traditional populations of Amazonas applied management practices direct to the natural regeneration of the species, making the young individuals benefit from the clearings of open lands in the midst of natural vegetation, same as the studies from Myers et al. (2000), when they observed the absence of *B. excelsa* tree seedlings under closed canopy. Additionally, evidence suggest that the availability of water can too influence the number of juvenile trees in *B. excelsa* populations. Zuidema and Boot (2002) highlighted that the differences between seedling densities were also influenced by season, in which the data were collected because the *B. excelsa* seeds germination takes place during the rainy season, the first dry season can bring mortality rates to 65% for new recruits.

Regarding to *B. excelsa* rates growth, studies have demonstrated that variability in growth is partly explained by variation in light availability (Zuidema 2000). Although the availability of light does not seem to influence the survival of *B. excelsa* seedlings, the growth rates of the species are strongly related to the availability of this resource (Zuidema 2000). *Bertholletia excelsa* grows best in large gaps during the initial stages of its life cycle (Mori and Prance 1990; Salomão 1991) and Cotta et al. (2008) have pointed that height and diameter growth of young *B. excelsa* individuals were positively related to light availability.

Table 2. Fruits and seeds production of *Bertholletia excelsa* trees in natural forest

Local	**DBH**	**Fruits production**	**Seeds production**		**Authors**
		(Fruits tree^{-1})	**(kg ha^{-1})**	**(kg tree^{-1})**	
São João Baliza – Roraima	65.9	24.8	32.7	4.3	Tonini et a. 2008
Caracaraí – Roraima	74.6	18.6	8.48	3.8	Tonini et a. 2008
Xapurí, Acre, Brazil				24	Viana et al. 1998
RESEX Chico Mendes, Acre, Brazil				10.28	Wadt et al. 2005
RESEX Chico Mendes, Acre, Brazil		65.5		9.7	Kainer et al. 2006
RESEX Chico Mendes, Acre, Brazil		72.2		10.7	Kainer et al. 2007
Cachoeira, Acre, Brazil		79.6			Wadt et al. 2008
Pindamonhangaba, Acre, Brazil		86.5			Wadt et al. 2008
Filipinas, Acre, Brazil		79.6			Wadt et al. 2008
Plato Almeida, Pará, Brazil					Salomão 2009
Plato Aviso, Pará, Brazil					Salomão 2009
RESEX Chico Mendes, Acre, Brazil		66			Kainer et al. 2007

* DBH = Diameter at breast height measured at 1.3 m above ground level

In addition to population structure and growth rates, light availability also appears to be associated with the reproductive age of trees and fruit production. Most reproductive Brazil nut trees have DBH higher than 40 cm, and the most productive trees have 0.8-1.60 m DBH (Viana et al. 1998; Zuidema and Boot 2002). With this diameter, trees are estimated to be 20-25 years old (Table 2). Wadt et al. (2005) related the production of fruits with tree diameter and the shape of the canopy, emphasizing that the presence of lianas reduces drastically the production of the trees. Kainer et al. (2014) found that the cutting of vines increased in 3 times the production of fruits of *B. excelsa* trees and suggested that lianas cutting increases fruit yield because it can reduce competition for below-ground resources, such as water and nutrients, and also by reducing competition above-ground for light. Kainer et al. (2007) demonstrate that cation exchange capacity (CEC) and foliar phosphorus were positively associated with fruits production by *B. excelsa* trees.

Although methodological differences and the absence of studies aimed at environmental factors that influence the characteristics of *B. excelsa* make it difficult to make inferences about the effect of the availability of resources on the characteristics of populations of *B. excelsa*, we found recurrent information in publications that allow us to infer that the population characteristics of *B. excelsa* in forests are influenced by the availability of resources (Table 3).

Table 3. Effects of availability of resource on population traits of *Bertholletia excelsa*.

Population traits	**Availability of resource**				
	Light	**Water**	**CEC**	**P**	**pH**
Survival		➚			
Aggregate distribution	➘				
Density	⬆				
Regeneration	⬆				
Diameter growth	⬆				
Life span					
Productive age					
Fruits production	➚		⬆	⬆	➚

⬆ indicates positive effects; ➚ indicates a predominantly positive trend; ➘ indicates a predominantly negative trend.

Survival rates appear not to be influenced by light but may be influenced by the availability of water at the early stage of the life cycle. Light also seems to influence the distribution pattern of the species, but there is little information on the subject in the literature. Density, regeneration and growth appear to be strongly influenced by the availability of light, whereas fruit production seems to be more associated with soil edaphic factors, especially CEC, phosphorus and pH (Table 3).

1.4 *Bertholletia excelsa* plantations

The cultivation of *B. excelsa* is not a recent activity, records of its cultivation by ancient populations date from 1,000 to 4,500 years ago (Pärssinen et al. 2009; Ranzi et al. 2007; Maezumi et al. 2018). However, the starting point of the cultivation of *B. excelsa* as an agricultural activity began in the 1930s in the cities of Tomé-Açu in the State of Pará and in Parintins in the State of Amazonas, by Japanese settlers who immigrated to these localities (Homma et al. 2014). Some reports indicate that there was doubt about the viability of *B. excelsa* plantations throughout the Amazon, most of them forecasted a less production due to the lack of pollinators, while others feared the possible difficulty in pure plantations due to the attack of pests and diseases:

> "*Bertholletia excelsa* monoculture plantations are an option for already capitalized investments, but in the future, it may be limited by pest and disease attack". (Andrade & Cardoso. 1984; Freire & Ponte, 1976)

> "Grow *Bertholletia excelsa* in plantations have not been very successful, probably due to the lack of efficient pollinators (Mori & Prance 1990), the risk of inbreeding and the economics of establishing plantations".

Other factors that also discouraged the plantations of *B. excelsa* were the low rate of germination of the species that reached approximately 25%, and took about 12 to 15 months, the long period of the production (Müller 1981). The problem of germination was overcome by removing the bark from the seeds and treating the almonds with fungicides before sowing them later in the wetland, thus raising germination rates from 25% to above 90% (Müller 1981). The problem with the very high size and long juvenile phase was solved through the Forkert method of grafting (Müller 1981).

The fact is that, currently considering the experiences with pure and mixed plantations of *B. excelsa* throughout the Amazon, it has been recognized that the Brazil nuts tree has excellent silvicultural characteristics and has been used for its rapid growth and economic potential in plantations for restoration of degraded areas, agroforestry systems, forest enrichment and homogenous plantations for the production of fruits and wood (Ferreira and Tonini 2009; Ferreira et al. 2012; Salomão et al. 2014; Scoles et al. 2014).

In all these planting systems, the species has presented high growth rates when planted in different regions of the Amazon (Table 4), with growth rates in diameter varying from 9.6 mm year^{-1} in mixed plantations for the restoration of degraded areas, to 31.6 mm year^{-1} in agroforestry systems, and with high growth rates varying from 0.34 m year^{-1} in plantations for the production of fruits with grafted *B. excelsa* trees, to 2.2 m year^{-1} in agroforestry systems (Table 4). Considering the experiments with *B. excelsa* plantations in different regions of Amazon, the average survival rate of the species is over 80% (Table 4). In addition, there are no reports of pests and diseases that impair the survival, growth and productivity of *B. excelsa* under planting conditions (Albuquerque 1960; Costa et al. 2009).

Table 4. Growth and survival of *Bertholletia excelsa* under different system of plantations along the Amazon regions.

City	State	Plantation system	Age	Spacing	Height	DBH	MAI		Survival
							DBH	Height	
			(years)	(m)	(m)	(m)	(cm ano^{-1})	(m ano^{-1})	(%)
Manaus[1]	AM	Agroforestry	12.0	12 x 12	20.90	0.38	3.16	1.74	78.00
Machadinho do Oeste[2]	RO	Agroforestry	10.0	12 x 12	12.95	0.21	3.20	2.23	89.63
Machadinho do Oeste[2]	RO	Monoculture	10.0	12 x 12	12.25	0.22	3.10	2.13	95.38
Manacapuru[3]	AM	Agroforestry	10.0	-	-	-	3.10	1.60	-
Manacapuru[3]	AM	Enrichment	10.0	-	-	-	1.80	1.30	-
Cantá[4]	RR	Agroforestry	10.0	-	14.00	0.26	-	-	98.60
Porto Velho[5]	RO	Agroforestry	25.0	-	20.60	0.41	-	-	-
Nova Mamoré[5]	RO	Monoculture	35.0	-	23.90	0.44	-	-	-
Manaus[6]	AM	Monoculture	40.0	10 x 10	23.90	0.69	-	-	-
Porto Velho[6]	RO	Monoculture	30.0	-	22.00	0.40	-	-	-
Macapá[6]	AP	Monoculture	30.0	10 x 10	20.40	0.45	-	-	-
Tomé-Açu[6]	PA	Monoculture	49.0	20 x 20	20.60	0.80	-	-	-
Belterra[7]	PA	Monoculture	6.5	3 x 3	7.50	0.12	1.80	1.20	66.70
Manaus[10]	AM	Monoculture	10.0	3 x 3			1.39	1.50	69.40
Saracá-Taquera	PA	RAD mixed	30.0	10 x 50	15.30	0.19	0.96	0.78	
Roçado[12]	PA	Enrichment	2.0	10 x 10				1.20	92.00
Capoeira[12]	PA	Enrichment	2.0	10 x 10				0.78	96.00
Castanhal[12]	PA	Monoculture	2.0	10 x 10				0.34	90.00

Obs.: traço (-) = uninformed data. DBH = diameter at breast height, measured at 1.30 m above ground. MAI: Mean annual increment. RAD = restoration of degraded areas. Authors: [1] = Costa et al. (2009) ; [2] = Vieira et al. (1998); [3] = Soares et al. (2004); [4]= Ferreira e Tonini (2009); [5] = Locatelli et al. (2013); [6] = Yared et al. (1993); [7] = Yared (1988); [8] Castro 2017, [10] = Fernandes e Alencar (1993), [11]=Tonini et al. 2008

The fruit production in forests normally begins at 25 years, in plantations the production usually starts at 10 or 13 years, however, there are reports of grafted trees producing in 3.4 years. Costa et al. (2009) observed that fruit production was initiated in the eighth year after the establishment of the species in the field, and after 12 years of planting, all individuals fruited. Under planting condition fruit production has varied from 6 to 30 fruits per tree, while seed production has varied from 7.5 to 24.0 kg of seeds per tree (Costa et al. 2009; Ferreira and Tonini et al. 2009).

1.4.1 Bertholletia excelsa in agroforestry system

Bertholletia excelsa has been indicated as being a key component for the formation of agroforestry system in the Amazon, due to its economic potential and by favor a positive interaction with other plants on the agroforestry system, possibly due to a vast cycling of nutrients (Vieira et al. 2002). *Bertholletia excelsa* trees produce: nuts, that are used in food or commercialized; oil, used on the production of soaps, creams, shampoos; burr, for use in handicrafts, toys, organic fertilizer; bark, used as medicine (tea) for intestinal complications, and wood.

Evidence suggests that *B. excelsa* is able to establish and grow in agroforestry system with different light availability, but high growth rates have been observed in areas with higher irradiance. On the other hand, in more closed plantations, the incidence of trunks with defects was smaller. Regarding to water, it was reported that the lower survival of *B. excelsa* in agroforestry system in Rondônia, may have been due to water competition with other plants of the system, mainly the banana tree, a species of faster growth (Vieira et al. 2002).

Costa et al. (2009) in Manaus, considered that the distinction in growth of *B. excelsa* trees in different agroforestry system may be due, mainly, to the chemical characteristics of the soil and to the history of use, and the growth of the species can be favored, mainly by the levels of phosphorus and organic matter in soil. Similar results were observed in fertilized agroforestry systems, in which trees have responded to increased levels of nitrogen, phosphorus and lime, with significantly increased foliar nutrient, contents of nutrients and growth, probably because of the improved availability of Mg and Ca, for which the species seems to have a relatively high demand. In addition, it was observed that, in contrast to *B. excelsa* trees grown in forests or dense plantations, the agroforestry trees invested a substantial part of their biomass and nutrients on large branches and developed wide crowns, to improve stem form, reducing competition with associated crops for light and nutrient recycling (Schroth et al. 2015).

1.4.2 Bertholletia excelsa in degraded areas

Among the native species, *Bertholletia excelsa* is one of the most promising for reforestation and recovery of degraded areas in the Amazon (Veillon et al. 1971). The silvicultural characteristics of the *B. excelsa* tree, according to Yared et al. (1993), qualify it as one of the most promising native species for use in reforestation of altered areas in the Amazon, considering its growth, shape, natural shedding, biomass production and absence of phytosanitary problems. In order to select species for reforestation of degraded areas, Tonini and Arco-Verde (2005) evaluated an experimental planting of *B. excelsa* trees in Roraima by analyzing the relationships between the main morphometric indexes of the crown, such as diameter, area and depth, and higher values were found for *B. excelsa* when compared to other species.

After 30 years of evaluations, the analysis of *B. excelsa* growth rates showed that, in areas that were heavily degraded by mining activity, the increases were generally lower, sometimes reaching 50% of the observed values for anthropogenic secondary forests, agriculture or livestock in the Amazon. Rarely the diametric growth rates of the mined areas were higher than those of the agricultural areas, but when compared to other species *B. excelsa* is a species with good growth potential in diameter, when considering an annual increment of 10.0 mm year^{-1} for use in forest restoration of mined areas (Salomão et al. 2014).

Bertholletia excelsa also showed capacity to recover a hard degraded area in the region of Manaus. The process of degradation of the area began in the late 1970s with deforestation followed by soil decoupling to a depth of 3 m. Subsequently, the area was terraced and compacted for the beginning of civil constructions, however, the area was abandoned and still in 2009 there were none vegetation cover. Thus, was performance *B. excelsa* plantation on area and this species showed better performance in survival and growth, when compared with other species planted in the same area (Ferreira 2009).

1.4.3 Bertholletia excelsa in plantations of enrichment

Several experimental enrichment studies have been carried out in the western Brazilian Amazon (Acre State) and northern Bolivia (Beni Department) under different canopy opening conditions, with positive results regarding the growth of *B. excelsa* in large clearings (Oliveira 2000), and in anthropogenic and open areas (Kainer et al. 1998). In the same way, several studies have shown that in managed forests or transformed by human action, the rates of regeneration of *B. excelsa* are higher (Pereira 1994; Cotta et al. 2008). However, in the areas of forest enrichment with logging, some results showed a high mortality rate (82.7%) in the first year (Oliveira 2000). In Bolivia, in an enrichment planting, after four years, the survival rate of seedlings varied between 59 and 94% (Peña-Claros et al. 2002). The results obtained in the Trombetas experimental plantations followed the pattern observed in some trees of enrichment experiments, where the degree of light exposure was determinant for the best performance of the plant, increasing the growth with the size of the canopy opening (Scoles et al 2011).

1.4.4 Bertholletia excelsa in monoculture for wood and/or fruit production

Rusticity, rapid growth and the possibility of producing multiple products make *B. excelsa* one of the most important species for the development of forest plantation production in the Amazon. In general, the rates of survival of *B. excelsa* in homogeneous plantations are high and usually range from 90 to 100% (Table 4). The high survival rate of *B. excelsa* is higher than that observed in other tropical trees in similar experimental situations (Peña-Claros et al. 2002; Souza et al. 2008).

At the Confidence Experimental Station, in Cantá in the State of Roraima, Tonini et al. (2005) evaluated the data of 21 *B. excelsa* trees that have been planted in plots of 180 m² in the spacing of 2.5 x 2.0 m. In this study, other species were also evaluated: *Carapa guianensis* Aubl (andiroba), *Tabebuiav avellanedae* Lorentz ex Griseb. (purple ipê) and *Hymenaea courbaril* L. (jatobá). At the age of seven, among all species, *B. excelsa* presented the highest values of mean annual increment in diameter (19.0 mm year^{-1}), height (1.6 m year^{-1}) and commercial volume (14.6 m³ ha^{-1} year^{-1}).

1.5 Plantations traits of *Bertholletia excelsa* and resource availability

Light availability has been the most investigated resource on the survival and growth of *B. excelsa* plantations. In general, what is observed is that increased light availability favors growth, especially in diameter in enrichment plantations, agroforestry systems and monocultures, but has no effects on the survival of the seedlings in the initial phase of the plantation (Table 5). In addition, edaphic soil factors, mainly the availability of Ca and Mg, were highlighted for the production of biomass of *B. excelsa* trees in agroforestry systems. On the other hand, some evidence suggests that the availability of water and nutrients may have an effect on the establishment of seedlings of *B. excelsa* in degraded areas (Table 5).

Table 5. Effects of availability of resources on plantations traits of *Bertholletia excelsa*.

Plantations traits	**Availability of resources**				
	Light	**Water**	**CEC**	**pH**	**P**
Survival	➡	↗			
Diameter growth	⬆		⬆		
Height growth	⬆		⬆		
Wood production	⬆		⬆		
Fruits production					↗

⬆ indicates positive effects; ↗ indicates predominantly positive trend; ➡ indicates no effects.

1.6 Effects of resource availability on *Bertholletia excelsa* ecophysiological traits

The high growth performance of *B. excelsa* has been attributed to the ecophysiological characteristics of this species. Studies evaluating the ecophysiological responses of the species when submitted to the variation in the availability of primary resources to the growth allowed to verify that *B. excelsa* is benefited by the increasing in availability of light, it is tolerant to the reduction of water availability and it has high efficiency in the use of nutrients (Table 6). These responses are associated with morpho-physiological strategies that the species develop and that favor the capture and use of these resources (Peña-Claros et al. 2002; Scoles et al. 2014).

B. excelsa brings together features that allow us to suggest that this species possesses high foliar plasticity in response to irradiance. Souza et al. (2019) verified in that the species is able to modulate all its functional traits according to the availability of light. Additionally, it is able to recover from the stress caused by changes in light environments, and that both, the physiological adjustment as well as the recovery from stress caused by irradiance, occur in a short time interval.

Table 6. Physiological responses of *Bertholletia excelsa* to the variation in the availability of light, water and nutrients.

Physiological traits	**Light[1]**		**Water[2]**		**Nutrients[3]**	
	Sun leaves	**Shadow leaves**	**Dry**	**Rainy**	**Without fertilization**	**Fertilized**
P_N (µmol m^{-2} s^{-1})	10.34	12.61	11.21	11.74	8.23	11.60
g_s (mmol m^{-2} s^{-1})	235.83	297.50	285.00	248.33	265.33	369.28
E (mmol m^{-2} s^{-1})	4.14	4.77	4.62	4.29	3.57	4.71
R_d (µmol m^{-2} s^{-1})	1.03	1.72	1.47	1.28	0.82	0.95
SLA (m^{-2} kg^{-1})	11.50	9.50	11.50	9.50	14.24	15.04
Chl *a*/Chl *b*	2.92	3.05	2.88	3.08	3.46	3.29
Chl_{total}/Car	2.83	2.65	2.72	2.77	3.07	2.50
F_V/F_M	0.82	0.78	0.81	0.80	0.74	0.76
NUE (mmol mol-1 s^{-1})	0.56	0.70	0.61	0.66	0.66	0.62
PUE (mmol mol-1 s^{-1})	11.02	14.45	10.83	14.64	9.27	10.30
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	2.63	2.69	2.53	2.79	2.28	2.50

Obs.: The values are means calculated from literature data. P_N = photosynthesis, g_s = stomatal conductance, E = transpiration, R_d = respiration, SLA = specific leaf area; Chltotal = chlorophyll total, Car = carotenoids, F_V/F_M = photochemical efficiency, NUE = nitrogen use efficiency, PUE = phosphorus use efficiency, WUE = water use efficiency. Authors: [1] = Morais et al. 2007 and Ferreira et al. 2009; [2] = Ferreira 2013 and Morais 2007; [3] = Ferreira et al. 2009, Gomes 2012 and Corrêa 2013.

The functional traits that most contribute to explaining the photosynthetic plasticity of *B. excelsa* to light are LMA, the F_V/F_M ratio, R_d, g_s and carbohydrates (Lopes et al. 2019, Souza et al. 2017). In addition, Souza et al. (2017), studying the ecophysiological responses of *B. excelsa* to the controlled variation of light, also noted that the species has the ability to modulate gas exchange and leaf mass per area. Similarly results also found by Ferreira et al. (2016) and Morais et al. (2007), observed that the availability of light exerts strong influence on photosynthetic rates and that this species possibly presents tolerance to high irradiance stress. Ferreira et al. (2016) observed that the light environment had a strong influence on the functional traits of *B. excelsa* particularly for P_N, g_s, F_V/F_M ratio, LMA and R_d.

Costa (2015) observed that the application of thinning resulted in increased availability of light (large canopy opening), which favored the stomatal conductance (*gs*), leaf mass per area (LMA) and nutritional status of *Bertholletia excelsa*. Those responses were associated to the increase of photosynthetic rates and growth in diameter. The results obtained by Costa (2015) suggest that the availability of light is a limiting factor for growth of *B. excelsa* under dense planting, requiring the implementation of thinning regime as a strategy to increase the availability of these resources with the resumption of the growth of trees.

Bertholletia excelsa have been shown to tolerate a reduction in the availability of water. Schimpl et al. (2019) observed that seedlings of *B. excelsa* were able to withstand up to 58 days without irrigation. During this time, photosynthesis reached values close to zero, but after this period, with the return of irrigation in about 16 days, the plants were able to recover values of photosynthesis similar to the control treatment plants, indicating that young plants of *B. excelsa* exhibit high physiological plasticity in relation to stress resulting from water deficiency.

Although *B. excelsa* is considered a tolerant species to the low availability of nutrients in the soil, results of some researches indicate that fertilization favors the ecophysiological performance and growth of *B. excelsa*. Corrêa (2013), for instance, found that phosphate fertilization favored growth rates and biomass accumulation in seedlings of *B. excelsa*, and that the photosynthetic performance of the species is strongly related to the phosphorus content in the leaves, influenced by the higher efficiency of uptake and use of energy.

2 CONCLUSION AND FUTURE PROSPECTS

In this review, we have considered aspects associated with the effects of the resources availability, mainly light, water and phosphorus, in *B. excelsa* trees traits in forests, plantations and seedlings under controlled conditions. These responses indicate that *B. excelsa* has phenotypical and functional plasticity to resource availability. But it seems that light factor is a major to determine the structure of populations and growth in both forests and plantations. In addition, we found evidence indicating that increase in irradiance can also increase fruit production; however, fruit production seems to be more influenced by edaphic factors, especially phosphorus availability. Regarding water, the species shows tolerance to the reduction of availability of water in soils. These results contribute with information that helps, not only the determination of management techniques for *B. excelsa*, which would induce Brazil nuts forest plantations, but also allow us to make inferences about sustainability and possible impacts of climate change on the amazon forest.

Bertholletia excelsa growth under thinning, liming and P fertilization: a linkage between traits below- and above-ground and resource use efficiency

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Abstract

A research recent suggested that liming, phosphorus fertilization and thinning may increase the growth in plantations of *B. excelsa*. Here, we investigated how these treatments influence the relationship between above and below tree traits, resource use efficiency and growth of *B. excelsa* plantations in Central Amazon. Liming has no significant effect on growth, but phosphorus and thinning have increased growth in 47% and 82%, respectively, and responses of trees to thinning were independently of liming and phosphorus addition. Thinning reduced specific leaf area (SLA) and has not altered belowground traits, while liming and phosphorus fertilization decreased fine root biomass, length, density and increased fine root diameter, leaf area and litterfall, but has not affected SLA. Specific leaf area was correlated with light use efficiency ($r = 0.61$, $p < 0.001$), while fine roots diameter was correlated to phosphorus use efficiency ($r = 0.34$, $p = 0.02$). Growth of *B. excelsa* was correlated with light use efficiency ($r = 0.77$, $p < 0.001$) and phosphorus use efficiency ($r = 0.41$, $p = 0.03$). Our results suggest that the changed in below and aboveground traits reflect not only the availability of resources on site, but also may favor the resource use efficiency for trees, and that thinning is sufficient to increase the growth of the and improve *B. excelsa* forest plantations.

Key-words: Brazil nuts; Fine-roots, Phosphorus; Tropical silviculture

1 INTRODUCTION

Tropical forests will not be able to sustain increasing domestic and international demands for wood due to low growth rates, environmental concerns and rapidly diminishing resources (FAO 2018). Thus, plantations are necessary to meet the demand for wood and to reduce pressure of deforestation on natural forests (Knoke et al. 2009). Although Brazil is prominent in the *Eucalyptus* silviculture, little is known about the silviculture of native species, especially from the Amazon forest. Among Amazon species, *Bertholletia excelsa* Bonpl. is as one of the most promising for the formation of forest plantations, due to its high economic value, high growth rates and excellent silvicultural characteristics (Scoles and Gribel 2012*a*, Costa et al. 2015).

Bertholleta excelsa plantations in Central Amazon, in general, have no soil treatment performed, and although it is usual to apply thinning, this is done empirically. Despite this, it has been observed that the plantations of *B. excelsa*, even under these conditions, have diameter growth rates and survival of 37.0 mm year^{-1} and 92%, respectively (Costa 2015). These values are high for Amazon species and are similar to those achieved by *Eucalyptus* when planted under the same conditions (Matos et al. 2012; Scoles et al. 2014). Although *B. excelsa* achieves high growth rates without the application of silvicultural treatments, researches showed that phosphorus fertilization, liming and thinning may increase yield plantation on this specie (Corrêa 2013, Costa 2015, Schroth et al. 2015). These silvicultural treatments may favor the plantations growth rates by altering trade-offs between below and above ground traits, for example, potential feedbacks between decomposition organic matter and nutrients turnover require efficient structure, a as fine roots.

Fine roots, traditionally defined as roots < 2 mm in diameter, are the most physiologically active component of the belowground plant system (Weemstra et al. 2016) and biomass and morphological traits have been linked to variation in resource availability (Iversen et al. 2017). In case of liming, it may occur an increase of fine root biomass and specific root length (SRL) due to a reduction in Al in the soil (Vondráčková et al. 2017), this can increase capacity of nutrients uptake (McCormack et al. 2012). On the other hand, the addition of phosphorus may reduce SRL and increase fine root diameter (Lamber et al. 2006, Ushio et al. 2015), that could result in a greater capacity for nutrient transporter (McCormack et al. 2012). Regarding thinning, the effects on fine roots are contradictory, it can favors traits associated with the acquisition of nutrients and water, like biomass, SRL, area and tips (Lopez et al. 2003), but may also not affect (Shen et al. 2017) or even influence negatively (Noguchi et al. 2011) and this response has been assigned to species, site and resource availability.

Specific leaf area or SLA, is an important above-ground indicator of plant strategies related to light availability but it is not a good indicator of soil fertility (Poorter et al. 2009). On the other hand, leaf area and litterfall have often been used to measure site fertility (Sayer and Tanner 2010). Thus, SLA, together with leaf area and litterfall, may provide information that allow us to understand strategies, regarded to resource availability, used by trees after liming, phosphorus fertilization and thinning. Effects of liming on above-ground traits are controversial. A positive, negative or absence effects of liming on SLA, leaf area and litter fall has been demonstrated (Forey et al. 2015*b*) and these responses vary depending on the site, species and may be related to some chemical factors (N, P). However, phosphorus fertilization normally favors leaf area, boosting litter fall and reducing SLA due to an increasing leaf turnover (Wright et al. 2001, Hidaka and Kitayama 2009). Thinning, in turn, results in reduction of SLA and leaf area due to the increase light availability (Gebauer et al. 2011), it also reduces litterfall as a response to a decreasing of basal area (Kunhamu et al. 2009).

Below and above-ground tree traits have been used in many correlative approaches to describe communities and demography (See chapter 1), it has remained unclear how and why traits should influence whole-plant growth (Falster et al. 2018). Regarding the questions: How traits influence plant growth? We know that LMA (or SLA) is positively and tightly correlated with relative growth rate in seedlings, in trees this relation is not clear (Falster et al. 2018). The relation between fine root traits and growth is poorly documented, but Meng et al. (2018) found a positive and strong relationship between trees growth and fine roots biomass, where these responses were associated to a greater capacity of trees for uptake of water and nutrients.

Still regarding questions: Why traits influence plant growth? Considering that the availability of resources on the site and the resource use efficiency by plants are elements of the ecological production equation (Binkley et al. 2010), we suspect that morphological traits, besides reflecting the availability of resources, are also associated with the resource use efficiency by plants, and also for this reason traits influence plant growth. For instance, the increase in leaf thickness and consequently decreasing of SLA, is usually associated with a lower light use efficiency that has been attributed an increase in diffusive limitations and low efficiency in light absorption (Terashima et al. 2011), while increasing in root diameter has been associated with root longevity and transport capacity of nutrients and water (Comas et al. 2002), which may contribute to the investment in biomass per unit of phosphorus usage, the most limiting nutrient in tropical soils (Davidson et al. 2004).

Considering that the factors responsible for plant growth contribute to the definition of more efficient silvicultural strategies for the management of forest plantations and also growing interest in understanding the factors that determine the growth of native species of the Amazon. Here, we investigated the effects of liming, phosphorus fertilization and thinning on below and above-ground of *B. excelsa* traits to test the following hypotheses: 1) *Bertholletia excelsa* under thinning, liming and phosphorus fertilization will change above- and below-ground traits with the purpose of favoring the acquisition and use efficiency of the most limiting resource; 2) Liming and phosphorus would be to increase the diameter growth of *B. excelsa* and these responses would be strong influenced by thinning.

2 MATERIAL AND METHODS

2.1 Site description

The studied *B. excelsa* plantation was located in Itacoatiara, Amazonas, Brazil (3°0'30.63" S, 58°50'1.50" E), which is 120 to 170 m above sea level, a mean annual temperature of 31.2 °C, and annual rainfall of 2539.6 mm (INMET 2018).

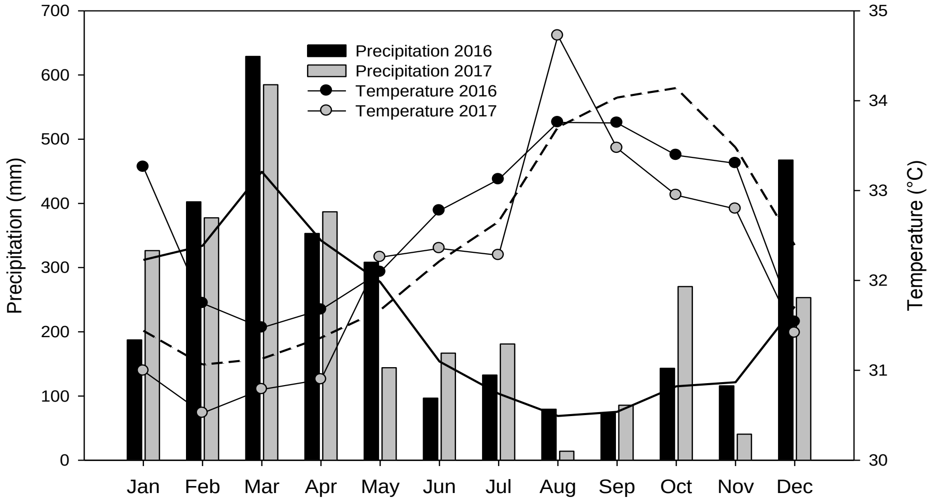

Figure 1. Precipitation and temperature in the study area. The solid and dashed lines represent the historical mean of precipitation and temperature, respectively.

Two well-defined seasons exist, the rainy season with precipitation > 300 mm from January to April and dry season with precipitation < 100 mm from August to September (Fig. 1). The dominant soil type is Ferrasol. The plantation was established in 2000, on area of 2.69 ha. Seven months-old seedlings with 150 mm of height were used, and the spacing between them was 2.5 x 1.5 m, resulting in a stocking density of 2,666 trees per hectare. Seedlings were not fertilized.

2.2 Experimental design

A randomized complete block design was established in this study. The number of blocks were defined from the variable diameter (measured at 1.3 m aboveground), obtained by a preliminary pilot inventory. We used the sample size for infinite populations equation to determine how many sample units were needed to have a degree of confidence of 95% and 10% of precision. Thus, eight blocks (1,102.5 m^2) were established and in each block the six treatments (Control; Liming; Liming + Phosphorus; Thinning; Thinning + Liming and Thinning + Liming + Phosphorus) were randomly applied. The treatments plots were separated by a buffer zone of approximately 4 m. Thus, the measurements were performed in plot interior of 12.5 m x 7.5 m (93.75 m^2) with 25 trees per plot (1,200 total trees).

2.3 Treatments application

Liming treatments were 0 and 2.0 Mg ha^{-1}, applied to the soil in the high precipitation season (14/03 to 01/04/2016), with applications of 824 g of limestone (PRNT 91%) per tree. Phosphorus treatments were 0 and 150 kg ha^{-1} of triple superphosphate (46% of P_2O_5), applied sixty days after the liming was assigned, and when the soil pH increased to 5.5, then it was applied more 122 g per tree. The estimation of the necessity of liming was performed based on the methodology proposed by Batista (2014), while the requirement for P_2O_5 was extrapolated from suggestions for *Eucalyptus* ssp. (CFSEMG 1999).

Liming and phosphorus were applied at a distance of 0.5 m from the stem base and 0.2 m depth from the soil surface, forming a circle around the plant (soil amendment circle). The distance of stem base, depth liming and phosphorus application was defined according to studies about root distribution, which showed that 70% of the total root biomass of adult trees are in 0-0.2 m depth from the soil surface and 61% are at a distance of 0.5 m from the stem base (Sudmeyer et al. 2004). Thus, all liming and phosphorus applied were within the influence of the root absorption zones.

After the phosphate fertilizer, a low thinning of heavy intensity was performed, where 50% of the basal area was removed from the stand and suppressed, tortuous and forked trees were eliminated. After thinning, all remaining leaves and branches of the trees were maintained in the area. The thinning intensity was defined considering recommendation by Costa (2015).

2.4 Data collected and trees samplings

Data collection was performed between 2016-2017, during peaks of high precipitation (precipitation > 300 mm) and low precipitation (precipitation < 100 mm), as well as in the transition between these seasons (Fig. 2).

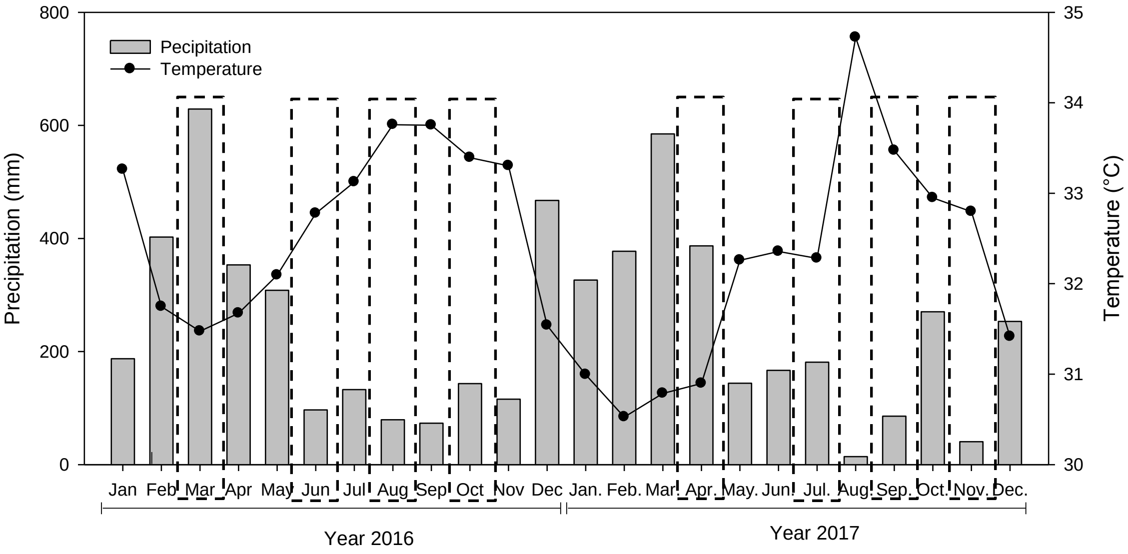

Figure 2. Precipitation and temperature over the period the data was collected in the field. Dashed bars indicate the months in which the data was collected.

Due to the impossibility of some measurements being taken from all the trees of the experiment (1,200 trees in total), three sample-trees were selected in each plot for data collection. The criteria for selected trees were representativeness in diameter, height and crown form in each plot.

2.5 Availability of resources: light, water and nutrients

Light availability was monitored from photosynthetically active radiation (PAR). PAR was obtained from a quantum sensor (ULM-500 and MQS-B of Walz) from 6:00

a.m. to 6:00 p.m. in intervals of 2 hours, and at 2 m of height from the ground level, in each plot were taken 3 random measurements of PAR over the period in which data collection was carried out in the field (on average 15 days). Thus, we calculated daily PAR per treatment from a mean of PAR values collected throughout the day.

Water and nutrients availability data were obtained from soil cores taken from each plot in the same place that was applied liming and phosphorus. Soil cores (50 mm inner diameter) were taken at 0.2 m depth from 3 randomly selected locations and combined to one composite sample. The litter layer was removed before the soil core was collected. The samples were air-dried then sieved by a 2 mm mesh, after removing the stones and roots by hand.

Thus, soil moisture content was determined by gravimetric method (Black 1965). Total N was determined by the Kjeldahl method (Bremner and Mulvaney 1982). Organic C was determined by the Walkley-Black method (Walkley and Black 1934). Al, Ca and Mg were extracted by 1 M KCl (Silva et al. 1999), while K, Fe, Zn and Mn were extracted by the Mehlich 1 solution (Mehlich 1953). Concentrations of these nutrients were determined by atomic absorption spectrometry (Perkin-Elmer 1100B, Uberlingen, Germany) (Silva et al. 1999). The available P was extracted using the Mehlich 1 solution (Mehlich 1953) and P concentrations were determined by spectrophotometry at 725 nm (Silva et al. 1999). The pH of the soil was potentiometrically measured in the supernatant suspension of a 1:2.5 soil:water and 1:2.5 soil:1M KCL solution (Silva et al. 1999).

2.6 Above and below-ground tree traits and phosphorus determination

Five healthy and fully expanded leaves were taken from the middle third of samples-trees. The leaf area (area of an individual leaf blade) was measured using a leaf area meter (CI-202, CID, Inc. Camas, WA, USA). Specific leaf area (SLA) was calculated by the ratio between the leaf area of 30 leaves disks (28.3 mm^2 each) and dry mass dried at 65 °C for 48 h (Ferreira et al. 2009*a*). Litterfall were taken monthly in one litter traps (0.25 m^2) that were installed and assigned in the center of each plot at 0.5 m above the ground. The collected litter samples were oven-dried at 65 °C to constant mass (Aragão et al. 2009).

Fine-roots were obtained from soil cores taken from the same places that were applied liming and phosphorus. Each sample-tree had one soil core (80 mm inner diameter), taken at 0.1 m depth, the litter layer was removed before the soil core was collected. This, cores were soaked for at least 12 h in deionized water and sieved through a 20 mesh with a water jet. All fine root (diameter < 2 mm) segments were carefully separated from the soil, cleaned with deionized water and classified as live or dead based on the appearance. Each root sample was scanned using a high-resolution flatbed scanner (800 DPI resolution, 256-level gray-scale, JPEG format; Epson Scanner Perfection V700 Photo, USA) and WinRhizo software (2007 Pro version, Instrument Regent, Quebec, Canada) to get roots surface area, roots length, roots diameter, roots tips and roots volume. After scanning, all samples were oven-dried for 48 h at 65 °C, weighed, and then we calculated specific root length (ratio between roots length and roots mass), specific root surface area (ratio between roots surface and roots mass) and root density (ratio between roots mass and root volume) (Valverde-Barrantes et al. 2015).

Phosphorus concentrations in trees was a mean between phosphorus in leaves and roots. Leaves and roots samples were dried in an oven at 65 °C to mass constant, thus phosphorus was extracted by digestion with 3:1 nitric-perchloric solution and was determined by spectrophotometry at 725 nm (Silva et al. 1999).

2.7 Monthly and annual tree growth

The annual growth was obtained from measuring diameter over bark at 1.3 m height (DBH) above the ground, taken for all trees (1,200 trees in total) to calculate the current annual increment of 2016-2017 and 2017-2018. The number and amplitude of diameter classes were calculated according to Sturges (1926) using growth data from period of 2017-2018. The growth monthly over period of 2017-2018 was obtained from a dendrometric band fixed to the stem of the samples-trees, the growth in circumference was measured with a digital caliper according to Muller-Landau and Dong (2010).

2.8 Light and phosphorus use efficiency

Light use efficiency was calculated by ratio between total biomass (g m^{-2}) and incident PAR (MJ m^{-2}) (Gitelson and Gamon 2015). Phosphorus use efficiency was calculated by ratio between biomass total of a tree (kg) and mean phosphorus concentration in a tree (g kg^{-1}) (Reich et al. 2014). Biomass total per compartments was calculated from ratio between fresh biomass (kg) and dry biomass (kg) of samples, multiplied by fresh total biomass (kg), also the total biomass of a tree was the sum of biomass compartments (Jaquetti et al. 2016).

2.9 Data analysis

A linear mixed model was performed to evaluate the effects of treatments on annual growth. Blocks and plots were included in the models as nested random effects to control for potential spatial autocorrelation. Principal components analysis (PCA) was performed to evaluate the effects of the seasonality and treatments on morphology of leaves and fine-roots. Pearson product-moment correlations were used to assess the influences of soil variables, PAR, leaves and roots and resource use efficiency on the growth variables. All statistical analyses were performed using the R environment (R Development Core Team 2017), adopting the nlme packages (Pinheiro 2018).

3 RESULTS

3.1 Availability of resource

Thinning increased the light availability from 51 to 423 µmol m^{-2} s^{-1}, and from rainy to dry season the daily PAR in unthinned plots increased 70%, while in thinning plots the increase was 200% (Fig. 3 A).

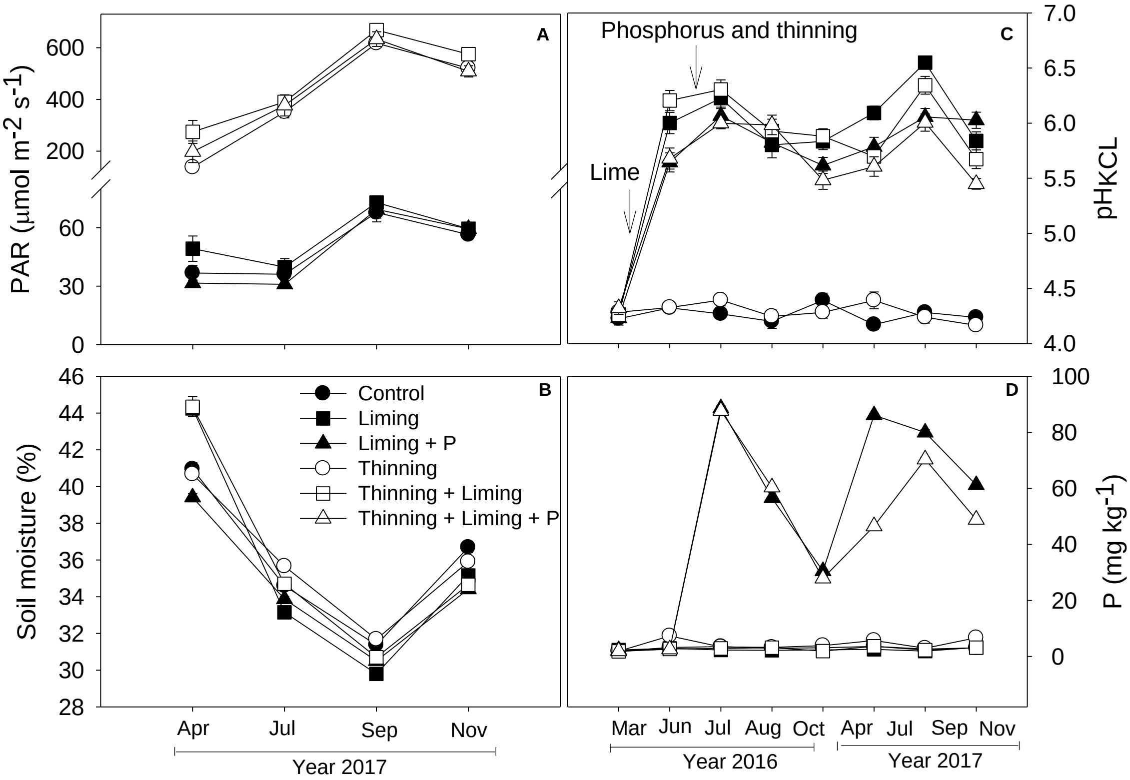

Figure 3. Changes in availability of light (A), water (B) and nutrients (C, D) in *Bertholletia excelsa* plantations under liming, phosphorus fertilization and thinning.

The treatments applied did not change the availability of water in the soil, but we observed a progressive reduction of soil moisture from rainy to dry season, a reduction of 24% (Fig. 3 B). Sixty days after lime application, the soil pH increased from 4.3 to 5.8, and thirty days after phosphorus application, the P in soil increased from 2.1 to 88.2 mg kg^{-1} (Fig. 3 C, D). Values of pH and P remained greater in these plots throughout the experimental period (Fig. 3 C, D). Thinning has not influenced the soil chemical attributes (Fig. 3 C, D). On the other hand, the soil responses to the addition of liming and phosphorus were influenced by seasonality and in the dry season it was observed an increase in pH from 5.5 to 6.5, and in P from 30 to 75 mg kg^{-1} (Fig. 3 C, D).

3.2 Below- and above-ground traits

The changes in soil attributes, due to liming and phosphorus fertilization, were determinant to separate fine-roots traits in two groups: without lime, or P and liming, or liming + P, independent of thinning (Fig. 4 A). On the other hand, the change in light availability, due to thinning, was determinant to separate leaves traits in two groups: without thinning and thinning, independent of liming, or liming + P (Fig. 4 C).

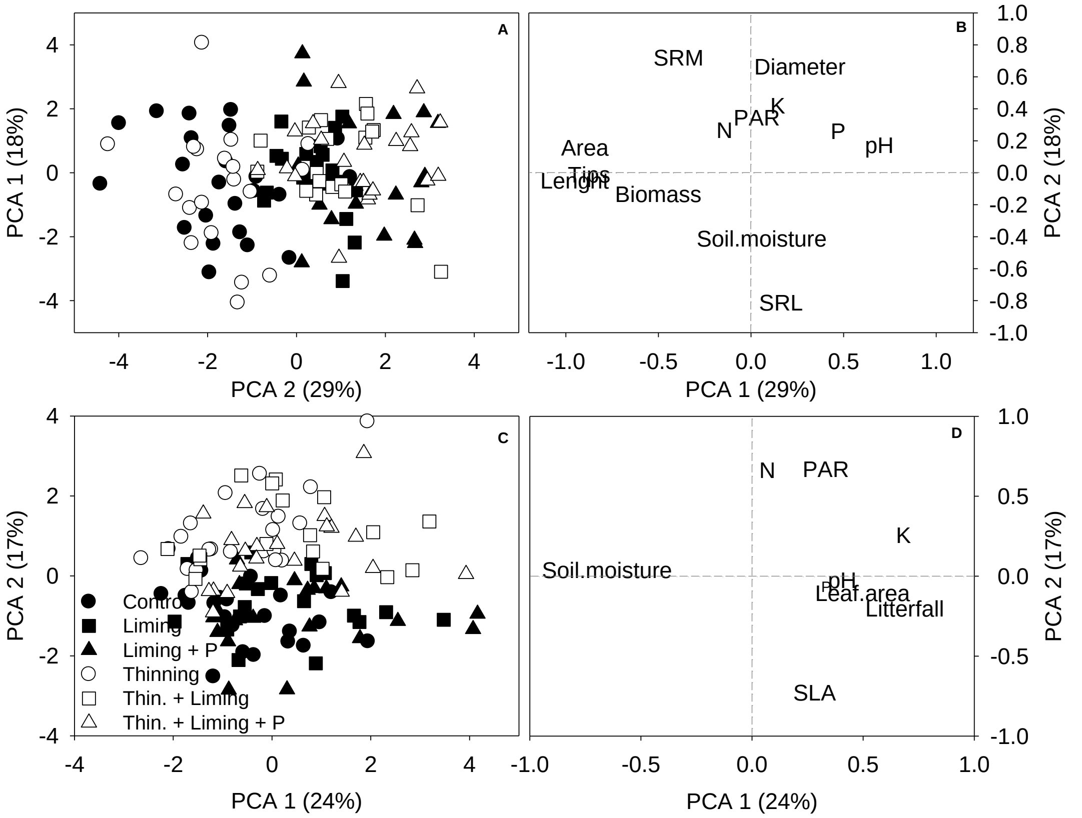

Figure 4. Below (A, B) and above-ground (C, D) of *Bertholletia excelsa* trees under thinning, liming and phosphorus fertilization.

When, we investigate the factors associated with changes in morphology of fine-roots (Fig. 4 B), the PCA explained 46% of the total variability, and the first axis was correlated to pH (r = 0.69, $p < 0.001$) and P (r = 0.47, $p < 0.001$), therefore the root traits most correlated (root mass, area, tips, length) to this axis, reflect the change in morphology associated to nutrient acquisition (Fig. 4 B). The pH and P were positively correlated to root diameter, and negatively correlated to fine-root biomass, tips, area and length (Fig. 4 B). The second axis was correlated to soil moisture (r = -0.41, $p < 0.001$) and PAR (r = 0.34, $p < 0.001$), therefore the root traits most correlated (specific root length, specific root mass and root dimeter) reflect the change in fine-roots morphology associated to seasonality and water availability. The soil moisture was positively correlated to specific root length (SRL) and negatively correlated to specific root mass (SRL) and root diameter (Fig. 4 B).

When we investigate the factors associated with changes in leaves traits (Fig 4 D), the PCA explained 41% of the total variability, and the first axis was correlated to soil moisture (r = -0.65, $p < 0.001$), P (r = 0.39, $p < 0.001$) and soil pH (r = 0.34, $p < 0.001$), therefore the leaf traits most correlated (leaf area and litterfall) to this axis, reflect the change associated to availability of nutrients and water in soil (Fig. 4 D). The pH and P were positively related to leaf area and litterfall, while soil moisture was negatively related to leaf area and litterfall. The second axis was correlated to PAR (r = 0.67, $p < 0.001$) and reflects the changes in leaf morphology associated with light availability, the PAR was negatively related to specific leaf mass (SLA) (Fig. 4 D).

3.3 Growth of trees

Already in the first year we observed increasing in growth in all treatments regarding control (Fig. 5 A). After two years, although liming increased by 30% the growth of *B. excelsa* trees, only effects of phosphorus and thinning were significant when compared with control. Phosphorus fertilization increased trees growth by 47%, while thinning increased, on average, tree growth by 82%, no differences were observed between thinned plots with or without liming, or liming + P (Fig. 5 A). When investigating the monthly growth of trees, we observed that significant growth rates occurred on rainy season, and a progressive reduction of growth was observed from rainy to dry season, a reduction of 94% (Fig. 6 B).

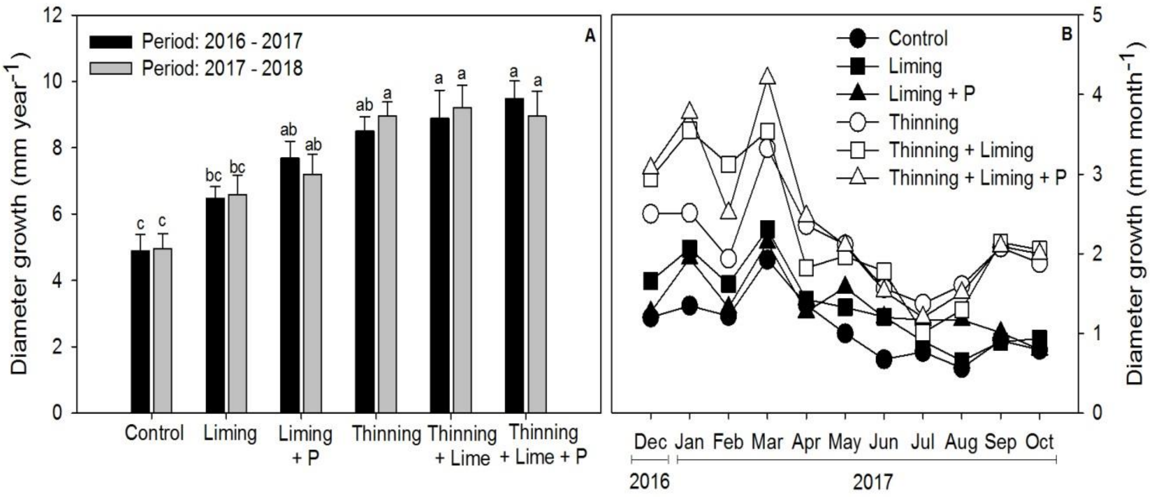

Figure 5. Annual (A) and monthly (B) growth of *Bertholletia excelsa* under thinning, liming and phosphorus fertilization.

The treatments changed the structure of the stand, we observed thinning, liming and liming + P reduced the frequency of trees in smaller diameter class and an increase in middle and larger class (Fig. 6 A, B), and also that the larger trees had growth rates higher than smaller trees (Fig. 6 C, D).

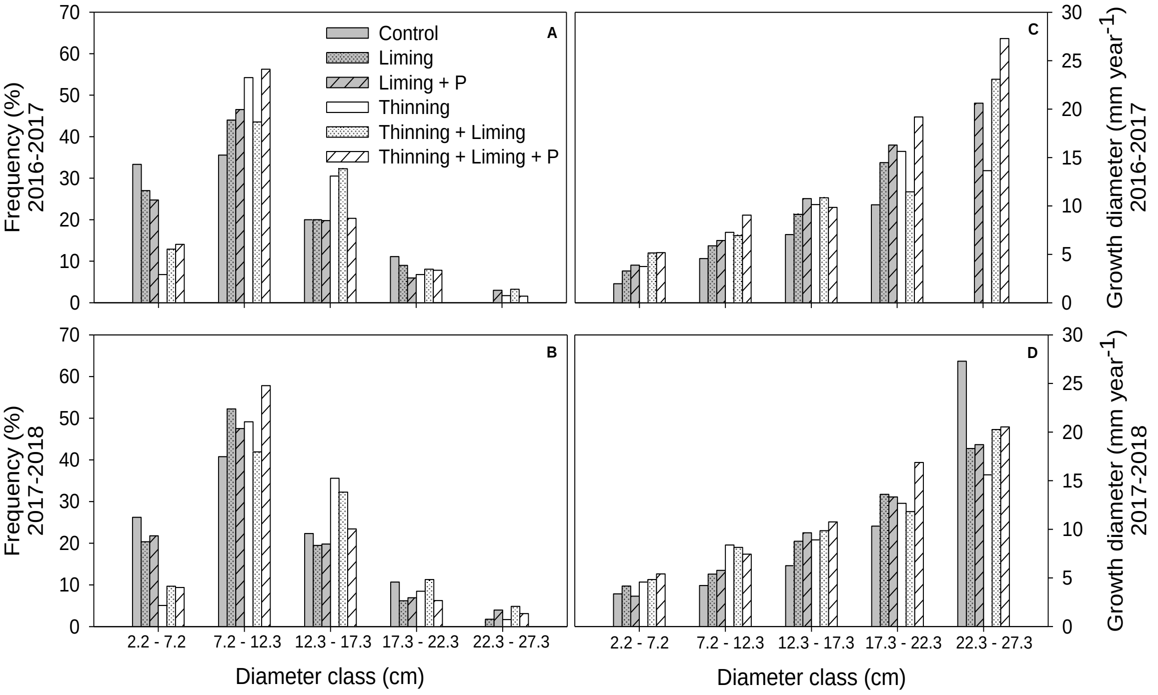

Figure 6. Frequency (A, B) and growth (C, D) of *Bertholletia ecxelsa* trees in different diameter class under thinning, liming and phosphorus fertilization

We found positive effects of PAR on growth only in thinned plots and soil pH and soil P were positively correlated with growth only in unthinned plots (Fig. 7 A-C). Growth rates were negatively correlated with specific leaf area independent of treatment (Fig. 7 D) and fine root diameter was positively correlated with growth regardless of treatment (Fig. 7 F). Fine root biomass was positively correlated with tree growth in plots that have not received liming or liming + P and was negatively correlated with tree growth in plots that received liming or liming + P (Fig. 7 E).

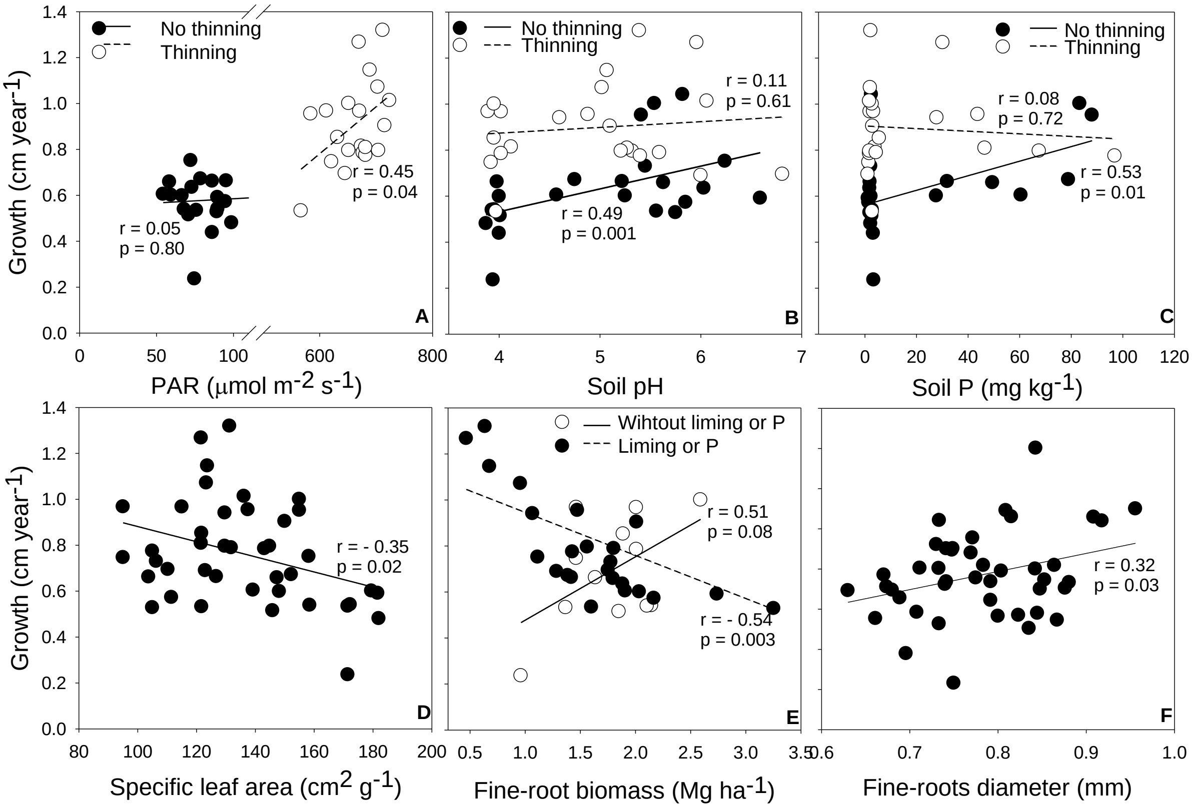

Figura 7. Relations of growth and availability of resource and traits above-and below-ground of *Bertholletia excelsa* under thinning, liming and phosphorus fertilization.

When investigating the relations between tree traits and resource use efficiency, we found that light use efficiency was positively correlated with specific leaf mass, regardless of treatment (Fig. 8 A), and phosphorus use efficiency was positively correlated with fine root diameter, regardless of treatment (Fig. 8 B). The increasing in light and phosphorus use efficiency, is turn, were positively correlated with growth of *Bertholletia excelsa* trees (Fig. 8 C, D).

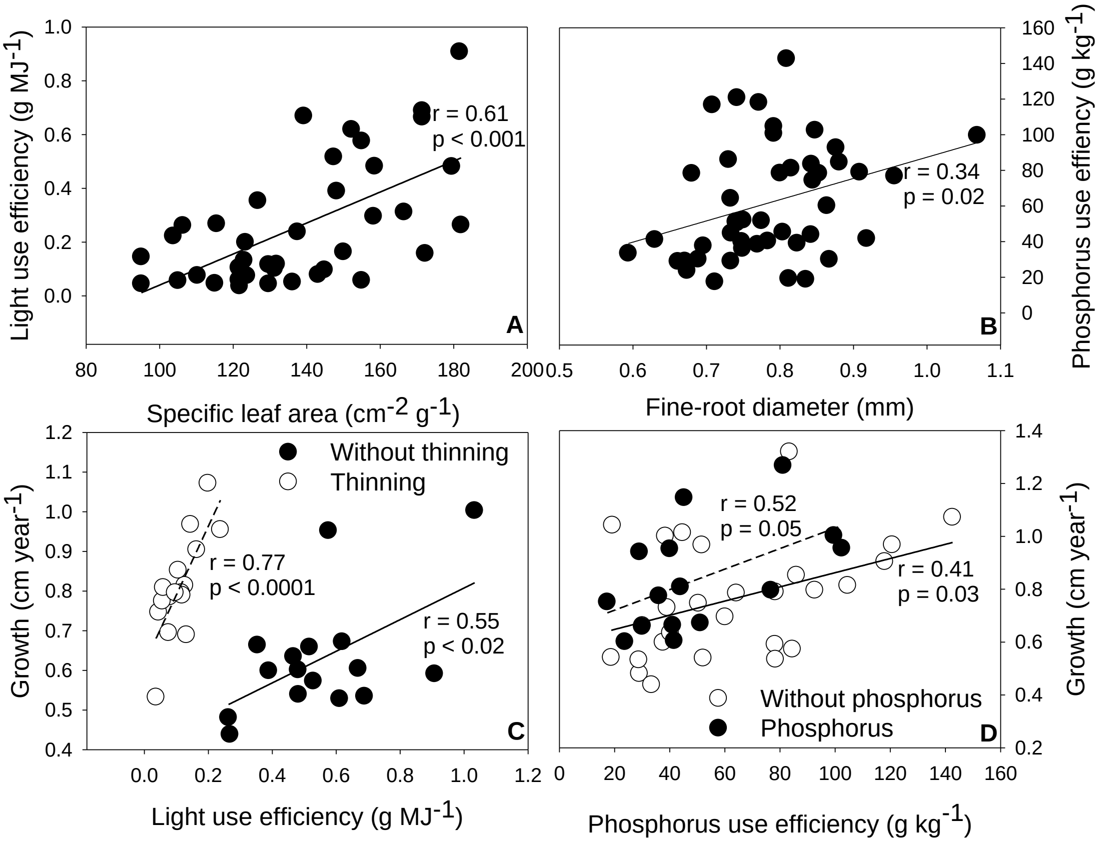

Figure 8. Relations between morphology traits and resource use efficiency, and relations between resource use efficiency and growth.

4 DISCUSSION

4.1 Liming, phosphorus and thinning change *B. excelsa* below and above-ground traits

We have found that liming, phosphorus fertilization and thinning effect the below- and above-ground traits of *B. excelsa* trees, corroborating with the first hypothesis of this study, but we observed that the treatments have affected the traits by different mechanisms. Thinning influenced only SLA, while liming and phosphorus had similar effects on all belowground traits and also on leaf area and litterfall.

The negative thinning effects on specific leaf area has been shown in several studies and are a response of an increasing in light availability (Wu et al. 2018*a*). However, in this study we also found that thinning has not influenced fine roots, leaf area and litterfall, these are unexpected results, because although the effects of thinning on biomass and root morphology are contradictory (Lopez et al. 2003, Noguchi et al.

2011), several studies have shown a negative effect on leaf area and litterfall (Inagaki et al. 2008, Park et al. 2009, Jiménez and Navarro 2016).

In general, the responses of trees to thinning, related to fine roots, when negative are due to an increase in fine root mortality (Noguchi et al. 2011), and when positive, due to an increase in biomass allocation in fine roots, to expand the area for nutrient acquisition (Shen et al. 2017). Regarding leaf area, normally, it is diminished to reduce the potential for loss of water by transpiration, especially in sites where water availability is a limiting factor (Wu et al. 2018*a*). Thinning also decreases litterfall due to a reduction of the number of trees in the stand, and consequently, decreasing of basal area (Jiménez and Navarro 2016).

The maintenance of leaf area, litterfall and fine root biomass in conditions similar to the control treatment after thinning, associated to specific leaf area reduction and higher growth rates, suggest that *B. excelsa* takes advantage of the significant availability of light after thinning to increase above-ground growth, and also that the availability of nutrients and water does not appear to be a limiting factor. Additionally, it has been pointed out that the availability of nutrients and water on the anatomy and, consequently, leaf morphology are weak and nutrient availability usually only changes foliar traits in situations where plants are severely limited by these resources (Poorter et al. 2009).

Liming and phosphorus fertilization increase fine root diameter and decrease fine root biomass, length, area and tips. This same standard of responses to nutrient availability showed in several studies for other species and sites (Zobel et al. 2007, Freschet et al. 2017). The increment in root biomass, length, area and tips in low fertile soils occurred to increase the acquisition of nutrients, while the increase in fine root diameter was associated to a significant capacity of transportation of water and nutrients and also to a high longevity of roots (Ushio et al. 2015, Weemstra et al. 2016). *Bertholletia excelsa* under liming and phosphorus fertilization also increased leaf area and litterfall. The increment in leaf area may have occurred to increase area for light capture and litterfall increase can be associated to a greater leaf turnover (Wright et al. 2011).

4.2 Specific leaf area (SLA) and fine root diameter favor light and phosphorus use efficiency

This study has produced two surprises, we found that specific leaf area (SLA) and fine root diameter favored light and phosphorus use efficiency, respectively. We found that thinning, decreased SLA and liming and phosphorus had no effect on this variable, while the increment in fine root diameter was due to pH and phosphorus increase, but was not affected by thinning. Thus, in this study we observed that changes in specific leaf area was due to an increase in light availability, while changes in fine roots diameter was due to an increase in soil pH and/or phosphorus availability.

The relationship between SLA and light use efficiency (LUE) has been little explored, but we know that the reduce of SLA, in general, is due to an increase in the thickness of the palisade cells (Wu et al. 2018*a*) and that thicker leaves have low efficiency in light absorption (Xiao et al. 2016) and has pointed that low efficiency of resource use may result from declines in acquisition of resources (Milla and Reich 2007, Binkley et al. 2010). Thicker leaves also have greater diffusive limitations (Terashima et al. 2009) and this favors the reduction on photosynthetic rates by unit of photon absorbed (Terashima et al. 2009). Additionally, the increase in leaf mass invested per unit leaf area corresponds directly to an increase in construction and maintenance cost (Milla and Reich 2007).

The relationship between below-ground traits and resource use efficiency has been poorly documented and in this study we surprisingly found a positive relation between phosphorus use efficiency (PUE) and fine root diameter. We know that increase in root diameter favors the uptake of phosphorus (Zobel et al. 2007) and that the increase in capacity of resource acquisition favors the resource use efficiency (Binkley et al. 2010). Additionally, fine root with greater diameter has more capacity to transport water and nutrients, and more longevity. More longevity implies a lower energy investment for the production of new roots, thus the plant may have to increase the part of energy drive to the growth of above-ground, this may explain the negative relationship between growth and fine root biomass in fertilized plots. *Bertholletia excelsa* growth is result of an increase in resource availability and light and phosphorus use efficiency.

Increase in light availability, due to thinning, affected growth rates, but the effects of liming and phosphorus addition were less pronounced than the effects of increased irradiance and the combination between liming or phosphorus fertilization and thinning had no effects on growth. This result came as a surprise, because although many studies have shown the positive effect of irradiance on *B. excelsa* growth (Myers et al. 2000, Scoles et al. 2011, Scoles and Gribel 2012*a*, Kainer et al. 2018), some studies also showed that *B. excelsa* is specie-demanding of phosphorus (P), calcium (Ca) and magnesium (Mg) (Corrêa 2013, Schroth et al. 2015).

The results found in this study indicate that light may be a more important limiting factor than pH and phosphorus on the growth of *B. excelsa* plantations, but we also found evidence of size-dependent responses to fertilization. We found that large trees were most responsive to fertilization than smaller trees, and for larger trees, the soil pH and phosphorus availability seemed to be the most limiting resources. The greater efficiency for larger trees to use the resource availability in the site has been discussed in several studies and has been attributed, especially, to the genetic potential (Campoe et al. 2013). In case of study, the responses of a large tree may also reflect the non-limitation by light.

Thus, if trees not limited by light can respond to fertilization, why the combination between liming or phosphorus addition and thinning had no effects on growth? First, due to the stand structure, which has a high number of smaller trees that have a low genetic potential for the resource use availability and, second, some studies have shown that trees adapted to low resource levels tend to have limited potential to boost growth rates in response to increased resource levels (Wright et al. 2018).

The increase in *B. excelsa* growth was due not only to the increased availability of resources, but also to the increased light and phosphorus use efficiency by trees. We found that light and phosphorus use efficiency favor the growth rates of *B. excelsa*. The higher light use efficiency is may the result of increasing of rate of photosynthesis per unit of absolved light, or from an increased partitioning of carbohydrates to stem growth (Campoe et al. 2013). In this study, we suspect that greater values of LUE are associated with both the photosynthetic rates and the carbon partition. Because LUE was correlated by SLA and the trees with considerable LUE showed greater allocation of biomass in stem.

A higher phosphorus use efficiency is a result of many processes that involved not only photosynthesis rate per unit of absolved phosphorus, or partitioning of carbohydrates, but also uptake efficiency, homeostatic relationships, and this study, have found that fine-root diameter influenced by phosphorus use efficiency is difficulty to indicate the factors associated to the relationship between growth and phosphorus use efficiency.

We found greater growth rates in the rainy season, which corroborate with other studies that also observed that the increasing in diameter growth of tropical forest is higher in the rainy season than dry season (Grogan and Schulze forthcoming, Nepstad et al. 2002, Wagner et al. 2012, 2013). This considerable growth in rainy season may be due to a strong influence by water availability of soils on biological processes (i.e. cell division in cambial tissues), and also may reflect a time lag in the use of carbohydrates synthesized during the whole year, though allocated into short-lived pools (leaves) in the dry season and into long-lived pools (wood) only during the wet season (Würth et al. 2005, Wagner et al. 2013).

5 CONCLUSION

Thinning reduced SLA and has not altered belowground traits, while liming and phosphorus fertilizations decreased fine root biomass, length, density and increased fine root diameter, leaf area and litterfall, but have no affected SLA. The effects of liming or phosphorus fertilization on diameter growth of *B. excelsa* do not depend of thinning and thinning, liming and phosphorus fertilization to favor the growth of *B. excelsa* by increasing availability of resource and resource use efficiency.

Soil-trees elemental stoichiometry and their effects on nutrients use efficiency and biomass of *Bertholletia excelsa* under thinning, liming and phosphorus addition

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Abstract

Forest plantations management necessarily contemplates the adoption of silvicultural treatments such as thinning, liming and fertilization, whose objective is to increase the growth rate of the trees. For this it is require making appropriate choices of species and silvicultural treatments to achieve high productivity. Plantations productivity reflect changes in below and above-ground tree traits and resource use efficiency (See chapter 2), which, in turn, are strictly related to nutrients stoichiometry in the soil-plant interface, but these information are still scares on species performance, especially for tropical trees. Thus, we investigate the effects of thinning, liming and phosphorus fertilization on soil-tree elemental stoichiometry and their effects on nutrients resorption, use efficiency and biomass of *B. excelsa* trees. The changes in availability of nutrients in soil modified the elemental stoichiometry of organs of *B. excelsa* trees. Fine-roots and litterfall were more responsive to changes in availability of nutrients in soil than mature leaves, but for phosphorus (P) this responses depend on the basis (area or mass) that the nutrient is expressed. Liming, phosphorus and thinning influenced the reduce of the resorption of Ca and Mg only in the dry season and thinning, liming and phosphorus increased N and P use efficiency. Biomass was correlated with PAR ($r = 0.38$), soil aluminum ($r = -40$), nitrogen in trees ($r = 0.52$) and phosphorus in trees ($r = 0.43$). Thus, we conclude that thinning, liming and phosphorus fertilization improve nutritional status of *B. excelsa* and use efficiency of N and P, resulting in increasing in biomass stocks.

Key-words: Brazil nuts, Forest nutrition; Silviculture Tropical, Soil Fertility.

1 INTRODUCTION

Recent researches had been suggesting that the application of thinning, liming and phosphorus fertilization could increase the yield plantations of *B. excelsa* (Corrêa 2013, Costa 2015, Schroth et al. 2015). However, the low number of studies specifically directed to answer these questions and that were carried out in plantations of this specie, still left many doubts about the effectiveness of these treatments. Studies have shown that thinning, liming and phosphorus fertilization resulted in a significant increase in growth of *B. excelsa* and the greater growth rates were associated with availability of phosphorus, light and increasing in pH of soil, but still to need confirmed changes in morphophysiology of below and above-ground tree traits.

Morphophysiology of below and above-ground tree traits are strictly related to nutrients stoichiometry in the soil-plant interface (Wright et al. 2004). Thinning, liming and phosphorus fertilization could increase the availability of multiple soil nutrients. Liming and thinning, for instance, could increase P availability in soil, because liming is weaker and reduces P retention capacity due to increasing in soil pH, while thinning increases organic matter and decreases competition between trees for below-ground resources (Antoniadis et al. 2015, Zhang et al. 2018*b*). The improvement of soil fertility by thinning, liming and phosphorus addition may thus to influence the tree nutrients stoichiometry (Fan et al. 2015).

Tree nutrient stoichiometry reflects the balance of multiple chemical elements associated to assimilation of carbon (C) and nutrient uptake during plant growth, and thus plays a fundamental role in the growth of plantations (Fan et al. 2015, Kou et al. 2018). Nitrogen (N) and phosphorus (P) are key elements to the growth of trees, and their stoichiometry (N:P ratio) is a useful indicator of nutrient limitation. Different plant organs may have variations in stoichiometric (Elser et al. 2010, Schreeg et al. 2014), for instance, in contrast to root and senescent leaves, new and mature leaves thus should have higher stoichiometric homeostasis with less sensitivity to soil nutrient availability (Minden and Kleyer 2014, Mo et al. 2015*b*).

Nutrient resorption plays a significant role in maintaining a balance in the stoichiometry of plants and influences many processes, including resource use efficiency (Brant and Chen 2015, Zhou et al. 2016). Nutrient resorption is most succinctly defined as the movement of nutrients in photosynthetic organisms from senescing tissues back to surviving tissues and is a reflection of the plant nutrient status relative to the environment (Brant and Chen 2015). Plants resorb relatively more of a particular nutrient, if the lack of it is limiting to its growth and globally, natural terrestrial ecosystems, recycled N accounts for nearly 90% of annual terrestrial plant demand, whereas recycled P sustains > 98% of global terrestrial plant productivity (Cleveland et al. 2013) .

Re-use of nutrients by new leaves reduces nutrient loss and thereby increases nutrients use efficiency. Once more nutrients tightly coupled to C gain and its efficient allocation from one leaf to another, contributes to optimal C fixation (Reich et al. 2014). In this process older leaves with declining nutrients use efficiency are exploited as a source for nutrient, which is reallocated to young leaves to promote their growth (Reich et al. 2014). This way, the nutrient is used efficiently for photosynthesis at a whole plant level and also NUE is increased, as loss is reduced.

The highest accumulation of biomass by plantations under thinning, liming and phosphorus fertilization are attributed to changes in the nutrients stoichiometry of the trees that are normally related to the increase of resources availability (light, water and nutrients) and also to the nutrients use efficiency (Forrester 2013, Silva et al. 2018). Additionally, the availability of resources can determine the proportion of biomass allocation in different organs of trees. In general, liming, phosphorus and thinning result in an increase in allocation of biomass in the stem, due to the lower need for investment in behaviors responsible for capturing nutrients and light (Hermans et al. 2006).

Soil-trees nutrients stoichiometry and resorption and use efficiency of nutrients by trees vary seasonality. In tropical soil was observed an increase of pH, phosphorus and exchange cations in rainy season due to increase activity of microorganisms and organic matter decomposition (Turner et al. 2015), but different patterns have also been found and have been explained by the growing season of trees (Campo et al. 1998). In trees, it refers generally to the effect of seasonality on leaves and in a general increase in macronutrients are observed in dry season due the greater photosynthetic capacity and transpiration rates (Silva et al. 2011).

Thus, to understand how tree-soil elemental stoichiometry influences nutrients resorption and uses efficiency and biomass of *B. excelsa* trees, we investigated the effects of thinning, liming and phosphorus fertilization on the interface soil-tree by testing the following hypotheses: (I) Liming and thinning would increase availability of phosphorus in soil; (II) Nutrients stoichiometry of roots would be more responsive to liming and phosphorus additions than leaves and litter, (III) Thinning, liming and phosphorus fertilization would decrease some nutrients resorption and use efficiency, and increase accumulation of biomass by trees.

2 MATERIAL AND METHODS

2.1 Site description

The studied *B. excelsa* plantation was located in Itacoatiara, Amazonas, Brazil (3°0'30.63" S, 58°50'1.50" E), which is 120 to 170 m above sea level, a mean annual temperature of 31.2 °C, and annual rainfall of 2539.6 mm (INMET 2018). Two well-defined seasons exist, the rainy season with precipitation of > 300 mm from January to April and the dry season with precipitation of < 100 mm from August to September (Fig. 1). The dominant soil type is Ferrasol. The plantation was established in 2000, on an area of 2.69 ha. Seven months-old seedlings with 15 cm of height were used and the spacing between them was 2.5 x 1.5 m, resulting in a stocking density of 2,666 trees per hectare. Seedlings were not fertilized.

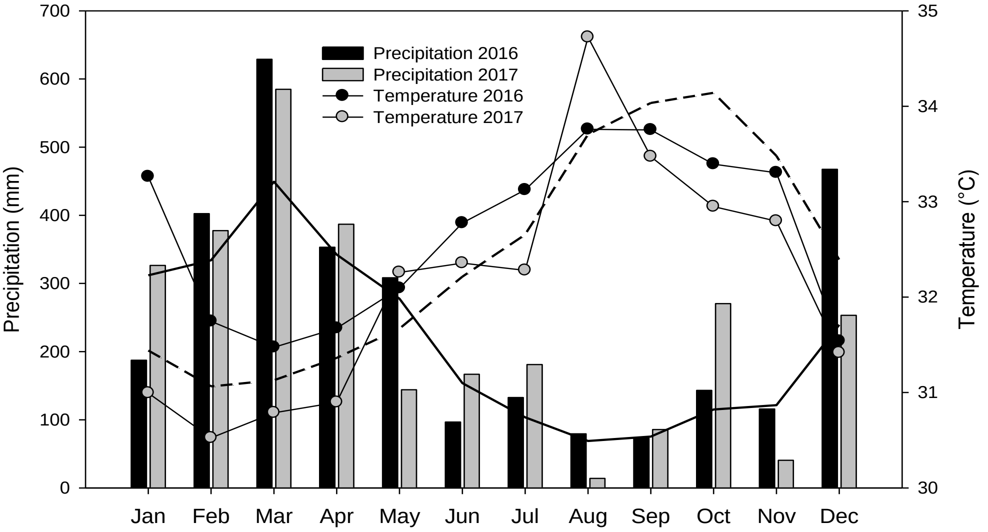


Figure 1. Precipitation and temperature in the study area. The solid and dashed lines represent the historical mean of precipitation and temperature, respectively.

2.2 Experimental design

A randomized complete block design was established in this study. The number of blocks was defined from the variable diameter (measured at 1.3 m above-ground), obtained by a preliminary pilot inventory. We used the sample size for infinite populations equation to determine how many sample units were needed to have a degree of confidence of 95% and 10% of precision. So, eight blocks (1,102.5 m^2) were established and in each block the six treatments (Control; Liming; Liming + Phosphorus; Thinning; Thinning + Liming and Thinning + Liming + Phosphorus) were randomly applied. The treatments plots were separated by a buffer zone of approximately 4 m. Thus, the measurements were performed in plot interior of 12.5 m x 7.5 m (93.75 m^2) with 25 trees per plot (1,200 total trees).

2.3 Treatments application

Liming levels were 0 and 2.0 Mg ha^{-1}, applied to the soil in the high precipitation season (14/03 to 01/04/2016), with applications of 824 g of limestone (PRNT 91%) per tree. Phosphorus levels were 0 and 150 kg ha^{-1} of triple superphosphate (46% of P_2O_5), applied sixty days after the liming was assigned, and when the soil pH increased to 5.5, then it was applied more 122 g per tree. The estimation of the necessity of liming was performed based on the methodology proposed by Batista (2014), while the requirement for P_2O_5 was extrapolated from suggestions for *Eucalyptus* ssp. (CFSEMG 1999).

Liming and phosphorus were applied at a distance of 50 cm from the stem base and 20 cm depth from the soil surface, forming a circle around the plant (soil amendment circle). The distance of stem base, depth liming and phosphorus application was defined according to studies about root distribution, which showed that 70% of the total root biomass of adult trees are in 0-20 cm depth from the soil surface and 61% are at a distance of 50 cm from the stem base (Sudmeyer et al. 2004). Thus, all liming and phosphorus applied were within the influence of the root absorption zones.

After the phosphate fertilizer, a low thinning of heavy intensity was performed, where 50% of the basal area was removed from the stand and suppressed, tortuous and forked trees were eliminated. After thinning, all remaining leaves and branches of the trees were maintained in the area. The thinning intensity was defined considering recommendation by Costa (2015).

2.4 Data collected and trees samplings

Data collection was performed between 2016-2017, during peaks of high precipitation (precipitation > 300 mm) and low precipitation (precipitation < 100 mm), as well as in the transition between these seasons (Fig. 2). Due to the impossibility of some measurements being taken from all the trees of the experiment (1,200 trees in total), three sample-trees were selected in each plot for data collection. The criteria for selected trees were representativeness in diameter, height and crown form in each plot.

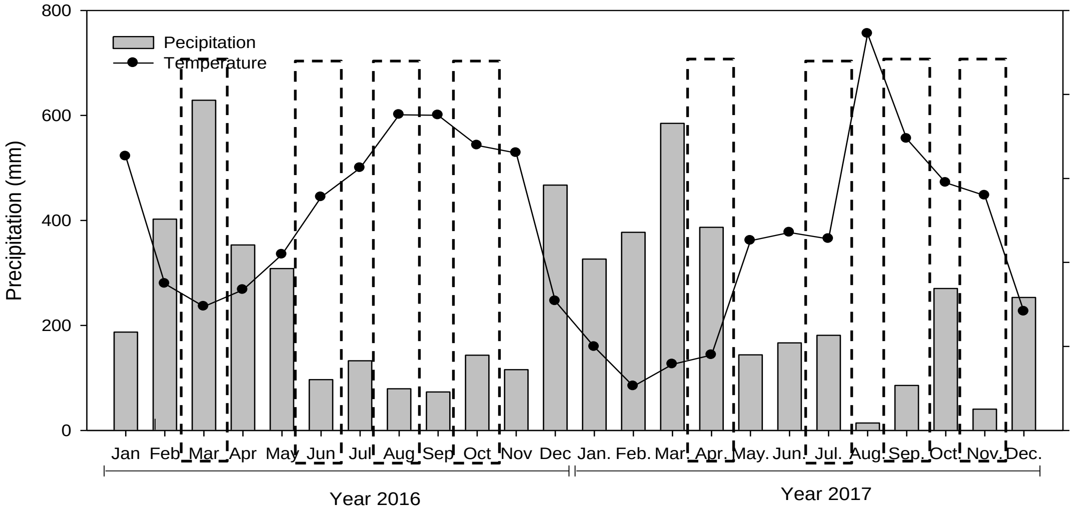


Figure 2. Precipitation and temperature over the period the data was collected in the field (2016-2017). Dashed bars indicate the months in which the data was collected.

2.5 Availability of resources: light, water and nutrients

Light availability was monitored from photosynthetically active radiation (PAR). PAR was obtained from a quantum sensor (ULM-500 and MQS-B of Walz) from 6:00 a.m. to 6:00 p.m. in intervals of 2 hours and at 2 m of height from ground level, in each plot were taken 3 random measurements of PAR over the period in which data collection was carried out in the field (on average of 15 days). Thus, we calculated daily PAR per treatment from mean of PAR values collected throughout the day.

Water and nutrients availability were obtained from soil cores taken from each plot in the same place that liming and phosphorus were applied. Soil cores (5 cm inner diameter) were taken at 20 cm depth from 3 randomly selected locations and combined to one composite sample. The samples were air-dried then sieved by a 2 mm mesh after removing the stones and roots by hand. Thus, soil moisture content was determined by gravimetric method (Black 1965). Total N was determined by the Kjeldahl method (Bremner and Mulvaney 1982). Organic C was determined by the Walkley-Black (1934). Al, Ca and Mg were extracted by 1 M KC, while K, Fe, Zn and Mn were extracted by the Mehlich 1 solution (Mehlich 1953). Concentrations of these nutrients were determined by atomic absorption spectrometry. The available P was extracted using the Mehlich 1 solution (Mehlich 1953) and P concentrations were determined by spectrophotometry at 725 nm. The pH of the soil was potentiometrically measured in the supernatant suspension of 1:2.5 soil:1M KCL solution (Silva et al. 1999)

2.6 Trees sampling and analysis

Five healthy and fully expanded leaves were taken from the middle third of sample-trees. Fine-roots were obtained from soil cores taken from the same places where liming and phosphorus were applied. Each sample-tree had one soil core (80 mm inner diameter) that was taken at 0.10 m depth. The cores were soaked for at least 12 h in deionized water and sieved through a 20 mesh with a water jet. All fine root (diameter < 2 mm) segments were carefully separated from the soil, cleaned with deionized water (Valverde-Barrantes et al. 2015). Litterfall were taken monthly in litter traps (0.25 m^2) that were installed and assigned in the center of each plot at 0.5 m above the ground (Aragão et al. 2009).

The leaves, roots and litter samples were dried in an oven at 65 °C. Total N was determined by the Kjeldahl method (Bremner and Mulvaney 1982). Organic C was determined by the Walkley-Black (1934) method. Macronutrients (Ca, Mg, P and K) and micronutrients (Fe, Zn, Cu and Mn) were extracted using 3:1 nitric-perchloric solution and concentrations were determined by atomic absorption spectrometry. P was determined by spectrophotometry at 725 nm. Ratios of C:N, C:P, N:P and Fe:Mn were considered in this study, due to their ecophysiological implications.

Nutrient resorption efficiency was defined as the proportional withdrawal of a nutrient during senescence and was calculated as the percent ratio of the differences between the nutrient concentrations (mass based) in mature leaves minus the concentrations in litter (Zhang et al. 2018*a*). Nutrient use efficiency relates to the net photosynthesis per unit of nutrients in leaves (Reich et al. 2014).

2.7 Data analysis

Principal component analysis (PCA) was performed to evaluate the effects of the treatments and seasonality on soil attributes. Linear mixed model was performed to evaluate the effects of treatments and seasonality on the elemental stoichiometry of leaves, litter and fine-roots, block and plots were included in the models as nested random effects. Pearson product-moment and Spearman rank correlations were used to assess the influences of soil variables, PAR and elemental stoichiometry on biomass. All statistical analyses were performed using the R environment.

3 RESULTS

Effects of liming and phosphorus addition in the soil were clearly showed by PCA, it is clear the separation between without fertilization, liming plots and phosphorus fertilized plots (Fig. 3 A).

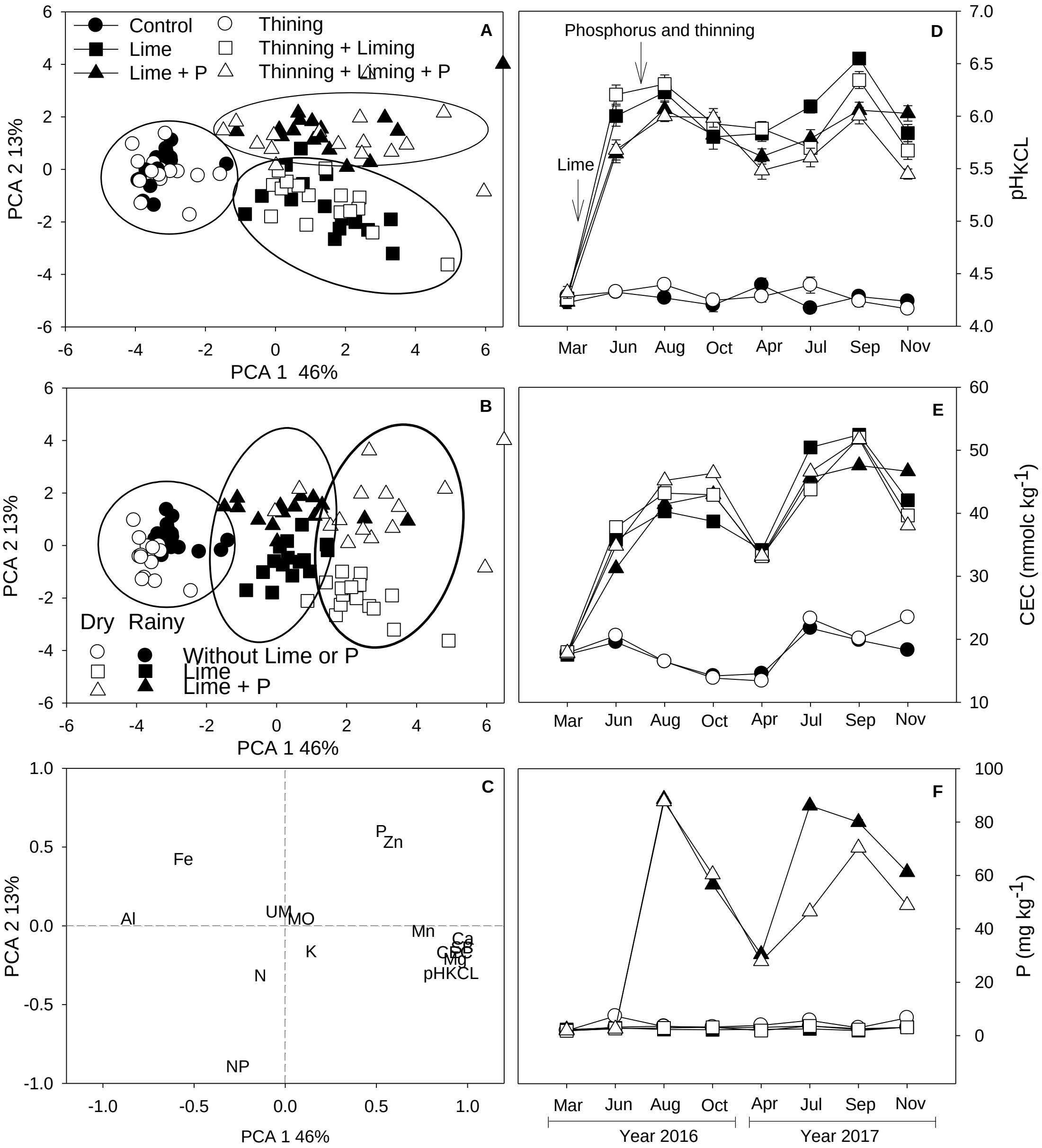


Figure 3. PCA ordination diagram of soil chemical attributes under liming, phosphorus and thinning at rainy and dry season of a *B. excelsa* plantation in Central Amazon.

The effects of liming on the soil were observed 60 days after the liming application, when liming addition increased soil pH from 4.26 to 5.86 and increased cation exchange capacity (CEC) from 17.7 to 35.1 mmolc kg^{-1} (Fig 3. D, E). Effects of phosphorus addition on soil were observed 30 days after the fertilizer application, with an increase of P from 2.08 to 88.17 mg kg^{-1} (Fig. 3 F). Values of pH, CEC and P have remained great in theses plots throughout the experimental period (Fig. 3 D-F).

Thinning is not influenced the soil chemical attributes (Fig. 3 A), the PCA is not separated between groups of thinning and no thinning plots (Fig. 3 A). On the other hand, the responses of soil to addition of liming and phosphorus were strong influenced by seasonality (Fig. 3 B-F), there was a clearly separation between without fertilization and fertilized plots and, in fertilized plots there were groups separated between rainy and dry season (Fig. 3 B). From rainy to dry season it was observed an increase of pH from 5.6 to 6.3 and phosphorus from 29.16 to 75.17 mg kg^{-1} (Fig. 3 D-F).

The treatments effects on elemental stoichiometry of *B. excelsa* trees varied according to the unit which these nutrients were calculated (Tab. 1). In mass unit, we observed the treatments effects in litter and roots, but not in leaves. In litter, were observed changes in Ca and Mg and in roots, no changes were observed in N (Tab. 1). In the area unit, we observed the same responses in litter and roots, but we also found effects of treatments on N, P and Ca in leaves (Tab. 1).

Table 1. F-value and significance (*** = 0.001; ** = 0.01; * = 0.05) of linear mixed model of elemental stoichiometry of *B. excelsa* leaf, litter and roots under liming, phosphorus and thinning application.

Elemental stoichiometry	Treatments effects					
	Leaf	Litter	Root	Leaf	Litter	Root
	--------- unit mass --------			----------- unit area ---------		
C	-	1.0	**7.8*** **	-	1.2	**2.5***
N	0.2	2.4	1.4	**4.1****	2.4	0.9
P	1.3	1.4	**16.0*****	**6.4****	**4.2****	**8.4*****
K	0.9	1.8	**3.4***	0.2	2.0	**4.7****
Ca	0.7	**6.3*****	**37.9*****	**8.3****	1.3	**30.2*****
Mg	1.7	**3.5***	**43.9*****	1.6	1.6	**23.7*****
Fe	2.1	1.6	**8.3*****	0.7	1.0	**3.6****
Zn	1.9	2.4	**9.6*****	**4.3****	1.8	**7.1*****
Mn	0.9	1.4	**9.9*****	0.9	1.6	**9.6****
C:N	-	2.0	**5.1****	-	2.0	**5.1****
C:P	-	1.0	**13.4*****	-	1.9	**13.2*****
N:P	0.8	0.9	**19.4*****	0.6	1.8	**13.4*****
Fe:Mn	1.9	1.6	**14.9*****	1.7	4.6	**7.6*****

The increase in N and Ca in mass unit in leaves, occurred only in plots that received thinning, but the increase in P concentrations was observed in both plots that received thinning and also in plots unthinned (Fig. 4). The responses for N and P occurred 20 days after thinning and 30 days after phosphorus fertilization. And for fine-roots, we found that, independently of the unit, the increase in Ca and P in fine-roots occurred immediately after liming and phosphorus addition (Fig. 4).

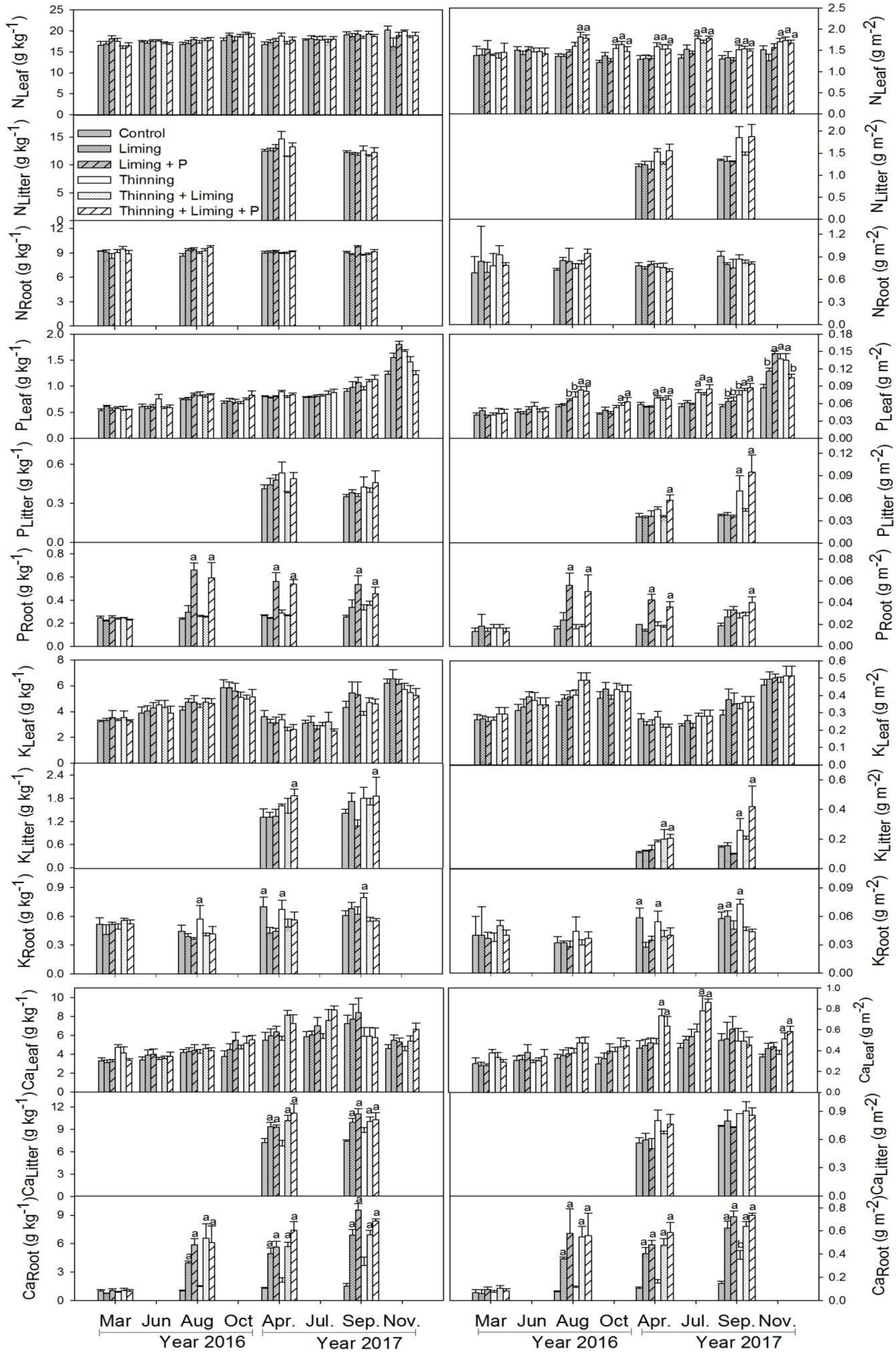


Figure 4. Seasonal variation of nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) in leaves, litter and fine-roots in *B. excelsa* plantation subjected thinning, liming and phosphorus fertilization.

Regarding seasonality, we observed regardless of nutrient basis, an increase of K (F = 37.34, $p < 0.0001$) and P (F = 134.28, $p < 0.001$) in dry season. In litter, seasonality has no effects on N, P, K and Ca concentrations, independently of nutrients basis, while in fine-roots there were only observed the effects of seasonality on Ca (F = 15, $p < 0.0001$), that was greater in the dry season, regardless of nutrients basis (Fig. 4).

The N:P ratio value was about 30 for leaves and litter, and 40 for roots at the beginning of the experiment. After the phosphorus addition, the N:P ratio decreased from 40 to 20 in fine-root, but had no changes in leaves and litter (Fig. 5). In leaves, N:P ratio decreased in the dry season in plots that received phosphorus fertilization, while in litter occurred the increase of N:P ratio in the same season (F=7.93, $p < 0.0001$). In fine-roots, no seasonal variation was observed (Fig. 5).

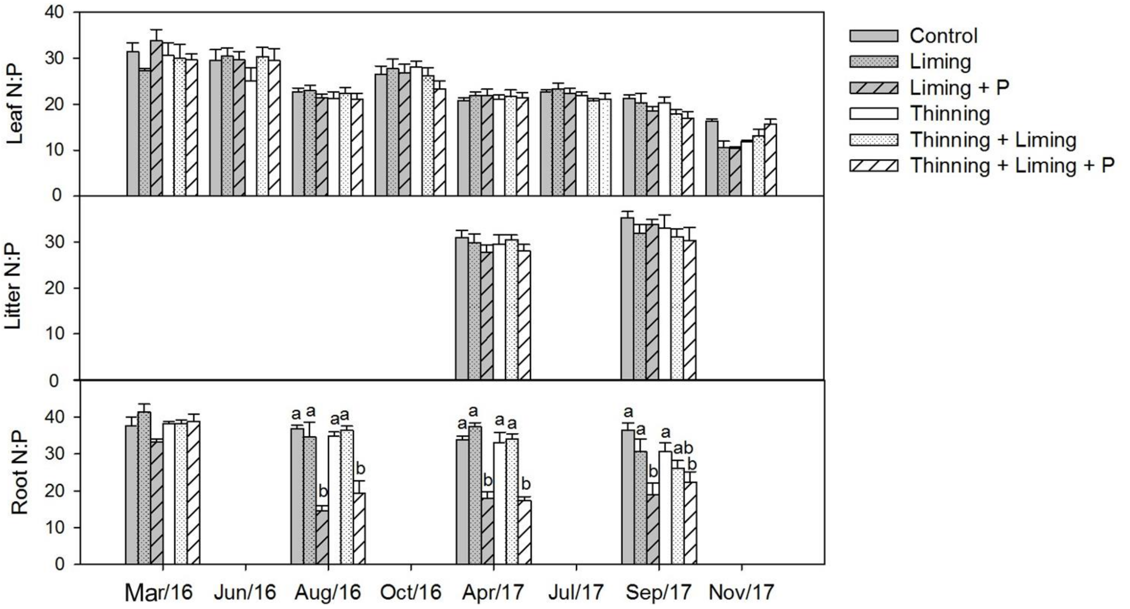


Figure 5. Seasonal variation of N:P ratio in leaves, litter and fine-roots in plantation of *Bertholletia excelsa* under thinning, liming and phosphorus fertilization.

We have found that, regardless of treatment or seasonality, the order for resorption efficiency was P = K > N > Mg > Ca. Liming, phosphorus and thinning reduced, only in dry season, Ca resorption efficiency in about 80% and Mg was reduced from 23% to -3%, approximately. For N (F = 19.98, $p < 0.0001$), P (F = 24.58, $p < 0.0001$), K (F = 12.80, $p = 0.001$) and Mg (F = 39.40 $p = 0.001$) the greater resorption efficiency rates occurred in the dry season (Fig 6 A-B).

Regarding nutrients use efficiency, we have found that, independently of treatment or seasonality, the order for use efficiency was P > K > Mg > Ca > N.

Thinning, liming and liming + P favor the N and P use efficiency in both rainy and dry season. In the dry season, the efficiency in the use of K (F = 8.6248, p = 0.0054) was reduced in all treatments. For N (F = 14.85, p = 0.0004) and Mg (F = 33.95, p < 0001), the greater use efficiency rates occurred in the dry season (Fig 6 C-D).

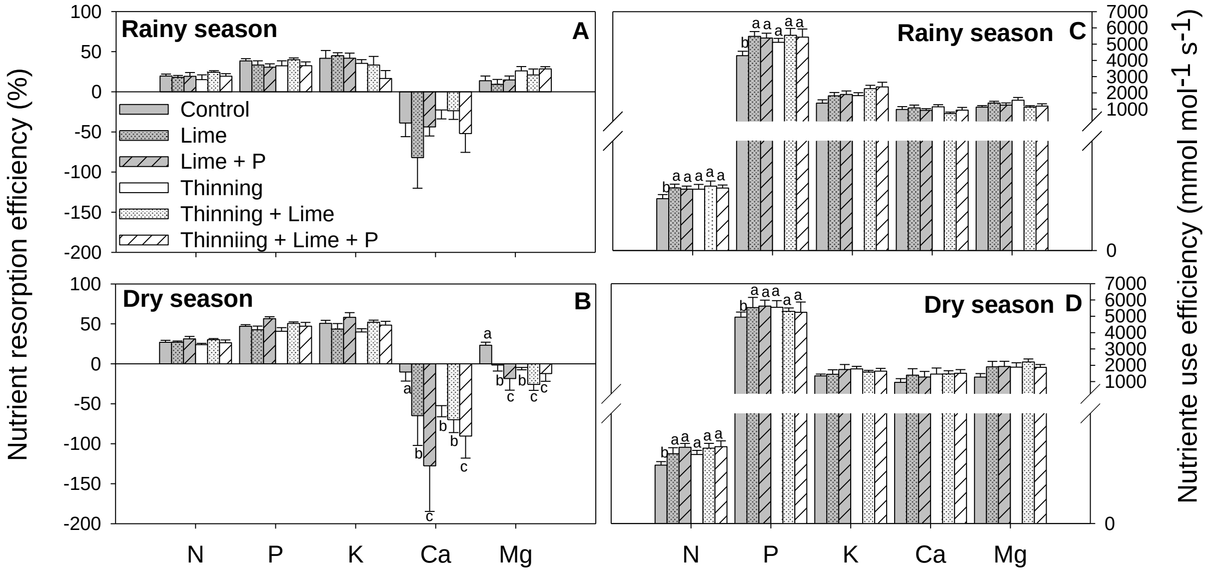


Figure 6. Nutrients resorption and use efficiency by *Bertholletia excelsa* trees under thinning, liming and phosphorus fertilization in rainy and dry seasons.

Treatments influenced accumulation and allocation of biomass in *B. excelsa* trees (Fig. 7 A). Trees that received liming and liming + P showed biomass accumulation of 30% greater than trees of control plots and 70% lower than trees of thinning + liming or liming + P. Only the thinning performed an increase of 52% in the biomass of the *B. excelsa,* when compared with control trees (Fig. 7 A). Trees under liming and liming + P have allocated more biomass to the stem (Fig. 7 B).

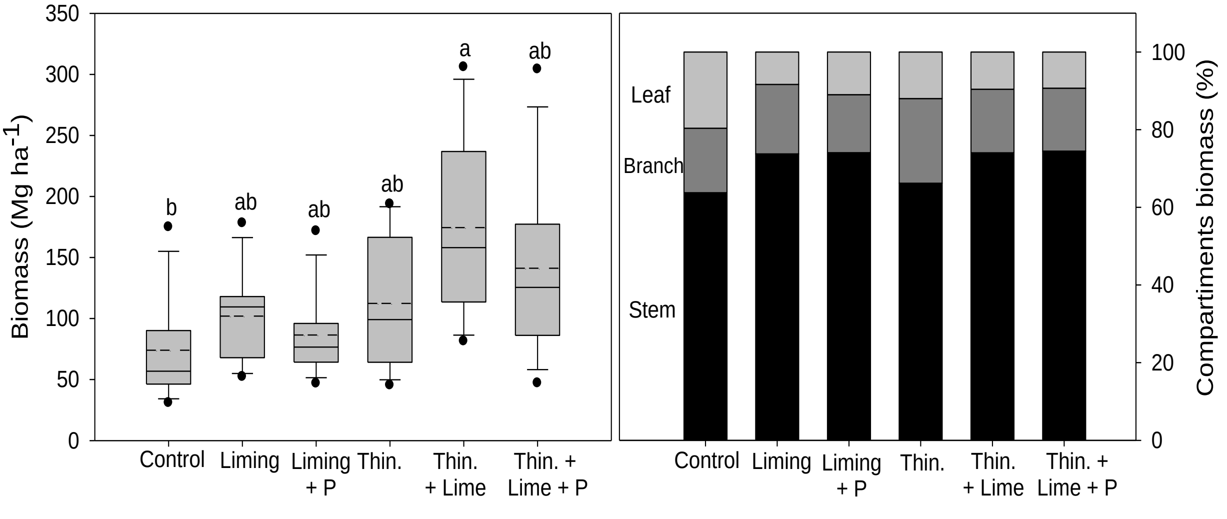


Figure 7. Accumulation (A) and allocation (B) of biomass by trees of *Bertholletia excelsa* plantations under thinning, liming and phosphorus fertilization.

When correlating all variables of soil-trees stoichiometry and nutrients resorption and use efficiency with biomass accumulation by trees, we have found that biomass of *B. excelsa* was positively correlated with PAR (r = 0.38, *p* = 0.02), soil sum-of-bases (r = 0.34, *p* = 0.03) and was negatively correlated with soil Al (r = -0.40, *p* = 0.01). The macro and micronutrients in trees (mean of nutrients in fine roots and leaves), leaves and fine roots were correlated with biomass only on the basis of area, with the exception of P and Fe that were also correlated with biomass in the mass basis, and only Fe were correlated negatively with biomass (Table 2).

Table 2. Significant correlations between biomass accumulation by trees of *B. excelsa* plantations and soil-trees nutrients stoichiometry, nutrient resorption efficiency and nutrient use efficiency.

Independente variables	Spearman rank		Pearson product-moment	
	rs	*p*	r	*P*
PAR (µmol m^{-2} s^{-1})	0.38	0.02	0.44	0.00
Al saturation (%)	-0.40	0.01	-0.23	0.15
SB (cmolc kg^{-1})	0.34	0.03	0.24	0.14
Mg_{Soil} (cmolc kg^{-1})	0.32	0.04	0.21	0.20
N_{Tree} (g m^{-2})	0.52	0.00	0.39	0.03
P_{Tree} (g m^{-2})	0.43	0.02	0.52	0.00
P_{Tree} (g kg^{-1})	0.19	0.28	0.34	0.05
Ca_{Tree} (g m^{-2})	0.46	0.01	0.32	0.09
Mg_{Tree} (g m^{-2})	0.37	0.05	0.23	0.22
Fe_{Tree} (mg kg^{-1})	-0.55	0.00	-0.51	0.00
Zn_{Tree} (mg m^{-2})	0.48	0.01	0.43	0.02
P_{Leaf} (g m^{-2})	0.27	0.12	0.42	0.02
C_{Root} (g m^{-2})	0.51	0.01	0.45	0.01
Ca_{Root} (g m^{-2})	0.50	0.01	0.46	0.01
$Mg_{Roo}t$ (g m^{-2})	0.59	0.00	0.55	0.00
Fe_{Root} (mg kg^{-1})	-0.54	0.00	-0.50	0.00
Zn_{Root} (mg m^{-2})	0.43	0.02	0.32	0.09
$FeMn_{Root}$	-0.33	0.05	-0.24	0.17

4 DISCUSSION

4.1 Soil-trees elemental stoichiometry of *Bertholletia excelsa* under thinning, liming and phosphorus addition

We found that thinning and liming have not influenced the P availability in soil, but the soil fertility was strongly influenced by seasonality. Thus, we accepted our first hypothesis partially. The absence of effects of liming on P-soil can be due to the low-P soil and the fast absorption by plants. In soils with low P, as for this study, the low amount of P released can be fast absorbed by plants and/or can be lost by leaching

(Antoniadis et al. 2015). Additionality, it has been observed that the liming can increase phosphorus availability only in soils with high concentration of P, but in soil with low phosphorus concentration would be needed phosphorus fertilization (Buni 2014).

Regarding thinning, its effects on soil nutrients are inconsistent, and appear to depend on a number of factors, including the intensity of thinning and time for litter decomposition. Lower thinning intensities are more favorable to improve the chemical quality of the soil, because higher thinning intensities, as a performance in this work, may favor the leaching of nutrients (Kim et al. 2016). Regarding the time for improvement of nutrients in soil by litter decomposition, in tropical forests the decomposition of litter, can vary from 1 to 3 years, and the time depends on the site and species (Hättenschwiler et al. 2011). In *Eucalyptus* plantations, for instance, were observed that only three years after the thinning, there was an increase in the concentration of C and N in the soil, which in turn is a result of both root death and incorporation of organic matter (Kim et al. 2016).

Although we have found no effects of liming and thinning on P-soil, we observed an increase of P, pH and CEC in the dry season only in plots that received liming or P. The results found in this study is different from what have been shown in the literature. In general, the greater values of P, pH and CEC occurred in the rainy season due, specially, to the decomposition of litter and input of nutrients (Turner et al. 2015) whether or not the soil was fertilized. However, other studies also found low values of pH and P-soil in the rainy season in tropical forest (Campo et al. 1998). In the case of pH, it was assigned for the increase in leach of basic cations (Ca, Mg and K) and in the case of P, it was assigned for the immobilization by microorganisms. Other hypothesis for low values of pH, CEC and P in the rainy season, is a massive uptake of nutrients by vegetation from soil during the growing season (Campo et al. 1998). In this study we suspected that the reduction of nutrients in the soil during the rainy season is due to great rates of growth of *B. excelsa*, that was observed in same season.

Despite that the thinning did not alter the nutrients in the soil, we have found that thinning, liming and phosphorus fertilization have influenced the elemental stoichiometry of *B. excelsa* and the responses varied between mass and basis area. We have found that in mass basis, the effects of the treatments were only observed in litter and fine roots, but not in mature leaves. These results corroborate with what has been shown in the literature, which has emphasized that old leaves and roots were better at reflecting the soil fertilization practices than mature leaves (Yan et al. 2016). According to these authors, these responses could be associated to several factors, among which the most substantial were the homeostatic regulation (Yan et al. 2016), tissue function (Minden and Kleyer 2014) and nutrients dilution effects (Jarrell and Beverly 1981). On the other hand, when calculating nutrients in area basis we also found changes in mature leaves by N_{area}, P_{area} and Ca_{area}. These results were expected in thinning plots due to the decrease in specific leaf area that was promoted by this treatment. But to our surprise, we have noted that an increase in phosphorus concentrations also occurred in unthinned plots that received P addition. In case of phosphorus, we suspected that a combination of subtle increasing in P_{mass} plus a subtle decrease in specific leaf area (SLA) may have resulted in a significant increase of P_{area} in leaves.

Seasonal variations in nutritional elements represent a dynamic growth response and may be related to the migration of nutrients caused by changes in the balance between the element uptake and utilization efficiency, which may lead to different nutrients concentrations in plant organs. In this study we have found that P, K and Ca have increased in the dry season, while N:P ratio decreased, in which the latter only in treatments that were fertilized with P. In this study, seasonal variations in P, Ca and N:P may reflect the occurrence of pools in soils that are also observed in dry season, and the dilution effects that may occurred in the rainy season when the trees presented the highest growth rates. On the other hand, we suspected that the increase in K in dry season may have been most associated to strategy of *B. excelsa* trees to maintain the stomatal opening and the high photosynthetic rates.

4.2 Soil-trees elemental stoichiometry and their effects on nutrient resorption and use efficiency

The increasing in resorption of nutrients in dry season, as observed in this work for N, P, K and Mg, are similar to what have been observed in other study. Previous studies have shown that variations in resorption of nutrients are related to soil conditions and climate (Aerts 1996, Chang et al. 2017, Zhang et al. 2018*a*) and is considered a strategy used by plants to compensate the difficulty in remove nutrients from the soil due to the lower availability of water (Ren et al. 2018). The resorption in the dry season may also reflect a great demand of these elements for leaf growth, and also for the role of stress tolerance due the increase of the availability of light and the reduction on the availability of water. Regarding the change in the tree nutrients stoichiometry caused by treatments application, it reduces only Ca and Mg resorption, in dry season. These responses reflect the significant availability of these nutrients in soil due to liming performance. The vast resorption of nutrients in the dry season favors the increase in nutrients use efficiency.

4.3 Soil-trees nutrients elemental stoichiometry and their effects on biomass

Responses to liming and phosphorus addition have not shown the clear effect on the accumulation of biomass by *B. excelsa* trees, but we observed that the site factors that most influenced the species growth were: the increase in availability of light (PAR), sum of bases (SB) and the reduction of aluminum concentrations in the soil. Increased availability of light and nutrients favors the growth of trees, supporting among other characteristics, the photosynthetic capacity of the trees. On the other hand, the reduction of the aluminum concentration in the soil, besides favoring the photosynthetic capacity, is also associated with the increase in capacity of capturing water and nutrients by the plants (Cai et al. 2008, Zheng 2010, Forey et al. 2015*a*). The effects of PAR, SB and Al on *B. excelsa* biomass were expected and similar results have been showed in other studies (Scoles and Gribel 2012, River et al. 2014, Schroth et al. 2015*b*, Lima et al. 2018). According to Lima et al. (2018), for instance, among the main edaphic factors related to the growth of *B. excelsa*, they are P, Al and Mg. Kainer et al. (2007) also observed that the cation exchange capacity, which is closely related to the SB and Al in Amazonian soils was positively associated with the increase in fruit production.

5 CONCLUSIONS

(I) Liming and thinning have not influenced the P availability in soil and an increase in Ca, CEC and P have occurred in the dry season.

(II) Fine roots and litter were more responsive to changes in availability of nutrients in soil than mature leaves, but for phosphorus (P) these responses depend on the basis (area or mass) that the nutrient is expressed.

(III) Liming, phosphorus and thinning influenced the reduce of the resorption of Ca and Mg only in the dry season and thinning increased all macronutrients use efficiency regardless of the availability of nutrients in the soil.

Photosynthesis traits of *Bertholletia excelsa* plantations following thinning, liming and phosphorus fertilization in Central Amazon

Abstract

The high performance of *B. excelsa* in plantations has been attributed, in part, to the ability of *B. excelsa* modular traits associated with the photosynthetic in order to favor their performance under certain environmental conditions. Here, we have evaluated the effects of thinning, liming and phosphorus fertilization on leaf pigments, chlorophyll *a* fluorescence, and the gas exchange of *B. excelsa* trees, with the objective of understand the associated mechanisms to its photosynthetic metabolism. Thinning increasing Chl*a*/Chl*b* ratio only in dry season. Immediately after thinning, photochemical performance (F_V/F_M and PI_{ABS}) decreases but the liming and phosphorus fertilization help to minimize stress caused by thinning and accelerates the recuperation of trees. After thinning, the remaining trees increase J-I phase and the formation of positive K-band occurs only in trees under thinning without liming or P. Additionally we have found, after thinning, a significant increase of 140% in DI_O/RC for trees without liming or P, while for trees under liming and P the increase was only of 47% and 79%, respectively. Photochemical performance (F_V/F_M and PI_{ABS}) of *B. excelsa* was positively correlated to Fe, Mn and Zn, and the reduction of F_V/F_M and PI_{ABS} was observed after the thinning in the remaining trees, which was not strong enough to reduce the photosynthetic rates (P_N) of *B. excelsa*. On the contrary, we observed an increase of 13% in P_N immediately after the application of thinning in trees and P_N was significant correlated with stomatal conductance (g_s), specific leaf area (SLA), nitrogen (N) and phosphorus (P).

Key-words: Leaf chlorophyll, Chlorophyll *a* fluorescence, Nutrition forest, Physiology of stress.

1 INTRODUCTION

Bertholletia excelsa is an Amazonian species of high commercial value that has occupied a prominent position in the silvicultural scenario of the region, because it presents high rates of survival and growth, even in sites with adverse conditions, which includes acid soils, with low availability of phosphorus and high irradiance incident (Scoles et al. 2011, 2014, Ferreira et al. 2015, Schroth et al. 2015, Gomes Costa et al. 2017). The performance of *B. excelsa* has been attributed, in part, to the ability of this species to tolerate the low availability of soil resources and also due to its high photosynthetic plasticity to irradiance (Ferreira et al. 2015, Shimpl et al. 2019). These characteristics reflect the ability of *B. excelsa* modular traits associated to photosynthetic metabolism in order to favor their performance under certain environmental conditions.

Photosynthesis is partially determined by chlorophyll (Chl) content and flux of energy in leaves. Plants should adjust Chl (Chl *a*+*b*, and Chl *a*/*b*) to adapt to a given environment and optimize photosynthesis. Thinning, for instance, can reduce the chlorophyll concentrations in leaves due to a large degradation of Chl by increasing the light availability (Wu et al. 2018*a*). Liming and phosphorus, in turn, can reduced the effects of stress due to the high irradiance caused by thinning application on leaf pigments altering the nutrients uptake of trees and the availability of nutrients in soil, mainly calcium (Ca), magnesium (Mg) and phosphorus (P) (Rahman et al. 2018). On the other hand, in light limiting condition, liming and phosphorus can increase leaf pigments to favor the capture of light (Filstrup and Downing 2017).

When a chlorophyll molecule absorbs light, approximately 80% of the absorbed light is used in photosynthesis, but when there is a stress condition, there may be an increase of light reemitted as fluorescence and this reflects changes in the shape of OJIP transient (Strasser et al. 2004). Plants under high irradiance, increasing initial fluorescence (Fo) and all the induction phases (O-J; J-I and I-P) may decrease, showing a much flatter curve (Kalaji et al. 2012) and also there may be the formation of positive K-band in O-J step, indicating the most severe stress. Regarding phosphorus, the effects of low P on OJIP transients seem contradictory and nonspecific, but it was reported that P deficiency affects the shape of the OJIP transient around the I step at 20 to 50 ms and causes the I step to gradually straighten and disappear (Carstensen et al. 2018*a*).

Changes in the shape of OJIP transients, reflect the alteration in energy flux in leaves. Plants in stress condition normally increase non-photochemical dissipation (DI_O) as a strategy to reduce the effect of energy excess on the photosynthetic apparatus and simultaneously there may be the reduction of all other steps of energy flux like absorption flux (ABS), trapped energy flux (TR_O) and electron transport flux (ET_O) (Strasser et al. 2004). The increase in non-photochemical dissipation, in general, results in the decrease of maximum quantum yield of photochemistry (F_V/F_M) and performance index (PI_{ABS}) (Strasser et al. 2004, Kalaji et al. 2012, Stirbet and Govindjee 2012).

Remaining trees, after the thinning, reduce F_V/F_M ratio as a response to greater light availability and this may cause the reduction of the trees photosynthetic rates (Wu et al. 2018*a*). However, liming and phosphorus fertilization can mitigate the effects of stress due to high irradiance and to accelerate the recovery of photochemical efficiency of trees. The impact of photochemical performance is associated to the photosynthetic capacity of plants, beside of stress characteristic, and also to species ability to acclimate to new environmental conditions. In the context, *Bertholletia excelsa* is a singular species and researches have pointed the capacity of this species to tolerate and recover from stress conditions (Shimpl et al. 2019).

Bertholletia excelsa seedlings, for instance, when exposed to high irradiance they reduced the F_V/F_M ratio, but the reduction was not enough to compromise the photosynthetic rates and in less than 10 days, *Bertholletia excelsa* reached values of F_V/F_M ratio similar to the non-stressed condition (Lopes et al. 2019). The increasing of photosynthesis rates of *B. excelsa* under high irradiance has been associated to the boost in stomatal conductance, the reduction of leaf specific area (SLA) and the tolerance to stress (Morais et al. 2007*a*, Ferreira et al. 2015, 2016). Traits associating tolerance to stress in *B. excelsa* are not clear, but recent evidences suggest that micronutrients (Fe, Zn and Mn) seem to play important role in the tolerance to stress by high irradiance in *B. excelsa* trees (Kador et al. 2015). Additionally it was verified that liming and phosphorus can benefit photosynthetic capacity of *B. excelsa* saplings, resulting in the increase of growth rates (Corrêa 2013).

Therefore, in this study, we have evaluated seasonally and during two years the effects of thinning, liming and phosphorus fertilization on leaf pigments, chlorophyll *a* fluorescence, gas exchange and specific leaf area (SLA) of *B. excelsa* trees to understand the mechanisms associated to changes in photosynthesis rates of *B. excelsa*.

The hypothesis of this research is that thinning, liming and phosphate fertilization influence the photosynthetic rates due to alteration on leaf pigments content and energy flux in the leaves and that liming and phosphate fertilization will alleviate the effects of stress by increasing the light availability after thinning.

2 MATERIAL AND METHODS

2.1 Site description

The studied *B. excelsa* plantation was located in Itacoatiara, Amazonas, Brazil (3°0'30.63" S, 58°50'1.50" E), which is 120 to 170 m above sea level, a mean annual temperature of 31.2 °C, and annual rainfall of 2539.6 mm (INMET 2018). Two well-defined seasons exist, the rainy season with precipitation of > 300 mm from January to April and the dry season with precipitation of < 100 mm from August to September (Fig. 1). The dominant soil type is Ferrasol. The plantation was established in 2000, on an area of 2.69 ha. Seven months-old seedlings with 15 cm of height were used and the spacing between them was 2.5 x 1.5 m, resulting in a stocking density of 2,666 trees per hectare. Seedlings were not fertilized.

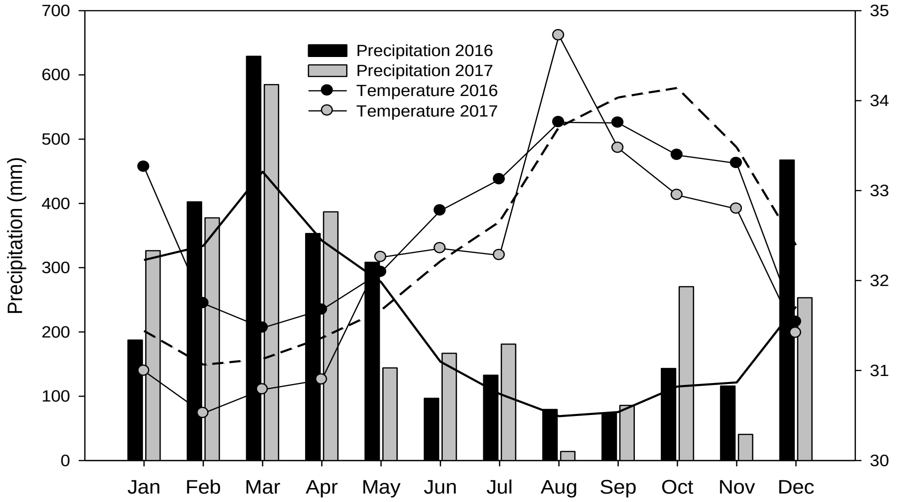


Figure 1. Precipitation and temperature in the study area. The solid and dashed lines represent the historical mean of precipitation and temperature, respectively.

2.2 Experimental design

A randomized complete block design was established in this study. The number of blocks was defined from the variable diameter (measured at 1.3 m aboveground), obtained by a preliminary pilot inventory. We used the sample size for infinite populations equation to determine how many sample units were needed to have a degree of confidence of 95% and 10% of precision. Thus, eight blocks (1,102.5 m^2) were established and in each block the six treatments (Control; Liming; Liming + Phosphorus; Thinning; Thinning + Liming and Thinning + Liming + Phosphorus) were randomly applied. The treatments plots were separated by a buffer zone of approximately 4 m. Thus, the measurements were performed in plot interior of 12.5 m x 7.5 m (93.75 m^2) with 25 trees per plot (1,200 total trees).

2.3 Treatments application

Liming levels were 0 and 2.0 Mg ha^{-1}, applied to the soil in the high precipitation season (14/03 to 01/04/2016), with applications of 824 g of limestone (PRNT 91%) per tree. Phosphorus levels were 0 and 150 kg ha^{-1} of triple superphosphate (46% of P_2O_5), applied sixty days after the liming was assigned, and when the soil pH increased to 5.5, then it was applied more 122 g per tree. The estimation of the necessity of liming was performed based on the methodology proposed by Batista (2014), while the requirement for P_2O_5 was extrapolated from suggestions for *Eucalyptus* ssp. (CFSEMG 1999).

Liming and phosphorus were applied at a distance of 50 cm from the stem base and 20 cm depth from the soil surface, forming a circle around the plant (soil amendment circle). The distance of stem base, depth liming and phosphorus application was defined according to studies about root distribution, which showed that 70% of the total root biomass of adult trees are in 0-20 cm depth from the soil surface and 61% are at a distance of 50 cm from the stem base (Sudmeyer et al. 2004). Thus, all liming and phosphorus applied were within the influence of the root absorption zones.

After the phosphate fertilizer, a low thinning of heavy intensity was performed, where 50% of the basal area was removed from the stand and suppressed, tortuous and forked trees were eliminated. After thinning, all remaining leaves and branches of the trees were maintained in the area. The thinning intensity was defined considering recommendation by Costa (2015).

2.4 Data collected and trees samplings

Data collection was performed between 2016-2017, during peaks of high precipitation (precipitation > 300 mm) and low precipitation (precipitation < 100 mm), as well as in the transition between these seasons (Fig. 2). Due to the impossibility of some measurements being taken from all the trees of the experiment (1,200 trees in total), three sample-trees were selected in each plot for data collection. The criteria for selected trees were representativeness in diameter, height and crown form in each plot.

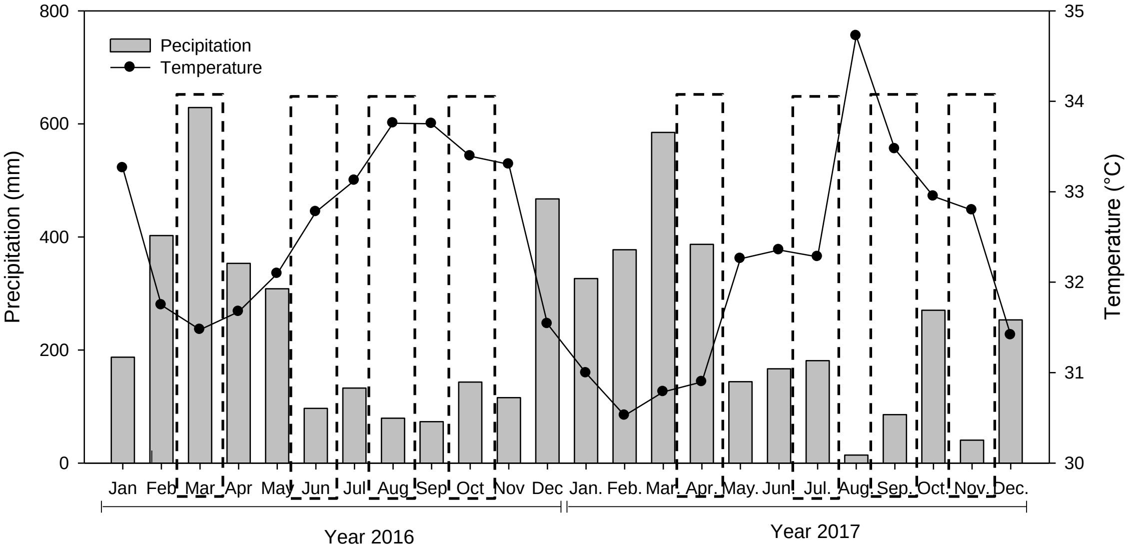


Figure 2. Precipitation and temperature over the period the data was collected in the field (2016-2017). Dashed bars indicate the months in which data were collected.

2.5 Availability of resources: light, water and nutrients

Light availability was monitored from photosynthetically active radiation (PAR). PAR was obtained from a quantum sensor (ULM-500 and MQS-B of Walz) from 6:00 a.m. to 6:00 p.m. in intervals of 2 hours and at 2 m of height from ground level, and in each plot was taken 3 random measurements of PAR over the period in which data collection was carried out in the field (on average of 15 days). Thus, we calculated daily PAR per treatment from mean of PAR values collected throughout the day.

Water and nutrients availability were obtained from soil cores taken from each plot in the same places where liming and phosphorus were applied. Soil cores (50 mm inner diameter) were taken at 0.2 m depth from 3 randomly selected locations and combined to one composite sample. The litter layer was removed before the soil core was collected. The samples were air-dried then it is sieved by 2 mm mesh after the removal of stones and roots by hand.

Thus, soil moisture content was determined by gravimetric method (Black 1965). Total N was determined by the Kjeldahl method (Bremner and Mulvaney 1982). Organic C was determined by the Walkley-Black method (Walkley and Black 1934). Al, Ca and Mg were extracted by 1 M KCl (Silva et al. 1999), while K, Fe, Zn and Mn were extracted by the Mehlich 1 solution (Mehlich 1953). Concentrations of these nutrients were determined by atomic absorption spectrometry (Perkin-Elmer 1100B, Uberlingen, Germany) (Silva et al. 1999). The available P was extracted using the Mehlich 1 solution (Mehlich 1953) and P concentrations were determined by spectrophotometry at 725 nm (Silva et al. 1999). The pH of the soil was potentiometrically measured in the supernatant suspension of a 1:2.5 soil:water and 1:2.5 soil: 1M KCL solution (Silva et al. 1999).

2.6 Nutrients tree

Five healthy and fully expanded leaves were taken in middle third of sample-trees. The leaves samples were dried in an oven at 65 °C for mass constant. The total N was determined by the Kjeldahl method (Bremner and Mulvaney 1982). Organic C was determined by the Walkley-Black method (Walkley and Black 1934). Macronutrients (Ca, Mg, P and K) and micronutrients (Fe, Zn, Cu and Mn) were extracted with a 3:1 nitric-perchloric solution, the concentrations of these nutrients were determined by atomic absorption spectrometry (Miyazawa et al., 1999) and P was determined by spectrophotometry at 725 nm Vitti and Ferreira (1997).

2.7 Chlorophyll

Chlorophyll contents were estimated from samples with 0.1 g of leaves (fresh material) healthy and fully expanded collected between 7:00 a.m. and 9:00 a.m. taken from the middle third of sample-trees. The pigments were extracted with 10 ml of 80% (V/V) acetone and 0.5% (W/V) magnesium carbonate ($MgCO_3$). The suspension was filtered (filter Quanty JP42) and the absorbance was determined using a spectrophotometer (Ultrospec 2100 pro., Armesham Biosciences UV/VIS spectrophotometer) with wavelengths at 663 nm (chlorophyll *a* - Chl*a*), 645 nm (chlorophyll *b* - Chl*b*) and total chlorophyll (Chl_{total}=Chl*a* + Chl*b*) (Arnon 1949). The calculations of the mass (µmol g^{-1}) of chlorophyll pigments were obtained using the equations of Hendry and Price (1993).

2.8 Chlorophyll a fluorescence and JIP-test

Chlorophyll *a* fluorescence induction curve was measured using Handy-PEA fluorimeter (Hansatech Instruments Ltd., UK). The data were collected between 9:00 a.m. and 12:00 p.m. in healthy and fully expanded leaves, that were taken from the middle third of sample-trees. The selected leaves were subjected to a 30-min period of adaptation to darkness. Immediately after the dark-adaptation period, the leaves were exposed to a pulse of saturated light at an intensity of 3000 μmol m^{-2} s^{-1} (peak 650 nm) for 1 s, thus fast fluorescence transients were obtained and were analyzed by the so-called ‘‘JIP test’’ (Strasser and Strasser 1995; Srivastava et al. 1999).

2.9 Photosynthesis and stomatal conductance

Leaf gas exchange was measured between 8:00 a.m. and 12:00 p.m in healthy and fully expanded leaves that were taken from the middle third of sample-trees. The net photosynthetic rate (P_N) and stomatal conductance (*gs*) were measured using a portable open system infrared gas analyzer (LI-6400). The fixed parameters were the CO_2 flux (400 μmol s^{-1}), temperature (31 °C) and water vapor (21 mmol mol^{-1}) (Shimpl et al. 2019).

2.10 Specific leaf area

Five healthy and fully expanded leaves were taken from the middle third of samples-trees and the specific leaf area (SLA) was calculated from the ratio between the leaf area of 30 leaf disks (0.283 cm^2 each) and dry mass dried at 65 °C for 48 h (Ferreira et al. 2009*a*).

2.11 Data analysis

Linear mixed model was performed to evaluate the effects of treatments and seasonality on leaf pigments content, chlorophyll *a* fluorescence, gas exchange and specific leaf area (SLA). Block and plots were included in the models as nested random effects to control for potential spatial autocorrelation. Pearson product-moment correlations were used to assess the influences of Chl and nutrients (mass basis) on chlorophyll a fluorescence. Regression analysis was performed to evaluate the relations of photosynthesis and important variables related to photosynthetic metabolism. All statistical analyses were performed using the R environment.

3 RESULTS

Differences in chlorophyll (F = 9.63, $p < 0.0001$) and chlorophyll a/b ratio (F = 5.97, $p < 0.0001$) between treatments were only observed in the dry season (Fig. 3 A-B). Trees in unthinned plots showed chlorophyll content by 60% higher and chlorophyll *a*/*b* ratio 25% lower than trees of thinned plots regardless of liming or phosphorus fertilization (Fig. 3 A-B). Leaf pigments varied seasonally, it has been found an increased pattern for chlorophyll (F = 5.92, $p < 0.001$) in rainy season followed by a decrease in dry season in the second year, while ratio chlorophyll *a*/*b* (F = 3.34, $p = 0.001$) showed the greater values in dry season (Fig. 3).

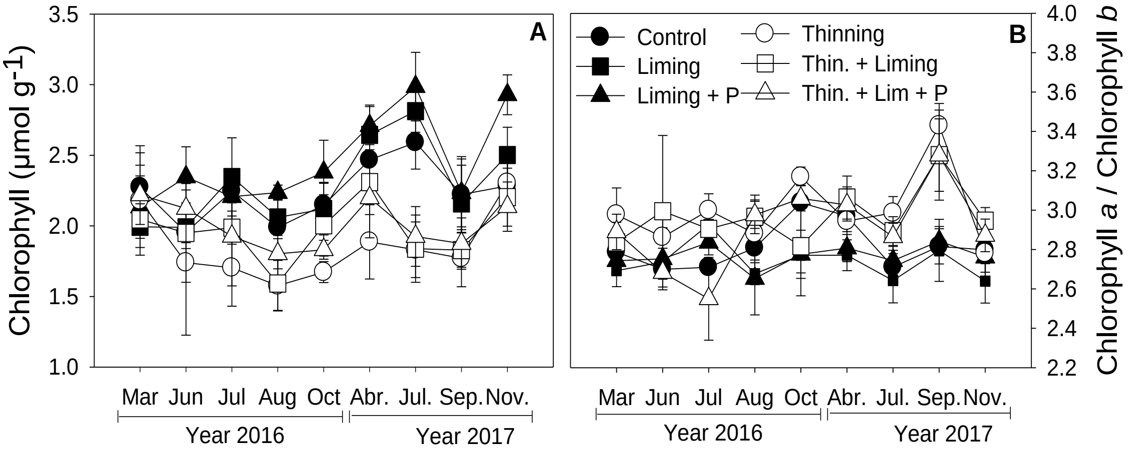


Figure 3. Seasonal content variation of chlorophyll and ratios in leaves of *Berthholetia excelsa* trees under thinning, liming and phosphorus fertilization.

Liming and phosphorus fertilization have not influenced the F_V/F_M ratio and PI_{ABS} of *B. excelsa* trees in unthinned plots (Fig. 4 A-B). However, immediately after thinning, the F_V/F_M and PI_{ABS} have decreased and liming and phosphorus fertilization helped to minimize the stress caused by thinning and accelerated the recuperation of trees (Fig. 4 A-B). After thinning, the reduce in F_V/F_M of trees under thinning + liming were 3% and thinning + liming + phosphorus were 7%, while in trees without liming or P the reduction were of 12% (Fig. 4 A). Seasonality only have influenced trees under thinning without liming or phosphorus fertilization, in these trees we found a decrease in F_V/F_M ratio of, approximately, 0.82 to 0.80 from rainy to dry season (Fig. 4 A).

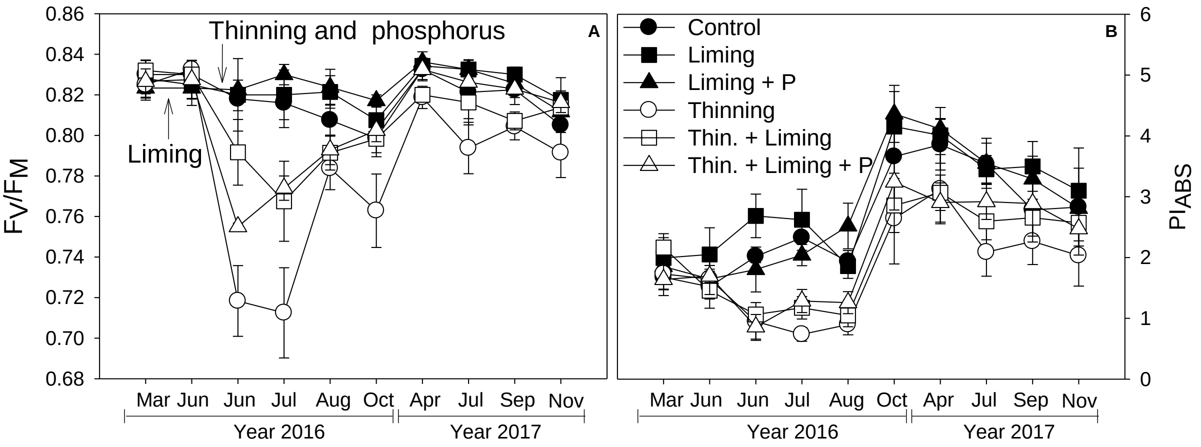


Figure 4. Seasonal variation of performance photochemical of *Bertholletia excelsa* plantations under thinning, liming and phosphorus fertilization. (A) photochemical efficiency PSII and (B) performance index.

To understand the factor associated to stress and recuperation of *B. excels*a after thinning, the OJIP transients were measured during the most severe stress stage (Jun/2016) and after the recovery from stress (Apr/2017). During the most severe stress we observed a decrease of I-P phase in all trees under thinning independent of liming or P, it was also observed an increase in J-I phase, and positive band-K formation only in trees under thinning without liming or P (Fig. 5 A-B). After recuperation from stress, non-visual differences were found between treatments for OJIP transients and not observed the formation of positive band-K for treatments (Fig. 5 C-D).

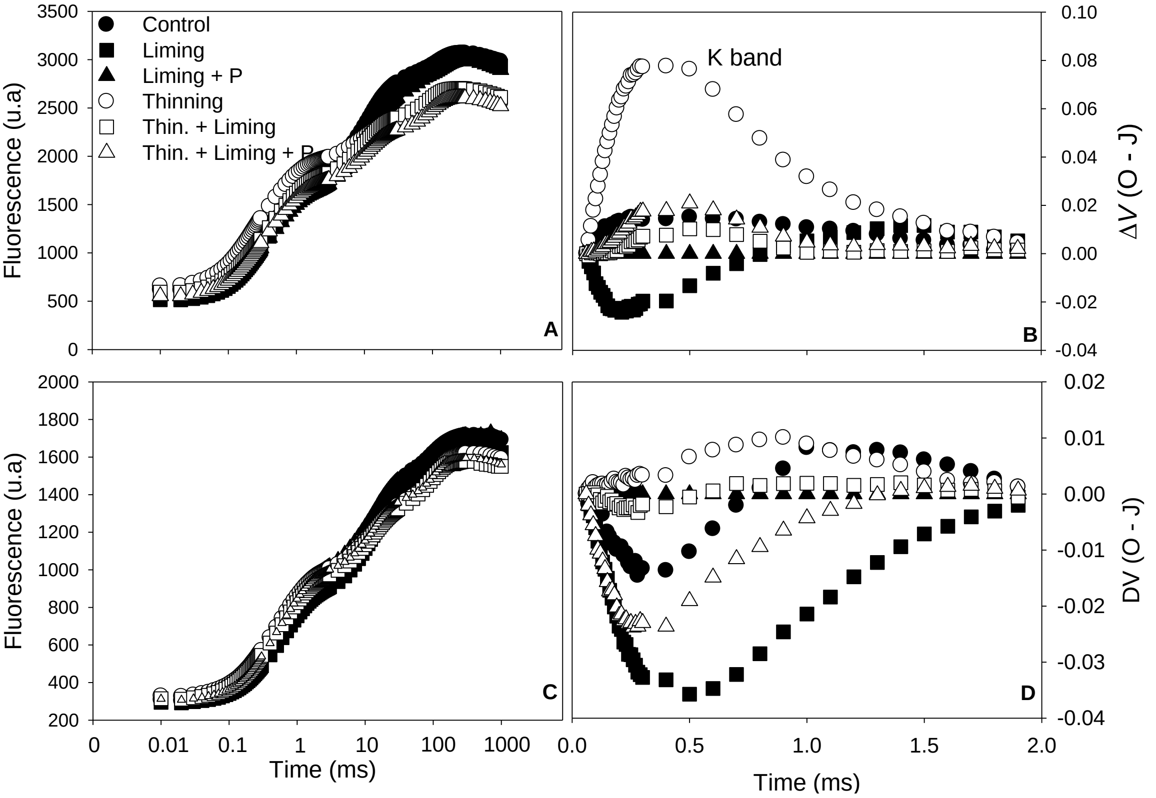


Figure 5. OJIP transient and K-band under stress after thinning performance (A-B) and after recuperation (C-D) of *Bertholletia excelsa* under thinning, liming and phosphorus fertilization.

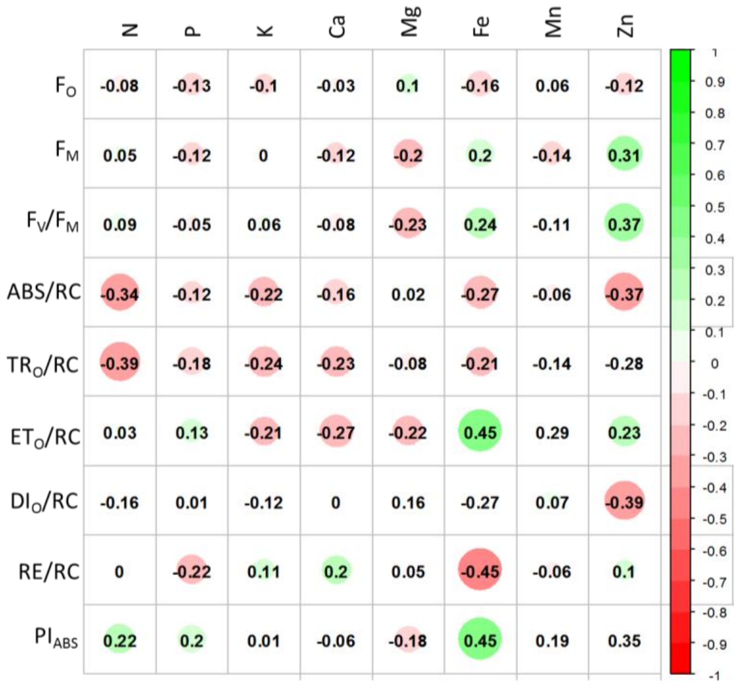
Additionally, we have found that during stress condition, after thinning, have an increase of 140% for no photochemical dissipation (DI_O/RC) in trees without liming or phosphorus and the increase for tree under liming was only 47% and under liming + P was only 79% (Table 1). We have also found that stress condition caused by thinning application increased absorption flux per PSII reaction center (ABS/RC), trapped energy flux per PSII reaction center (TR_O/RC), non-photochemical dissipation per PSII reaction center (DI_O/ABS), but not differences were found for electron transport flux from Q_A to Q_B per PSII (ET_O/RC).

Table 3. Specific flux of energy per reaction center (RC) in leaves of *B. excelsa* plantations under liming, phosphorus and thinning.

Fluorescence Parameter		Liming	Liming+P	Thinning	Thinning+ Liming	Thinning+ Liming +P	R^2	*p*
Fo	Stress	6.45	-0.57	36.56	7.56	10.75	0.22	0.05
	Recupered	-3.47	-1.09	1.46	-5.49	-5.22	0.01	0.50
F_M	Stress	8.11	1.32	-9.13	-4.49	-14.3	0.26	0.03
	Recupered	-1.79	1.16	-5.23	-10.74	-5.21	0.09	0.11
ABS/RC	Stress	-5.16	10.64	39.66	22.74	28.9	0.42	0.00
	Recupered	-6.85	-0.82	1.84	-0.86	-5.51	0.05	0.75
DI_O/RC	Stress	-5.61	9.69	140.65	47.11	78.57	0.38	0.00
	Recupered	-8.86	-2.11	10.97	5.61	-4.22	0.01	0.40
TR_O/RC	Stress	-5.19	10.68	18.29	17.66	18.51	0.38	0.00
	Recupered	-6.45	-0.49	0.33	-1.99	-5.61	0.06	0.79
ET_O/RC	Stress	2.2	4.63	0.95	-2.35	-8.87	0.01	0.76
	Recupered	-9.13	3.17	-8.08	-11.77	-19.77	0.20	0.01

*R^2 = determination coefficient

Investigating the effects of nutrients on photochemical performance of *B. excelsa*, we found positive effects of Fe and Zn micronutrients, in mass basis, on maximum quantum yield (F_V/F_M) and performance index (PI_{ABS}) (Fig. 6). Macronutrients (N, P, K Ca) were not significant correlated to $F_V//F_M$ rates, but N and P have influenced positively PI_{ABS} (Fig. 6).



	N	P	K	Ca	Mg	Fe	Mn	Zn
F_O	-0.08	-0.13	-0.1	-0.03	0.1	-0.16	0.06	-0.12
F_M	0.05	-0.12	0	-0.12	-0.2	0.2	-0.14	0.31
F_V/F_M	0.09	-0.05	0.06	-0.08	-0.23	0.24	-0.11	0.37
ABS/RC	-0.34	-0.12	-0.22	-0.16	0.02	-0.27	-0.06	-0.37
TR_O/RC	-0.39	-0.18	-0.24	-0.23	-0.08	-0.21	-0.14	-0.28
ET_O/RC	0.03	0.13	-0.21	-0.27	-0.22	0.45	0.29	0.23
DI_O/RC	-0.16	0.01	-0.12	0	0.16	-0.27	0.07	-0.39
RE/RC	0	-0.22	0.11	0.2	0.05	-0.45	-0.06	0.1
PI_{ABS}	0.22	0.2	0.01	-0.06	-0.18	0.45	0.19	0.35

Figure 6. Correlations between nutrients in mass basis and specific flux of energy per reaction center (RC) in leaves of *Bertholletia excelsa* plantations under liming, phosphorus and thinning.

The reduction of photochemical efficiency (F_V/F_M and PI_{ABS}) observed after the thinning in remaining trees was not strong enough to reduce the photosynthetic rates of *B. excelsa* (Fig. 7 A). On the contrary, we observed an increase of 13% in P_N immediately after the application of thinning in trees of plots under liming and liming + P, and for trees in thinned plots without liming or liming + P were observed a significant increase in only 50 days after thinning application (Fig. 7 A). The greater values of P_N were accompanied by an increase of approximately 12% in stomatal conductance and a decrease of 30% in specific leaf area (SLA) (Fig. 7 B-C). The P_N and g_s values remained higher in the thinning trees throughout the experimental period, while the SLA remained lower (Fig. 7 A-C).

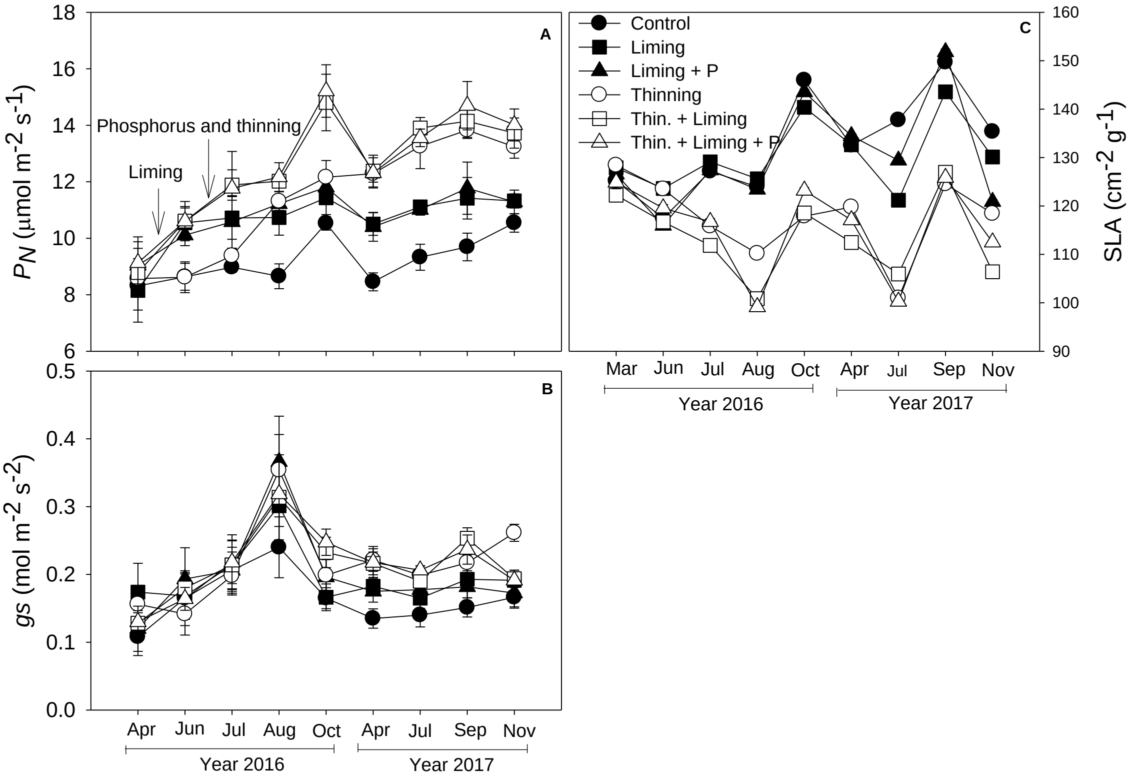


Figure 7. Photosynthesis (A), stomatal conductance (B) and specific leaf area (C) of *Bertholletia excelsa* plantations under thinning, liming and phosphorus fertilization.

Significant relations were found for photosynthesis and stomatal conductance (g_s), specific leaf area (SLA), nitrogen (N) and phosphorus (P) when trees were grouped in unthinned and thinned plots independently of liming and liming + phosphorus treatments (Fig. 8 A-D).

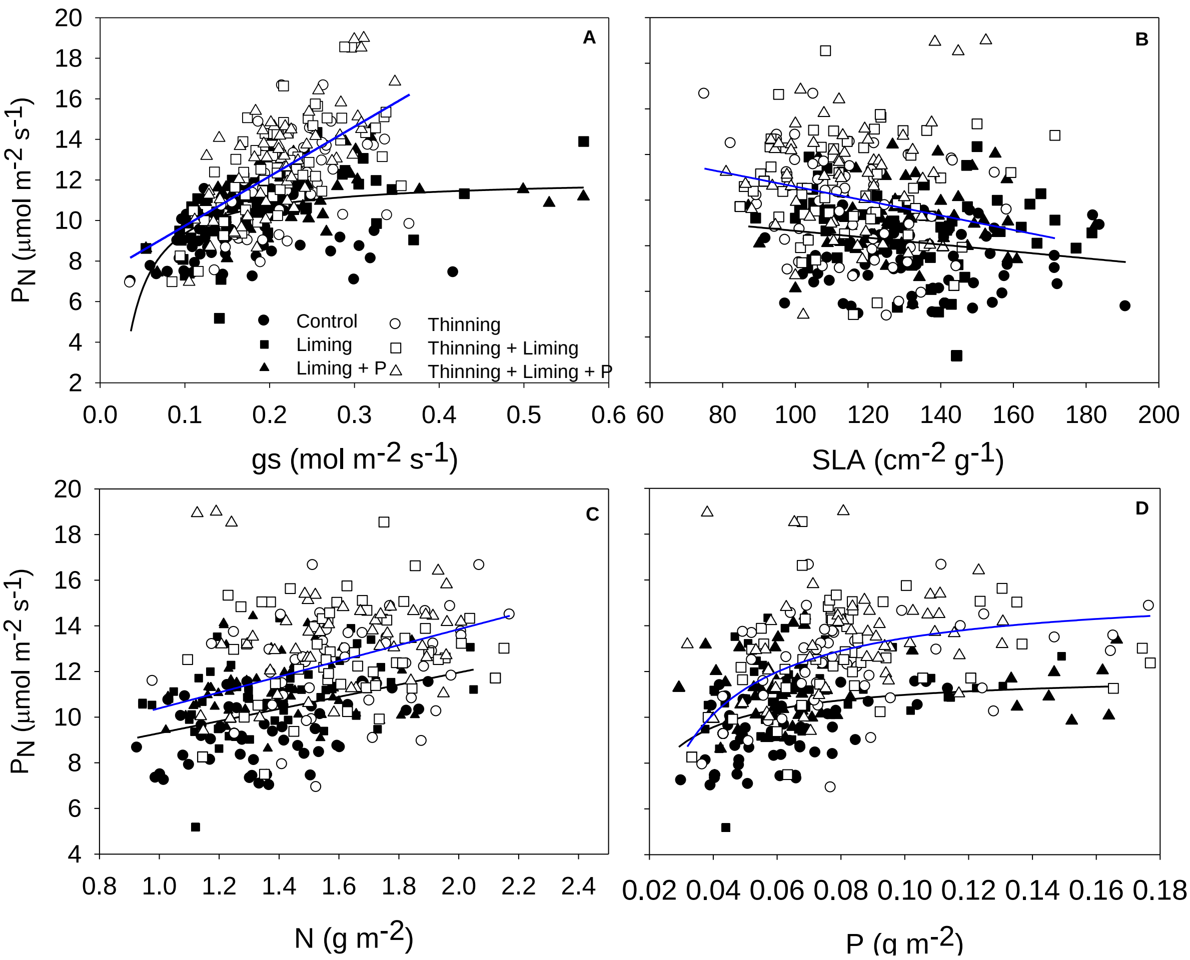


Figure 8. Relations between photosynthesis and stomatal conductance (A), specific leaf area (B), nitrogen (C) and phosphorus (D).

4 DISCUSSION

We have found that thinning influenced Chl and Chl*a*/Ch*b* ratio independently of liming or liming + P, but only during dry season. Differences between thinning and unthinned plots for Chl and Chl*a*/Chl*b* in the dry season, probably occurred due to the sum of the following factors: lowest water availability in soil plus the high incident light on thinned trees. The degradation of chlorophyll under these conditions is due to the formation of reactive oxygen species (ROS) that are responsible for a faster degradation of Chl (Croft and Chen 2017). In addition, the reduction of chlorophyll contents suggests a reduction of light harvesting complex, which may prevent the absorption of excessive light energy and avoid oxidative damages.

Chlorophyll *b* is present mainly in the light harvesting complexes of photosystems I and II, however chlorophyll *a* is present in both the light harvesting complexes and also in the reaction centers of the photosystems. Leaves under high irradiance tend to degrade the light harvesting complexes to avoid damage to the photosynthetic apparatus due to the excess irradiance and tend to increase the number of photosystems II to increase the photosynthetic capacity of the plants (Zivcak et al. 2014). Although the differences in pigment concentration and Chl*a*/Chl*b* ratio occurred only during the dry season, the increase in ABS/RC values went immediately after thinning indicates a substantial amount of the absorbing Chl*a* molecules per active RC. In this case, this response of ABS/RC after thinning may reflect a strategy of *B. excelsa* to increase the use of available energy.

The greater availability of light associated to the increase of ABS/RC and TR_O/RC, have not changed ET_O/RC. The increase in TR_O/RC and the constant in ET_O/RC indicate that the electron transferred from pheophytin (Pheo) to QA (reduction of Q_A to Q^-_A) during photosynthesis in the leaves of *B. excelsa* was increased, while the rates to transport electrons was minimally affected by sudden increase of light availability. The increase in TR_O/RC and maintenance of ET_O/RC in trees of *B. excelsa*, seems to be associated to micronutrients (Fe). Typically, approximately 80% of iron is found in photosynthetic cells where it is essential for the biosynthesis of cytochromes and other heme molecules, including chlorophyll, the electron transport system, and the construction of Fe-S clusters. It is also an active cofactor of many enzymes that are necessary for synthesis of plant hormone (Rout and Sahoo 2015).

The increasing of light availability, after thinning, also led to the boost of non-photochemical energy dissipation (DI_O/RC). The increase of DI_O/RC in plants when transferred from low to high irradiance environment is common and usually represents a mechanism of defense of the photosynthetic apparatus against photoinhibition-damage (Anand Gururani et al. 2015, Derks et al. 2015). Thus, the increase of DI_O/RC after thinning, may be the main factor that led the remaining trees to reduced F_V/F_M ration from 0.83 to 0.72 and the PI_{ABS} from 1.7 to 0.8. This association between DI_O/RC and photochemical efficiency (F_V/F_M and PI_{ABS}) has been demonstrated by other studies (Boisvert et al. 2006, Souza et al. 2010). However, the result of this experiment indicates that phosphorus and liming alleviate the effects of stress by high irradiance after thinning, because the decreasing in F_V/F_M was lowest in trees under liming and liming + P, than trees only under thinning without liming or liming + P.

Pi deficiency renders the light income excessive for their actual capacity to process it, thereby leading to photoinhibition. ATP production is affected under low P availability. The photophosphorylation of ADP is impaired under Pi deprivation due to low availability of Pi in the stroma, therefore causing limited RuBP regeneration. Furthermore, decreased utilization of NADPH and reduced ferredoxin due to decreased activity of the Calvin cycle, together with enhanced acidification of the lumen pH due to restrained ATP synthesis, lead to the accumulation of reduced electron transporters such as plastoquinone or ferredoxin. As a consequence of the feedback inhibition of photosynthesis, Pi deficiency causes chlorophyll loss and decreased of F_V/F_M, which are symptoms of photo-oxidative stress (Hernández and Munné-Bosch 2015).

In addition, we found increasing in J-I phase and positive K-band formation only trees under thinning without liming or P. Increasing in J-I phase and positive K-band formation are considered indicative of stress in plants (Frydenvang et al. 2015, Luo et al. 2016). Changes in J-I phase has been associated with responses of plants under low phosphorus availability, and K-band formation is normally observed in plants subjected to stress by raising the temperature (Sengupta et al. 2013, Carstensen et al. 2018*a*, *b*). However, plants under other factors of stress, such as high irradiance, water and nutrients limitation, are also associated with the shape alteration of J-I phase and positive K-band formation (Cendrero-Mateo et al. 2015).

The increase in J-I phase can occur due to the reduction of ATP synthase and accumulate protons in the thylakoid lumen causing acidification, which can decrease the oxidation of the plastoquinol (PQH2) pool at the cytochrome b6f (Cyt b6f) complex in the electron transport chain. The decreased oxidation will change the flow of electrons towards photosystem I (PSI), which is reflected by the shape of the J-I step (Carstensen et al. 2018*a*, *b*). The presence of the positive K band, in turn, indicates an imbalance between the donation of electrons from the oxygen-evolving complex (OEC) to the oxidized PSII reaction center chlorophyll (P680+) and the re-oxidation of reduced PSII acceptors (QA−). This is usually due to the impaired OEC, which is very sensitive to suboptimal conditions (Strasser 1997).

The reduction of photochemical efficiency (F_V/F_M and PI_{ABS}) observed after the thinning in the remaining trees, was not strong enough to reduce the photosynthetic rates of *B. excelsa*. On the other hand, photosynthesis is increasing these trees. This response has been commonly observed in studies on the ecophysiology of *B. excelsa*

(Ferreira et al. 2009*b*, 2016, Costa et al. 2015). In this study, we suggest that the increase in photosynthetic rates, even with the reduction of F_V/F_M and PI_{ABS}, may be associated with the stability of electron transport flux (ET_O/RC), associating to a boost in stomatal conductance (g_s) and a reduction in specific leaf area (SLA). The boost in gs as a response to thinning treatment, has also been a consequence of increased light availability, which is associated with the rise in temperature, which favors the increase of the pressure deficit. The fact that there are no changes in the water status of the under thinning, allow us to infer that, instead of the availability of water in the soil.

Decreasing in leaf specific area (SLA) of trees under thinning has been associated mainly with higher investment in the mesophyll. It has also been reported that acclimated plants at low irradiance, when exposed to high irradiance, may also increase the thickness of the epidermis (Poorter et al. 2009). Increase in the proportion of the mesophyll results in a higher number of chloroplasts on the basis of the foliar area, which favors the photosynthetic capacity of the plants (Oguchi et al. 2005), while increasing the thickness of the epidermis that protects the leaf against the damage caused by exposure to high irradiance (Poorter et al. 2009).

5 CONCLUSIONS

Although thinning reduces the photochemical efficiency of *B. excess*, this reduction is not strong enough to cause photosynthetic rates to decrease. In addition, the species is able to recover from stress caused by the sudden increase in light availability after thinning, and liming and phosphate fertilization alleviate the effects of high irradiance stress and accelerate tree recovery.

Seasonal responses of physiological traits and growth of *Bertholletia excelsa* subjected thinning, liming and phosphorus fertilization

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Abstract

Tree growth reflects the seasonality of litterfall, roots production and their linkage to the availability of resources and physiological tree traits. This linkage may be altered by silvicultural treatments application. Therefore, with the objective of understand the effects of seasonality on growth and physiological traits of *B. excelsa* trees under silvicultural treatments, we evaluated the physiological traits, litterfall and fine roots production in *B. excelsa* plantations under thinning, liming and phosphorus fertilization. The predawn and midday leaf water potential decreased from rainy to dry season and P addition minimizes the effects of the reduction of water availability on midday leaf water potential. Photosynthesis increased in the dry season and this was attributed to a high stomatal conductance favored by osmotic adjust through accumulation of proline, carbohydrates and potassium in leaves and fine roots. Beside stomatal conductance, variation in $\mathrm{Chl}_a/\mathrm{Chl}_b$ ratio, F_V/F_M ratio, V_{cmax}, J_{max}, light use efficiency and phosphorus also influenced the variations in photosynthesis responses to seasonality, but the mechanisms of regulation for these variables differ between treatments according to the availability of light and phosphorus. In the dry season we found an increase in litterfall, but a decrease in growth and not effects on fine-roots. Thus, seasonality has stronger effects on *B. excelsa* physiological traits and growth, while liming, thinning and phosphorus favors the capturing of resources and the stress tolerance caused by the seasonality.

Key-words: Fine-roots, Litterfall, Photosynthesis, Silvicultural treatments.

1 INTRODUCTION

Bertholletia excelsa is a key species, not only due to its importance in the scenario of the silviculture in Amazonia, but also because of its role in the provision of environmental services and the understanding of processes in an ecosystem scale, since *B. excelsa* plays such a fundamental role, it is occupying the first score among species economically important, in carbon sequestration and for support human livelihoods through Brazil nut seed that sustains the multimillion dollar extractive economy in Bolivia, Brazil and Peru (Vieira et al. 2005, Ferreira et al. 2012, Selaya et al. 2017, Thomas et al. 2018). Therefore, the understanding of the factors associated with the growth of *B. excelsa* contributes not only to information that helps with the definition of management techniques, but also allow us to make inferences about conservation practices and possible impacts of climate change on the Amazon forest.

Tree growth reflects, among other factors, the seasonality of litterfall and fine root production and its linkage to the availability of resources and functional tree traits (Saleska et al. 2003, Wu et al. 2016). In forest plantations this linkage has been little explored, but it is known that in tropical rain forest, during dry season an increase on the photosynthetic capacity occurs, simultaneously to the flush of new leaves and litterfall (Saleska et al. 2003; Wagner et al. 2013). In this season also has been observed peaks of fine-roots biomass that is associated to a boost of the capacity to water uptake. On the other hand, high growth rates have been observed in rainy season and may reflect the dynamic of synthesis and allocation of carbohydrates (Wagner et al. 2013).

The relationship between growth plantations dynamics and functional tree traits can be altered by the application of silvicultural treatments, since the availability of resources may influence the leaf production, carbon assimilation rate and stem growth. Thinning, liming and phosphorus fertilization has been indicated to increase growth rates of plantations due to a rise in the photosynthetic capacity and for promote tolerance to stress as a result of the variation in resource availability, as water and light in sites (Forrester et al. 2012).

During rainy season, for instance, when experiencing limited availability of light, liming and phosphorus fertilization can increase light capture via increment in leaf area, pigments concentrations, light use efficiency. On the other hand, in dry season liming increases the tolerance to lowest water availability reduced by the effects of aluminum toxicity on roots. Phosphorus fertilization increases the capacity of water uptake by boosting root diameter and increasing concentrations of osmolytes in leaves. While thinning reduces the competition for water and increases water use efficiency (D'Amato et al. 2013; Magruder et al. 2013).

Considering the growing interest in understanding the growth dynamics of the most important native commercial tree species in the Amazon, the aim of this study was to investigate the effects of seasonality on availability of resource, physiological tree traits, litterfall, growth and fine-roots production in *B. excelsa* plantations under thinning, liming and phosphorus fertilization to answer the following questions: I) Could seasonality influence physiological traits and growth in *B. excelsa*? II) How liming, thinning and phosphorus fertilization influence the responses of *B. excelsa* to seasonal variation of resources?

2 MATERIAL AND METHODS

2.1 Site description

The studied *B. excelsa* plantation was located in Itacoatiara, Amazonas, Brazil (3°0'30.63" S, 58°50'1.50" E), which is 120 to 170 m above sea level, a mean annual temperature of 31.2 °C, and annual rainfall of 2539.6 mm (INMET 2018). Two well-defined seasons exist, the rainy season with precipitation of > 300 mm from January to April and the dry season with precipitation of < 100 mm from August to September (Fig. 1). The dominant soil type is Ferrasol. The plantation was established in 2000, on an area of 2.69 ha. Seven months-old seedlings with 15 cm of height were used and the spacing between them was 2.5 x 1.5 m, resulting in a stocking density of 2,666 trees per hectare. Seedlings were not fertilized.

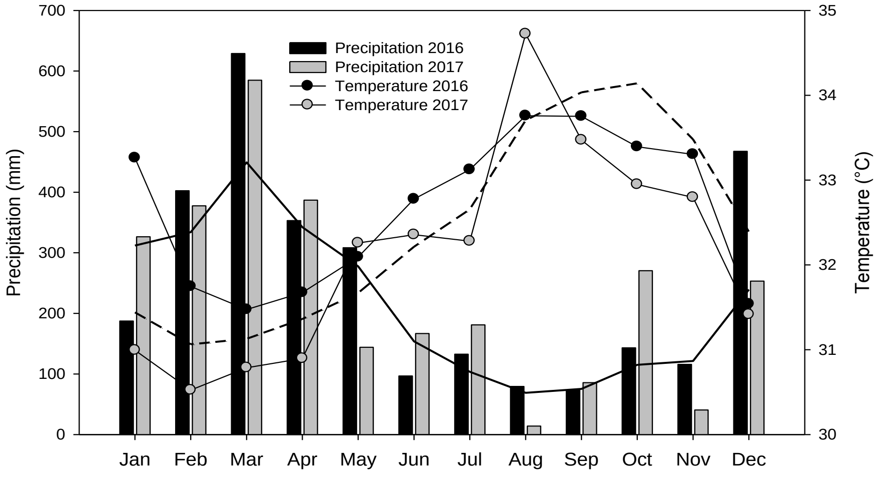

Figure 1. Precipitation and temperature in the study area. The solid and dashed lines represent the historical mean of precipitation and temperature, respectively.

2.2 Experimental design

A randomized complete block design was established in this study. The number of blocks was defined from the variable diameter (measured at 1.3 m aboveground), obtained by a preliminary pilot inventory. We used the sample size for infinite populations equation to determine how many sample units were needed to have a degree of confidence of 95% and 10% of precision. Thus, eight blocks (1,102.5 m^2) were established and in each block the six treatments (Control; Liming; Liming + Phosphorus; Thinning; Thinning + Liming and Thinning + Liming + Phosphorus) were randomly applied. The treatments plots were separated by a buffer zone of approximately 4 m. Thus, the measurements were performed in plot interior of 12.5 m x 7.5 m (93.75 m^2) with 25 trees per plot (1,200 total trees).

2.3 Treatments application

Liming levels were 0 and 2.0 Mg ha^{-1}, applied to the soil in the high precipitation season (14/03 to 01/04/2016), with applications of 824 g of limestone (PRNT 91%) per tree. Phosphorus levels were 0 and 150 kg ha^{-1} of triple superphosphate (46% of P_2O_5), applied sixty days after the liming was assigned, and when the soil pH increased to 5.5, then it was applied more 122 g per tree. The estimation of the necessity of liming was performed based on the methodology proposed by Batista (2014), while the requirement for P_2O_5 was extrapolated from suggestions for *Eucalyptus* ssp. (CFSEMG 1999).

Liming and phosphorus were applied at a distance of 0.5 m from the stem base and 0.2 m depth from the soil surface, forming a circle around the plant (soil amendment circle). The distance of stem base, depth liming and phosphorus application was defined according to studies about root distribution, which showed that 70% of the total root biomass of adult trees are in 0-0.2 m depth from the soil surface and 61% are at a distance of 0.5 m from the stem base (Sudmeyer et al. 2004). Thus, all liming and phosphorus applied were within the influence of the root absorption zones.

After the phosphate fertilizer, a low thinning of heavy intensity was performed, where 50% of the basal area was removed from the stand and suppressed, tortuous and forked trees were eliminated. After thinning, all remaining leaves and branches of the trees were maintained in the area. The thinning intensity was defined considering recommendation by Costa (2015).

2.4 Data collected and trees samplings

Data collection was performed between 2016-2017, during peaks of high precipitation (precipitation > 300 mm) and low precipitation (precipitation < 100 mm), as well as in the transition between these seasons (Fig. 2).

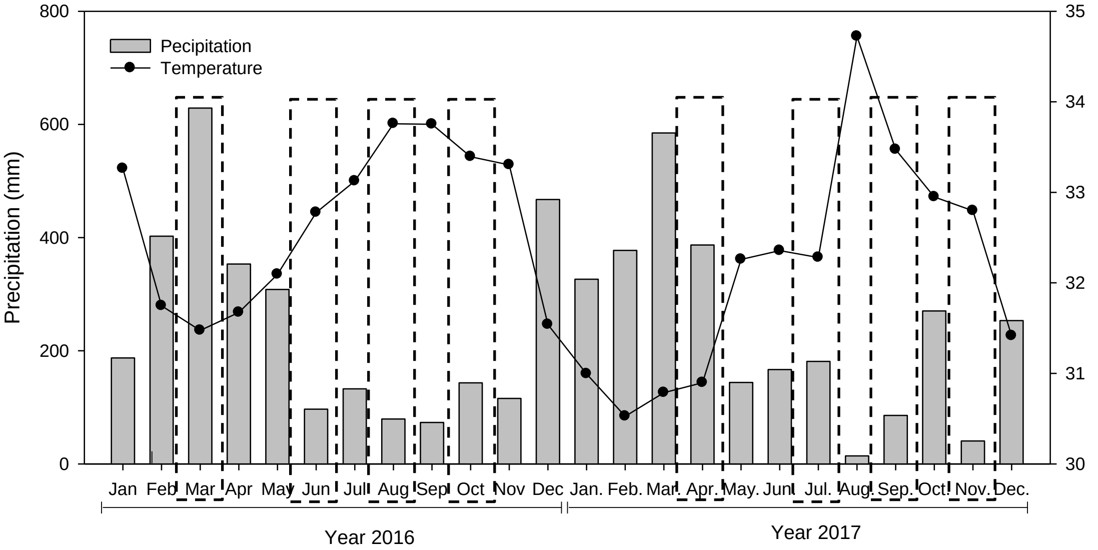

Figure 2. Precipitation and temperature over the period the data was collected in the field (2016-2017). Dashed bars indicate the months in which data were collected.

Due to the impossibility of some measurements being taken from all the trees of the experiment (1,200 trees in total), three sample-trees were selected in each plot for data collection. The criteria for selected trees were representativeness in diameter, height and crown form in each plot.

2.5 Light availability

Light availability was monitored from photosynthetically active radiation (PAR). PAR was obtained from a quantum sensor (ULM-500 and MQS-B of Walz) from 6:00 a.m. to 6:00 p.m. in intervals of 2 hours and at 2 m of height of the level of the ground, in each plot was taken 3 random measures of PAR over the period in which data collection was carried out in the field (on average 15 days). Thus, we calculated daily PAR per treatment.

2.6 Soil P, pH and moisture

Water and nutrients availability were obtained from soil cores taken from each plot in the same places where liming and phosphorus were applied. Soil cores (50 mm inner diameter) were taken at 0.2 m depth from 3 randomly selected locations and combined to one composite sample. The litter layer was removed before the soil core was collected. The samples were air-dried then it is sieved by 2 mm mesh after the removal of stones and roots by hand.

Soil moisture content was determined by gravimetric method (Silva et al. 1999). The available P was extracted using the Mehlich 1 solution (Mehlich 1953) and P concentrations were determined by spectrophotometry at 725 nm (Silva et al. 2009). The pH of the soil was potentiometrically measured in the supernatant suspension of a 1:2.5 soil:water and 1:2.5 soil: 1M KCL solution (Silva et al. 2009).

2.7 Water potential

Leaf water potential was measured at pre-dawn and midday, which typically represent minimum and maximum periods of diurnal water potential respectively. In healthy and fully expanded leaves were taken from the middle third of sample-trees using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA).

2.8 Gas exchange

Leaf gas exchange was measured between 8:00 a.m. and 12:00 p.m in healthy and fully expanded leaves were taken from the middle third of sample-trees. The net photosynthetic rate (P_N), dark respiration (R_d), transpiration rate (E) and stomatal conductance (gs) were measured using a portable open system infrared gas analyzer (LI-6400). The fixed parameters were CO_2 flux (400 µmol s^{-1}), temperature (31 °C) and water vapor (21 mmol mol^{-1}). We have measured photosynthetic responses to irradiance (A/I curves) with a CO_2 concentration of 400 µmol mol^{-1} and 11 photosynthetic photon flux density (PPFD) levels (0, 25, 50, 75, 100, 250, 500, 750, 1000, 1500 and 2000 µmolm^{-2} s^{-1}) in decreasing order. Following Iqbal et al. (1997), the A/I curves were fitted with an exponential model to obtain the light use efficiency (LUE). The photosynthetic responses to CO_2 concentration (A/Ci curves) were determined for a PPFD of 2000 µmolm^{-2} s^{-1} (saturating light) in 13 CO_2 levels (400, 300, 250, 200, 150, 100, 50, 400, 500, 600, 700, 800, 1000 and 1200 µmol mol^{-1}). We used Farquhar's model (Farquhar et al., 1980) to estimate V_{cmax} (µmolm^{-2} s^{-1}) and J_{max} (µmol m^{-2} s^{-1}) parameters utilizing the Sharkey et al. (2007) curve fitting utility. The instantaneous water use efficiency (WUE) and intrinsic water use efficiency (WIUE) were calculated as the ratios between P_N and E and between P_N and g_s, respectively (Nogueira et al., 2004).

2.9 Leaves and fine - roots nutrients

Samples of healthy and fully expanded leaves were taken from the middle third of samples-trees. Samples of fine-roots were obtained from soil cores taken from the same places where liming and phosphorus were applied. Each sample-tree had one soil core (80 mm inner diameter) taken at 0.10 m depth, the litter layer was removed before the soil core was collected. These cores were soaked for at least 12 h in deionized water and sieved through a 20 mesh with a water jet. All fine root (diameter < 2 mm) segments were carefully separated from the soil, cleaned with deionized water and classified as live or dead based on appearance.

The leaves and fine-roots samples were dried in an oven at 65 °C for mass constant. The total N was determined by the Kjeldahl method (Bremner and Mulvaney 1982). Organic C was determined by the Walkley-Black method (Walkley and Black 1934). Ca, P and K were extracted by digestion with 3:1 nitric-perchloric solution, concentrations of these nutrients were determined by atomic absorption spectrometry (Perkin-Elmer 1100B, Uberlingen, Germany) (Miyazawa et al. 1999) and P was determined by spectrophotometry at 725 nm Vitti and Ferreira (1997).

2.10 Proline

The proline content was determined in healthy and fully expanded leaves taken from the middle third of sample-trees by the acid ninhydrin method (Bates 1993). The extraction was performed using the acid solution of ninhydrin (1.25 g of ninhydrin, 30 mL of glacial acetic acid) and 2 mL of glacial acetic acid. The quantification was determined spectrophotometrically at 520 nm using toluene as white. Absorbances were compared to the standard proline curve (0 to 100 $\mu g\ mL^{-1}$).

2.11 Carbohydrates

Samples of healthy and fully expanded leaves were taken from the middle third of samples-trees. Samples of fine-roots were obtained from soil cores taken from the same places where liming and phosphorus were applied. Each sample-tree had one soil core (80 mm inner diameter) taken at 10 cm depth, the litter layer was removed before the soil core was collected. This, cores were soaked for at least 12 h in deionized water and sieved through a 20 mesh with a water jet. All fine root (diameter < 2 mm) segments were carefully separated from the soil, cleaned with deionized water and classified as live or dead based on the appearance.

Soluble sugars were extracted from 0.2 g of ground leaves and fine roots (dry material at 65 °C) with 1 mL of 80% (V/V) ethanol and incubated in a water bath at 75 °C for 30 min, followed by centrifugation (10,000 rpm, 10 °C, 5 minutes). Extraction and centrifugation were repeated four times and thus supernatant and precipitate were dried at 65° C. The residual material supernatant was resuspended in 1 mL deionized water for analysis of total soluble sugars by the phenol-sulfuric method (DuBois et al. 1956). The precipitate was submitted to starch hydrolyzed with 1 mL of 35% (V/V) perchloric acid followed by centrifugation (10,000 rpm, 5 °C, 5 minutes), according to McCready (1950). The absorbance at 490 nm was measured in a spectrophotometer (Ultrospec 2100 pro., Armesham Biosciences UV/VIS spectrophotometer). A standard curve was established with D-glucose.

2.12 Litterfall and fine-roots produtcion

Litterfall were taken monthly in one litter traps (0.25 m^2) that were installed and assigned in the center of each plot at 0.5 m above the ground. The collected litter samples were oven-dried at 65 °C to constant mass (Aragão et al. 2009). Fine-roots biomass were obtained from soil cores taken from the same places that were applied liming and phosphorus. Each sample-tree had one soil core (80 mm inner diameter), taken at 0.10 m depth, the litter layer was removed before the soil core was collected. This, cores were soaked for at least 12 h in deionized water and sieved through a 20 mesh with a water jet. All fine root (diameter < 2 mm) segments were carefully separated from the soil, cleaned with deionized water and classified as live or dead based on the appearance. Thus, were oven-dried at 65 °C to constant mass.

2.13 Monthly tree growth

The growth monthly over period of 2017-2018 was obtained from a dendrometric band fixed to the stem of the samples-trees, the growth in circumference was measured with a digital caliper according to Muller-Landau and Dong (2010).

2.14 Data analysis

Linear mixed model was performed to evaluate the effects of the treatments and seasonality on physiological traits and litterfall, fine-root and growth. Block and plots were included in the models as nested random effects to control for potential spatial autocorrelation. All statistical analyses were performed using the R environment (R Development Core Team 2017), adopting the nlme packages (Bates et al., 2015).

3 RESULTS

The thinning increased the light availability from 51 to 423 µmol m^{-2} s^{-1} and from rainy to dry season the daily PAR in unthinned plots increased by 70%, while in thinning plots the increased was of 200% (Fig. 3 A). On the other hand, the treatments applied did not change the availability of water in soil, but we observed a progressive reduction of soil moisture from rainy to dry season of 24% (Fig. 3 B).

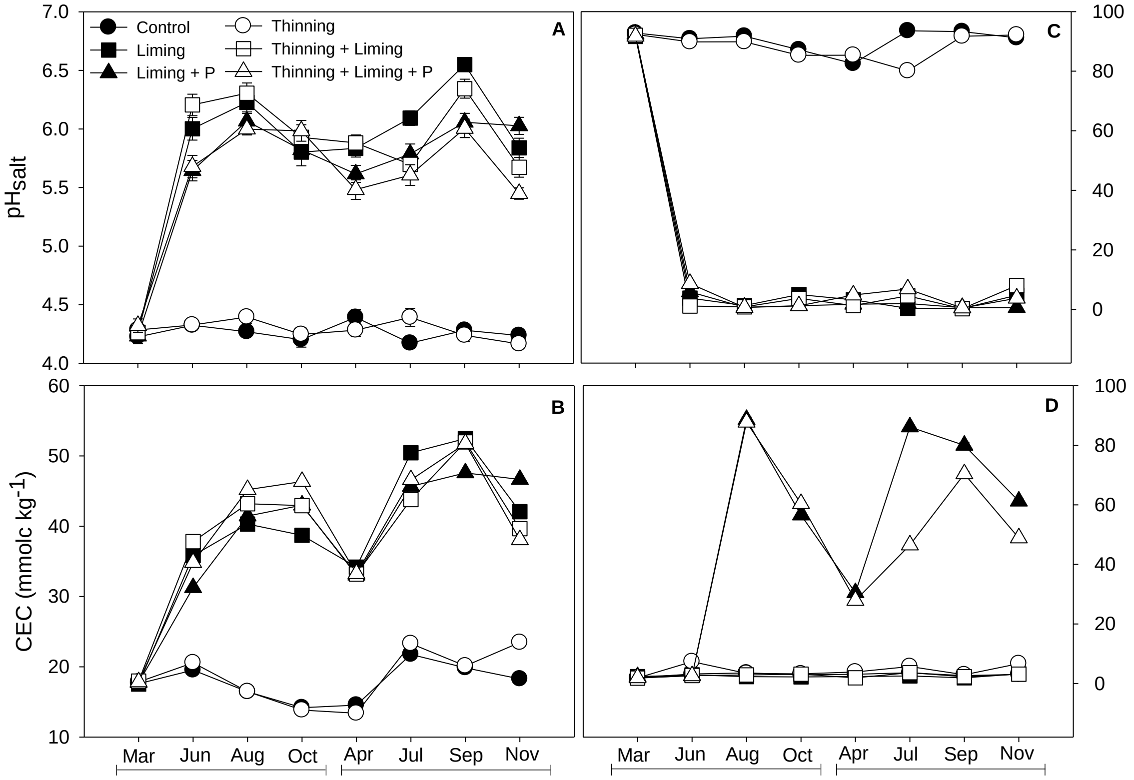

Figure 3. Changes in availability of light (A), water (B) and nutrients (C, D) in *Bertholletia excelsa* plantations under liming, phosphorus fertilization and thinning.

Sixty days after lime application, the soil pH increased from 4.3 to 5.8, and thirty days after phosphorus application the P in soil increased from 2.1 to 88.2 mg kg^{-1} (Fig. 3 C, D). Values of pH and P remained greater in this plots throughout the experimental period (Fig. 3 C, D). Thinning had no influence in the soil chemical attributes (Fig. 3 C, D). On the other hand, the responses of soil to the addition of liming and phosphorus were influenced by seasonality and in the dry season it was observed an increase in pH from 5.5 to 6.5 and in P from 30 to 75 mg kg^{-1} (Fig. 3 C, D).

Thinning, liming and phosphorus fertilization have not changed the predawn leaf water potential of *B. excelsa* (Fig. 4 A), but we have found that trees under phosphorus fertilization showed highest midday water potential in the dry season (Fig. 4 B). The predawn potential decreased from -0.16 in the rainy season to -0.29 MPa in the dry season, while the midday water potential decreased from -0.52 in the rainy season to -1.18 MPa in the dry season (Fig. 4 A-B). However, the reduction in midday water potential in the dry season was the lowest in trees under phosphorus fertilization, where there was a reduction of 145% for other treatments, while in trees from unthinned plots under phosphorus fertilization there was a reduction of 75% and between thinning treatments the reduction of trees under phosphorus fertilization was of 108% (Fig. 4 B).

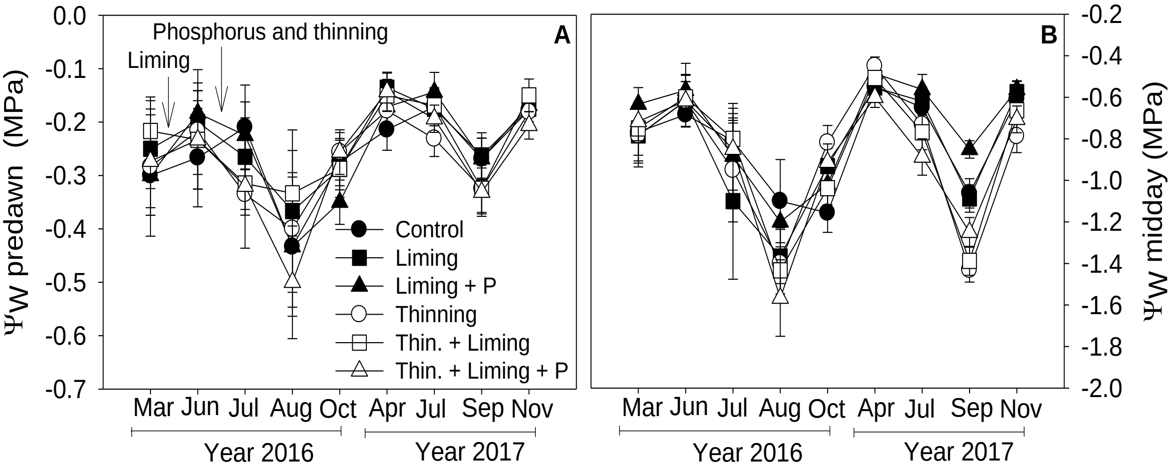

Figure 4. Seasonal variation of predawn (A) and midday (B) leaf water potential of *Bertholletia excelsa* under thinning, liming and phosphorus fertilization

Thirty days after liming application was observed an increase of 21% in P_N, but not in R_d, g_s and E, but from October 2016 (120 days after liming application) we found that values of *Rd*, *gs* and *E* were higher in plots that received liming than control plots. Phosphorus addition resulted in no difference for P_N, R_d, g_s and E in relation to plots only under liming, but the values were always higher than control and at the end of the two years of monitoring, we observed that in these plots the increase in relation to control for P_N, g_s and R_d were 20%, 10% and 60% approximately (Fig. 5 A-D).

The liming and phosphorus addition accelerated the responses of the trees to the thinning, in these plots an increase of 13% in P_N were observed immediately after the application of thinning, on the other hand only 50 days after the thinning performance changes were observed in the P_N of trees without liming or phosphorus. From October

2016 the values of P_N, g_s, R_d and E of trees under thinning reached similar values of trees under thinned + liming and thinning + liming + phosphorus. At the end of two years of monitoring, P_N, g_s and R_d values were 30%, 29% and 77% higher than the control and 21%, 20% and 34% higher than unthinned plots under limning or P (Fig. 5 A-D).

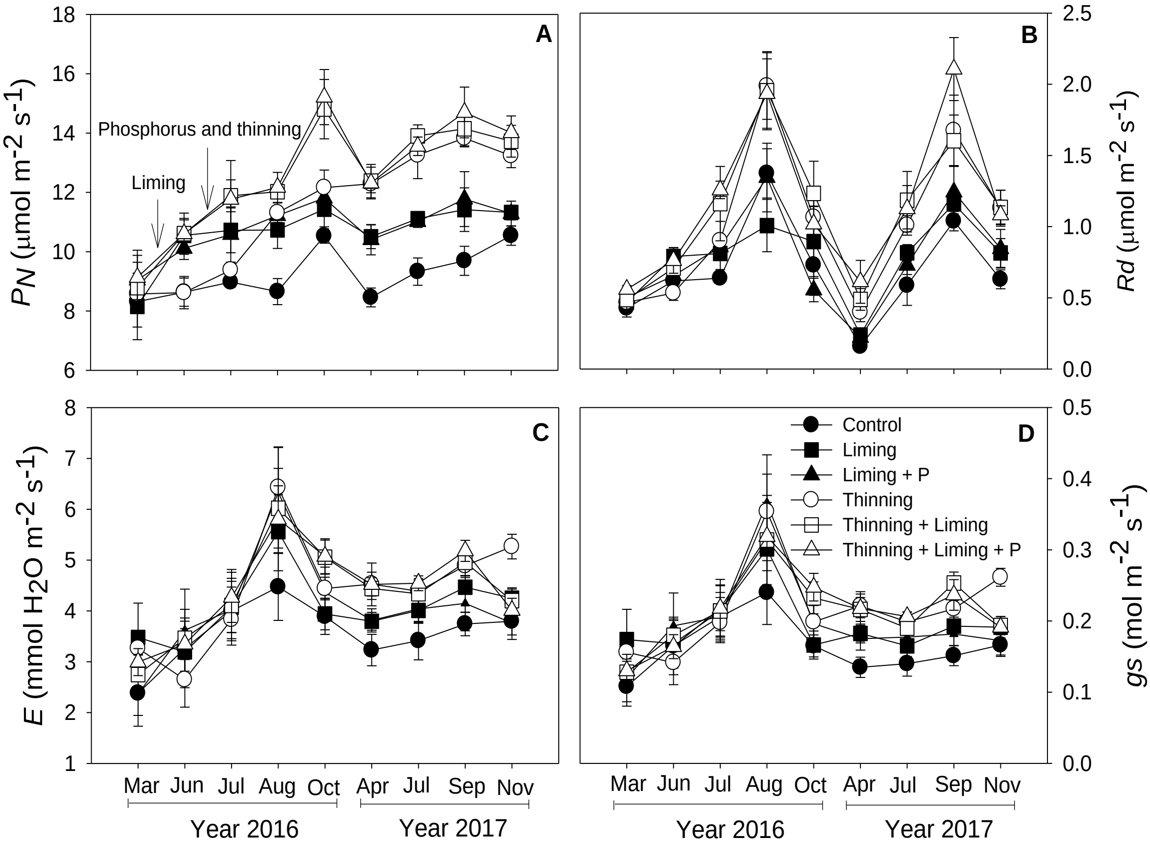

Figure 5. Seasonal variation of photosynthesis (A), dark respiration (B), transpiration (C) and stomatal conductance (D) of *Bertholletia excelsa* plantations under thinning, liming and phosphorus fertilization.

For P_N, g_s, E and R_d independent of silvicultural treatments, higher values were found in the dry season, while lowest values were observed in the rainy season (Fig. 5 A-D). Although gas exchange and leaf water potential were influenced by treatments or seasonality, differences were not observed for water intrinsic use efficiency (WIUE) between treatments (F = 0.79, p = 0.56) or between the seasonal periods (F = 0.01, p = 0.90) and also for water use efficiency (WUE) between treatments (F = 0.34, p = 0.88) and seasonal periods (F = 0.01, p = 0.98) (Table 1).

Table 1. Effects from rainy to dry season on tree traits of *Bertholletia excelsa* under thinning, liming and phosphorus fertilization. The values are percentage of increase or decrease (-) in relation to the rainy season for each treatment.

Tree traits	Effects from rainy to dry season					
	Control	Liming	Liming + P	Thinning	Thinning + Liming	Thinning + Liming + P
Chl*a*/*b*	(2.97) -5.2	(2.77) 0.9	(2.80) 1.2	(2.95) 16.4*	(3.06) 7.0*	(3.02) 8.4*
F_V/F_M	(0.83) -1.2	(0.83) -0.5	(0.84) -1.3	(0.82) -1.6*	(0.82) -1.7*	(0.83) -1.2
V_{cmax}	(57.2) 84.8*	(90.1) 51.7*	(74.6) 60.7*	(101) 21.9	(105) 17.1	(97) 26.6
LUE	(0.03) 55.5*	(0.05) 30.0*	(0.04) 38.5*	(0.04) 16.2	(0.04) 16.3	(0.04) 15.3
J_{max}	(83) 19.4	(101) 21.6	(95) 29.5*	(132) 4.8	(129) 3.4	(119) 23.9*
WUE	(2.7) -2.9	(2.8) -3.4	(2.8) 8.5	(2.7) 5.7	(2.8) 1.6	(2.9) -1.2
WIUE	(62.9) 4.0	(59.5) 6.9	(60.8) 9.1	(55.6) 13.8	(57.5) 3.1	(60.6) 2.5
Proline	(0.04) 86.7*	(0.06) 72.7*	(0.07) 38.1*	(0.07) 64.3*	(0.08) 39.1*	(0.06) 27.6*
P_{Leaf}	(0.06) -6.38	(0.05) 19.6*	(0.05) 19.60*	(0.07) 7.14	(0.07) 25.1*	(0.07) 29.62*
K_{leaf}	(0.3) 8.5	(0.2) 62.5*	(0.2) 51.9*	(0.3) 17.1	(0.2) 65.9*	(0.2) 67.6*
Ca_{leaf}	(0.4) 17.7	(0.5) 13.9	(0.5) 27.5	(0.5) 5.45	(0.7) -33.4	(0.6) -28.7
P_{root}	(0.02) -6.25	(0.01) 93.0*	(0.04) -21.0	(0.02) 39.9	(0.02) 60.0*	(0.04) 10.3
K_{root}	(0.06) -2.12	(0.03) 66.7*	(0.04) 33.3*	(0.05) 34.4	(0.04) 16.12	(0.04) 9.4
Ca_{root}	(0.11) 38.8	(0.41) 53.9	(0.49) 51.1 *	(0.15) 93.0*	(0.48) 34.3	(0.55) 25.6
SLA	(132.5) 12.9	(132.6) 8.2	(134.5) 12.8	(119.8) 3.9	(112.4) 12.9	(117.0) 7.5
SRL	(5.8) -9.6	(5.5) -13.4	(5.1) 17.1	(5.9) -3.8	(4.9) -2.1	(5.6) -16.9
SRA	(12.1) -6.3	(12.8) -11.7	(11.8) 12.9	(12.2) 6.5	(12.4) -2	(13.8) -13.3

Chl_a/Chl_b = ratio between chlorophyll *a* and chorophyll *b*; F_V/F_M = photochemical efficiency of photosystem II; WUE = water use efficiency; WIUE = water intrinsic user efficiency; LUE = Light use efficiency; Vc_{max} = maximum rate of carboxylation; J_{max} = maximum rate of electron transport; SLA = specific leaf area; SRL = specific root length: SRA = specific root area.

Seasonality influenced biochemical traits of *B. excelsa*, but the effects were different between treatments. We found that in the dry season there was an increase in Chl*a*/Chl*b* ratio but only in thinning plots, while there was a decrease of F_V/F_M ratio only in thinning plots that did not receive phosphorus fertilization (Table 1). On the other hand, V_{cmax} only increased in trees of control and liming unthinned plots, while it was verified that J_{max} only increased in plots that received phosphorus, independent of thinning (Table 1).

In the dry season, proline increased in all treatments (Table 1). Liming and liming + phosphorus favored a rise of phosphorus (P) and potassium (K) in leaves and fine roots of trees in the dry season and also, seasonality has no influence in the morphological traits of *B. excelsa*, regardless of treatment (Table 1).

Starch concentrations in leaves was not influenced by treatments (F = 1.18, p = 0.34), but had an increase by around 27% from rainy to dry season (F = 26.91, p <

0.0001). On the other hand, we found an increasing trend in soluble sugar concentration in leaves of trees under thinning (F = 2.70, p = 0.03) and as for starch, soluble sugar there was also an increase from rainy to dry season (F = 46.14, $p < 0.0001$). For fine roots no effects of seasonality were observed in starch (F = 0.57, p = 0.72), but we have also found an increasing trend in soluble sugar in dry season in trees without liming or phosphorus (Fig. 6 A-D)

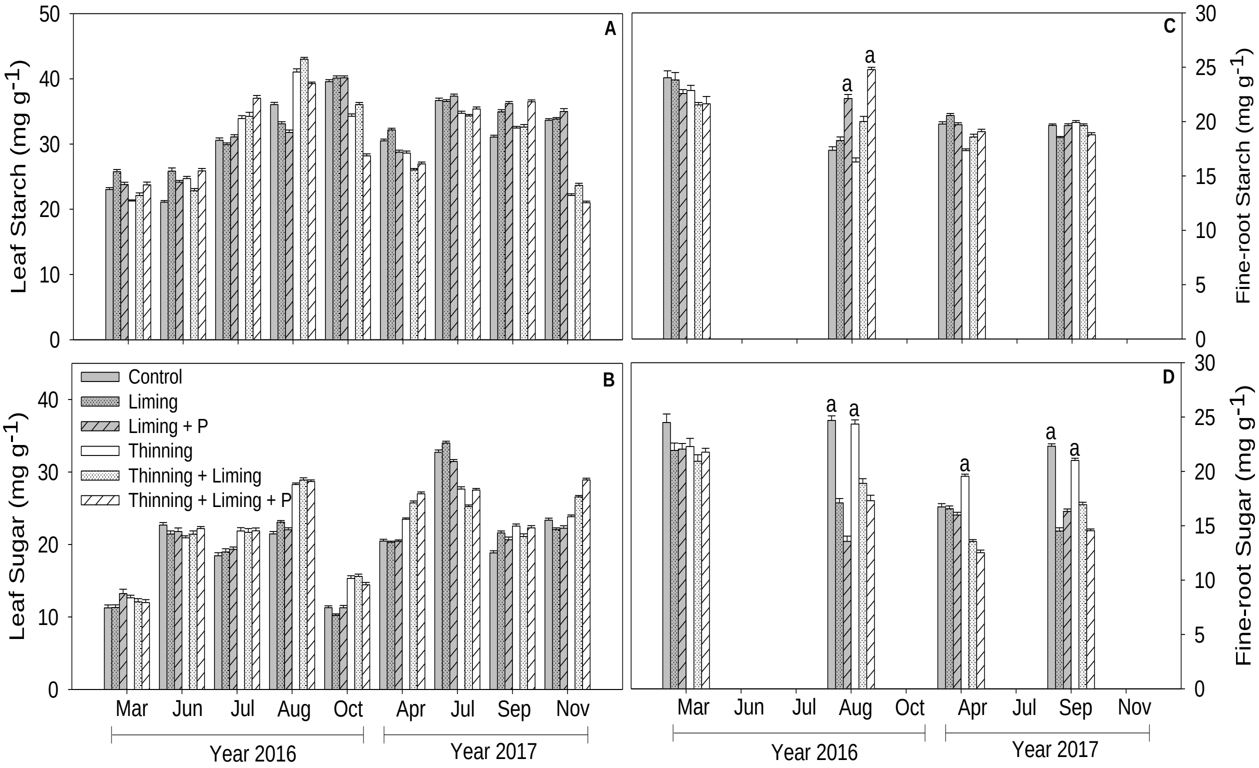

Figure 6. Seasonal variation in starch and soluble sugar in leaves and fine roots of *Bertholletia excelsa* subjected thinning, liming and phosphorus addition.

Although we found treatments effects on the annual growth of *B. excelsa*, we did not find differences between treatments on monthly growth, however, the monthly growth of *B. excelsa* was strongly influencing the seasonality and we found a reduction of 94% from rainy to dry season (Fig. 7 A).

Higher litterfall concentration was found in the dry season (Fig. 7 B). Biomass of fine-roots was greater in plots without liming or P independent of thinning and effects of seasonality were not observed in the production of fine-root (Fig. 7 C).

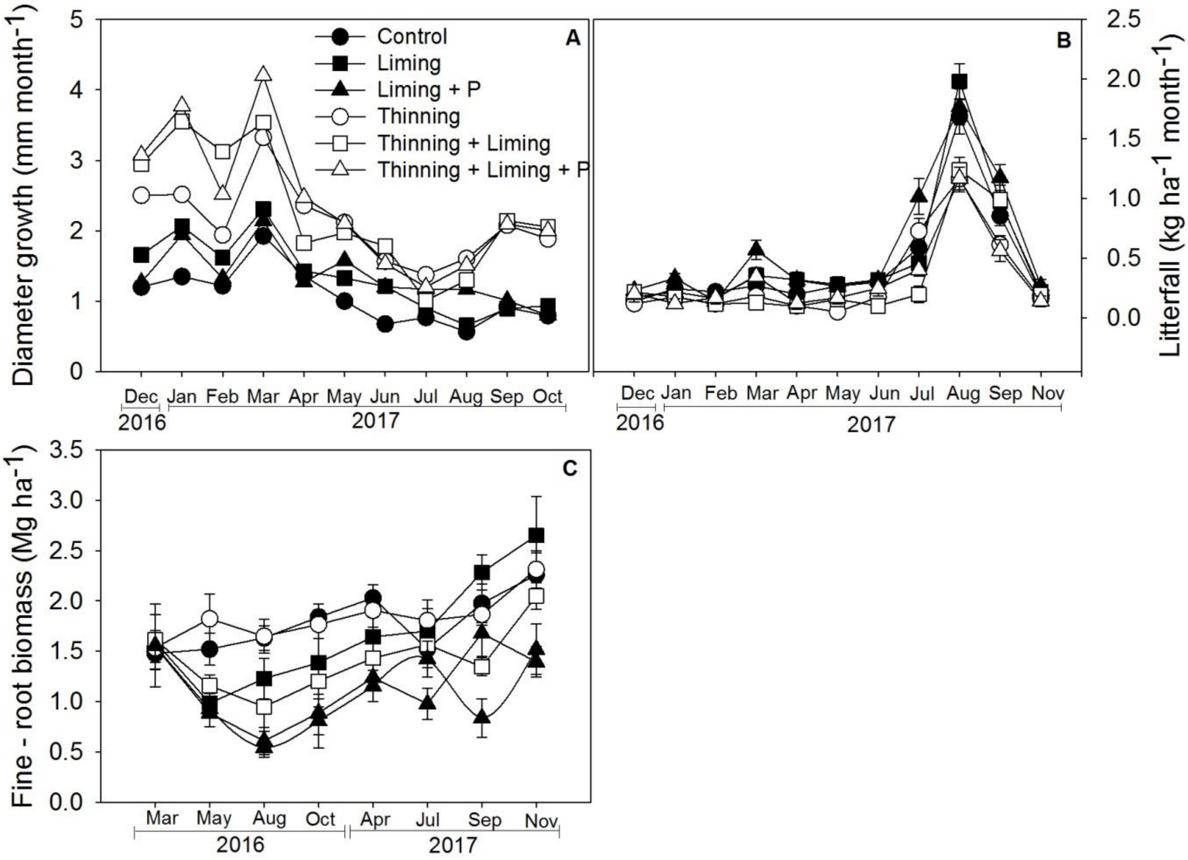

Figure 7. Seasonal variation in litter, wood and fine root production in *Bertholletia excelsa* plantations subjected thinning, liming and phosphorus fertilization.

4 DISCUSSION

The predawn and midday leaf water potential decreased from rainy to dry season, indicating the effects of reduced precipitation and soil moisture on the hydric status of *B. excelsa.* These results corroborate with several studies which demonstrate that seasonal variation of precipitation in the Amazon has effects on hydric status of trees (Domingues et al. 2014, Fontes et al. 2018, Manoli et al. 2018), but are different from the results found by Ferreira et al. (2016), where, studying clonal plantations of *B. excelsa* in the same area of this study, found no indications of the effects of seasonality on leaf water potential. A possible hypothesis for the difference between these results can be associated to the density of the stand. In this study the plantation have 2,600 trees ha^{-1}, while in the plantations studied by Ferreira et al. (2016) have 100 trees ha^{-1}, higher density of trees in a stand increases the competition for water and reduce water availability of trees (Bello et al. 2019, Navarro-Cerrillo et al. 2019).

Although no differences were observed in predawn leaf water potential between treatments, we observed that phosphorus addition minimizes the effects on the reduction of water availability in midday leaf water potential of *B. excelsa* in the dry season. The effects of phosphorus fertilization only in midday leaf water could be a result of the higher intensity of dry season on this variable due to the sum of factor that includes the low water soil, high evaporative demand, that normally occur in the period of day, and also high values of water loss by transpirations. Additionally, several studies have demonstrating that phosphorus fertilization alleviation the drought stress in plants (Basu et al. 2016, Wu et al. 2018*b*).

The main mechanisms that explain the role of P in water-stress tolerance are the rise in fine-root biomass, accumulate of osmolytes, like proline and carbohydrates, and also the increase in water use efficiency (Köhler et al. 2016, Wu et al. 2018*b*). In this work we found that *B. excelsa*, independently of treatment from rainy to dry season, increased proline and carbohydrates in leaves, though not changing water use efficiency nor SLA and SRA. Therefore, is difficult to point out a factor that explain the unique phosphorus responses found in this study, but has been discovered that trees fertilized with phosphorus have higher hydraulic efficiency due to the high porosity of the xylem tissue (Faustino et al. 2013, Goldstein and Bucci 2013) which exhibit a more efficient water transport system. Recent evidence also suggest that phosphorus can also improve embolism resistance in tropical trees as a response to the role of this nutrient in providing energy to invest in water transporting structures and maintain metabolic processes (Oliveira et al. 2019).

Despite the dry season have negative impacts on status hydric of *B. excelsa*, the effects were not sufficiently strong for that to cause the reduction of photosynthesis, on the contrary, we found that photosynthesis has increased in the dry season. The increasing in photosynthesis in the dry season can be attributed to an increase in stomatal conductance that occurred in this period in all treatments and also to changes in biochemical traits.

The high values of stomatal conductance, even with the decrease in leaf water potential in dry season, can be associated to accumulation of osmoregulators. We found that in the dry season, tree of *B. excelsa* increased concentrations of proline and carbohydrates in all treatments, while K only boosted in trees under liming and phosphorus fertilization. Proline, carbohydrates and K are common osmotic adjustment mechanisms under water stress (Jinyou et al. 2004, Akinci and Lösel 2006). The main physiological role of accumulated osmolytes in drought resistance is decreasing osmotic potential of cell, thus maintaining hydration of protoplasm (Thakvr and Rai 1985). In addition to the regulation function of osmotic potential, the osmolytes also play another role in drought stress in plants. Proline, for instance, is a metal chelator, an antioxidative defense molecule and a signaling molecule. Proline also contributes to stabilizing subcellular structures (e.g. membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions (Hayat et al. 2012). A part of their role in osmotic adjustment, soluble sugars also function in many other aspects such as maintaining stabilization of protein (Jinyou et al. 2004).

Potassium is considered a vital regulator of plant responses and tolerance to abiotic stresses and is not only a constituent of the plant structure but it also has a regulatory function in several biochemical processes related to protein synthesis, carbohydrate metabolism, and enzyme activation. K regulates stomatal opening and helps plants adapt to water deficits. In the presence of K, stomatal guard cells are swollen by absorbing water followed by stomatal opening and the allowance of gaseous movement in between plants and the environment. In addition to these effects, the osmotic gradient produced due to the accumulation of K in the roots helps to draw water into the root cells (Hasanuzzaman et al. 2018).

The increasing of K in leaves and fine-roots can occur only in plants under liming and liming + P due to the high values of Ca in fine roots. Ca plays an important role in ionic absorption, the Ca particularly in correcting the unfavorable effect of excessive hydrogen ion concentration, and it is essential, for the effect of not diminish the absorption of nutrients, since it is indispensable for the maintenance of the structure of the cellular membranes and the ATPases of the membrane, that participate in ionic absorption, are activities for Ca (White and Broadley forthcoming).

Regarding biochemical traits, we found that trees of unthinned plots, independent of liming or P from rainy to dry season, maintained F_V/F_M and increased V_{cmax} and LUE, while only tree under phosphorus fertilization increased J_{max}. These results indicate that trees are co-limited by light and phosphorus availability and that the increase in light availability favors Vc_{max}, while phosphorus benefits J_{max}. Light can favor Vc_{max} to stimulate the rubisco activity and increase the carboxylation rates (Bota et al. 2004). While phosphorus can favor J_{max} because of the many transformations of phosphorus-rich molecules (ATP, NADP and sugar-phosphates from the Calvin cycle) that occur for the regeneration of ribulose-1, 5-bisphosphate (RuBP). In thinned plot, on the other hand, seasonal variation can be most associated to change in stomatal conductance, although it is also being limited by phosphorus.

In relation to F_V/F_M, studies in the Amazon forest indicate a reduction in the dry season, with response to stress caused by the increase in irradiance and temperature, the maintaining of F_V/F_M values demonstrate high tolerance of *B. excelsa* support to variation in availability of water and irradiance that were showed in some studies (Morais et al. 2007*b*, Ferreira 2009, Ferreira et al. 2015, 2016).

Liming and phosphorus fertilization accelerated plant responses to thinning, but did not potentiated the photosynthetic rates, since we observed that the thinning plants that received liming or phosphate fertilization presented higher initial gains, but from the first leaf flush to photosynthetic capacity of these trees, they equaled. These responses together with the largest photosynthetic stacks of the trees only with thinning in relation to the ones that were not thinned but that received liming and phosphate fertilization, indicated a greater limitation by light than by phosphorus.

The high carbohydrate concentrations in the dry season also has been observed for other trees from the tropical forest and may reflect both the higher photosynthetic rates during this period and changes in carbohydrate allocation associated to metabolism and osmotic requirements (Würth et al. 2005). In this study we presumed that the increase in carbohydrates are most associated to high allocation in leaves for the maintenance of cellular turgor. On the other hand, high rates of growth in the rainy season can be associated to greater allocation of carbohydrates in the stem (Würth et al. 2005).

The decreasing in stem growth rates from rainy to dry season were also observed in some works in tropical forest and different hypotheses have been established to explain this behavior. Some studies considered that the increment in the stem growth in rainy season did not represent secondary growth, but only reflected the turgidity of the cells due to the high availability of water, while others suggest that the stem growth in rainy season occurs with resulted the greater allocation of carbohydrates in stem during this season (Wagner et al. 2013, Rowland et al. 2014, Girardin et al. 2016).

Results found in this work suggest that higher growth rates of the stem during the rainy season represents biomass gain, since we observed a reduction of nutrient contents in the soil during this season, and this has also been associated with the tree growth and carbohydrate reduction during the rainy season with a subsequent increase during drought in leaves and fine roots, suggesting that during the dry season the plant invests in carbohydrates for the production of new leaves as it has been verified in tropical forests and also for the production of roots that aid the capture of water and nutrients. In addition, we observed higher production of litter during the dry season, which is indicative of high level of leaf production.

5 CONCLUSION

Liming, thinning and phosphorus fertilization favors the capture of resources and the stress tolerance caused by the seasonal variation in resource availability. Phosphorus addition minimizes the effects of the reduction of water availability on midday leaf water potential. Photosynthesis increases in the dry season and this increase can be attributed to a high stomatal conductance favored by osmotic adjust through accumulation of proline, carbohydrates and potassium. Beside stomatal conductance, variation in Chl_a/Chl_b ratio, F_V/F_M ratio, V_{cmax}, J_{max}, LUE and P_a also influenced the variations in photosynthesis responses to seasonality. In the dry season we found an increase in carbohydrates in leaves, fine roots and litterfall, but a decrease in growth. On the other hand, fine root production is not influenced by seasonality, but was higher in trees without liming or P.

GENERAL CONCLUSION

The treatments of thinning, liming and phosphate fertilization influenced the growth of the trees by different mechanisms: The thinning increased the light availability and favored the efficiency in the use of light and phosphorus promoted by changes in the characteristics above-ground. The liming increased the pH, the availability of Ca and Mg and favored the efficiency in the use of light and P promoted by changes below and above-ground and phosphate fertilization increased the availability of Ca and Mg, favored light efficiency and water status promoted by changes in the characteristics below and above-ground. Although soil conditions (pH and P availability) have effect on the growth of *B. excelsa*, light is the most limiting factor, and thinning is indicated to increase the yield of the plantations formed by this species.

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