RESPONSES OF NET PRIMARY PRODUCTION AND PLANT-AVAILABLE SOIL NUTRIENTS TO ELEVATED NUTRIENT INPUT IN A TROPICAL FOREST ON HIGHLY WEATHERED SOIL

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“In questions of science, the authority of a thousand is not worth the humble reasoning of a single individual.”

- Galileo Galilei (1564–1642) -

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Summary

Globally, tropical forests are highly productive ecosystems and play a critical role in sequestering anthropogenic carbon dioxide (CO_2) from the atmosphere, accounting for up to half of the terrestrial biosphere's carbon sink. For reasons not yet fully reconciled, there are indications that the carbon sink strength of these forests is slowly declining, thereby decreasing the buffering capacity that these forests offer in mitigating global climate change. It is recognized that ecosystem nutrient limitations play an important regulatory role in plant growth, therein affecting ecosystem carbon assimilation and specifically net primary production (NPP). Furthermore, the direction and magnitude of these limitations are poorly understood, especially in understudied African tropical forests, on highly weathered soils. This dissertation consists of three studies (Chapters 2−4) aimed at elucidating the mechanistic roles of nitrogen (N), phosphorus (P), potassium (K) and their interactions on different components of NPP (tree stem growth, fine litter production, foliar chemistry, and fine root production) and plant-available soil nutrients. Following a full factorial design, we established 32 (eight treatments × four replicates) experimental plots of 40 m × 40 m each in a semi-deciduous tropical forest in northwestern Uganda. We added N, P, K, their combinations (N+P, N+K, P+K, and N+P+K) and control at the rates of 125 kg N ha^{-1} yr^{-1}, 50 kg P ha^{-1} yr^{-1} and 50 kg K ha^{-1} yr^{-1}, split into four equal applications.

In our first study, we measured tree growth responses among different tree sizes, taxonomic species, leaf habits, and at the community level to nutrient additions. After two years, the response of tree stem growth to nutrient additions was dependent on tree sizes, species, and leaf habit but not community-wide. First, tree stem growth increased under N additions, primarily among medium-sized trees (10−30 cm DBH) and in trees of *Lasiodiscus mildbraedii* in the second year of the experiment. Second, K limitation was evident in semi-deciduous trees, which increased stem growth by 46 % in +K than –K treatments, following a strong, prolonged dry season during the first year of the experiment. This highlights the key role of K in stomatal regulation and maintenance of water balance in trees, particularly under water-stressed conditions. Third, the role of P in promoting tree growth and carbon accumulation rates in this forest on highly weathered soils was rather not pronounced. Our results underscore the fact that, in a highly diverse forest ecosystem, multiple nutrients and not one single nutrient regulate tree growth and aboveground carbon uptake due to varying nutrient requirements and acquisition strategies of different tree sizes, species and leaf habits.

For our second study, we assessed the effect of the nutrient additions on fine litter production and foliar quality. To do this, we placed four-leaf litter collectors (0.75 m × 0.75 m in size) at random locations in each experimental plot, emptied them for dry mass determination every two weeks for three consecutive years (May 2018−April 2021). Our data suggest that: (1) Although annual fine litter production was not significantly affected by nutrient additions in the short-term (3 years), an observed trend towards higher annual fine-litter production in the N addition plots may become stronger with continued nutrient additions. (2) Following a prolonged dry season in the first year of the experiment, leaf litterfall reduced significantly with P and K additions. This observed effects of K in leaf litterfall corroborate the increased stem growth among semi-deciduous trees in our first study and highlights the roles of K as well as P in maintaining water balance in trees, thereby ensuring stress tolerance during water-deficit conditions. (3) Both leaf litter and foliar nutrient contents were affected by the elevated availability of all three nutrients in both positive and negative directions but varied considerably among different tree species. Even though the long-term effects of nutrient perturbation on this ecosystem are yet to be known, the concept of multiple nutrients rather than a single nutrient regulation of litter production and foliar quality was supported in this second study.

Our third study evaluated the effects of nutrient additions on below-ground processes including fine root biomass production and plant available soil nutrients. First, we quantified fine root biomass (0−10 cm soil depth) at the end of the first and second years of the experiment by excavating soil monoliths (20 cm × 20 cm) at six random locations within each plot. Next, fine root production in the top 30 cm soil depth was estimated using the sequential coring technique. We found that the addition of N reduced fine root biomass by 35% after the first year of the experiment whereas K addition was associated with reduced fine root production, suggestive of an alleviated N and K limitation in this site as found in our first study. This rapid reduction in fine root biomass in the N and K treatments supports the idea that trees will scale back their energy-intensive root network or production when they have adequate resources available. Additionally, nutrient additions resulted in a cascade of biochemical responses in the soil nutrient availability. Specifically, (1) Net N mineralization and nitrification rates were enhanced by the interaction effects of all three nutrients (N × P × K), highlighting the complementary roles of these nutrients in regulating plant and soil processes in this species-rich ecosystem. (2) Microbial biomass C increased with P additions but was dependent on the season (Wet or dry). Lastly, P additions increased plant-available P by 80%. This large increase indicates high P availability and explains the lack of plant growth response to P additions (as shown in our first study).

Overall, this dissertation provides credence to the concept of multiple nutrients (co)regulation of NPP and other ecosystem processes; further substantiating the growing pool of evidence that productivity in tropical forests does not follow Liebig's Law of the Minimum. Instead, resource and nutrient requirements (and their limitations) vary in different ecological processes or components of NPP in this forest, ranging from tree growth (N and K), leaf litterfall (P and K), root biomass, and production (N and K). These observations are indeed consistent with the multiple resource limitation theory. Considering that most large-scale experimental research to date has focused only on the roles of N and P availability in limiting plant productivity, our data show that other nutrients, specifically K, can be equally important in the functional and biochemical roles related to ecosystem carbon uptake. More such research is undoubtedly needed particularly for the African tropical region, which is the least researched worldwide.

Zusammenfassung

Tropische Wälder sind weltweit hochproduktive Ökosysteme. Sie spielen eine entscheidende Rolle bei der Bindung von anthropogenem Kohlenstoffdioxid (CO_2) aus der Atmosphäre und machen bis zur Hälfte der Kohlenstoffsenke der terrestrischen Biosphäre aus. Es gibt Anzeichen dafür, dass diese Kohlenstoffsenkenfunktion langsam abnimmt und damit die Pufferkapazität, die diese Wälder zur Abschwächung des Klimawandels beitragen. Es ist bekannt, dass Nährstofflimitierungen in Ökosystemen eine wichtige Rolle bei der Regulierung des Pflanzenwachstums spielen und damit die Kohlenstoffassimilation im Ökosystem, insbesondere die Nettoprimärproduktion (NPP) beeinflussen. Die Richtung und das Ausmaß dieser Limitierungen sind nur unzureichend bekannt, speziell in den wenig untersuchten afrikanischen Tropenwäldern auf stark verwitterten Böden. Diese Dissertation besteht aus drei Studien (Kapitel 2-4), die darauf abzielen, die mechanistische Rolle von Stickstoff (N), Phosphor (P), Kalium (K) und deren Wechselwirkungen auf verschiedene Komponenten der Nettoprimärproduktion (Stammwachstum, Produktion von Feinstreu, Blattchemie und Feinwurzelproduktion) und pflanzenverfügbare Bodennährstoffe zu klären. Nach einem vollfaktoriellen Versuchsplan legten wir 32 (acht Behandlungen × vier Wiederholungen) Versuchsparzellen von je 40 m × 40 m in einem halb-laubabwerfend tropischen Wald im Nordwesten Ugandas an. Wir fügten N, P, K und ihre Kombinationen (N+P, N+K, P+K und N+P+K) sowie die Kontrolle in den Mengen von 125 kg N ha^{-1} a^{-1}, 50 kg P ha^{-1} a^{-1} und 50 kg K ha^{-1} a^{-1} zu, aufgeteilt in vier gleiche Anwendungen.

In unserer ersten Studie untersuchen wir die Reaktion des Baumwachstums auf Nährstoffzugaben bei verschiedenen Baumgrößen, taxonomischen Arten, Blattwuchsformen und auf Bestandesebene. Nach zwei Jahren war die Reaktion des Stammwachstums auf die Nährstoffzugabe abhängig von der Baumgröße, -art und der Blattwuchsstellung, jedoch nicht auf der Bestandesebene. (1) Das Stammwachstum nahm unter N-Zugabe zu, vor allem bei mittelgroßen Bäumen (10−30 cm DBH) und bei Bäumen von *Lasiodiscus mildbraedii* im zweiten Jahr des Experiments. (2) Es zeigte sich eine K-Limitierung bei Laubbäumen, deren Stammwachstum bei +K-Behandlungen um 46 % höher war als bei −K-Behandlungen, nachdem im ersten Versuchsjahr eine starke, langanhaltende Trockenzeit herrschte. Dies unterstreicht die Schlüsselrolle von K bei der Regulierung der Stomata und des Wasserhaushalts von Bäumen, insbesondere unter Trockenstressbedingungen. (3) Die Rolle von P bei der Förderung des Baumwachstums und der Kohlenstoffakkumulationsraten in diesem Wald auf stark verwitterten Böden war eher unauffällig. Unsere Ergebnisse unterstreichen die Tatsache, dass in einem

hochdiversen Waldökosystem mehrere Nährstoffe und nicht nur ein einziger Nährstoff das Baumwachstum und die oberirdische Kohlenstoffaufnahme regulieren. Dies ist auf die unterschiedlichen Nährstoffanforderungen und Aufnahmestrategien der verschiedenen Baumgrößen, Arten und Blattformen zurückzuführen.

In unserer zweiten Studie untersuchten wir die Auswirkungen der Nährstoffzugaben auf die Produktion von Feinstreu und die Streuqualität. Zu diesem Zweck haben wir an zufälligen Stellen in jeder Versuchsparzelle vier Streusammler (0,75 m × 0,75 m groß) aufgestellt, die in drei aufeinanderfolgenden Jahren (Mai 2018-April 2021) alle zwei Wochen zur Bestimmung der Trockenmasse geleert wurden. Unsere Daten legen nahe, dass: (1) Obwohl die jährliche Feinstreuproduktion kurzfristig (3 Jahre) nicht signifikant durch die Nährstoffzugabe beeinflusst wurde, könnte sich der beobachtete Trend zu einer höheren jährlichen Feinstreuproduktion in den Parzellen mit N-Zugabe bei fortgesetzter Nährstoffzugabe verstärken. (2) Nach einer längeren Trockenzeit im ersten Versuchsjahr ging der Blattstreufall durch P- und K-Zugaben deutlich zurück. Diese beobachteten Auswirkungen von K auf den Blattstreufall bestätigen das verstärkte Stammwachstum der Laubbäume in unserer ersten Studie. Außerdem unterstreichen sie die Rolle von K und P bei der Regulierung des Wasserhaushalts der Bäume unter Trockenstressbedingungen. (3) Sowohl die Blattstreu als auch die Blattnährstoffgehalte wurden durch die erhöhte Verfügbarkeit aller drei Nährstoffe sowohl in positiver als auch in negativer Richtung beeinflusst, variierten jedoch erheblich zwischen den verschiedenen Baumarten. Auch wenn die langfristigen Auswirkungen der Nährstoffstörung auf dieses Ökosystem noch nicht bekannt sind, wurde in dieser zweiten Studie das Konzept der Regulierung der Streuproduktion und der Blattqualität durch mehrere Nährstoffe anstelle eines einzelnen Nährstoffs bestätigt.

In der dritten Studie wurden die Auswirkungen der Nährstoffzugabe auf unterirdische Prozesse wie die Produktion von Feinwurzelbiomasse und pflanzenverfügbare Bodennährstoffe untersucht. Zunächst wurde die Feinwurzelbiomasse (0−10 cm Bodentiefe) am Ende des ersten und zweiten Versuchsjahres durch Ausheben von Bodenmonolithen (20 cm × 20 cm) an sechs zufälligen Stellen innerhalb jeder Parzelle quantifiziert. Anschließend wurde die Feinwurzelproduktion in den obersten 30 cm Bodentiefe mithilfe der sequenziellen Entkernungstechnik geschätzt. Wir stellten fest, dass die Zugabe von Stickstoff die Feinwurzelbiomasse nach dem ersten Versuchsjahr um 35 % verringerte. Die Zugabe von K mit ging ebenfalls einer verringerten Feinwurzelproduktion einher. Dies deutet auf eine geringere N- und K-Limitierung an diesem Standort hin, wie sie bereits in unserer ersten Studie festgestellt wurde. Dieser rasche Rückgang der Feinwurzelbiomasse bei den N- und K-Zugaben unterstützt die Idee, dass Bäume ihr energieintensives Wurzelgeflecht bzw. -produktion zurückfahren, wenn sie über ausreichende Ressourcen verfügen. Darüber hinaus führten die Nährstoffzugaben zu einer Kaskade von biochemischen Reaktionen auf die Nährstoffverfügbarkeit im Boden. Im Einzelnen: (1) Die Netto-N-Mineralisierung und Nitrifikationsraten wurden durch die Interaktionseffekte aller drei Nährstoffe (N × P × K) erhöht, was die komplementäre Rolle dieser Nährstoffe bei der Regulierung von Pflanzen- und Bodenprozessen in diesem artenreichen Ökosystem verdeutlicht. (2) Die mikrobielle Biomasse C nahm mit der P-Zugabe zu, war jedoch abhängig von der Jahreszeit (Regen- oder Trockenzeit). Schließlich erhöhte sich durch die P-Zugabe der pflanzenverfügbare P-Gehalt um 80 %. Dieser starke Anstieg deutet auf eine hohe P-Verfügbarkeit hin und erklärt die fehlende Reaktion des Pflanzenwachstums auf P-Zugaben (wie in unserer ersten Studie gezeigt).

Insgesamt verdeutlicht diese Dissertation das Konzept der (Ko-)Regulierung der NPP und anderer Ökosystemprozesse durch mehrere Nährstoffe und bestärkt die zunehmende Zahl von Belegen dafür, dass die Produktivität in tropischen Wäldern nicht dem Liebigschen Minimumgesetz folgt. Stattdessen variieren die Ressourcen- und Nährstoffanforderungen (und ihre Grenzen) bei verschiedenen ökologischen Prozessen oder Komponenten der NPP in diesem Wald, angefangen beim Baumwachstum (N und K), dem Laubstreufall (P und K) bis hin zur Wurzelbiomasse und -produktion (N und K). Diese Beobachtungen stehen in der Tat im Einklang mit der Theorie der mehrfachen Ressourcenbegrenzung. In Anbetracht der Tatsache, dass sich die meisten groß angelegten experimentellen Untersuchungen bisher nur auf die Rolle der N- und P-Verfügbarkeit bei der Begrenzung der Pflanzenproduktivität konzentriert haben, zeigen unsere Daten, dass andere Nährstoffe, insbesondere K, für die funktionellen und biochemischen Aufgaben im Zusammenhang mit der Kohlenstoffaufnahme im Ökosystem ebenso wichtig sein können. Zweifellos sind weitere Forschungsarbeiten dieser Art erforderlich, insbesondere für die tropische Region Afrikas, die weltweit am wenigsten erforscht ist.

Dissertation Outline

This dissertation consists of five chapters aimed at revealing the nature of nutrient limitation and identity of nutrients potentially limiting ecosystem productivity and processes by measuring the ecosystem's responses to elevated nutrient inputs. Chapter 1 provides a brief overview of the concepts of nutrient limitations (core theme of this dissertation) in tropical forest ecosystems and approaches to assessing them. Furthermore, the justification, objectives and hypotheses of this research are presented here along with general methodologies common to all studies within this dissertation (site description, experimental design, and an introduction to the statistical method used). The individual studies (chapters 2−4) address questions related to different compartments of the forest ecosystem (aboveground i.e. stem and canopy, and belowground i.e. roots and soil nutrients). Chapter 2 investigates whether or not nutrients limit stem growth in the entire tree community or subgroups within the community (e.g. small trees or N-fixing trees). Chapter 3 explores how nutrient availability regulates fine litterfall and foliar chemistry in the tree canopy. Chapter 4 examines the effects of nutrient availability on fine root production and plant available nutrients in the soil. In Chapter 5, NPP is estimated based on data presented in chapters 2−4 and compared to NPPs from other tropical forests. The responses of the ecosystem to the addition of different nutrients (N, P and K) are discussed and a revisit to the term 'nutrient limitation' in the context of our results (chapters 2−4), nutrient manipulation experiments and the pervasiveness of its use in literature is made.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Resource limitations of net primary production in tropical forest

Net primary production (NPP), which refers to the amount of carbon that is fixed from atmospheric carbon dioxide (CO_2) into new organic matter (per unit area and per unit time) is fundamental to all life on Earth (Saugier et al. 2001). In terrestrial ecosystems, NPP is composed of several components, including above-ground wood productivity, leaf production, below-ground wood productivity, fine root production but also the production of root exudates and volatile organic carbon compounds (Saugier et al. 2001, Malhi et al. 2011). Under increasing anthropogenic CO_2 emissions, sustained NPP is critical in reducing and eventually stabilizing CO_2 concentrations in the atmosphere (IPCC 2007). Tropical forests play a crucial role in this respect, regulating the exchange of water, carbon and nutrients between the atmosphere and the terrestrial biosphere. Tropical forests store approximately 55% (471 ± 93 Pg C) of the world's forest carbon pool compared to the 32% (272 ± 23 Pg C) in boreal and 14% (119 ± 6 Pg C) in temperate forests (Pan et al. 2011); nearly one-third of the world's soil carbon (Jobbágy and Jackson 2000) and 30 to 50% of terrestrial productivity (Field et al. 1998, Phillips et al. 1998). Whereas higher atmospheric CO_2 concentrations can improve plant growth through "CO_2 fertilization", ecosystem productivity may be capped by other resource limitations (Beedlow et al. 2004).

The growth of all plants is contingent on the availability of water, light and nutrients as essential resources (Coley et al. 1985). Resource constraints on plant life have long been studied in agricultural production to prevent food insecurity (Naylor 1996). An increase in unsustainable agricultural production methods in the past and industrial activities have led to climate change and transformations of biogeochemical cycles throughout the biosphere. How the Earth's ecosystems will respond in future, in part, depend on our improved understanding of resource limitations not only of agricultural systems but in natural ecosystems too. Unlike agricultural ecosystems or other biomes, tropical forests are generally unique for their high local diversity in species and structural composition (John et al. 2007). Consequently, and at any given time, this diversity can result in uneven availability, distribution and accessibility of the essential resources required for primary productivity. In recent years, there has been a growing concern that the sink strength of tropical forests is declining (Brienen et al. 2015, Hubau et al. 2020, Maia et al. 2020, Rammig and Lapola I 2021). This potentially indicates that these forests are either constrained by essential resources or becoming carbon saturated. Meanwhile, it is widely recognized that ecosystem nutrient limitations play a critical regulatory role in plant growth, therein affecting ecosystem carbon assimilation and NPP (Vitousek and Farrington 1997, Porder et al. 2007, Wright et al. 2011, Powers et al. 2015, Wright 2019). How much carbon tropical forests will be able to store and sequester in the future remains highly uncertain. This uncertainty is in large part due to our limited understanding of how nutrients control NPP in tropical forests (Oren et al. 2006, Gerber et al. 2013, Hedin 2015). Understanding the factors or resources that limit tree growth in these highly productive forests is necessary to predict future changes in terrestrial carbon stocks and possible future threats to these ecosystems.

1.2 The concepts of nutrient limitation in tropical forests

Nutrient limitation, as an organizing principle in contemporary biogeochemistry, originated from 19th-century agricultural chemistry (Perakis 2002). According to Vitousek (2010), nutrient limitations occur when meaningful inputs of essential elements in biologically available forms cause an increase in the rate of a biological process (e.g. primary productivity) and/or in the size of an important ecosystem compartment (e.g. biomass). Gibson (1971), on the other hand, defined (nutrient) limitation in three succinct ways: (1) When an organism is not growing as fast as it is theoretically able to grow then it is limited. (2) When a factor or nutrient is in short supply such that no growth is possible, that factor or nutrient is believed to be limiting growth. Analogous to these two is (3) if no effect on growth is observed when a factor or nutrient is increased then the factor or nutrient is not limiting growth. Although the growth of individual organisms, net primary production of ecosystems, and net ecosystem production (NEP) are all potentially being limited by nutrients (Howarth 1988), "nutrient limitation" in this dissertation is applied only to the scales of individual plant growths or net primary production and its related ecological processes. It excludes NEP response to nutrient additions, which conforms with the above definitions and other synthesized literature (Vitousek and Howarth 1991, LeBauer and Treseder 2008, Elser et al. 2007).

Two working concepts are often associated with nutrient limitation studies: First, the single "Liebigs" concept or the Liebigs law of the minimum. Indeed, it is one of the early landmark theories of plant nutrition and soil fertility, which has held the view, that the scarcest nutrient controls the productivity of any given ecosystem. In recent years, however, the application of this theory in natural (tropical) forest ecosystems, which are inherently diverse and complex, have been questioned, presented new hypothesis and attracted increasing research attention (Mirmanto et al. 1999, Elser et al. 2007, Kaspari et al. 2008, Vitousek et al. 2010, Cleveland et al. 2011, Wright et al. 2011, Waring et al. 2019, Du et al. 2020). This new hypothesis represents the second concept called multiple nutrient co-limitation or the "non-Liebig" concept. It postulates that different ecosystem or growth processes may be limited by different nutrients resulting in simultaneous multiple co-limitations of plant growth (Kaspari and Powers 2016). Possible mechanisms supporting these co-limitations of NPP include (1) positive interactions or synergy in resource use and supply, for instance, when one nutrient stimulates the mineralization of another (Rietra et al. 2017). (2) substitution of chemical compounds e.g. the use of sulfolipids or galactolipids in place of phospholipids in cellular membranes (Lambers et al. 2012). (3) Physiological plant processes e.g. adjustments of root/shoot allocations (Bloom et al. 1985). And (4) limitation of different functional groups or species by different nutrients within one ecosystem e.g. non-N-fixers limited by N whilst N fixers are limited by P (Vitousek et al. 2010).

Furthermore, the productivity of tropical forests underlain by highly weathered soils have been widely recognised to be P limited (Walker and Syers 1976, Vitousek 1984, Vitousek and Sanford 1986, Crews et al. 1995, Vitousek and Farrington 1997, Elser et al. 2007, Vitousek et al 2010, Turner et al. 2018). Reasons underpinning this recognition are: First, the low availability of P in tropical soils triggered by the fixing of soil P to iron (Fe) and aluminium (Al) oxides and hydroxides, which in turn becomes occluded or inaccessible to plant uptake (Cross and Schlesinger 1995). Second, the potentially rapid loss of soil P (which is rock-derived) through leaching than can be replenished through weathering (Walker and Syers 1976, Vitousek and Farrington 1997, Vitousek et al. 2010). On the other hand, temperate forests (before industrial-driven N depositions), tropical forests on high altitudes and other terrestrial ecosystems on young substrates were considered to be N limited (Vitousek and Howarth 1991, Vitousek and Farrington 1997, Hedin et al. 2009, Vitousek et al. 2010). This determination is made because N, unlike P, accumulates through N-fixation, which are mainly enhanced in wetter and warmer climates (Vitousek and Farrington 1997, LeBauer and Treseder 2008, Hedin et al. 2009). Nevertheless, the results of nutrient addition experiments, under the assumption that the addition of a limiting nutrient would increase primary production, have not only been geographically biased, spatio-temporarily heterogenous but also inconclusive so far (Mirmanto et al. 1999, Newbery et al. 2002, Davidson et al. 2004, Santiago et al. 2012, Alvarez- Clare et al. 2013, Wright et al. 2011, 2018, 2019).

1.3 Approaches of assessing nutrient limitation in tropical forest

We can distinguish between direct and indirect assessment of nutrient limitation (Fig.1.1). Direct evidence of nutrient limitations is shown if the addition or fertilization of nutrients leads to an increase in the ecosystem process being measured (Tanner et al. 1998) and can only be rigorously evaluated through experimentation. Powers, et al. (2015) wrote that "… *the 'gold standard' of ecosystem ecology remains large-scale fertilization experiments".* According to them, such experiments provide the possibility of resolving nutrient addition effects and the mechanisms of nutrient limitations across a hierarchy of scales from microbial to trees. On the other hand, nutrient limitation can be inferred by indirect methods often based on the availability of nutrients in the soil (Powers 1980), element concentrations or ratios in plant tissues (Koerselman and Meuleman 1996), and investments by plants in acquiring specific nutrients (Harrison and Helliwell 1979). Although laborious and logistically challenging in a tropical setting, this dissertation is based on the former (direct or experimental) approach with all conducted activities highlighted in red ink (Fig.1.1). It is also worth noting that the expressions; fertilization, nutrient addition, and elevated nutrient inputs are synonymous and are used interchangeably throughout this dissertation.

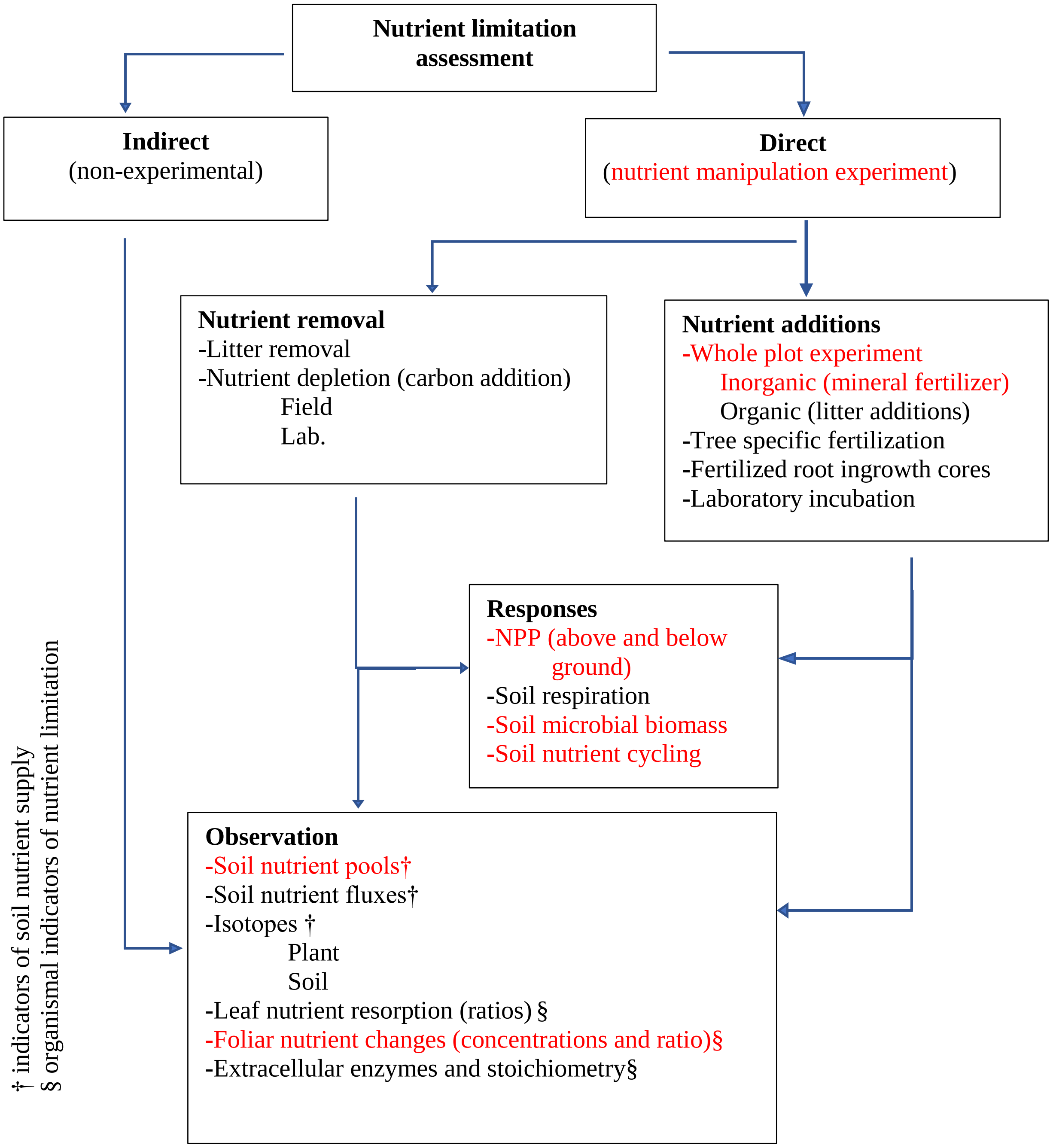


Figure 1.1: Approaches of nutrient limitation assessment (toolbox). Although laborious, a direct experimental approach (highlighted in red ink) was adopted in this dissertation since our research involved only one site. If the research has multiple research sites and the objective is the relative difference in nutrient limitation among the sites, then observational or experimental methods may be considered. Adopted and modified from (Sullivan et al. 2014).

1.4 Study framework, justification and objectives

This dissertation forms part of a larger project code-named “RELIANCE”; a DFG-funded project with the overarching aim of elucidating the mechanistic controls of nutrient availability on ecosystem carbon assimilation. In doing so, we established a large-scale nutrient manipulation experiment (NME) in 2018 in a semi-deciduous tropical forest in Uganda (Africa) using a replicated factorial experimental design. To date, only one NME of P (without N or K) has been conducted in tropical Africa (Newbery et al. 2002). The selected study location, therefore, exemplifies this underrepresented tropical region with likely P or K-depleted soils. Because these experiments are uncommon in Africa, our knowledge of how nutrient availability control ecosystem productivity globally in the tropics remain unclear and geographically selective (with most of the few existing NMEs concentrated in the Americas and Asia). A most recent meta-analysis involving 48 NMEs in tropical forests were conducted in the neotropics (32), South-East Asia (8) and Hawaii (8), with no representation from Africa or Australia (Wright 2019). This paucity of NMEs contributes to the inconclusiveness of working ecological concepts or mechanisms on the magnitude and direction of nutrient limitations of tropical NPP.

Furthermore, in contrast to N and P, the role of K on ecosystem processes has largely been overlooked in the few tropical NMEs. To date, the only long-term, ecosystem-scale NME in the lowland tropics that included a K treatment found that K is particularly limiting for the growth of young trees (Wright et al. 2011). To the best of our knowledge, this experiment is the second NME to equally consider K in a full factorial ecosystem-scale NME and the only experiment to be sited on sandy (~55 % sand) soil. Here, this dissertation aimed to investigate the roles of N, P and K and their interactions on ecosystem processes across a hierarchy of scales from the NPP of a standing forest trees to microbial nutrient cycling processes and consists two main objectives divided into three separate studies (Chapter 2−4).

The first objective is to evaluate the effects of elevated nutrient inputs on different components of NPP by quantifying changes in forest aboveground biomass, tree diameter increments, fine litter biomass, and foliar (leaf-litter and sun-lit leaves) chemistry. We hypothesized that there would be multiple nutrient co-limitations rather than one single nutrient limitation on NPP, as this forest has a high species diversity (~126 tree species), suggesting different nutrient acquisition strategies of different species or functional groups at the same site and different nutrient demand by different components of NPP (Wright et al. 2011, Kaspari et al. 2008).

The second objective is to assess the impact of elevated nutrient inputs on belowground processes i.e. fine root production and levels of plant-available nutrients in the soil (by measuring net N cycling rates, plant-available P and microbial biomass). Here we hypothesized that the increased nutrient availability will alter fine root architecture whilst soil biochemical responses would differ among the nutrient addition treatments (Yavitt et al. 2011). The combined additions of either two or three of these nutrients will have positive effects on plant nutrient availability rather than their single nutrient addition because of their complementarity in supplying the stoichiometric nutrient requirements of trees and soil organisms.

1.5 Study location and site description

1.5.1 Study location and soils

The research was conducted in the Budongo Forest Reserve in northwestern Uganda (1°43'29''N 31°32'21''E; Fig. 1.2). This species-diverse, moist semi-deciduous tropical forest is located on the African shield on heavily weathered soils, likely classified as Lixisols (FAO World Reference Base 2014). Lixisols are polygenetic soils that experienced strong weathering (feralization) during earlier stages of development under wetter climates of the past, which was then followed by the deposition of base-rich aeolian dust and ash from biomass burning. Accordingly, the soils have a relatively high pH with relatively high exchangeable bases (especially calcium).

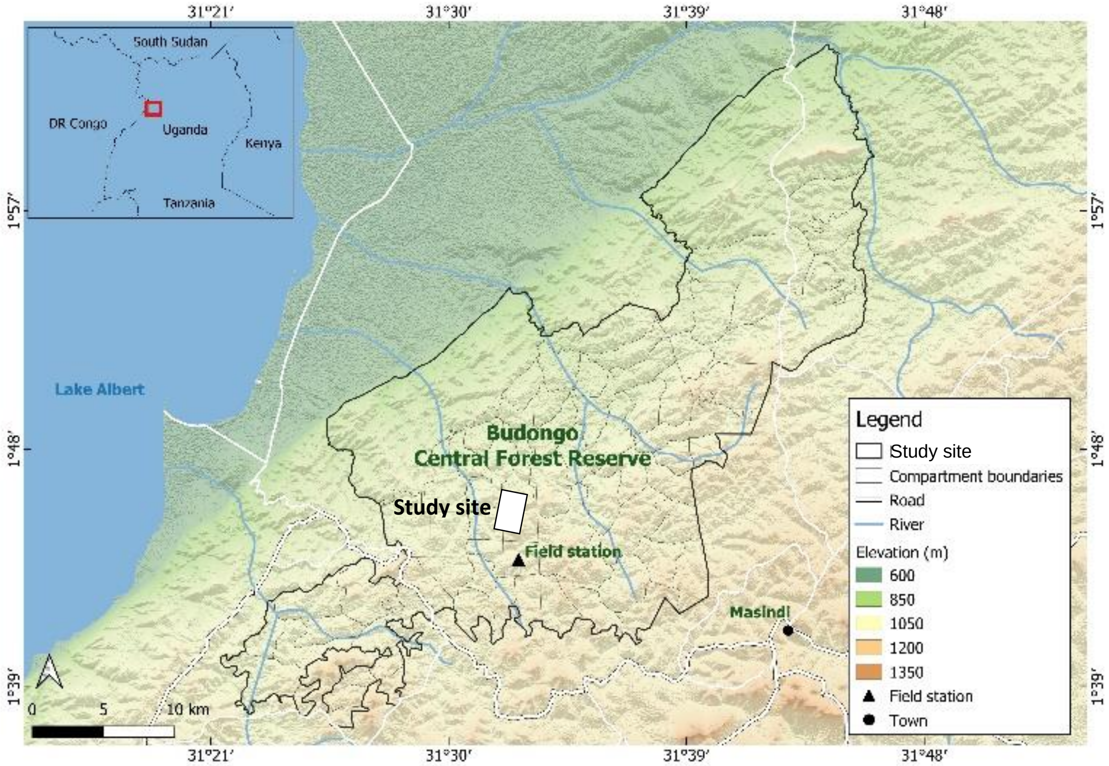


Figure 1.2: Location of study site within the Budongo Forest Reserve in Uganda.

1.5.2 Climate

The region experiences a bimodal rainfall pattern between March to June and August to November (Fig. 1.3). Its mean annual air temperature is 22.8 ± 0.1 ◦C and annual mean precipitation of 1670 ± 50 mm (2000–2019; Budongo Conservation Field Station).

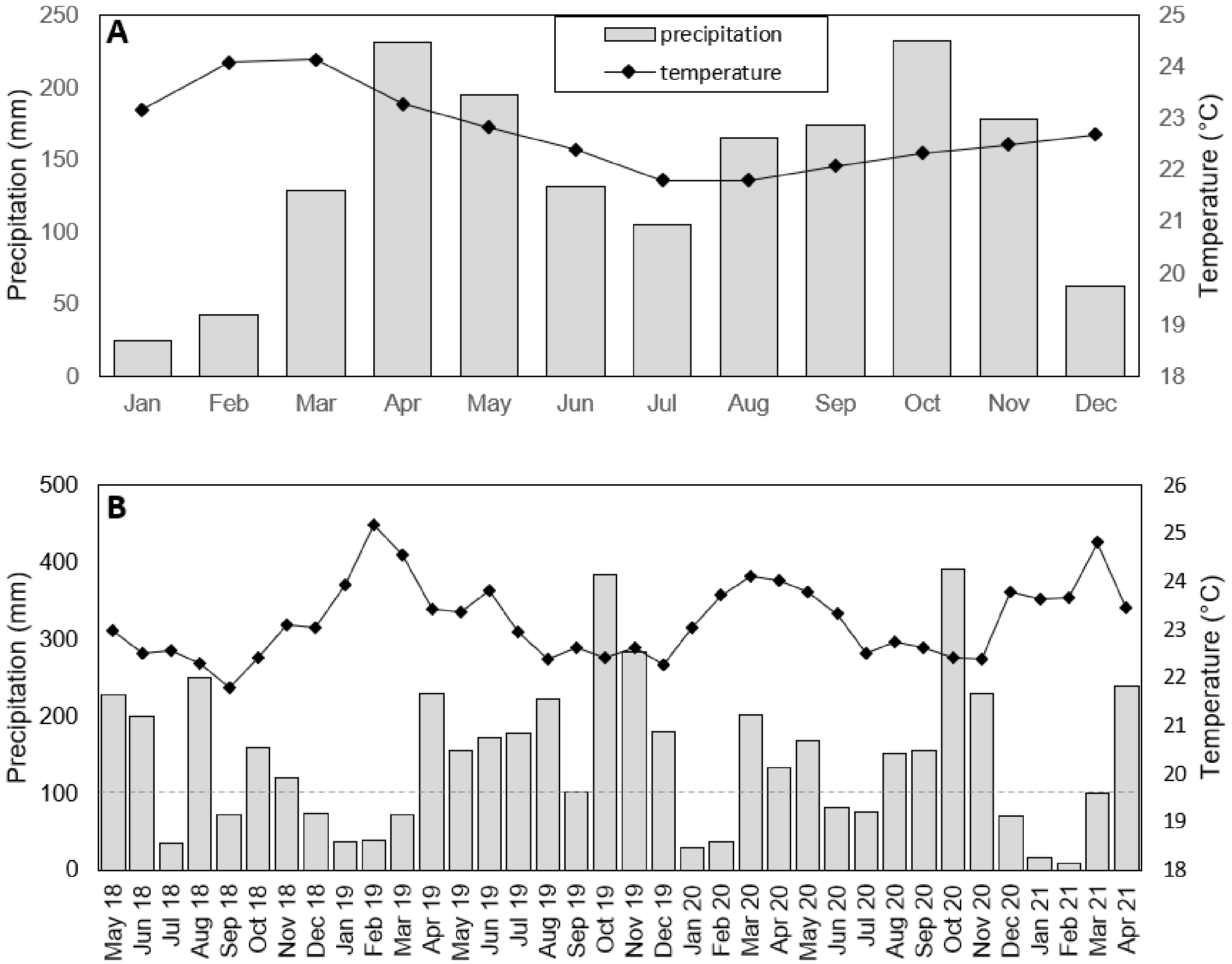


Figure 1.3: Monthly rainfall pattern at the study area based on long-term (2000-2019) climate data (A) and during the study period (B) from the Budongo Conservation Field Station (2 km from experimental site) in Uganda.

1.5.3 Logging history of the Budongo Forest Reserve

The Budongo Forest Reserve is the largest in Uganda and despite its selective logging history, has remained undisturbed for nearly 60 years now. Selective logging started in 1911 on a negligible scale but increased markedly until the 1960s. The experimental site (located in compartment N4; 341 ha) was selectively logged, between 1952 and 1954, with a total volume of 94.0 m^3 ha^{-1} removed (Plumptre 1996). This resulted in a higher abundance of mid-stage succession tree species (e.g. *Funtumia elastica*). Logging was mainly on trees of the Meliaceae family which happen to be marketable at the time (Bahati 1998). Following these logging activities, replanting of economically important species (Khaya and Entandrophragma) were carried out in the logged areas. Between 1960 and 1962, trees that were not marketable, particularly *Cynometra spp*., were treated with arboricides to open up the canopy and encourage species richness through natural regeneration (Philip 1965, Plumptre 1996). The site has since been designated for research purposes. The most noticeable effect of this past logging was an increased species richness compared to an unlogged compartment. The geographical location of a compartment within the forest explained more of the variation in species distribution than the variation between adjacent logged and unlogged compartments (Plumptre 1996).

1.6 Experimental design

We established thirty-two 40 m × 40 m experimental plots, separated by at least 40 m, in a factorial design with eight randomly assigned treatments, each replicated by four plots. The treatments included the sole additions of N, P, K, their combinations (NP, NK, PK, and NPK) and a control (Fig. 1.4). Within each 40 m × 40 m plot (Fig. 1.5), we also laid out a 30 m × 30 m core measurement zone where all subsequent response measurements were conducted (to minimize edge effects) and sixteen 10 m × 10 m quadrats to facilitate fertilizer additions. Nitrogen was added as urea ($(NH_2)_2CO$) at a rate of 125 kg N ha^{-1} yr^{-1}, P as triple superphosphate ($Ca(H_2PO4)_2$) at a rate of 50 kg P ha^{-1} yr^{-1} and K as muriate of potash (KCl) at a rate of 50 kg K ha^{-1} yr^{-1}. The experimental design and nutrient addition rates were consistent with Wright et al. (2011). Pre-packaged fertilizers for each 10 m × 10 m quadrat were mixed with soil directly adjacent to the plot (as filler material) and broadcasted by hand within each quadrat, walking forward and back and subsequently changing directions (north to south and east to west). We fertilized four times a year in equal doses during the wet season (beginning from 17th May 2018).

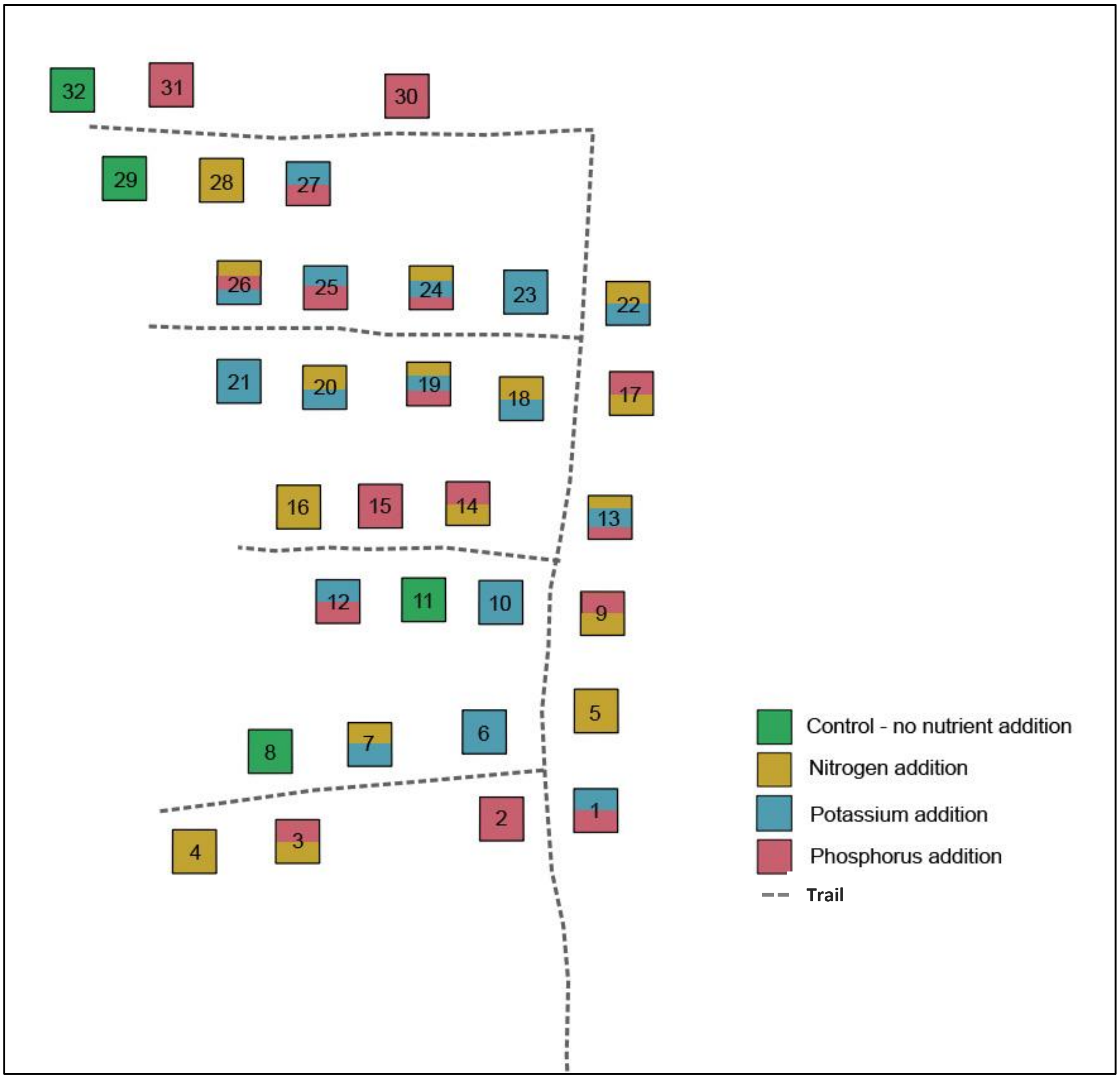


Figure 1.4: Layout of the 32 experimental plots with randomly assigned treatments (b): control, N, P, K, NP, NK, PK, and NPK with four replicate each. Plots are 40 m × 40 m in size and are at least 40 m apart.

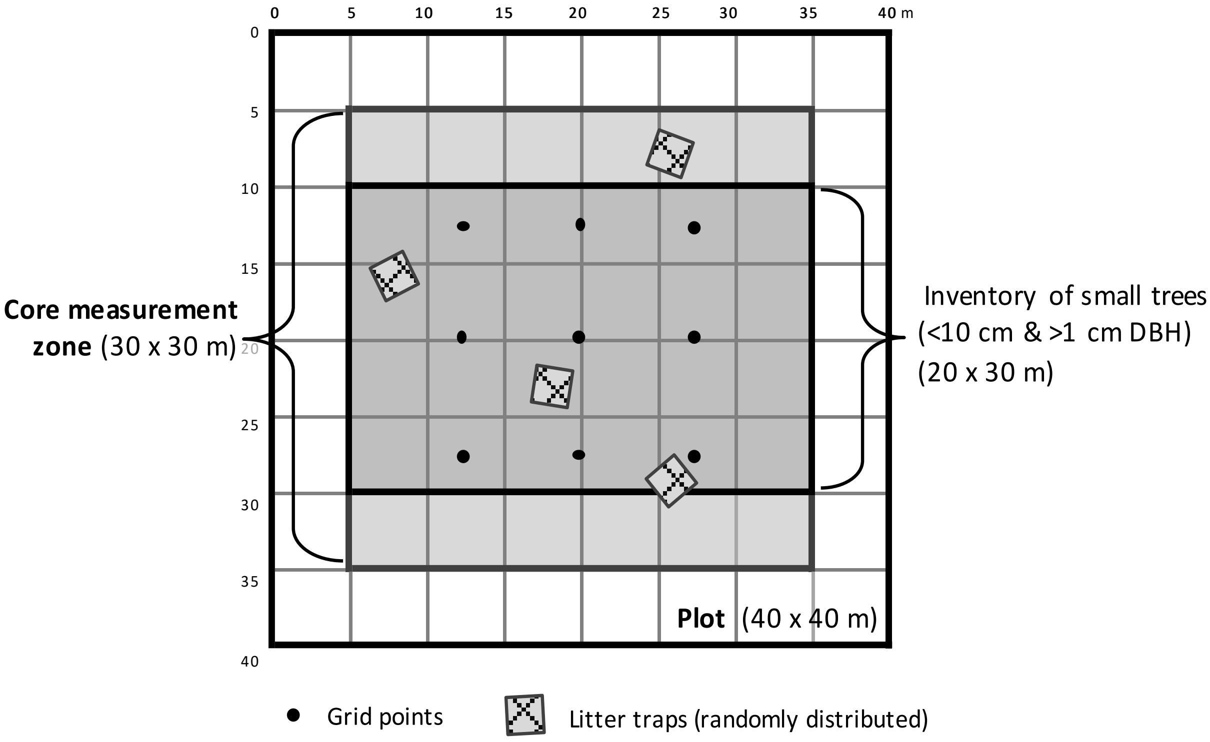
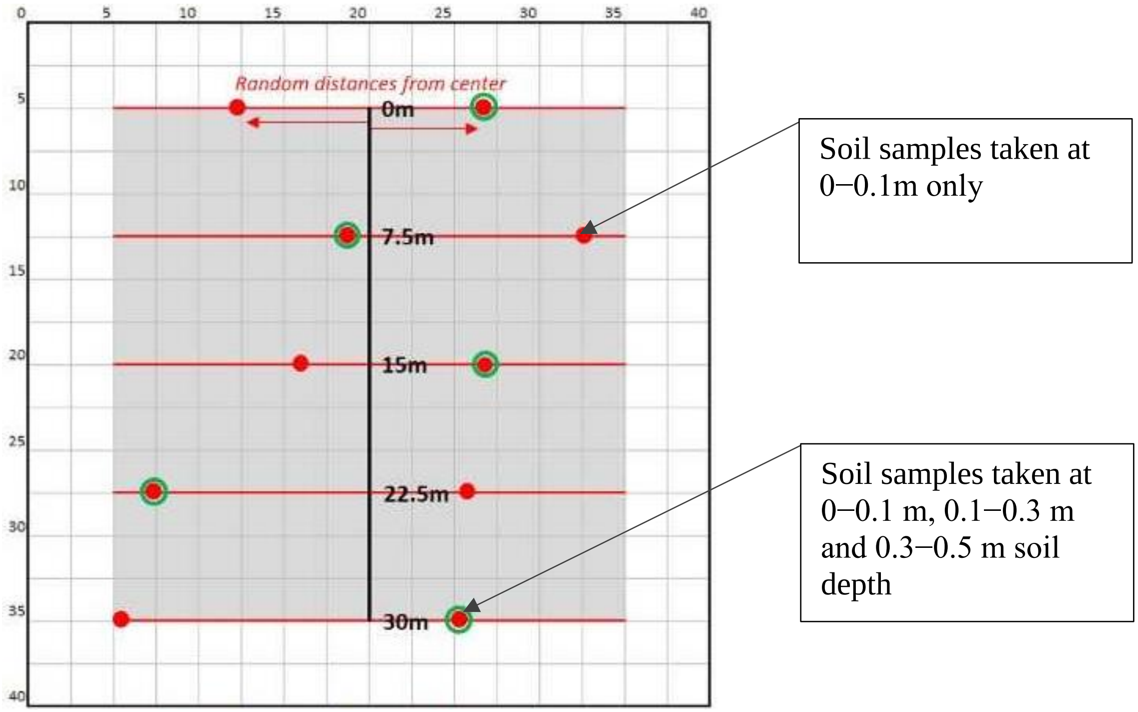


Figure 1.5: Experimental design within each of the 32 plots in the nutrient manipulation experiment

1.7 Soil physical and biochemical analysis prior to nutrient additions

In April 2018 before nutrient additions, soil samples were taken from 10 randomly placed locations per plot at fixed depth intervals of 0−0.1 m, 0.1−0.3 m and 0.3−0.5 m in all the 32 plots (Fig. 1.6). This was done to assess whether or not inherent differences in soil physical and biochemical characteristics existed among the treatment plots prior to nutrient additions. A summary of initial soil characteristics is presented in Tables 1.1 and 1.2. The lack of statistical differences between the treatment plots prior to initial nutrient addition (Tables 1.1) provides the basis of attributing differences in response measurement to the effects of nutrient addition (*ceteris paribus*).



Soil samples taken at 0−0.1m only

Soil samples taken at 0−0.1 m, 0.1−0.3 m and 0.3−0.5 m soil depth

Figure 1.6: Illustration of random soil sampling locations within each of the 32 plots at the nutrient manipulation experimental site in the Budongo Forest Reserve.

Table 1.1: Soil physical and chemical characteristics (mean ± SE; n = 32 plots) at 0−0.1 m, 0.1−0.3 m and 0.3−0.5 m, measured in April 2018 prior to nutrient additions.

Site characteristics	Soil depth		
	0−0.1 m	0.1−0.3 m	0.3−0.5 m
Bulk density (g/cm^3)	1.23 ± 0.03	1.53 ± 0.03	1.38 ± 0.03
Soil pH (1:2.5 H_2O)	6.43 ± 0.04	6.08 ± 0.09	5.90 ± 0.10
Total soil nitrogen (kg/m^2)	0.42 ± 0.01	0.57 ± 0.02	0.56 ± 0.02
Total organic carbon (kg/m^2)	4.02 ± 0.13	4.59 ± 0.20	4.09 ± 0.19
Soil C:N ratio	9.54 ± 0.10	7.99 ± 0.08	7.22 ± 0.11
^{15}N natural abundance (‰)	7.79 ± 0.06	9.22 ± 0.13	9.51 ± 0.16
Bray II phosphorus (g/m^2) †	1.80 ± 0.24	0.85 ± 0.08	0.65 ± 0.06
Effective cation exchange capacity ($mmol^+/kg$)	149.2 ± 8.3	63.0 ± 4.1 †	51.9 ± 2.6 †
Base saturation (%)	98.2 ± 0.2	97.5 ± 1.0 †	97.2 ±1.3†
Soil texture: Sand (%)	54.8 ± 1.6	55.3 ± 1.6	48.7 ± 1.4
Silt (%)	27.0 ± 1.7	21.3 ± 1.2	13.7 ± 0.9
Clay (%)	18.2 ± 0.8	23.4 ± 1.1	37.6 ± 1.3

Note: Methods of soil analysis are described in Chapters 3 and 4

† Parameter was measured from 16 plots

Table 1.2: Soil physical and chemical characteristics (mean ± SE; n = 4 plots) in the top 0−0.1 m, measured in April 2018 prior to nutrient additions. Within rows, there were no differences in initial soil characteristics among plots that were randomly assigned to the eight treatments (one-way ANOVA at $P < 0.05$).

Site									Statistics	
characteristics	Contol	N	P	K	NP	NK	PK	NPK	$F_{(7, 24)}$	*P value*
Bulk density (g/cm^3)	1.34 ± 0.11	1.31 ± 0.06	1.10 ± 0.08	1.27 ± 0.05	1.20 ± 0.17	1.15 ± 0.17	1.19 ± 0.05	1.25 ± 0.12	*0.71*	*0.66*
Soil pH (1:2.5 H_2O)	6.38 ± 0.09	6.49 ± 0.14	6.58 ± 0.08	6.37 ± 0.10	6.27 ± 0.02	6.57 ± 0.02	6.46 ± 0.12	6.33 ± 0.09	*1.24*	*0.32*
Total soil nitrogen (kg/m^2)	0.41± 0.03	0.48 ± 0.03	0.41± 0.03	0.46 ± 0.04	0.40 ± 0.07	0.38 ± 0.07	0.41± 0.02	0.39 ± 0.03	*0.86*	*0.55*
Total organic carbon (kg/m^2)	4.02 ± 0.39	4.68 ± 0.17	3.88 ± 0.42	4.40 ± 0.53	3.81 ± 0.54	3.62 ± 0.54	3.89 ± 0.09	3.86 ± 0.30	*0.88*	*0.53*
Soil C:N ratio	9.71 ± 0.14	9.68 ± 0.28	9.34 ± 0.41	9.26 ± 0.32	9.42 ± 0.33	9.58 ± 0.33	9.46 ± 0.39	9.86 ± 0.10	*0.51*	*0.82*
^{15}N natural abundance (‰)	7.92 ± 0.19	7.62 ± 0.21	8.15 ± 0.24	7.90 ± 0.12	8.05 ± 0.18	8.16 ± 0.18	7.91± 0.18	8.07 ± 0.13	*0.99*	*0.46*
ECEC (mmol$^+$/kg)	148 ± 29	153 ± 23	199 ± 40	146 ± 21	136 ± 10	134 ± 10	158 ± 25	119 ± 17	*0.92*	*0.51*
Base saturation (%)	98.2 ± 0.5	98.1 ± 0.5	99.2 ± 0.2	98.0 ± 0.4	97.4 ± 0.2	98.4 ± 0.2	98.8 ± 0.3	97.9 ± 0.4	*1.60*	*0.18*
Sand (%)	58 ± 2	53 ± 4	45 ± 7	54 ± 5	54 ± 3	57 ± 3	57 ± 3	61 ± 4	*1.15*	*0.37*
Silt (%)	26 ± 2	31 ± 3	38 ± 8	28 ± 6	24 ± 1	24 ± 1	26 ± 5	19 ± 3	*1.26*	*0.36*
Clay (%)	16 ± 3	16 ± 2	17 ± 1	18 ± 2	22 ± 2	20 ± 2	17 ± 2	20 ± 2	*1.06*	*0.42*

1.8 Statistical analyses

For the most part, all response variables in this dissertation are analysed based on the 2^3 or $2 \times 2 \times 2$ factorial N-P-K design, unless otherwise mentioned, and are consistent with other nutrient manipulation studies (Wright et al. 2011, Yavitt et al. 2011, Santiago et al. 2012, Wurzburger and Wright 2015). This approach of analyses allowed us to test the main effects of each nutrient treatment (i.e. N, P or K) as well as their interaction effect on the measured responses (e.g. stem growth, litter biomass etc.) to nutrient additions. Generally, this type of analysis is referred to as a 2^k factorial design (For a detailed illustration of this experimental design, see Montgomery 2012), where k represents the number of factors being studied in the experiment (in this case three factors: N, P and K), each having only two levels (i.e. nutrient addition coded as “1” and no nutrient addition coded as "0"), the regression model for estimating three fixed factors (N, P and K) are generally expressed as in equation (1). Let main effects of the N, P and K additions be represented by α, β and γ, then the overall ANOVA model (y_{ijkl}) takes the form:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{ik} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl} \qquad (1)$$

where:

μ is the global mean of the response,

α_i is the main effect of factor N at level *i,*

β_j is the main effect of factor P at level *j*,

γ_k is the main effect of factor K at level *k*,

$(\alpha\beta)_{ij}$ is the interaction effect of factors N and P at levels *i* and *j*,

$(\alpha\gamma)_{ik}$ is the interaction effect of factors N and K at levels *i* and *k*,

$(\beta\gamma)_{ik}$ is the interaction effect of factors P and K at levels *j* and *k*,

$(\alpha\beta\gamma)_{ijk}$ is the interaction effect of factors N, P and K at levels *i*, *j* and *k*,

ε_{ijkl} is the random error of the model

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CHAPTER 2

Responses of tree growth and biomass production to nutrient addition in a semi-deciduous tropical forest in Uganda

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Abstract:

Experimental evidence of nutrient limitations on primary productivity in Afrotropical forests is rare and globally underrepresented, yet are crucial for understanding constraints to terrestrial carbon uptake. In an ecosystem-scale nutrient manipulation experiment, we assessed the early responses of tree growth rates among different tree sizes, taxonomic species and at a community level in a humid tropical forest in Uganda. Following a full factorial design, we established 32 (eight treatments × four replicates) experimental plots of 40 m × 40 m each. We added nitrogen (N), phosphorus (P), potassium (K), their combinations (NP, NK, PK, and NPK) and control at the rates of 125 kg N ha^{-1} yr^{-1}, 50 kg P ha^{-1} yr^{-1} and 50 kg K ha^{-1} yr^{-1}, split into four equal applications, and measured stem growth of more than 15,000 trees with diameter at breast height (DBH) $\geq$ 1 cm. After two years, the response of tree stem growth to nutrient additions was dependent on tree sizes, species and leaf habit but not community-wide. First, tree stem growth increased under N additions, primarily among medium-sized trees (10−30 cm DBH), and in trees of *Lasiodiscus mildbraedii* in the second year of the experiment. Second, K limitation was evident in semi-deciduous trees, which increased stem growth by 46 % in +K than –K treatments, following a strong, prolonged dry season during the first year of the experiment. This highlights the key role of K in stomatal regulation and maintenance of water balance in trees, particularly under water-stressed conditions. Third, the role of P in promoting tree growth and carbon accumulation rates in this forest on highly weathered soils was rather not pronounced; nonetheless, mortality among saplings (1–5 cm DBH) was reduced by 30 % in +P than in –P treatments. Although stem growth responses to nutrient interaction effects were positive or negative (likely depending on nutrient combinations and climate variability), our results underscore the fact that, in a highly diverse forest ecosystem, multiple nutrients and not one single nutrient regulate tree growth and aboveground carbon uptake due to varying nutrient requirements and acquisition strategies of different tree sizes, species and leaf habits.

Keywords: *Budongo forest, carbon stock, fertilisation, nitrogen, nutrient limitations, phosphorus, potassium, primary productivity, relative growth rate, Uganda*

2.1 Introduction

Nutrient limitations play an important role in constraining plant growth and ecosystem productivity across all terrestrial biomes. Under increasing global atmospheric CO_2 concentrations, tropical forests remain one of the largest mitigants of climate change, storing nearly 55 % of the world's forest carbon stock and having the highest productivity compared to other biomes (Pan et al. 2011). The photosynthetic and carbon-fixation capacity of these forests relies largely on essential resources (light, water and nutrients) in sufficient quantities. It is therefore axiomatic that inadequate supply of any one or more resources will impose limits on the capacity of these forests to assimilate CO_2 efficiently and produce new plant biomass (Danger et al. 2008). Brienen et al. (2015) reported that the aboveground carbon sequestration rates of the Amazon rainforest decreased by about one-third between 2000 and 2010 in comparison to the 1990s. This potentially indicates that, among other adverse global changes, carbon saturation or nutrient limitations could be the constraining factor of the growth and productivity of these forests. How much carbon tropical forests will be able to store and sequester in the future remains uncertain particularly for underrepresented tropical regions (Wieder et al. 2015). Moreover, many reviews and observations, thus far, have partly attributed these uncertainties to gaps in our knowledge of how nutrient availability control forest carbon assimilation and dynamics, which represents a major challenge for ecologists in modelling terrestrial ecosystem response to global changes (Oren et al. 2006, Hedin et al. 2009, Gerber et al. 2013).

Pathways of nutrient input in forest ecosystems (biological nitrogen fixation (BNF), rock weathering and atmospheric deposition) are variable as are nutrient requirements, acquisition and availability to different ecosystem processes (e.g. Hedin et al. 2009). Apart from N, all other nutrients primarily originate from the weathering of soil parent material and then cycled in the forest ecosystem (soil-biomass-litter-soil). In both direct and indirect observations, N and phosphorus (P) are commonly recognized to limit tree growth and other ecosystem processes in most terrestrial ecosystems (Elser et al. 2007, Tamale et al. 2021). Soil age and climatic regimes are known large-scale controllers of nutrient limitations in tropical forests (Walker and Syers 1976, Cai et al. 2009). Young soils have a large supply of rock-derived nutrients, e.g. P and potassium (K), which diminishes as soils weather with age, whereas N accumulates as organic matter builds up with time (Walker and Syers 1976, Tanner et al. 1998, Fisher et al. 2013). In older, highly weathered soils under warm and humid climates, P and other rock-derived nutrients decrease as a result of excessive nutrient leaching (Veldkamp et al. 2020). In such soils, rock-derived nutrients may limit BNF and decomposition processes (Barron et al. 2009), which possibly down-regulates N availability (Hedin et al. 2009). On one hand, it is postulated that tropical lowland forests on highly weathered soils are P-limited but have high bioavailability of N due to the high abundance and diversity of N-fixing organisms (Hedin et al. 2009, Barron et al. 2011). Such postulation was, however, not supported by findings from a 15-year experiment of tropical lowland forest in Panama (Wright et al. 2018). On the other hand, N limitation on plant productivity is more prevalent in tropical montane forests (Adamek et al. 2009, Wolf et al. 2011, Homeier et al. 2012) and become more pronounced with elevation (Tanner et al. 1998, Graefe et al. 2010). Notwithstanding, a more recent meta-analysis of 48 nutrient addition experiments showed that both N and P are equally likely to limit plant function in tropical forests regardless of elevation (Wright 2019).

In contrast to N and P, the role of K on ecosystem processes has largely been overlooked in tropical forests. To date, the only long-term, ecosystem-scale nutrient manipulation experiment in the lowland tropics that included a K treatment found that K is particularly limiting for the growth of young trees (Wright et al. 2011). Furthermore, indirect evidence has shown that K limitations likely affect ecosystem below- vs above-ground carbon allocations in the Congo basin (Doetterl et al. 2015). A meta-analysis of 38 K addition experiments, involving 26 different tree species revealed that many forest trees (in 69 % of the experiments) responded positively to increased K availability (Tripler et al. 2006). The spatial distributions of tree species at local scales has also been reported to be associated with K availability (John et al. 2007b). It has therefore become imperative that the role of K on tree growth and development in highly diverse natural forest ecosystems is revisited.

In recent years, there are increasing evidence that different ecosystem or growth processes are limited by different nutrients, resulting in simultaneous multiple limitations on plant growth (Kaspari and Powers 2016). Nutrient addition experiments in lowland tropical forests reveal (co)limitations of N, P or K on tree growth of different size classes, components of net primary production (NPP) and succession groups (Wright et al. 2011, 2018). Most nutrient manipulation studies have been conducted on relatively young soils, and there is a serious underrepresentation in regions with likely P- and K-depleted soils such as Ferralsols, Acrisols, Nitisols and Lixisols. To date, only one nutrient manipulation experiment of P (without N and K) has been conducted in tropical Africa (Newbery et al. 2002). In our present study, we established an ecosystem-scale nutrient manipulation experiment in a moist semi-deciduous tropical forest in Uganda, which exemplifies the underrepresented transition zone between African humid and dry tropical forests using a replicated full factorial experimental design. To our knowledge, this study represents the first ecosystem-scale nutrient manipulation experiment to be conducted on sandy soil (sand > 50 %), in contrast to the nutrient addition studies that have been conducted on clay soils (Davidson et al. 2004, Siddique et al. 2010, Alvarez-Clare et al. 2013, Wright 2019, Du et al. 2020). Our first objective was to investigate whether or not N, P and K or their interactions (co-) limit community forest growth. Here we hypothesize that there will be multiple nutrient co-limitations rather than a single nutrient limitation on community forest growth and biomass productivity, as this forest has a high species diversity (126 tree species), which will have many different nutrient acquisition strategies (Wright et al. 2011, Detto et al. 2018). Our second objective was to evaluate the response of tree diameter growth to nutrient additions by different tree size classes. Here, we predict that nutrient acquisition strategies will change over the life span of a tree, whereby small-sized trees that are still actively growing will require more nutrients than large canopy trees that have a low nutrient demand (Adamek et al. 2009, Alvarez-Clare et al. 2013). A small but important part of these trees may have a high demand for N or P (e.g. reproductive organs), which may surpass nutrient availability in the soil (Kaspari et al. 2008, Fortier and Wright 2021). We, therefore, expect that the alleviation of nutrient co-limitations will lead to a growth response of trees with small to medium stem diameters that had previously experienced high nutrient resource competition. The addition of K may alter biomass allocations (Wright et al. 2011, Doetterl et al. 2015), stimulate processes responsible for tree growth as K plays an important regulatory role in the guard cells of leaves that control stomatal aperture and potentially limit CO_2 assimilation, particularly under drought stress conditions. Our last objective was to evaluate the response of stem diameter growth of different tree species, leaf habits (deciduous, semi-deciduous and evergreen) and functional traits (N-fixers and non-N fixers) to nutrient additions. We predict that N-fixing tree species will increase in stem diameter growth in response to P but not to N additions (Waring et al. 2019).

2.2 Materials and methods

2.2.1 Site description

This experiment was conducted in the Budongo Forest Reserve in northwestern Uganda (1°44'28.4"N, 31°32'11.0"E; elevation range: 1050−1070 m; Fig. 1.2). The Budongo forest is a moist, semi-deciduous tropical rainforest situated on an uplifted Shield, specifically on a Precambrian gneissic-basaltic basement complex (van Straaten 1976). Annual precipitation and air temperature average 1670 ± 50 mm and 22.8 ± 0.1 °C, respectively (2000–2019; Budongo Conservation Field Station). The region experiences two dry seasons (< 100 mm per month) from December to February and in July. Annual nutrient depositions from rainfall are 8.5 kg N ha^{-1} yr^{-1}, 0.03 kg P ha^{-1} yr^{-1} and 4.3 kg K ha^{-1} yr^{-1}. The soil is classified as Lixisol (IUSS Working Group WRB 2014), a well-drained (> 50 % sand), highly weathered soil commonly found in a transition zone between tropical forests and savannahs. Unlike other highly weathered soils, this Lixisol's high base saturation and pH (Tables 1.1) are contributed by depositions of aeolian dust and ashes from agricultural biomass burning (Boy and Wilcke, 2008; Bauters et al., 2018) and by weathering of its parent material that consists of coarse-grained basaltic granulites with ~10 % CaO and 6−7 % MgO.

Vegetation at the site is composed of 126 tree species (Shannon-diversity index H': 2.53 ± 0.04 and canopy heights reaching up to 50 m). Among trees with diameter at breast height (DBH) ≥ 10 cm, N-fixing trees (Family: Fabaceae or Leguminosae) constitute 6 % in stem abundance (Table S2.1) but account for 16 % of the forest's basal area and 25 % of aboveground wood biomass (Table 2.1). Leaf area index averaged 3.3 ± 0.0 m^2 m^{-2} (determined in April 2018 and November 2019) in the control plots. The site was selectively logged in the 1950s but has remained undisturbed for nearly 60 years (Plumptre 1996). As a result of the past logging, there is a higher abundance of some mid-stage succession tree species (e.g. *Funtumia elastica*). The ten most dominant species together represent 78 % of all trees ≥ 10 cm DBH in the experimental plots (Tables 2.1 and S2.1). Larger trees (> 30 cm DBH) contributed a large proportion (73 %) of the total wood biomass (395 ± 17 Mg ha^{-1}) and 66 % of carbon storage (annual wood biomass productivity of 5.04 ± 0.74 Mg C ha^{-1} yr^{-1}). Out of 93 tree species ≥ 10 cm DBH (Table S2.1), five species (*Cynometra alexandri* (19 %), *Celtis durandii* (13 %), *Funtumia elastica* (8 %), *Celtis mildbraedii* (8 %), and *Khaya anthoteca* (7 %)) could be considered biomass hyperdominant (i.e. those tree species which collectively account for > 50 % of biomass; Fauset et al. 2015) (Fig. 2.1) and just eight tree species (listed in Table 2.1) were responsible for about half the total wood biomass productivity at this site (Table 2.1).

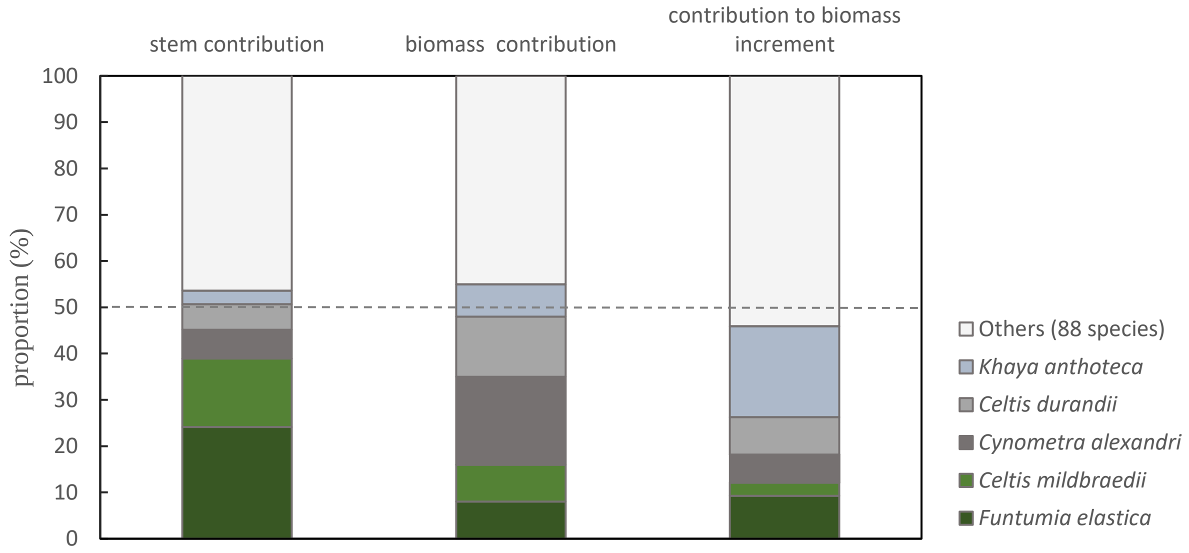

Figure 2.1: Contribution of dominant species to stem density, biomass stocks and productivity in the Budongo Forest Reserve.

2.2.2 Experimental design

We established a full factorial NPK experiment with eight treatments: control, N, P, K, NP, NK, PK, and NPK (Fig. 1.4). These treatments had four replicates and were randomly assigned to

32 plots, 40 m × 40 m each and separated by at least 40 m. Within each 40 m × 40 m plot, we laid out sixteen 10 m × 10 m quadrats to facilitate fertilizer additions. Nitrogen was added as urea ($(NH_2)_2CO$) at a rate of 125 kg N ha^{-1} yr^{-1}, P as triple superphosphate ($Ca(H_2PO4)_2$) at a rate of 50 kg P ha^{-1} yr^{-1} and K as muriate of potash (KCl) at a rate of 50 kg K ha^{-1} yr^{-1}. The experimental design and nutrient addition rates were consistent with Wright et al. (2011). Pre-packaged fertilizers for each 10 m × 10 m quadrat were mixed with soil directly adjacent to the plot (as filler material) and broadcasted by hand within each quadrat, walking forward and back and subsequently changing directions (north to south and east to west). We fertilized four times in a year in equal doses during the wet season (beginning from 17th May 2018, then August, November and March).

2.2.3 Tree growth measurements and biomass productivity

We conducted censuses of all trees ≥ 10 cm DBH in each 40 m × 40 m plot, and trees 1 cm ≤ DBH < 10 cm within an inner 30 m × 20 m subplot. All measured trees (3180 trees ≥ 10 cm DBH; 12,604 trees 1 cm ≤ DBH < 10 cm) were tagged with identification numbers and taxonomically identified to species level. Non-cylindrical stems at breast height due to buttresses or deformity were measured 50 cm above buttresses or deformity. All points of measurement were marked with spray paint to ensure that subsequent censuses were taken at the same point. We repeated censuses four times within the two-year experiment period, i.e. April 2018 (pre-treatment), April 2019 (after 1 year), October 2019 (after 1.5 years) and April 2020 (after 2 years).

To assess the effect of nutrient addition and seasonal pattern on individual tree stem growth to a finer temporal scale, we installed dendrometer bands (D1, UMS, Munich, Germany; with increment-sensitivity of 0.1 mm) on 20 selected trees ≥ 10 cm DBH in an inner 30 m × 30 m measurement zone of each plot. The selection of the 20 trees (which was ~ 40 % of the trees in this inner zone) was based on species composition and DBH distribution. First, in each plot, the importance value index (IVI) of a tree species was determined: sum of relative density, relative frequency and relative basal area (Curtis and McIntosh 1950). Based on the IVI ranking and species proportions, the most important species and the corresponding number of individuals to be sampled were determined. Next, we randomly sampled the individuals (based on tree tag numbers) of the selected species. A backup list of trees was prepared in advance for each species in case the sampled tree died during the experiment (this happened 16 times during the two-year study). The installed dendrometer bands were allowed to settle for a month before our first measurement (May 22, 2018), followed by two consecutive bi-monthly dendrometer measurements (July 22, September 22). Thereafter, all measurements were taken every month on the same date (22nd).

We calculated relative growth rate (RGR; Eq. (1)) (Hoffmann and Poorter 2002) as a metric for tree growth. We separately analyzed four DBH classes (1–5 cm, 5–10 cm, 10–30 cm and >30 cm) as these classes likely experience contrasting light availability and may accordingly differ in their response to nutrient additions (e.g. Wright et al. 2011, 2018). For all analyses of tree growth response to nutrient additions, we only included trees measured in the inner 30 m × 30 m zone in each plot to minimize edge effects.

$$\mathrm{RGR}\ (\mathrm{cm.cm^{-1}.t^{-1}}) = (\ln(\mathrm{DBH}_f) - \ln(\mathrm{DBH}_i)) / \Delta t) \qquad (1)$$

where f and i represent final and initial measurement periods, respectively, and Δt represents the change in time (year or month). For a specific DBH class, the RGR value for each plot was the mean RGR of trees belonging to that class. To assess community-level response to nutrient addition, we calculated community-level RGR (Eq. 2) for each plot by weighting the RGR of each DBH class with the corresponding number of trees (n) belonging to that size class.

$$\text{Community level RGR} = ((\text{RGR}_{(1\text{-}5\text{cm})} \times n_1) + (\text{RGR}_{(5\text{-}10\text{cm})} \times n_2) + (\text{RGR}_{(10\text{-}30\text{cm})} \times n_3) + (\text{RGR}_{(>30\text{cm})} \times n_4)) / (n_1 + n_2 + n_3 + n_4) \qquad (2)$$

Furthermore, tree growth largely depends on biomass allocations to its various organs (roots, stem and leaves), which may differ among different species, functional groups (N fixers and non-N fixers) and different leaf habits (evergreen, semi-deciduous and deciduous) (Appendix S1: Table S3). For instance, deciduous tree species (which shed their leaves during the dry season) may have to resorb and reallocate nutrients in the leaf before shedding them, whereas evergreen species may not. Therefore, the RGR responses of these groups of species (Table S2.1) under elevated nutrient supply may differ. Thus, we evaluated plot-level RGR responses of different tree species, leaf habits and functional groups to nutrient additions. For species-level analysis, we included five dominant tree species that were present in at least three replicate plots for each treatment.

We estimated the aboveground wood biomass (AWB; Eq. (3)) of each tree for the four census periods during the two years, using a pan-tropical allometric equation (Chave et al. 2014; Eq. 3), $\text{AWB} = 0.0673 \times (\rho D^2 H)^{0.976}$ (3)

where D refers to DBH (cm), H is tree height (m) and ρ represents wood density (g/cm^3). Heights of 783 trees (representing all species and at least 20 trees ≥ 10 cm DBH per plot) were measured using a Vertex III ultrasonic hypsometer and a T3 transponder (Haglöf, Sweden). The heights of all other trees ≥ 10 cm DBH were estimated using diameter-height allometry (Chave et al. 2005). Species-specific wood density was determined for 764 trees by driving an increment borer (Mora, Sweden) into the tree stem ~20 cm above the DBH measurement point. Wood density was calculated by dividing the oven-dry mass (60 °C, 72 h) of the wood core by its fresh volume (Chave 2005). Wood biomass productivity for each tree was the change in AWB during 2018−2020, and summed for all the trees in each plot for each year; when expressed in terms of carbon, we assumed 50 % carbon in wood biomass (Chave et al. 2005, Lewis et al. 2009).

2.2.4 Statistical analyses

We used a factorial analysis of variance (ANOVA) to test the effect of nutrient additions on community-level RGR as well as RGR of different DBH classes, species, leaf habits, and functional groups for each year of the experiment. We separately analysed for each year to isolate possible effects of climate variation or lag response of the RGR to nutrient additions. All parameters were first tested for normal distribution using Shapiro-Wilk's test and equality of variance using Levene's test. Logarithmic or square root transformation was applied when an assumption of the aforementioned tests is violated. For the monthly measured RGR response to nutrient additions, linear mixed-effects (LME) models ('lme'-function in the nlme package) were used to test the fixed effects of treatments (N, P, K, each with two levels) and their interactions. The spatial replication (for the plot-level RGR; n = 4 plots) and time (months) were included in the LME as random effects. A function that allows different variances of the response variable per level of the fixed effect and/or a first-order temporal autoregressive process was included, if this improved the relative goodness of the model fit based on Akaike Information Criterion (AIC). The significance of the fixed effect was evaluated using ANOVA (Crawley, 2009). The LME analyses were performed for the entire period of the experiment as well as for the first and second years separately. If residual plots revealed non-normal distribution or non-homogenous variance, we log-transformed the data and then repeated the analyses. To assess the short-term influence of nutrient addition on tree mortality events among the different DBH classes, the number of dead stems per DBH class in each plot for the entire experimental period (2018−2020) were analyzed using Poisson regression (a generalized linear model appropriate for count data with correction for overdispersion (link = quasipoisson)). Nutrient addition treatment was the predictor and counted mortality event was the response variable. For all analyses, nutrient addition effects were considered significant at $P \leq 0.05$. All statistical analyses were conducted using the R statistical software version 3.6.2 (R Development Core Team 2018).

2.3 Results

We report results of both monthly dendrometer band monitoring of 20 selected individual trees per plot (Fig. 2.2) and growth rates based on annual censuses of all trees in each of the 32 plots (Figs. 2.3, 2.4 and 2.5). Data obtained from dendrometer measurements strongly correlates with the census data ($R^2 = 0.72$, $P < 0.001$; Fig. S2.1). There were no differences in surface soil characteristics among treatment plots before nutrient additions (Table 1.2) and accordingly, differences in tree growth rates can be attributed to nutrient addition treatments and not to inherent differences in soil biochemical characteristics. Overall, we found no treatment effects of nutrient additions on community-level RGR in either the first or second year of the experiment ($F_{1,\ 24}$ = 0.40, 0.37 and P = 0.892, 0.909; Fig. S2.2). Community-level RGR was, however, higher in the second year (0.046 ± 0.006 cm cm^{-1} yr^{-1}) than in the first year (0.025 ± 0.005 cm cm^{-1} yr^{-1}) in the control plots as well as in all other treatments ($t_{(31)} =$ 9.00, $P < 0.0001$; Fig. S2.2). In contrast to wood biomass productivity (Table 2.1), RGR tended to decrease with increasing DHB classes (Table 2.1).

Table 2.1: Vegetation characteristics of the experimental site (mean ± SE; n = 32 or for tree species, n = 27−32 plots). Proportions in the bracket represent the contribution of individual species to the total aboveground wood biomass productivity of the forest.

DBH classes; Species	Tree density (trees ha^{-1})	Mean height (m)	Basal area (m^2 ha^{-1})	AWB † (Mg ha^{-1})	Rel. growth rate †† (cm cm^{-1} yr^{-1})	Wood biomass productivity†† (Mg C ha^{-1} yr^{-1})
1−5 cm	5938 ± 269	−	2.4 ± 0.1	6.1 ± 0.3	0.042 ± 0.007	0.32 ± 0.09
5−10 cm	627 ± 30	−	2.3 ± 0.1	10.4 ± 0.5	0.020 ± 0.005	0.33 ± 0.04
10−30 cm	514 ± 13	16.3 ± 0.1	11.8 ± 0.3	90.8 ± 3.1	0.012 ± 0.001	1.04 ± 0.05
> 30 cm	108 ± 5	30.0 ± 0.4	22.0 ± 1.0	287 ± 17	0.011 ± 0.000	3.35 ± 0.89
Tree species§						
Funtumia elastica	150 ± 13	17.6 ± 0.2	4.2 ± 0.4	29.2 ± 3.1	0.011 ± 0.001	0.41 ± 0.10 (9.3%)
Celtis mildbraedii	92 ± 10	15.2 ± 0.4	2.7 ± 0.4	29.6 ± 5.2	0.007 ± 0.003	0.12 ± 0.07 (2.8%)
Rinorea ardisiaeflora	42 ± 8	13.2 ± 0.3	0.9 ± 0.3	8.8 ± 4.4	0.017	0.06 (1.4%)
Cynometra alexandri	39 ± 4	21.3 ± 1.2	4.2 ± 0.7	70 ± 12.9	0.021 ± 0.003	0.27 ± 0.16 (6.1%)
Celtis zenkeri	39 ± 4	18.0 ± 0.6	1.4 ± 0.2	14.1 ± 2.1	0.008 ± 0.002	0.14 ± 0.06 (3.3%)
Celtis durandii	37 ± 4	28.4 ± 0.6	4.3 ± 0.4	51.0 ± 5.5	0.004 ± 0.002	0.36 ± 0.13 (8.1%)
Lasiodiscus mildbraedii	36 ± 6	14.5 ± 0.5	0.8 ± 0.2	8.1 ± 1.9	0.009 ± 0.004	0.06 ± 0.04 (1.5%)
Trichilia rubescens	28 ± 5	15.5 ± 0.5	0.7 ± 0.1	5.0 ± 1.2	0.026 ± 0.015	0.04 ± 0.01 (0.9%)
Khaya anthoteca	20 ± 2	22.8 ± 1.4	2.4 ± 0.4	28.0 ± 2.5	0.021 ± 0.004	0.86 ± 0.31 (19.6%)
Trichilia prieuriana	15 ± 2	19.1 ± 0.5	0.6 ± 0.1	5.5 ± 1.1	0.033 ± 0.010	0.10 ± 0.04 (2.4%)

[†] AWB: aboveground woody biomass, measured in all plots in April 2018 prior to nutrient addition

[††] Measured from the control plots (n = 4) during the 2018−2020 measurement period

[§] Tree species listed here includes only trees ≥ 10 cm DBH and are the most dominant species in the experimental site (Table S2.1).

2.3.1 Seasonal pattern in tree growth rate

Monthly RGR was highly seasonal and correlated strongly with monthly rainfall ($R^2 = 0.52$, $P < 0.001$; Fig. S2.2), with higher RGR recorded in the wet months than in the dry months (Figs. 2.2, and S2.3). In the first year (June 2018–May 2019) of the experiment, the region experienced a longer dry season (five dry months; annual rainfall = 1695 mm) than in the second year (three dry months; annual rainfall = 2168 mm) with ~ 30 % more rainfall recorded (Fig. 2.2d).

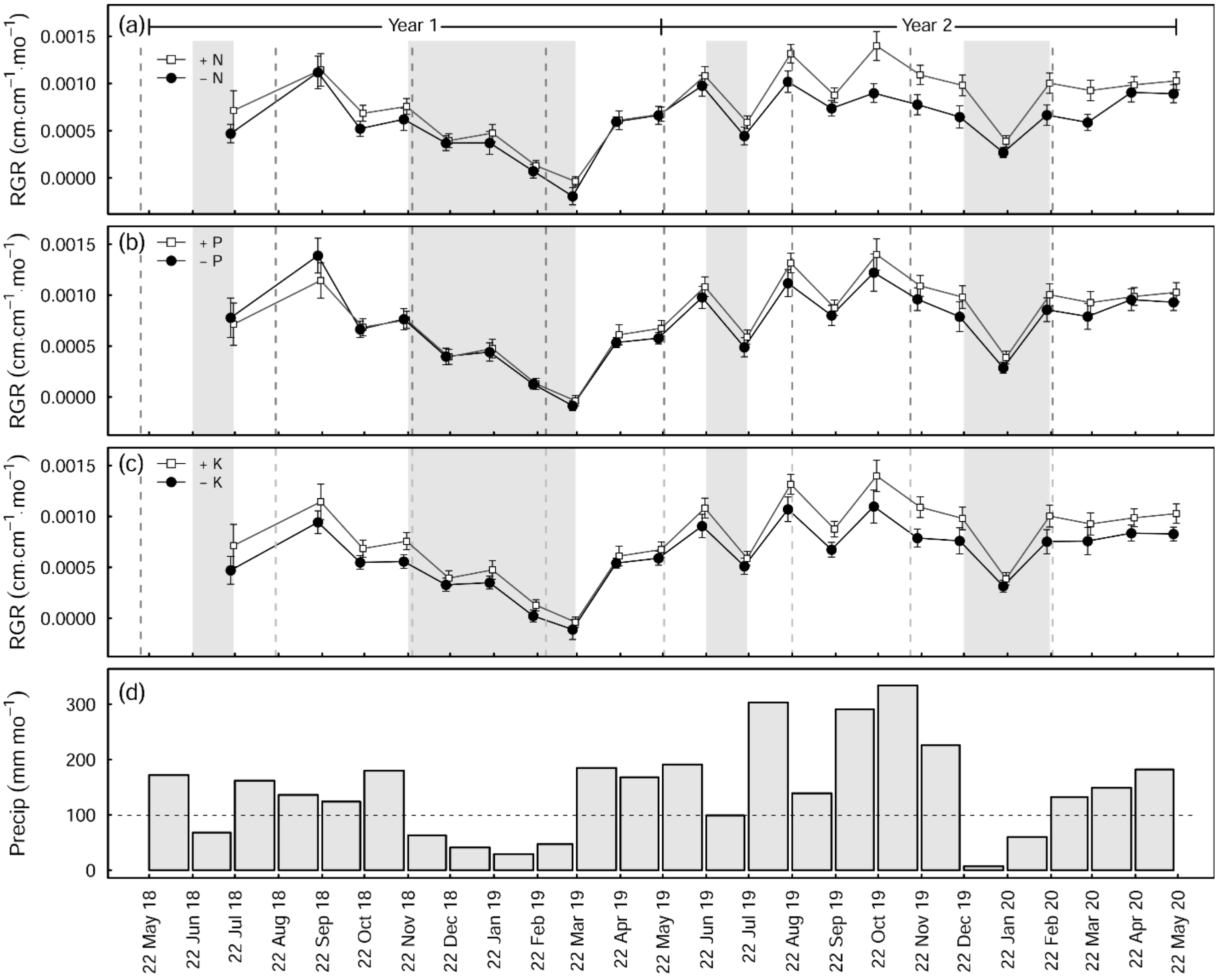

Figure 2.2: Relative growth rate (RGR) responses of trees 10–30 cm diameter at breast height (82 % of 656 trees on which dendrometer bands were installed) to nitrogen (N), phosphorus (P) and potassium (K) additions. Presented are pooled +N (N, N + P, N + K and N + P + K) and −N (control, K, P and P + K) in panel (a); +P (P, N + P, P + K, and N + P + K) and −P (control, N, K and N + K) in panel (b); +K (K, P + K, N + K and N + P + K) and −K (control, N, P and N + P) in panel (c); and monthly precipitation (panel d). Error bars are standard errors of the mean for each treatment aggregation, n = 16 plots. Statistical analysis was based on the eight treatments in the full factorial NPK design where the main effect of N additions was found in the second year (linear mixed-effects model, $F_{1,\,24} = 6.14$, $P = 0.021$). Dotted vertical lines correspond to dates of nutrient additions; grey shades represent dry months (<100 mm mo^{-1} precipitation, shown by the horizontal dotted line in panel d).

2.3.2 Tree growth response to nutrient addition by different tree diameter classes

The response of RGR to nutrient addition was dependent on tree diameter classes (Fig. 2.3). After separate analysis of different diameter classes (1–5 cm, 5–10 cm, 10–30 cm and > 30 cm DBH), no observable nutrient addition effect on the growth rates of young trees (1–10 cm DBH; Table. S2.2) was found. There was, however, an increase in RGR of medium-sized trees (10–30 cm DBH) under N additions in the second year of the experiment ($F_{1, 24} = 4.76$, $P = 0.039$; Fig. 2.3a and Table S2.2), which was well corroborated by the temporal RGR response observed from the trees measured more intensively with dendrometer bands ($F_{1, 24} = 6.14$, $P = 0.021$; Fig. 2.2a). Additionally, there was a negative N × K interaction effect on tree growth, resulting in a decreased RGR among medium-sized trees in the second year ($F_{1, 24} = 4.71$, $P = 0.040$; Fig 2.3b and Table S2.2). Furthermore, while no single nutrient addition was associated with increased stem growth among larger trees (> 30 cm DBH), a positive N × P × K interaction effect on stem growth was found among these larger trees in the second year of the experiment ($F_{1, 24} = 5.56$, $P = 0.027$; Table S2.2). Additions of P or K alone did not have any observable effects on stem growth (Figs. 2.2 and 2.3) in either the first or second year of the experiment, although P additions reduced mortality among the smallest class of saplings (1–5 cm DBH; $P = 0.047$) and increased their survival by 30 %.

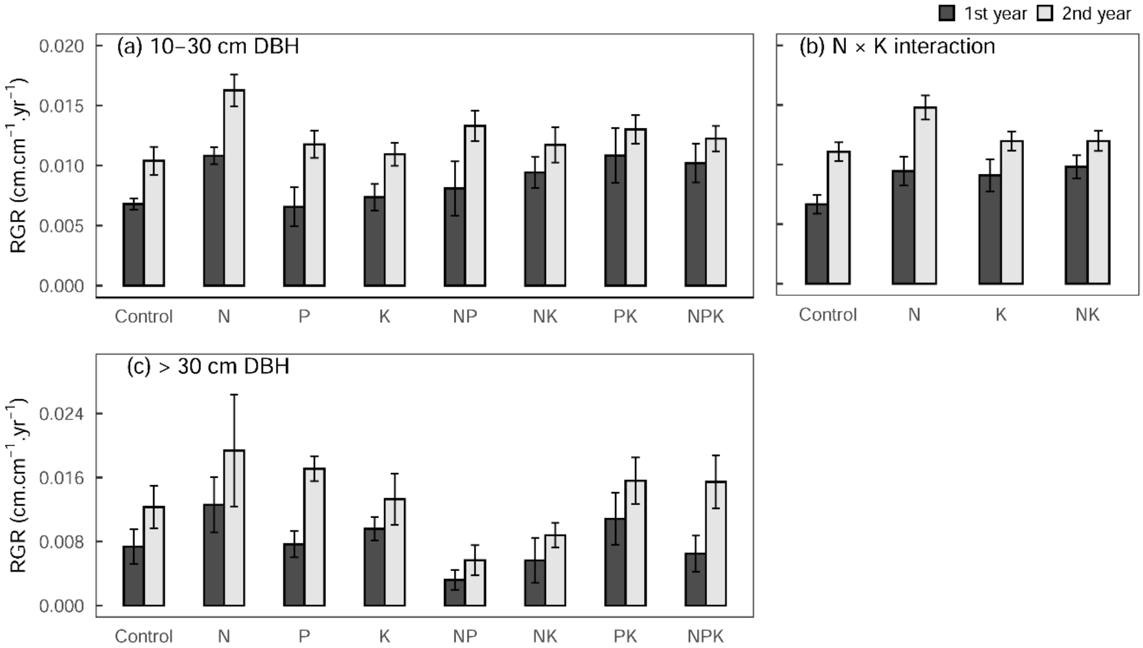

Figure 2.3: Relative growth rate (RGR; mean ± SE) responses of different tree size classes to nutrient additions for trees ≥ 10 cm diameter at breast height (DBH). Census intervals for 2018−2019 and 2019−2020 are reported as first and second years of growth, respectively. Panel (a) presents RGR of trees 10−30 cm DBH, where we found a positive RGR response to N additions in the second year ($F_{1, 24} = 4.76$, $P = 0.039$; n = 4 plots with full factorial ANOVA). Panel (b) illustrates a negative response to N × K interaction for trees 10−30 cm DBH in the second year ($F_{1, 24} = 4.71$, $P = 0.040$; n = 8 plots: Control (control, P), N (N, N + P), K (K, P + K) and NK (N + K, N + P + K), factorial ANOVA). In panel (c), a positive response to N × P × K interaction for trees ≥ 30 cm DBH was found in the second year ($F_{1, 24} = 5.56$, $P = 0.027$; n = 4 plots with full factorial ANOVA).

2.3.3 Tree growth response to nutrient addition by different species, leaf habits and functional groups

Tree growth response to nutrient additions by different tree species was variable and time-dependent (Fig. 2.4). The RGR of *Lasiodiscus mildbraedii* increased significantly under N additions in the second year ($F_{1, 20} = 8.06$, $P = 0.010$) and under P × K interaction effect in both the first and second years of the experiment ($F_{1, 20} = 10.12, 5.06$; $P = 0.005, 036$) (Fig. 2.4e). All other species combined (apart from the five species presented in Fig. 2.4) also had a positive RGR response to P × K interaction effect in the first year ($F_{1, 24} = 5.12$, $P = 0.033$; Fig. 2.4f). In contrast, the RGR of *Funtumia elastica* declined in response to N × P interaction effect in the second year of nutrient additions ($F_{1,\,24}$ = 5.45, P = 0.028; Fig. 2.4a) as did the negative effect of K additions on the RGR of *Lasiodiscus mildbraedii* in the first and second years of the experiment ($F_{1,\,20}$ = 5.68, 13.03; P = 0.027, 0.002; Fig. 2.4e). Among different leaf habits, K additions were associated with increased RGR of semi-deciduous trees in the first year of the experiment when the site experienced a prolonged dry season (Fig. 2.2d). Specifically, we found that the RGR of this leaf habit increased under K additions and P × K interaction effect only in the first year ($F_{1,\,24}$ = 7.48, 4.80 and P = 0.012, 0.042; Fig. 2.5; Table S2.3). Tree growth response to nutrient additions among N-fixers nor non-N-fixer were insignificant in both years of the experiment.

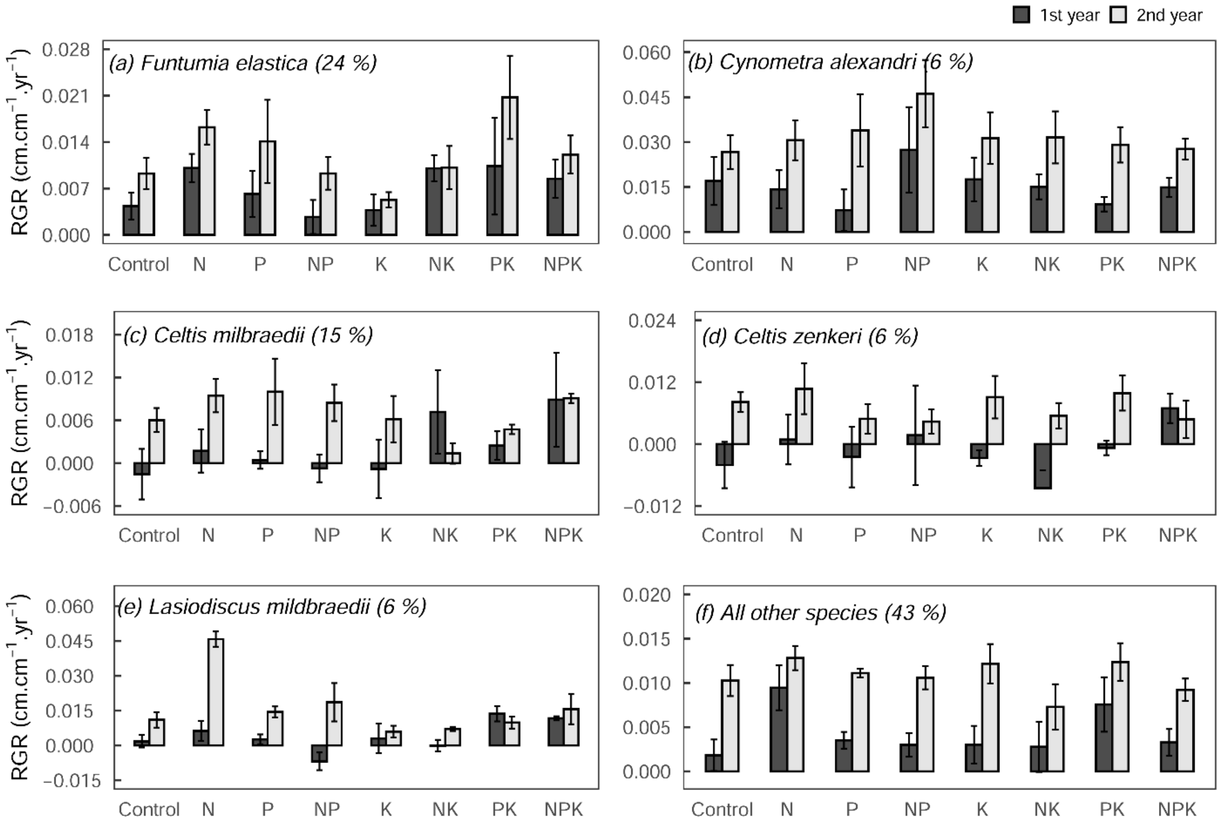

Figure 2.4: Relative growth rate (RGR; mean ± SE) response of different tree species (a−e; representing 57 % of tree abundance and all other species combined (f)) to nutrient additions, for trees ≥ 10 cm diameter at breast height (DBH). Census intervals for 2018−2019 and 2019−2020 are reported as first and second years of growth. All panels present the eight treatments of the full

factorial NPK design. Bars represent the RGR of at least three replicate plots per treatment. The RGR of Funtumia elastica decreased under N × P interaction effects in the second year (panel a; $F_{1, 24} = 5.45$, $P = 0.028$). The RGR of Lasiodiscus mildbraedii increased under N additions in the second year (panel e; $F_{1, 20} = 8.06$, $P = 0.010$), and P × K interactions in the first and second years of nutrient additions (panel e; $F_{1, 20} = 10.12, 5.06$; $P = 0.005, 0.036$), and a declined RGR response to K addition in both years of nutrient addition (panel e; $F_{1, 20} = 5.68, 13.03$; $P = 0.027, 0.002$). The RGR of the remaining species increased under P × K interaction effect in the first year (panel f; $F_{1, 24} = 5.12$, $P = 0.033$).

2.4 Discussion

2.4.1 Community-level tree growth and productivity response to nutrient additions

The tree growth rate in this forest (trees ≥ 10 cm DBH in the control plots; 0.011 ± 0.001 $cm.cm^{-1}.yr^{-1}$) was comparable to those reported from humid tropical forests in Panama (Wright et al. 2011, 2018) but lower than those in Costa Rica (Alvarez-Clare et al. 2013, Waring et al. 2019). Carbon accumulation rate (2.2 ± 0.4 Mg $C.ha^{-1}.yr^{-1}$) in this forest was also comparable to those measured in Panama (Adamek et al. 2009, Wright et al. 2011), Brazil, Colombia and Peru (Aragõo et al. 2009, Malhi et al. 2009, 2011), but was lower than those reported from Costa Rica (Russell et al. 2010). The difference in tree growth and productivity with these other sites may be due to several factors related to species diversity and forest composition (Ammer, 2019), soil fertility (John et al. 2007b), allocation of sequestered carbon between above- and below-ground tree components (Malhi et al. 2004) and climatic regimes (Toledo et al. 2011).

In this forest, as in other seasonal tropical forests, rainfall was the primary driver of tree growth (Toledo et al. 2011, Doughty et al. 2014, Wagner et al. 2014, Guan et al. 2015). This was evident in the close correlation between monthly tree growth and monthly rainfall ($P < 0.001$; Fig. S2.3), where tree growth rates were slow during the dry season and increased in the wet season (Figs. 2.2 and S2.4). Moreover, relative growth rates were consistently higher in the second (wetter) year of the experiment where rainfall was ~ 30 % more than the drier first year (Figs. 2.3−2.5 and Fig. S2.2).

The role of soil nutrients in regulating tree growth at this site was complex and heterogeneous as revealed in this field experiment. Overall, we did not detect a positive community-wide tree growth response to any of the nutrients added (Fig. S2.2), which is consistent with other nutrient manipulation experiments conducted in tropical forests (Alvarez-Clare et al. 2013, Jiang et al. 2018, Wright et al. 2018, Waring et al. 2019). The lack of a community-wide response can be explained by the diverse nature of the forest and varying nutrient acquisition strategies of (1) the many different species (126 species) that exist at the site (Turner et al., 2018); (2) the different tree sizes and phenological stages and (3) tree position in the canopy (shaded understory to large trees in the overstory; Zemunik et al. 2018). Moreover, tree species at this forest are adapted to the soil's nutrient supply and may accordingly have limited potential to increase stem growth rates in response to nutrient enrichment in the short term (Coley et al. 1985, Wright et al. 2018). Although this could mean a longer time is needed to observe significant community-wide tree growth responses to nutrient additions (Townsend et al. 2008, Vitousek et al. 2010, Wright et al. 2011, Alvarez-Clare et al. 2013), a longer experimental period does not guarantee tree growth responses, possibly due to confounding effects of climate patterns and/or pest susceptibility, as was the case for the nutrient manipulation experiment in Gigante, Panama (Wright et al. 2018), where they did not observe community-scale growth response despite 15 years of chronic nutrient additions.

2.4.2 Tree growth response to nutrient additions by different tree diameter classes

The DBH-dependent response of stem growth to nutrient additions at our site was similar to those observed in other tropical forests (Adamek et al. 2009, Wright et al. 2011, Alvarez-Clare et al. 2013). Large trees, which have low relative growth rates yet accounted for a large portion of the forest's wood biomass productivity (Table 2.1), did not respond to individual nutrient additions (Fig. 2.3c). These large trees, however, did exhibit a significant response to the N × P × K interaction effect (Table S2.2), which highlights the intrinsic complementary link among the functions of these nutrients in the production of new woody and reproductive tissues (flowers, fruits and seeds) (Rietra et al. 2017).

It was the medium-sized trees (10−30 cm DBH) that responded positively to N addition (Fig.1a and 2a), similar to the study of Adamek et al. (2009). This N limitation became evident approximately 1.25 years after the experiment began (August 2019), after which stem growth rates remained consistently higher for the duration of the experiment (Fig. 2.2a). We suspect that at the onset of the rainy season (April 2019) there was sufficient nutrient availability for tree growth in all treatments (Fig. S2.4) as a result of the rapidly decomposing litter (Kagezi et al. 2016), most of which fell during the dry season (~ 50 % of annual litterfall). However, when nutrient release from decomposition subsided (leaf litter had decomposed after about 5 months, based on ancillary data) and the plant-available N decreased, tree growth limitations in N addition plots became pronounced (Figs. 2.2a and S2.4). Considering it was still the rainy season, and these medium-sized trees have their leaves in the upper part of the canopy, tree growth would not have been constrained by either moisture or light, but mainly by N availability.

Surprisingly, the diameter increment of saplings and poles (1−10 cm DBH) did not respond to either N, P or K addition as others have observed (Table S2.2; Alvarez-Clare et al. 2013, Wright et al. 2011). We did however find that P additions reduced tree mortality among the smallest trees sizes (1−5 cm DBH), a trend also observed by Alvarez-Clare et al. (2013). We suspect an intense competition in the dense understory (Table 2.1) for resources such as nutrients and light. Considering the relative importance of P for sapling survival (Alvarez-Clare et al. 2013), the growth of these small trees may be potentially co-limited by both P and light availability (Chou et al. 2018), which could explain why the addition of P alone did not increase growth.

2.4.3 Tree growth response to nutrient additions by different species, leaf habits and functional trait

The varied growth responses of different species to nutrient additions (Fig. 2.4) was similar to those reported for other species-rich tropical forests (Cárate-Tandalla et al. 2018, Turner et al. 2018). In this study, only two of the five most abundant tree species exhibited significant stem growth increases or decreases in response to nutrient addition (Fig. 2.4). These two species (*Funtumia elastica and Lasiodiscus mildbraedii)* account for 30 % of the individuals analyzed. Such early responses by a few species are however not uncommon in highly species-diverse tropical forests (characterised by high abundance of a few species and many rare species), where different tree species have varying nutrient requirements, resource acquisition and adaptation strategies (Chou et al. 2018, Detto et al. 2018, Waring et al. 2019).

Nitrogen addition increased tree growth rates by 80 % in *Lasiodiscus mildbraedii*, which suggests that this species was N-limited. All individuals of this species were medium-sized; again, highlighting that even among different species N-limitation is predominantly evident in this tree size class.

Notably, in the drier first year, semi-deciduous tree species increased stem growth by 46 % (Fig. 4c) under +K compared to –K additions. This positive RGR response in the drier year (Fig.

2.5 and Table S2.3) indicate that these semi-deciduous tree species, which would normally have lost many leaves under the prolonged dry months, might have been able to delay leaf shedding (R. Manu, *unpublished data*), maintain photosynthesis during this period and enhance stem growth under elevated K availability (Hasanuzzaman et al. 2018, Wu et al. 2020). Furthermore, it is recognized that K, in particular, plays an important role in controlling cell signaling (e.g. activation of reactive oxygen species) and stomatal regulation in plants, particularly under water-deficit conditions, thereby alleviating drought stress (Detto et al. 2018, Hasanuzzaman et al. 2018, Wu et al. 2020).

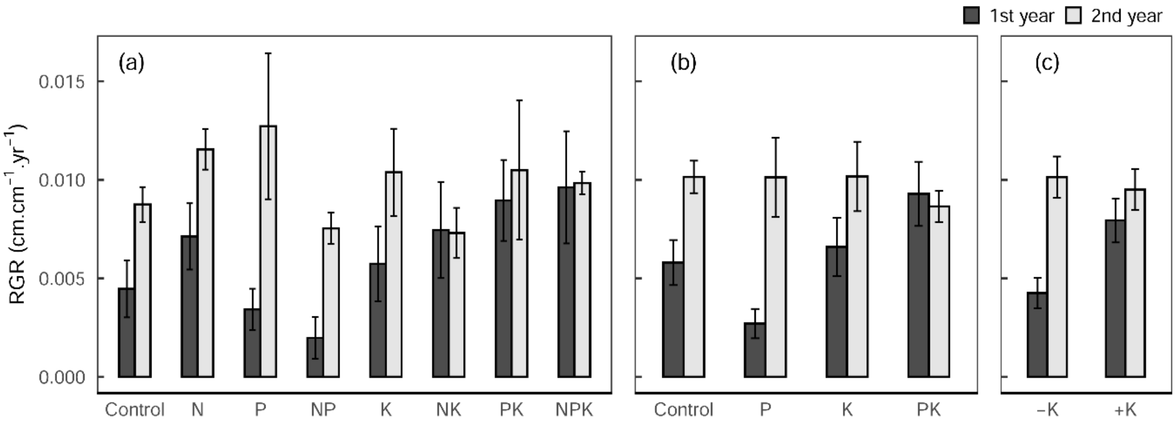

Figure 2.5: Relative growth rate (RGR; mean ± SE) responses of semi-deciduous trees (≥ 10 cm diameter at breast height) to nutrient additions. Census intervals for 2018−2019 and 2019−2020 are reported as first and second years of growth. Panel (a) presents the eight treatments of the full factorial NPK design (n = 4 plots). To the right are the four treatments with K additions, illustrating the positive effect of K addition on RGR in the first year ($F_{1, 24} = 7.48$, $P = 0.012$). Panel (b) presents the increased RGR under P × K interaction effect in the first year ($F_{1, 24} = 4.80$, $P = 0.042$). Each treatment represents eight plots: Control (control, N), P (P, P + K), K (K, P + K) and PK (P + K, N + P + K)). Panel (c) presents the significant effect of K addition for the first year. Treatments without added K (control, +N, +P, +NP) are pooled (denoted -K on horizontal axis) and treatments with added K (+K, +NK, +PK, +NPK) are pooled. Thus, the sample sizes are (a) 4 plots, (b) 8 plots and (c) 16 plots for each bar.”

In contrast to our hypothesis, that P would generally limit tree growth (Walker and Syers 1976, Vitousek et al. 2010) and specifically among N-fixing tree species (Waring et al. 2019), there was no positive response in stem growth as a result of P additions, as evident in the growth rates of any species (Figs. 2.2−2.4). This is not particularly surprising, however, considering that extractable P at this forest site was higher or comparable to other tropical forests (Allen et al. 2015, Newbery et al. 2002). The higher extractable P is attributed to the near-neutral soil pH at this site (Table 1.1), which means that P is not fixed by hydrous oxides of Fe and Al, and hence sufficient P could be available for plant uptake. Therefore, in accordance with the recent review by Wright (2019), neither the prediction that P-limitation is widespread on old, highly weathered soils (Cárate-Tandalla et al. 2018, Turner et al. 2018, Vitousek et al. 2010, Walker and Syers 1976) nor a generalized P-limitation in N-fixing tree species (Waring et al. 2019) is supported.

2.4.4 Nutrient interaction effects on tree growth

The rationale of nutrient additions in this experiment was to identify which nutrient(s) would cause a positive stem growth response and thereby reveal nutrient (co-)limitations on tree growth. However, while most observed growth responses to nutrient additions were positive, there were a few cases, in which reduced RGR were observed. This was the case in two tree species (*Funtumia elastica and Lasiodiscus mildbraedii)*) under different nutrient additions (Fig. 2.4). Although, decreased stem growth response to nutrient additions have been reported elsewhere (Báez and Homeier 2018, Braun et al. 2010, Pedas et al. 2011), it is not entirely clear what mechanisms could explain this observation at our site. We suspect that among other things, intense seasonal variability (prolonged dry period in the first year; Figs. 2.2, 2.4c−d and S2.4) and nutrient interaction effects may have contributed to these observations. Nutrient interaction effects may arise when the addition of one nutrient affects the availability, uptake, function and distribution of another nutrient. Specifically, the effect of N × K interactions resulted in decreased relative growth rates in the medium-sized trees (Fig 2.3b), where gains would normally have been made if N alone had been applied. A similar trend was also observed by Jiang et al. (2018), where tree stem growth rates in N + P plots were lower than those in either N or P addition plots. This could also be caused by nutrient antagonism (Rietra et al. 2017); nutritional imbalances (Boccuzzi et al. 2021) or an adjustment in biomass allocation to other organs other than for stem growth (leaves, roots or reproductive organs) (Wright et al. 2011, Alvarez-Clare et al. 2013).

2.5 Conclusions

The response of tree growth and wood biomass productivity to nutrient additions offers many important insights into understanding the magnitude and direction of nutrient limitations in this Afrotropical forest with implications on previous widely adopted hypotheses. First, our results strongly suggest that, in addition to rainfall, tree growth in this semi-deciduous forest was dominantly controlled by N availability, occasionally by K availability (climate-dependent), but not P availability. While the concept of multiple nutrient limitation was supported, neither the prediction that P-limitation is widespread on old, highly weathered soils nor P-limitation on N-fixing tree species was supported in this diverse forest ecosystem. Second, the response of tree growth to nutrient additions was dependent on tree sizes, in which neither small nor large trees but medium-sized trees positively responded to N additions suggesting that these medium-sized trees, under conditions of sufficient rainfall and light, were indeed N limited. Third, tree growth response to nutrient addition at our site was species-dependent and not community-wide. Our data suggest that rainfall and leaf habits mediate tree growth responses to nutrient additions and that seasonal and inter-annual changes in rainfall may regulate the relative importance of nutrients and their requirement by forest plants. Finally, considering that to our knowledge, this study is the first in tropical Africa and the second worldwide to include N, P and K in a factorial large-scale ecosystem nutrient manipulation experiment, additional studies are crucial to advance our understanding of the mechanisms of nutrient control on carbon assimilation.

2.6 Acknowledgements

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2.7 Appendix S2

Table S2.1: Ecological characteristics and functional traits of all tree species identified in all 32 plots at the Budongo Forest Reserve, Uganda. The assignment of tree species into a family, leaf habits and light guilds were based on an extensive literature survey (Hawthorne, 1995; Sheil, 1996, and http://tropical.theferns.info/query.php; accessed in May 2019) as well as expert knowledge from botanists in Uganda. The N-fixing tree species were identified from a world database of legumes "International Legume Database & Information Service (https://ildis.org/LegumeWeb/; accessed on 14.06.2019)".

Species†	**Stem contribution (%)**	**Family**	**Light guild**	**Leaf habits**	**N-fixing species?**	**Wood density (g/cm^3)**	**IVI*** **(%)**
Funtumia elastica	24.12	Apocynaceae	NPLD	Evergreen	No	0.48	40.24
Celtis mildbraedii	14.81	Cannabaceae	Shade bearer	Semi-deciduous	No	0.68	26.39
Cynometra alexandri	6.23	Fabaceae	Shade bearer	Evergreen	Yes	0.71	23.07
Celtis zenkeri	6.07	Cannabaceae	NPLD	Deciduous	No	0.64	13.81
Lasiodiscus mildbraedii	5.85	Rhamnaceae	Shade bearer	Evergreen	No	0.80	12.18
Celtis durandii/gomphophylla	5.53	Cannabaceae	NPLD	Semi-deciduous	No	0.58	21.20
Rinorea ardisiaeflora	5.50	Violaceae	Shade bearer	Evergreen	No	0.65	10.91
Trichilia rubescens	4.06	Meliaceae	NPLD	Evergreen	No	0.55	9.31
Khaya anthoteca	2.89	Meliaceae	NPLD	Semi-deciduous	No	0.53	12.80
Tapura fischeri	1.64	Dichapetalaceae	Shade bearer	Evergreen	No	0.68	5.79
Trichilia prieuriana	1.60	Meliaceae	Shade bearer	Evergreen	No	0.63	5.34
Margeritaria (Phyllanthus) discoideus	1.26	Phyllanthaceae	Pioneer	Deciduous	No	0.68	7.57
Trilepisium madagascarensis	1.16	Moraceae	NPLD	Evergreen	No	0.52	5.11
Aningeria altissima	1.10	Sapotaceae	NPLD	Deciduous	No	0.60	4.53
Croton sylvaticus	1.04	Euphorbiaceae	Pioneer	Semi-deciduous	No	0.54	5.01
Alstonia boonei	1.01	Apocynaceae	Pioneer	Deciduous	No	0.33	9.27
Antiaris toxicaria	1.01	Moraceae	NPLD	Deciduous	No	0.43	3.92
Caloncoba schweinfurthii	0.97	Flacourtiaceae	Pioneer	Deciduous	No	0.57	3.84
Myrianthus holstii (forest pineapple)	0.82	Urticaceae	Shade bearer	Deciduous	No	0.54	2.78
Chrysophyllum albidum	0.69	Chrysophylloideae	Shade bearer	Evergreen	No	0.64	3.47
Desplatsia dewevrei	0.69	Malvaceae	Shade bearer	Evergreen	No	0.49	2.96
Maesopsis eminii	0.69	Rhamnaceae	Pioneer	Evergreen	No	0.43	5.40
Lychnodiscus cerospermus	0.57	Sapindaceae	Shade bearer	Unclassified	No	0.71	2.15
Ehretia cymosa	0.53	Boraginaceae	Pioneer	Deciduous	No	0.48	2.50
Tabernaemontana holstii	0.41	Apocynaceae	Shade bearer	Evergreen	No	0.53	1.79
Tetrapleura tetraptera	0.41	Fabaceae	Pioneer	Deciduous	Yes	0.62	2.14
Entandrophragama angolense	0.38	Meliaceae	NPLD	Deciduous	No	0.59	1.31
Blighia unijugata	0.31	Sapindaceae	Shade bearer	Evergreen	No	0.55	1.59
Chrysophyllum perpulchrum	0.31	Chrysophylloideae	Shade bearer	Evergreen	No	0.67	1.65
Guarea cedrata	0.31	Meliaceae	Shade bearer	Evergreen	No	0.57	1.50
Greenwayodendron suaveolens	0.28	Annonaceae	Shade bearer	Deciduous	No	0.66	1.31

Monodora lactea / mesozygia	0.28	Annonaceae	Shade bearer	Deciduous	No	0.58	1.42
Psidium guajava	0.28	Myrtaceae	Savanna	Evergreen	No	0.55	0.74
Vitex amboniensis	0.28	Lamiaceae	Unclassified	Evergreen	No	0.53	1.26
Belonophora hypoglauca	0.25	Rubiaceae	Pioneer	Unclassified	No	0.72	1.07
Milicia (Chlorophora) excelsa	0.25	Moraceae	Unclassified	Deciduous	No	0.50	1.88
Monodora angolensis	0.25	Annonaceae	Shade bearer	Deciduous	No	0.51	1.09
Drypetes sp.	0.22	Euphorbiaceae	Shade bearer	Deciduous	No	0.72	1.05
Putranjiva ugandensis	0.22	Putranjivaceae	Unclassified	Evergreen	No	0.74	0.91
Ficus exasperata	0.22	Moraceae	Pioneer	Deciduous	No	0.36	1.75
Ficus sur (capensis/vogelana)	0.22	Moraceae	Pioneer	Evergreen	No	0.37	1.35
Leptonychia mildbraedii	0.22	Malvaceae	Shade bearer	Unclassified	No	0.57	0.79
Ricinodendron heudelotii	0.22	Euphorbiaceae	Pioneer	Deciduous	No	0.29	3.65
Antidesma laciniatum	0.19	Euphorbiaceae	Shade bearer	Unclassified	No	0.63	1.01
Baphia wollastonii	0.19	Fabaceae	Shade bearer	Evergreen	Yes	0.72	0.82
Celtis wightii / philippensis	0.19	Cannabaceae	Shade bearer	Evergreen	No	0.68	1.42
Strombosia scheffleri	0.19	Olacaceae	Shade bearer	Evergreen	No	0.70	0.96
Alangium chinense	0.16	Cornaceae	Pioneer	Evergreen	No	0.50	0.98
Bequaerhodendron oblanceolatum	0.16	Sapotaceae	Shade bearer	Unclassified	No	0.62	0.45
Chrysophyllum muerense	0.16	Chrysophylloideae	Shade bearer	Unclassified	No	0.53	0.66
Cleistopholis patens	0.16	Annonaceae	Pioneer	Unclassified	No	0.24	1.05
Glyphaea brevis	0.16	Tiliaceae	Shade bearer	Deciduous	No	0.63	0.69
Holoptelea grandis	0.16	Ulmaceae	Pioneer	Deciduous	No	0.54	0.48
Klainedoxa gabonensis	0.16	Irvingiaceae	NPLD	Evergreen	No	0.72	1.52
Trichilia dregeana	0.16	Meliaceae	Unclassified	Evergreen	No	0.60	0.85
Alchornea laxiflora	0.13	Euphorbiaceae	Shade bearer	Deciduous	No	0.41	0.54
Aphania senegalensis	0.13	Sapindaceae	Unclassified	Evergreen	No	0.76	0.55
Fangara angolensis	0.13	Rutaceae	Unclassified	Deciduous	No	0.51	1.07
Lovoa trichilioides	0.13	Meliaceae	NPLD	Evergreen	No	0.61	0.68
Mildbraediodendron excelsum	0.13	Fabaceae	NPLD	Deciduous	Yes	0.83	2.17
Pycnanthus angolensis	0.13	Myristicaceae	NPLD	Evergreen	No	0.54	0.68
Tetrorchidium didymostemon	0.13	Euphorbiaceae	Pioneer	Evergreen	No	0.53	0.97
Unknown spp. ‡	**0.13**	**Unknown spp.**	**Unclassified**	**Unclassified**	**No**	**0.60**	**0.40**
Albizia glaberrima	0.09	Leguminosae	Pioneer	Deciduous	Yes	0.63	1.46
Albizia zygia	0.09	Leguminosae	NPLD	Deciduous	Yes	0.55	0.51
Bridelia micrantha	0.09	Euphorbiaceae	Pioneer	Deciduous	No	0.61	0.49
Coffea canephora	0.09	Rubiaceae	Shade bearer	Evergreen	No	0.53	0.50
Canarium scheinfurthii	0.09	Burseraceae	NPLD	Unclassified	No	0.64	0.77
Erythrophleum suaveolens	0.09	Fabaceae	NPLD	Evergreen	Yes	0.70	1.45
Ficus variifolia	0.09	Moraceae	Pioneer	Unclassified	No	0.44	0.88
Melanodiscus sp.	0.09	Sapindaceae	Unclassified	Unclassified	No	0.59	0.50
Macaranga monandra	0.09	Euphorbiaceae	Pioneer	Evergreen	No	0.33	0.62
Teclea nobillis	0.09	Rutaceae	Shade bearer	Evergreen	No	0.70	0.51
Balsamocitrus dawei	0.06	Rutaceae	Shade bearer	Deciduous	No	0.49	0.35

Coffea robusta	0.06	Rubiaceae	Shade bearer	Evergreen	No	0.69	0.33
Entandrophragama utile	0.06	Meliaceae	NPLD	Unclassified	No	0.59	0.36
Fagaropsis angolensis	0.06	Rutaceae	Unclassified	Deciduous	No	0.68	0.46
Leptaulus daphnoides	0.06	Cardiopteridaceae	Shade bearer	Evergreen	No	0.49	0.37
Lannea welwitchii	0.06	Anacardiaceae	Pioneer	Evergreen	No	0.54	0.50
Antiocarion spp	0.03	Antiocarion spp	NPLD	Unclassified	No	0.55	0.44
Antidesma membrenensi	0.03	Euphorbiaceae	Shade bearer	Unclassified	No	0.67	0.16
Cola gigantea	0.03	Sterculiaceae	NPLD	Deciduous	No	0.46	0.17
Cordia millenii	0.03	Boraginaceae	Pioneer	Deciduous	No	0.38	0.22
Ficus polita	0.03	Moraceae	Savanna	Evergreen	No	0.39	0.67
Mallotus oppositifolia	0.03	Euphorbiaceae	Unclassified	Unclassified	No	0.53	0.16
Maerua duchesnei	0.03	Capparaceae	Shade bearer	Evergreen	No	0.56	0.17
Mitragyna stipulosa	0.03	Rubiaceae	Unclassified	Evergreen	No	0.52	0.18
Rothmannia urcellifomis	0.03	Rubiaceae	Shade bearer	Evergreen	No	0.78	0.16
Rauvolfia vomitoria	0.03	Apocynaceae	Pioneer	Evergreen	No	0.46	0.17
Sachrebera arborea	0.03	Oleaceae	Pioneer	Evergreen	No	0.61	0.77
Staudtia kamerunensis	0.03	Myristicaceae	Unclassified	Evergreen	No	0.42	0.17
Trema orientalis	0.03	Cannabaceae	Pioneer	Evergreen	No	0.26	0.18
Trichilia africana	0.03	Meliaceae	Unclassified	Evergreen	No	0.61	0.16
Uvariopsis congensis	0.03	Annonaceae	Shade bearer	Evergreen	No	0.65	0.18

NPLD: non-pioneer light demander, *IVI: importance value index (Curtis and McIntosh 1950) is based on all trees in all plots.

† These species represent trees ≥ 10 cm in diameter at breast height. ‡ Four individual trees (0.13 %) could not be identified to the species level (***Unknown spp.***). A few species contributing ~ 2 % of the total tree count were 'Unclassified' under light guild or leaf habit.

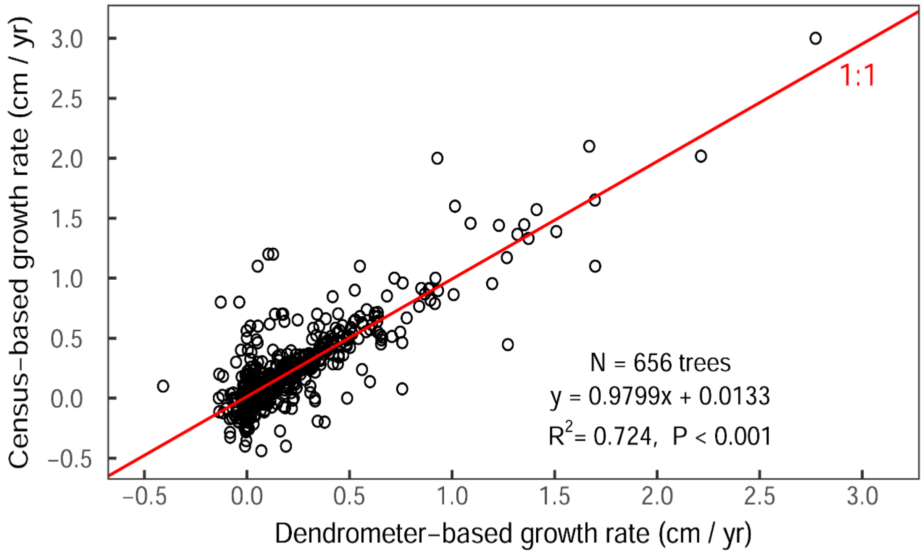

Figure S2.1: Relationship between census-based tree growth rate (using diameter tape) and dendrometer band-based growth rate on selected trees. Data points are trees with ≥ 10 cm diameter at breast height, measured with both dendrometer bands and diameter tape (during censuses) during the two-year measurement period.

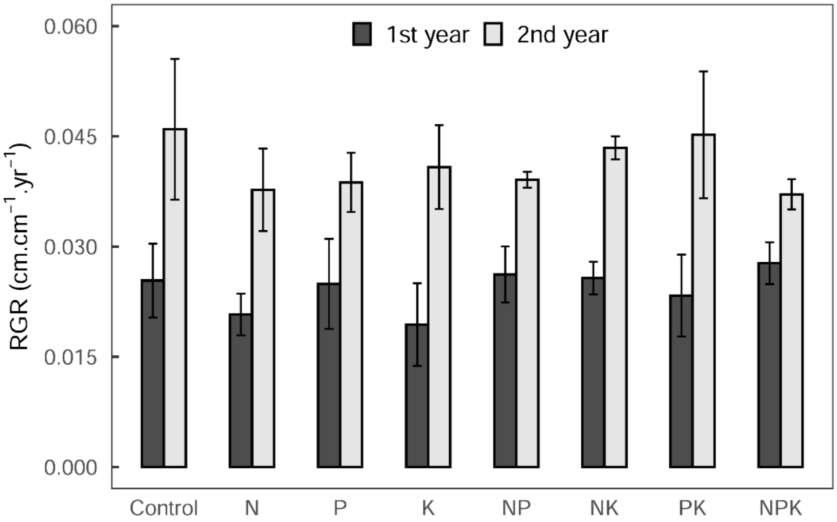

Figure S2.2: Community-level relative growth rate (RGR; mean ± SE; n = 4 plots) of all trees ≥ 1 cm diameter at breast height. Census intervals for the first and second years are 2018−2019 and 2019−2020, respectively. No treatment effects on community-level RGR was found in the first and second years of the experiment.

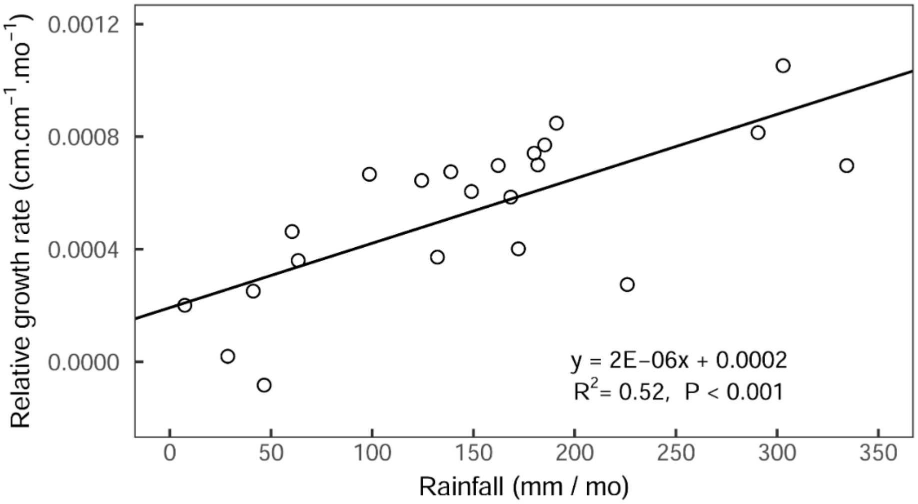

Figure S2.3: Relationship between monthly relative growth rate in the control plots and monthly rainfall. Data points are trees ≥ 10 cm diameter at breast height, on which dendrometer bands were installed.

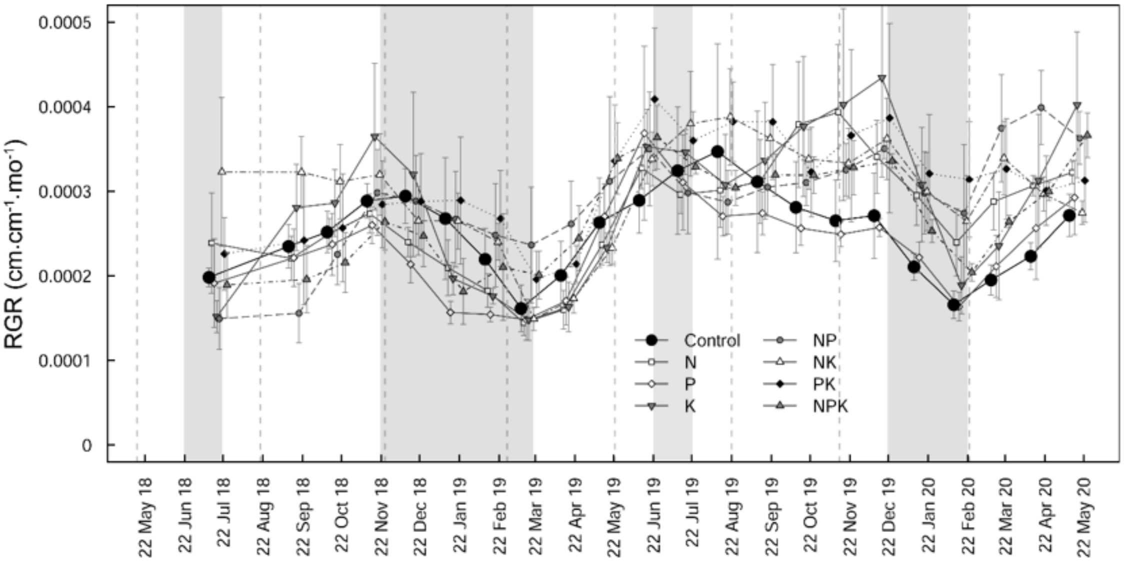

Figure S2.4: Monthly relative growth rates (RGR; mean ± SE; n = 4) of trees ≥ 10 cm diameter at breast height, measured on 20 trees in each replicate plot with dendrometer bands. No treatment effects on RGR was found across both years of measurement; statistical analysis was based on the nutrient addition treatments in the full factorial NPK design

Table S2.2: Factorial ANOVA on relative growth rate response to nutrient addition for different tree size classes in the first and second years of nutrient additions. Given are F statistics and P values. Bold P values indicate significant effects of treatment.

Treatment	1−5 cm DBH		5−10 cm DBH		10−30 cm DBH		>30 cm DBH	
	$F_{(1, 24)}$	P value	$F_{(1, 24)}$	P value	$F_{(1, 24)}$	P value	$F_{(1, 24)}$	P value
2018−2019								
N	0.125	0.726	0.138	0.714	2.559	0.123	1.224	0.279
P	1.353	0.256	0.527	0.475	0.088	0.770	1.059	0.314
K	0.011	0.917	0.002	0.966	1.632	0.214	0.066	0.799
N × P	0.085	0.773	0.007	0.935	1.406	0.247	2.207	0.150
N × K	1.250	0.275	0.279	0.602	0.891	0.355	1.792	0.193
P × K	0.007	0.932	0.204	0.656	2.710	0.113	2.710	0.113
N × P × K	0.478	0.496	0.653	0.427	0.002	0.966	1.874	0.184
2019−2020								
N	1.461	0.239	0.032	0.860	4.764	**0.039**	0.865	0.362
P	0.123	0.729	0.611	0.442	0.088	0.769	0.000	0.999
K	0.098	0.757	0.035	0.854	1.250	0.275	0.018	0.894
N × P	0.113	0.740	0.004	0.954	2.965	0.098	2.124	0.158
N × K	0.045	0.834	0.287	0.598	4.705	**0.040**	0.001	0.978
P × K	0.207	0.653	0.047	0.831	1.497	0.233	3.407	0.077
N × P × K	1.700	0.205	0.167	0.686	0.656	0.426	5.555	**0.027**

Table S2.3: Factorial ANOVA on relative growth rate response to nutrient addition by different leaf habits in the first and second years of nutrient additions. Given are F statistics and P values. Bold P values indicate significant effects of treatment.

Treatment	Relative growth rate (cm.cm^{-1}.yr^{-1})			
	2018−2019		2019−2020	
Evergreen	F $_{(1, 24)}$	P value	F $_{(1, 24)}$	P value
N	1.614	0.206	0.299	0.590
P	0.516	0.480	0.178	0.677
K	0.798	0.380	0.008	0.929
N × P	0.901	0.352	1.497	0.233
N × K	0.482	0.494	0.870	0.360
P × K	0.079	0.871	0.007	0.934
N × P × K	0.182	0.673	2.241	0.147
Semi-deciduous				
N	0.443	0.512	0.370	0.544
P	0.023	0.882	0.060	0.809
K	7.480	**0.012**	0.371	0.548
N × P	0.913	0.349	0.316	0.579
N × K	0.047	0.831	0.021	0.886
P × K	4.800	**0.042**	0.684	0.416
N × P × K	0.319	0.577	3.508	0.073
Deciduous				
N	3.622	0.069	0.233	0.634
P	1.152	0.294	0.264	0.612
K	0.464	0.502	0.146	0.704
N × P	0.376	0.546	0.207	0.654
N × K	0.531	0.473	0.119	0.734
P × K	1.384	0.251	0.113	0.302
N × P × K	1.043	0.317	1.517	0.230

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CHAPTER 3

Multiple nutrients regulate leaf litter production and foliar quality in a semi-deciduous tropical forest in Africa

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Manuscript in preparation

Abstract:

Identifying nutrient limitations on primary productivity in tropical forests remain an important task in ecology, particularly, as these forests play a critical role in storing and sequestering large quantities of carbon. Here, we report the results of a large-scale, full factorial, nutrient manipulation experiment conducted in a humid semi-deciduous tropical forest in northwestern Uganda. We added nitrogen (N), phosphorus (P), potassium (K), their combinations (N+P, N+K, P+K, and N+P+K) and control at the rates of 125 kg N ha^{-1} yr^{-1}, 50 kg P ha^{-1} yr^{-1} and 50 kg K ha^{-1} yr^{-1}, split into four equal applications and investigated the effects of N, P and K on litter biomass productivity and foliar quality. After three years (May 2018−April 2021) of bi-weekly litter collections: (1) Total annual fine litter production was not significantly affected by nutrient fertilization in the short term, although we observed a trend towards higher annual fine-litter production in the N addition plots, which may become stronger with continued nutrient additions. (2) Leaf litterfall reduced significantly with P and K additions markedly following a prolonged dry season in the first year of the experiment. This highlights the key role of K in stomatal regulation in plant leaves and P in maintaining the water balance in trees, thereby ensuring stress tolerance during water-deficit conditions. (3) Foliar N (sunlit leaves) and leaf litter C content increased with all three nutrient additions (N × P × K interactions) suggesting that all three nutrients played complementary roles in enhancing C assimilations in this site. Additionally, foliar nutrient content and their response to fertilization varied considerably among tree species, specifically, nitrogen additions increased foliar contents of N (7%) and C (5%) in CMI. Phosphorus additions increased foliar P content by 33 % in CDU. Foliar N content of CYA (a legume) was enhanced under N × P interaction effects. Lastly, the effect of K additions on foliar K content was insignificant among the four dominant species studied. Overall, our results show that multiple nutrients rather than a single nutrient regulated leaf litter production rates and foliar chemistry.

Keywords: *nutrient limitation, leaf litter, sun-lit leaves, Budongo forest, highly weathered soils, Lixisols*

3.1 Introduction

Litter production and their subsequent decomposition represent an important pathway of nutrient release into soils and play a major role in regulating nutrient cycling and energy flow for the functioning of terrestrial ecosystems. These processes are even more important for primary productivity in tropical forests where most underlying soils are old, highly weathered and generally nutrient-poor (Hedin et al. 2009, Wright et al. 2011). The return of organic matter to soil surfaces through litterfall does not only have a bearing on soil fertility but also on saprotrophs in the food chain, soil formation and other biogeochemical processes in the forest (Swift et al. 1979). Species composition and climatic conditions such as temperature and rainfall are known controllers of the amount and pattern of litterfall worldwide (Parsons et al. 2014, Zhang et al. 2014). Although litterfall constitutes about two-third of aboveground net primary productivity (ANPP) in most tropical forests, how nutrient availability controls litter production and other related ecosystem processes are still poorly understood (Sollins 1998, Vitousek et al. 2004). Only a few direct experimental evidence exist for a geographically selective region (the Americas), which have revealed nutrient (co-) limitations to tree stem growth, litter production, decomposition and microbial processes (Kaspari et al. 2008, Adamek et al. 2009, Vitousek et al. 2010, Wright et al. 2011). However, the spatial patterns of these nutrient limitations remain unclear, particularly for the understudied African tropical forests. It is believed that in species-diverse forests as in the tropics, interspecific differences in adaptation to nutrient supplies, investment in leaf vitality or defences and different tree sizes (Coley et al. 1985, Grime et al. 1996a) may generate a mismatch among soil-, plant woody tissues-, and foliar (fresh or litter)- mineral nutrients.

The pioneering work of Redfield (1958) in which he argued that the elemental composition i.e. carbon (C) nitrogen (N), and phosphorus (P) of marine phytoplankton occur in a specific atomic ratio (C_{106}: N_{16}: P_1) and that the abundance of these elements is regulated by mutual interactions occurring between the organism and its environment has triggered increased research in ecological stoichiometry. These principles have and continue to provide insight into the nutrient status, the nature of nutrient limitations to primary productivity and biogeochemistry in both aquatic and terrestrial ecosystems. Altered nutrient stoichiometry in plants and microbes could in turn influence nutrient balance, energy flow and consequently carbon storage in living and non-living organic matter. This is because, the relationships between C, N and P cycling, for example, are fundamentally linked in living organisms and the biosphere as a whole (Redfield 1958, Kaspari and Powers 2016). For instance, an increase in atmospheric carbon dioxide can lead to increased rates of photosynthesis (C fixation) and a higher C:N ratio in primary producers. The C:N of litter can in turn alter rates of mineralization by microbes (Aber and Melillo 1980) and thus nutrient availability for plant uptake. Understanding how these nutrients are coupled with each other either in different species or at the ecosystem level is crucial for the accurate prediction of the consequences of anthropogenic perturbations to terrestrial nutrient biogeochemistry.

Through a large-scale factorial N, P, K fertilization experiment in a semi-deciduous African tropical forest in Uganda, we found that multiple nutrients (co)regulate tree stem growth (Chapter 2), further substantiating the growing pool of evidence that tropical forests are a non-Liebig’s world (Kaspari et al. 2008, Wright et al. 2011). Instead, nutrient requirements, as well as their limitations, vary depending on the tree species and tree sizes. Specifically, tree growth in this semi-deciduous forest was dominantly controlled by N-availability, occasionally by K (climate-dependent) but not P-limited. In this paper, however, we evaluate the nutrient controls of litter productivity and changes in foliar (sunlit leaves and leaf litter) chemistry using the same factorial N, P, K fertilization design. We hypothesize that (1) there will be multiple nutrient co-limitations rather than one single nutrient regulation of litter production rates and quantities, which is a significant component of ANPP (Kaspari et al. 2008, Wright et al. 2011). (2) foliar quality will change with elevated nutrient availability to the plants and will vary with tree species due to varying nutrient requirements and acquisition strategies. Our data will contribute to the scarce knowledge on nutrient limitation of litter production and foliar vitality in an African tropical forest.

3.2 Materials and methods

3.2.1 Study site and experimental design

We conducted this research in the Budongo Forest Reserve in northwestern Uganda (31° 32'E, 1° 44' N; elevation = 1050 m a.s.l.). It is the largest forest reserve in Uganda spanning an area of 825 km^2 (Hamilton 1984) and lies east of Lake Albert, on the Albertine Rift in the Masindi District. The area has a bimodal rainfall pattern, which varies between 1200 mm and 2200 mm annually. The rainy season is usually between April−June and August−November. The minimum and maximum temperatures vary between 23−29 °C and 29−32 ºC respectively. Geologically, very old rocks of the Precambrian gneissic-granulitic basement complex (van Straaten 1976) underlie most parts of the forest. Soils at the site are well-drained, highly weathered, with low activity clay and are classified as Lixisols (IUSS Working Group WRB, 2014; Veldkamp et al., 2020). The soils also have high base saturation and pH as well as the calcium-dominated cation exchange capacity (Table 3.1), which are likely related to the weathering of geological parent material, which consist of coarse-grained basaltic granulites with ~ 10 % calcium oxide (CaO) and 6−7 % magnesium oxide (MgO) as well as depositions of aeolian dust and ash from agricultural biomass burning outside the forest (Fabian et al. 2005, Bauters et al. 2018). The ten most dominant tree species at the site are *Funtumia elastica (24 %), Celtis mildbraedii (15 %), Cynometra alexandri (6 %), Celtis zenkeri (6 %), Lasiodiscus mildbraedii (6 %), Celtis durandii (6 %), Rinorea* *ardisiaeflora (6 %), Trichilia rubescens (4 %), Khaya anthoteca (3 %), Tapura fischeri (2 %)* (Table S2.1).

We laid out thirty-two 40 m × 40 m experimental plots, which are at least 40m apart following a factorial plot design with eight fertilization treatments and four replicates. The treatments included the addition of Nitrogen (N), Phosphorus (P), Potassium (K), N + P, N + K, P + K, N + P + K and Control. Within each 40 × 40 m plot, we also laid out a 30 x 30 m inner core measurement zone (effective plot size) and sixteen 10 × 10 m quadrats to facilitate fertilizer addition. Nitrogen was added as urea ($(NH_2)_2CO$) at a rate of 125 kg N ha^{-1} yr^{-1}, P as triple superphosphate (Ca(H2PO4)2) at a rate of 50 kg P ha^{-1} yr^{-1} and K as muriate of potash (KCl) at a rate of 50 kg K ha^{-1} yr^{-1} consistent with earlier studies (Kaspari et al. 2008, Wright et al. 2011). Pre-packaged fertilizer for each 10 × 10m quadrat is mixed with soil and broadcasted by hand, walking forward and back and subsequently changing directions (North to South and East to West). We fertilized four times a year in equal doses during the rainy season (starting from May 2018).

Prior to the fertilization, we conducted baseline measurements of the litter and soil biochemical characteristics within the 30 m × 30 m measurement zone of our experimental plots (Fig. 1.6 and Table 1.1). Soil samples were taken from a total of 320 soil sampling locations in all the 32 plots thus, 10 randomly placed locations per plot at 0−0.1m soil depth. Soil samples were air-dried at room temperature, sieved (with 2 mm sieves) and analyzed. Soil organic carbon and total N were analysed on finely ground samples using a CN elemental analyser (VARIO EL Cube, Elementar Analysis Systems GmbH, Hanau, Germany). Soil pH was analysed in 1:2.5 of soil-to-distilled water ratio. Soil ^{15}N natural abundance was analyzed using isotope ratio mass spectrometry (Delta Plus, Finnigan MAT, Bremen, Germany). Soil extractable P was determined using the Bray II method (Bray and Kurtz 1945) and analysed with ICP-AES. Exchangeable cations (Ca, Mg, K, Na, Al, Fe, Mn) were determined by percolating the soil samples with unbuffered 1M NH_4Cl and cation concentrations in percolate were determined using the inductively coupled plasma-atomic emission spectrometer (ICP-AES; iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH, Dreich, Germany).

Table 3.1: Foliar and soil chemical characteristics (mean ± SE; n = 32). Soil, leaf litter and tree species characteristics were measured before the start of the experiment. There were no statistical differences in soil characteristics among treatment plots prior to fertilization (ANOVA with Tukey's HSD test or Kruskal Wallis ANOVA with multiple comparison test at $P < 0.05$).

Site characteristic	Sunlit leaves*	Leaf litter	Soil (0−10 cm depth)
Organic Carbon			
Concentration (%)	41.3 ± 0.3	41.2 ± 0.1	3.3 ± 0.1
Stocks (kg C m^{-2})	-	-	4.0 ± 0.1
Nitrogen			
Concentration (%)	2.7 ± 0.1	1.7 ± 1.0	0.34 ± 0.01
Stocks (kg C m^{-2})	-	-	0.42 ± 0.01
C:N ratio	16 ± 1.1	25.1 ± 2.4	9.54 ± 0.10
Soil pH (1:2.5 H_2O)	-	-	6.43 ± 0.04
^{15}N natural abundance (‰)	6.7 ± 0.1	6.6 ± 0.1	7.79 ± 0.06
Bray II phosphorus (g P m^{-2})	-	-	1.80 ± 0.24
Effective cation exchange capacity (mmol^{+} kg^{-1})	-	-	149.2 ± 8.3
Base saturation (%)	-	-	98.2 ± 0.2

* Measurement were taken from the control plots only (n = 4 plots).

Table 3.2: Characteristics of tree species further analysed for their foliar nutrient response to NPK factorial experiment.

Tree Species	Tree density (trees ha^{-1})	Basal area (m^2 ha^{-1})	Foliar C mg g^{-1}	Foliar N mg g^{-1}	Foliar P mg g^{-1}	^{15}N natural abundance in sunlit leaves (‰)*
Celtis durandii	37 ± 4	4.3 ± 0.4	384 ± 17	27.5 ± 4.0	1.9 ± 0.3	7.3 ± 1.0
Celtis mildbraedii	92 ± 10	2.7 ± 0.4	362 ± 19	31.6 ± 1.6	1.2 ± 0.2	7.8 ± 1.2
Cynometra alexandri	39 ± 4	4.2 ± 0.7	451 ± 4	25.6 ± 0.6	1.6 ± 0.1	7.5 ± 0.5
Funtumia elastica	150 ± 13	4.2 ± 0.4	462 ± 6	31.1 ± 0.1	2.4 ± 0.2	5.8 ± 0.5

3.2.2 Fine litter production and leaf litter chemistry

Fine litter production was quantified using four randomly placed litter collectors per plot (Fig. S3.1). The 0.75 m × 0.75 m collectors were constructed from PVC pipe frames and 1mm plastic mesh (similar to methods used by Adamek et al., (2009)). Fine litter was collected bi-weekly and separated into seven categories: leaves, flowers, fruits, wood ≤ 2 cm diameter, epiphytes (Fig. S3.2), and unidentified (hereafter called "other"), following established methods (Clark et al. 2001). Subsequently, the samples were oven-dried at 60°C until the constant mass is achieved usually 48−72 hours (season-dependent) and then weighed. We calculated annual litter production as a sum of all litterfall for 12 consecutive months (May 1 to April 30).

We collected pooled samples of leaf litter in May 2018 (pre-treatment), September 2018 (four months after initial fertilization) and September 2019 (16 months into the experiment). Litter samples analyzed for nutrient content (C, N, P, K, Mg, Ca, Al) using nitric acid pressure digestion with analysis in an inductively coupled plasma-atomic emission spectrometer (ICP-AES; iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH, Dreieich, Germany) and ^{15}N signatures using an isotope ratio mass spectrometer (IRMS; IRMS; Delta Plus, Finnigan MAT, Bremen, Germany).

3.2.3 Sunlit leaves and hemispherical photographs

Based on the importance value index (IVI; Curtis and McIntosh 1950), we collected matured sunlit leaves (Mo et al. 2015) from the four important tree species (FUE-*Funtumia elastica,* CMI-*Celtis mildbraedii*, CDU-*Celtis durandii*, CYA-*Cynometra alexandri;* Table 2) after 1.5 years of initial fertilization (November 2019) in all plots to determine their nutrient contents and ^{15}N signatures (just as the leaf litter samples above). The stoichiometric relationships (i.e., N:P or N:K ratios) in leaves gives a good indication of nutrient limitations (Aber et al. 1998), whereas the ^{15}N signature in fresh leaves and litterfall is a good indicator of ecosystem N losses (Corre et al. 2010, Wolf et al. 2011). We ensured that each of the selected tree species was present in at least three replicate plots of each treatment to allow for statistically valid comparisons between treatments. Tree heights at this site reach up to 50 m aboveground, which made it challenging to sample sunlit leaves. Notwithstanding, a big slingshot was used as a throw-line launcher (Fig. S3.3), the line is equipped with a throw-weight and portable chain-saw for cutting down small tree branches from which we sampled the sunlit leaves approximately 1.5 years after initial fertilization.

Additionally, in April 2018 (prior to initial fertilization) and October 2019 (after 1.5 years), hemispherical photographs (using a Solariscope) from the centre of each of the 16 quadrats (10 m × 10 m) per plot were taken to compare canopy closure and Leaf Area Index (LAI) between the control and the fertilized plots. Photographs were taken under sky-overcast conditions between 12h00 to 14h00 at 1 m above the ground facing skyward.

3.2.4 Statistical analyses

For repeated measurement data (fine litter production, leaf litter chemistry), linear mixed-effect models (‘lme’ function in the ‘nlme’ package) were used to test the effect of nutrient additions and their interaction in the factorial design (N × P × K). The presence or absence of each of the main nutrients and time were used as a fixed factor whereas replicate plots were included in the model as random factors. The significance of the fixed effect was evaluated using ANOVA (Crawley, 2009). The LME analyses were performed for the entire period of the experiment as well as for the three years separately. If residual plots revealed non-normal distribution or non-homogenous variance, we log-transformed the data and then repeated the analyses. Treatment effects on parameters measured only once after fertilization (sunlit leaf chemistry and LAI) were analysed using a three-way ANOVA whereas baseline measurements (in leaf litter chemistry, LAI, soil physicochemical characteristics) were analysed using one-way ANOVA with Tukey HSD test (eight assigned treatments) to examine if inherent chemical differences existed proir to fertilisation. All parameters were first tested for normal distribution (Shapiro-Wilk's test) and equality of variance (Levene's test; Sokal and Rohlf 1981). Logarithmic or square root transformation was applied when these assumptions are violated. In cases where no significant interaction effects were detected between the different fertilization nutrients and to graphically assess the main effects of specific nutrients, results are shown all plots where a specific nutrient was not added (e.g. −N; n = 16) compared with all plots where that nutrient was added (e.g. +N; n = 16) (Wright et al. 2011, Lugli et al. 2021). In all tests, statistical significance was set at $P \leq 0.05$. All statistical analyses were performed using the statistical package R version 3.6.2 (R Development Core Team, 2018).

3.3 Results

3.3.1 Fine litter production, LAI and leaf litter chemistry

Total fine litter production averaged 12.9 ± 0.8 Mg ha^{-1} yr^{-1} in the control plots, of which leaf litter constituted 66 %, 19 % fell as twigs (wood ≤ 2 cm diameter), 10 % as reproductive litter (fruits and flowers) and the remaining 7 % represented tree bark, epiphytes and unidentified materials (Table 3.3). Fine litter production was highly seasonal with higher bi-weekly litterfall (73 ± 7 g m^{-2}) occurring during the major dry season of the year (December−March) than in the wet season (38 ± 2 g m^{-2}), with a distinct peak varying from January to March in the control plots (Fig. S3.4).

Table 3.3: Annual fine litter production (mean ± SE; n = 4 plots; in Mg ha^{-1} y^{-1}) in response to N-P-K fertilization in the Budongo Forest based on three-year monitoring (2018−2020). Analysis was based on the full factorial NPK experimental design.

Treatment	Twigs ≤ 2 cm	Leaves	Bark	Flowers	Fruit	Epiphyte	Other	Total fine litterfall
Control	2.5 ± 0.3	8.5 ± 0.3	0.3 ± 0.1	0.36 ± 0.16	0.9 ± 0.1	0.02 ± 0.01	0.3 ± 0.06	12.9 ± 0.8
N	2.7 ± 0.1	9.1 ± 0.1	1.2 ± 0.7	0.53 ± 0.11	1.0 ± 0.2	0.01 ± 0.00	0.4 ± 0.04	14.9 ± 0.9
P	2.4 ± 0.1	8.8 ± 0.2	0.5 ± 0.2	0.34 ± 0.11	1.3 ± 0.6	0.02 ± 0.01	0.4 ± 0.05	13.8 ± 1.1
K	2.4 ± 0.2	8.7 ± 0.5	0.4 ± 0.1	0.45 ± 0.11	1.6 ± 0.6	0.06 ± 0.04	0.4 ± 0.03	14.0 ± 1.2
NP	2.5 ± 0.1	8.7 ± 0.2	0.7 ± 0.2	0.38 ± 0.13	0.7 ± 0.2	0.02 ± 0.01	0.4 ± 0.06	13.4 ± 0.7
NK	2.1 ± 0.3	8.2 ± 0.5	0.3 ± 0.1	0.33 ± 0.14	0.9 ± 0.3	0.06 ± 0.05	0.3 ± 0.07	12.3 ± 1.1
PK	2.2 ± 0.3	8.1 ± 0.4	0.4 ± 0.2	0.18 ± 0.04	0.5 ± 0.1	0.01 ± 0.00	0.3 ± 0.05	11.8 ± 0.7
NPK	2.4 ± 0.3	8.4 ± 0.5	0.3 ± 0.2	0.22 ± 0.04	0.7 ± 0.3	0.01 ± 0.01	0.4 ± 0.08	12.4 ± 0.5

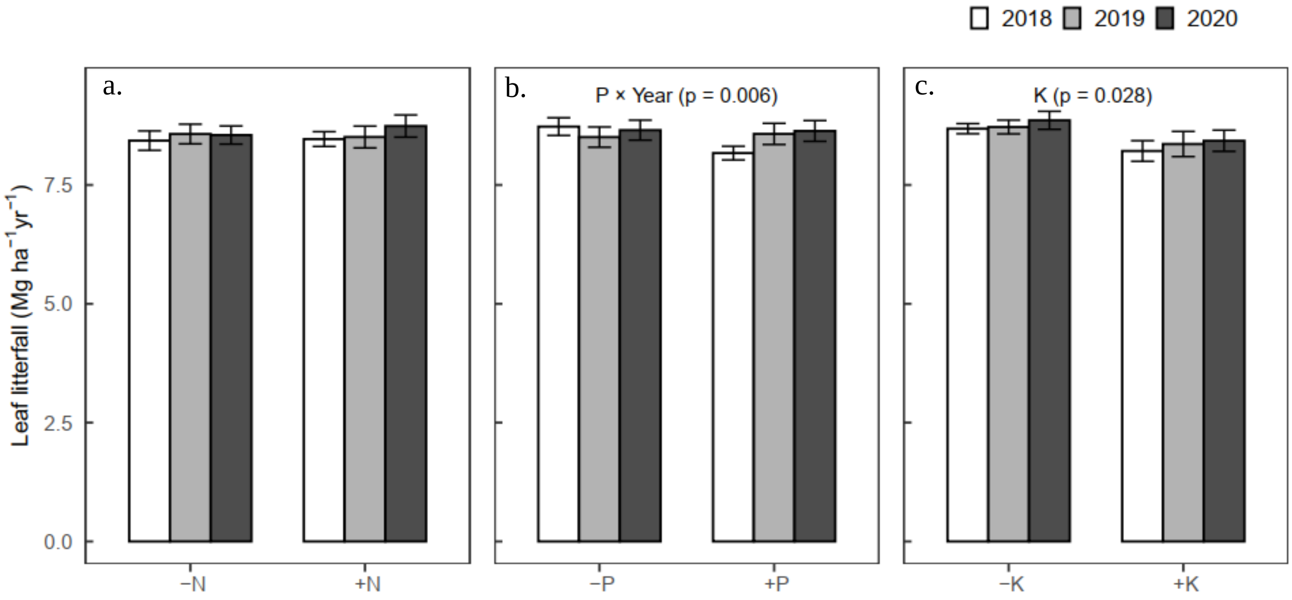


Figure 3.1: Responses of annual leaf litter production (mean ± SE; n = 16 plots) in plots with or without nitrogen (panel a; +N vs −N), phosphorus (panel b; +P vs −P) and potassium (panel c; +K vs −K). Statistical analysis was based on the N × P × K factorial design. Treatments without added nutrient e.g. in panel (a) −N (control, +P, +K, +PK) are pooled (denoted −N on horizontal axis) and treatments with added +N (+N, +NP, +NK, +NPK) are pooled. Panel (b) and (c) follow a similar approach.

In the first year of nutrient addition, there was a four-week delay in the peak of leaf litterfall in almost all the K addition. Although there were no significant effects of nutrient additions on total fine litter production for the three-year measurement period, P and K additions significantly reduced leaf litterfall. This was evident in the effects P × Year interactions ($F_{2, 48} = 5.8$, $P = 0.006$; Fig. 3.1b) and of K additions ($F_{1, 24} = 5.4$, $P = 0.028$; Fig. 3.1c) on leaf litter production across the three years of measurement. Reproductive litterfall, twigs, bark and epiphytes were invariant across nutrient addition treatments. Nutrient additions had no detectable effects on canopy closure as determined by the LAI.

There were no differences in baseline leaf litter chemistry prior to fertilization among treatment plots. The response of leaf litter chemistry to nutrient addition was dependent on the nutrient in question (Tables 3.4 and S3.1). Increased leaf litter C content was associated with N × P × K interaction effects ($F_{1, 24} = 6.0$, $P = 0.022$; Table 3.4), leaf litter Ca and Mg content decreased with N × P × K interaction effects ($F_{1, 24} = 8.9$, 4.9 and $P = 0.007$, 0.036; Table 3.4). Within the one year measurement interval (September 2018−September 2019), Mg content in leaf litter decreased by 10 % (Time effect; $F_{1, 24} = 21$, $P < 0.001$) and by the same proportion in +K compared to –K addition plots ($F_{1, 24} = 5.9$, $P = 0.023$; Table 3.4). We also found an increased leaf litter ^{15}N natural abundance with K additions ($F_{1, 24} = 4.6$, $P = 0.043$). Leaf litter N, P and K were however not affected by nutrient additions as were leaf litter C:N and N:P ratios.

Table 3.4: The *F* statistics of the factorial NPK analysis of the responses to nutrient addition by different leaf litter nutrients, based on measurements of September 2018 and 2019

Treatment	C mg/g	N mg/g	P mg/g	K mg/g	Ca mg/g	Mg mg/g	C:N	N:P
N	0.35	2.94	2.12	0.99	0.20	0.90	1.93	0.52
P	0.18	0.83	0.34	0.20	0.54	0.23	0.22	0.05
K	0.49	3.84	0.13	0.00	1.06	**5.87***	2.24	3.69
N × P	2.03	2.09	0.32	0.00	0.67	0.10	0.48	0.01
N × K	2.86	1.01	0.06	0.39	0.04	0.30	0.33	1.10
P × K	0.09	2.55	0.16	0.87	0.23	0.21	1.91	0.29
N × P × K	**5.97***	0.54	1.33	2.12	**8.89****	**4.92***	2.92	0.58

Within columns, bolded F vlaues indicate significant response at $P < 0.01$** or $P < 0.05$*

3.3.2 Foliar chemistry of different tree species

The response of a composite foliar (sunlit leaves from species representing 67 % of tree abundance, with plot averages in Table S3.2) nutrient content to fertilization with each nutrient (N, P, K) did not directly elicited an increase in foliar content of that nutrient but was consistently affected by N × P × K interaction effects. Specifically, the interaction of N × P × K resulted in increased foliar N content ($F_{1,\,24} = 6.2$, $P = 0.020$) but decreased foliar C:N ($F_{1,\,24} = 5.4$, $P = 0.029$) and C:K ($F_{1,\,24} = 4.5$, $P = 0.045$) ratios. However, foliar nutrient content and their response to fertilization varied among tree species (Fig. 3.2 and S3.3). Nitrogen additions increased foliar N content by 7 % and C content by 5 % in CMI ($F_{1,\,15} = 5.5$, 6.1 and $P = 0.033$, 0.027; Fig. 3.2a and d) whereas the foliar N content of CYA was enhanced under N × P interaction effects ($F_{1,\,22} = 5.3$, $P = 0.031$). Phosphorus additions increased P content by 33 % (Fig. 3.2h), ^{15}N natural abundance by 10 % ($F_{1,\,20} = 7.0$, 9.2 and $P = 0.015$, 0.006) and as expected, decreased N:P ratio by 16 % (Fig. 3.2q) in CDU. The addition of K was associated with decreased foliar N content by 5 % ($F_{1,\,24} = 9.8$, $P = 0.005$; Fig. 3.2f), increased foliar Ca content by 24 % ($F_{1,\,24} = 9.2$, $P = 0.006$, Fig. 3.2l) and C:N ratio by 4 % ($F_{1,\,24} = 4.9$, $P = 0.036$; Fig. 3.2o) in FUE.

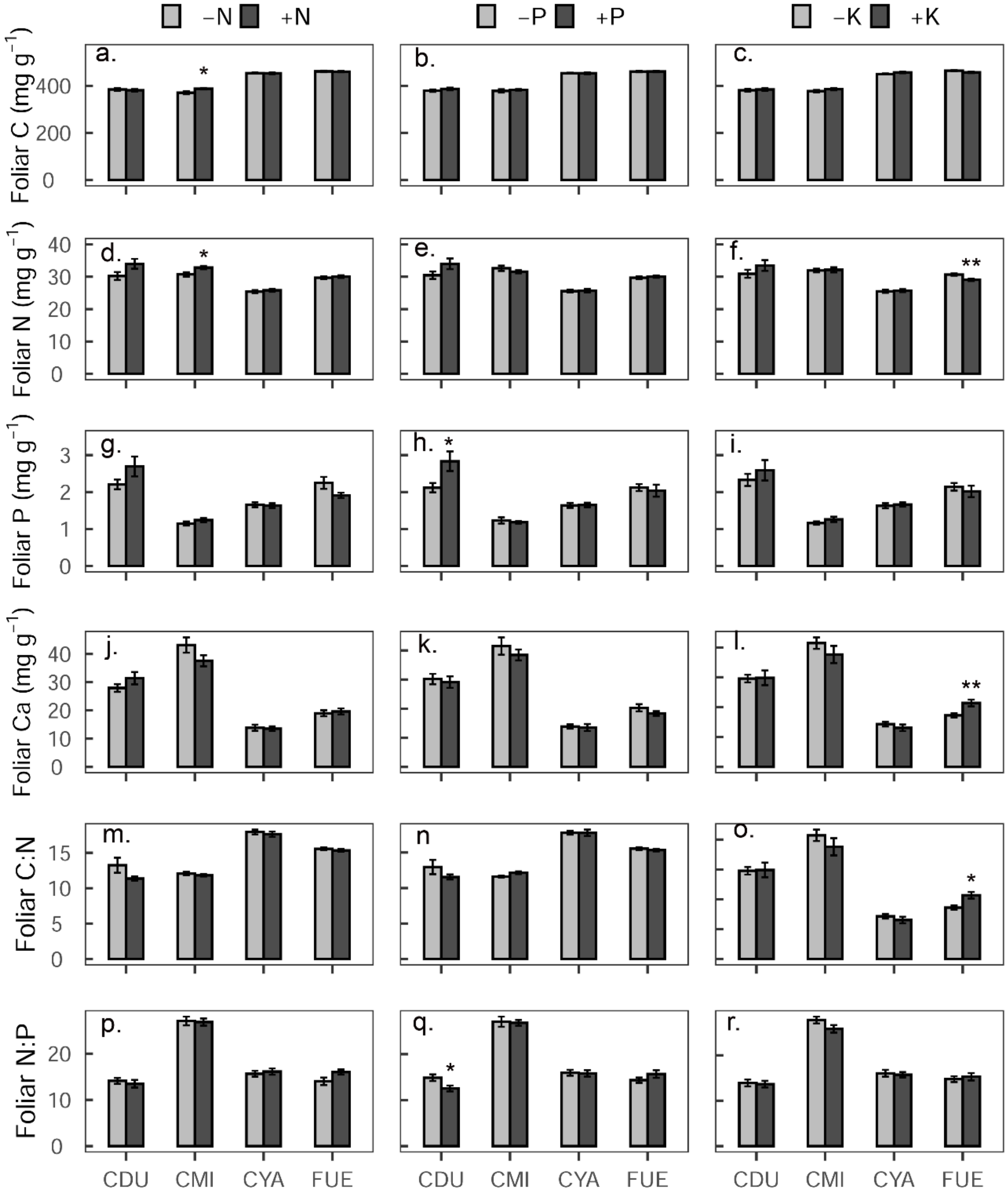


Figure 3.2: Response of the foliar chemistry of different species after 1.5 years of fertilization. Analyses were based on the factorial N-P-K fertilization design. Presented are mean values (± SE) in plots with or without nitrogen (+N vs −N), phosphorus (+P vs −P) and potassium (+K vs −K) for four dominant species (CDU = *Celtis durandii,* CMI = *Celtis mildbraedii,* CYA= *Cynometra alexandri and* FUE = *Funtumia elastica*) at the site. Asterisks represent the significant main effect of a Three-Way ANOVA ($P < 0.01$** and $P \leq 0.05$*).

3.4 Discussion

3.4.1 Seasonality of litterfall in the Budongo experimental site

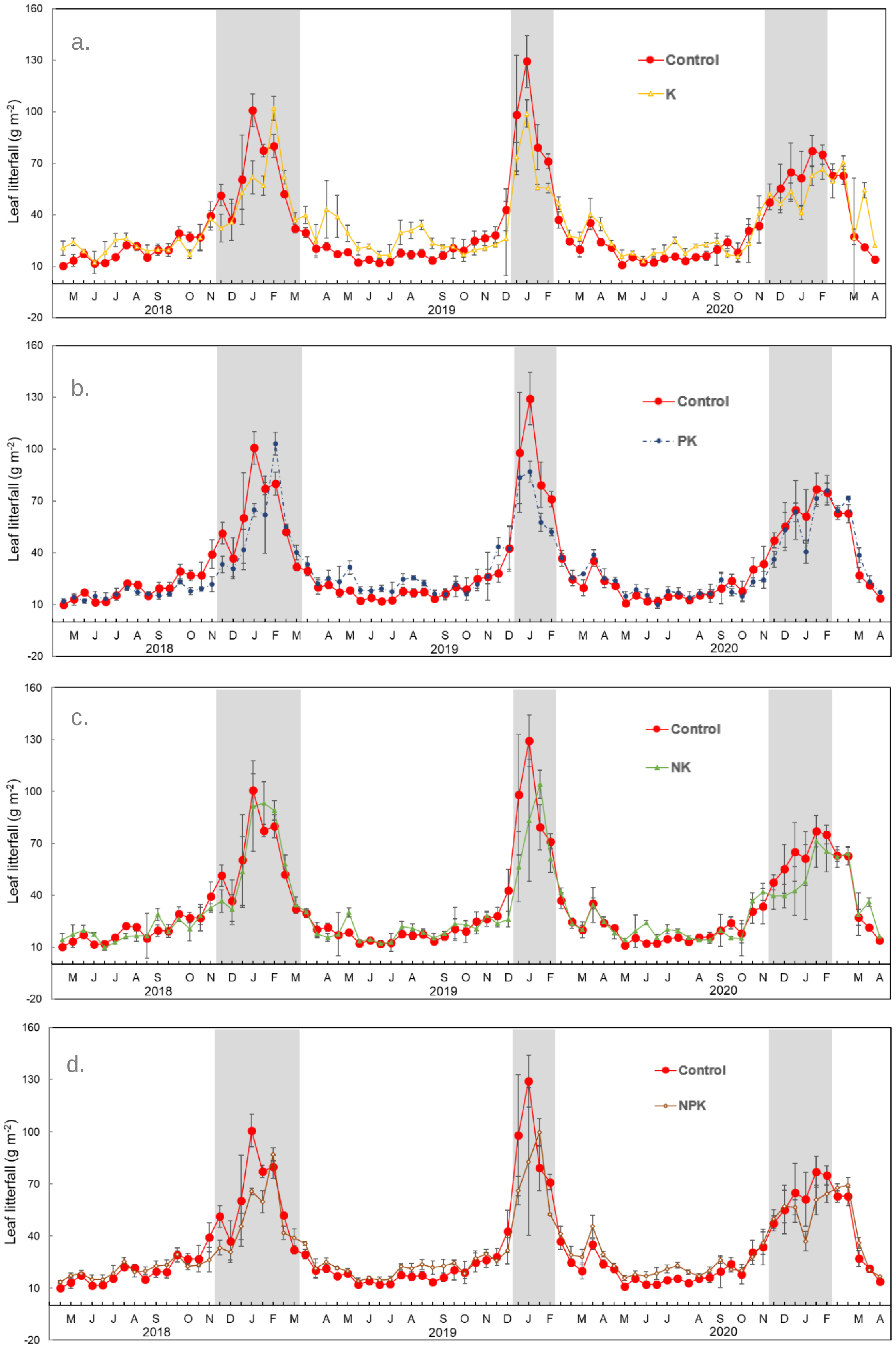
A varied seasonal pattern of fine litterfall have been reported worldwide and is dependent on temperature, rainfall, species composition and nutrient availability (Parsons et al. 2014, Zhang et al. 2014). For instance, in tropical forests, litterfall peaks have mostly occurred in the drier months of the year in response to soil moisture deficit (Tanner et al. 1992, Chave et al. 2010, Parsons et al. 2014); in wettest or warmest months of the year potentially due to strong winds or soil fertility coupled with rainfall (Congdon and Herbohn 1993, Dawoe et al. 2010); and in transition periods between the dry and wet seasons (Hopkins and Graham 1989, Congdon and Herbohn 1993, Zhang et al. 2014). In this moist semi-deciduous tropical forest site, about double the litterfall rate in the wet season occurred in the major dry season (December−March), where leaf senescence and abscission in plants are most prevailing (Lian and Zhang 1998). The observed seasonality in litterfall reflects the seasonal rainfall pattern and is largely dependent on the factors. During the dry periods, these plants shed leaves as a physiological response to moisture stress and/or temperature alteration, an adaptive mechanism with which excessive water loss through transpiration is reduced and energy is conserved to maintain necessary life processes (e.g. photosynthesis, respiration etc.), and ensures survival throughout the stress period. This pattern is consistent with seasonal tropical forests (with seasonal climates) (Tanner et al. 1992, Wieder and Wright 1995, Zhang et al. 2014), but in contrast to forests under climates without distinct dry seasons, such as Australian and Atlantic rain forests, where the litterfall peaks occur in the rainy season, often mechanically driven (Parsons et al. 2014).

3.4.2 The response of litter productivity to NPK fertilization

Annual fine litter production (12.9 ± 0.8 Mg ha^{-1} yr^{-1}) from the control plots of this seasonal forest site was at the upper end of the range reported in other humid tropical forests (Tanner et al. 1992, Paoli and Curran 2007, Adamek et al. 2009, Chave et al. 2010, Wright et al. 2011, Kho et al. 2013, Zhang et al. 2014, Kotowska et al. 2016). Overall, there were no significant effects of nutrient additions on total fine litter production, although the highest litterfall (14.9 ± 0.9 Mg ha^{-1} yr^{-1}) was recorded in the N fertilization plots. This lack of detectable response in fine litterfall may be due to the short duration of this fertilization experiment as it took four years after initial fertilization for a significant response to N + P fertilization to be detected in a Venezuelan montane forest despite higher rates of nutrient addition (225 kg N ha^{-1} yr^{-1} and 75 kg P ha^{-1} yr^{-1}) compared to our site (Tanner et al. 1992). The same is true for a six-year fertilization trial (with a similar nutrient addition rate as ours) in a tropical lowland forest in Panama, where only reproductive (flowers and fruits) litter significantly responded to N fertilization (Kaspari et al. 2008). It is therefore likely that continued fertilization with time (beyond the present experimental duration) may elicit a significant increase in fine litterfall.

That notwithstanding, the results of this large-scale factorial fertilization experiment suggest that P and K additions influenced leaf litter production rates (Figs. 3.1 and 3.3). Specifically, while K addition was consistently associated with a reduced leaf litterfall over the three-year experimental period (Fig. 3.1c), the response to P addition was time-dependent (P × Year interactions; Fig. 3.1b) and mainly in the first year following a prolonged and intense dry period in the first year of the experiment (May 2018−April 2019) with about 30 % less rainfall recorded compared to the subsequent year (Chapter 2). Moreover, unlike in the control and other nutrient fertilization plots, there was a trend of four-week delay in the peak of leaf litterfall in the

K addition plots in the intense drier months of the first year, which is indicative of delayed leaf shedding or inhibited activation of reactive oxygen species (reactive chemical for cell signalling) in the leaves of these plant. A plausible explanation for the decreased leaf litterfall in response to P and K is that, physiologically, deciduous trees will shed leaves upon the activation of reactive oxygen species during water-deficit conditions. However, the increased availability of K mitigates the early formation of this reactive oxygen species in the leaf (Hasanuzzaman et al. 2018, Wu et al. 2020). This prolongs leaf life span, which otherwise would have wilted and fallen. Furthermore, Whereas P is known for its mitigating effects on plant growth during water deficit conditions (Tariq et al. 2017, 2018), K availability in plants is recognised for its important stomatal regulatory role played in the guard cells of these leaves, allowing gaseous exchange and conserving moisture, thereby sustaining photosynthesis (or at least mitigate the stress) during the moisture stress conditions (Hasanuzzaman et al. 2018, Wu et al. 2020). It does not however mean that these leaves whose shedding was delayed will eventually not fall to the ground or new leaves will not be produced to replace older leaves, particularly, when adequate resources (e.g. water, nutrient and sunlight) become available.


a.
Control
K
b.
Control
PK
c.
Control
NK
d.
Control
NPK
Leaf litterfall (g m^{-2})
160
130
100
70
40
10
-20
M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A
2018
2019
2020

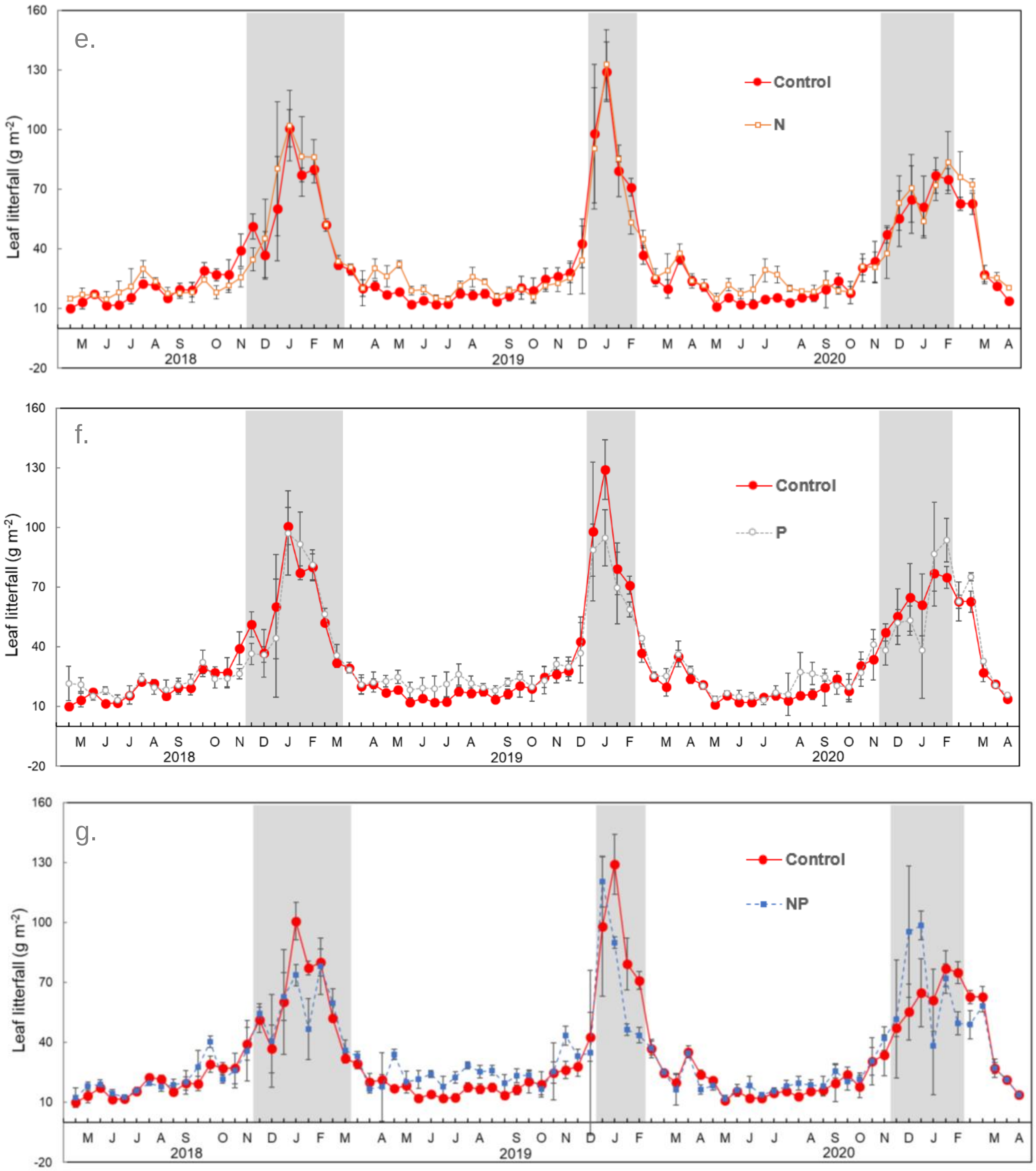


Figure 3.3: Temporal response pattern of bi-weekly leaf litterfall (mean ± SE, n = 4 plots) to N-P-K fertilization experiment in the Budongo Forest Reserve in Uganda.

3.4.3 Foliar chemistry and stoichiometric response to fertilization

The rationale of this fertilization experiment was to reveal possible nutrient (co-) limitations to ecosystem carbon assimilation. This limitation becomes evident when elevated input of limiting nutrients results in increased plant growth (C accumulation) or a process that is being measured (Tanner et al. 1998). Moreover, a growing pool of evidence have shown that tree growth, litterfall, decomposition and forest structure are controlled, in one way or the other, by multiple soil nutrients, including N, P, K, and even Ca (Wright et al. 2011, Chapter 2). This evidence, although spatially variable and selective, are consistent with the concept of simultaneous multiple nutrient limitation in tropical forests (Kaspari et al. 2008, Eskelinen and Harrison 2015). To this end, the addition of nutrients to the soil is expected to cause changes in soil nutrient concentrations or its related processes (e.g. increased nitrification and nitrate leaching with N additions; Corre et al. 2010), biomass accumulations (e.g. enhance tree growth with N, P or K additions; Adamek et al. 2009, Wright et al. 2011, Alvarez-Clare et al. 2013, Chapter 2), and plant tissue nutrient concentrations (e.g. increased litter N concentrations with N additions; Kaspari et al. 2008).

Our data showed that Foliar N (sunlit leaves) and leaf litter C content increased with all three nutrient additions (N × P × K interactions; Fig. 3.2) suggesting that all three nutrients played complementary roles in enhancing C assimilations in this site. This result is consistent with earlier observations at this site where N and K were found to limit tree growth (Chapter 2) while P and K became potentially crucial for moisture stress tolerance (Fig. 3.1). This enhanced leaf litter C, which came about through photosynthesis (atmospheric CO_2 fixation into plant biomass) will contribute to enriched organic matter for decomposers, enhance soil carbon stock and the quality of soil as a whole (Ontl and Schulte 2012). Unlike in temperate forests where climate and/ or leaf litter recalcitrants (which can vary with plant species) can slow down the process of decomposition and eventual soil C storage, there is shorter residence time for leaf litter due to the humid and warmer climate in tropical forests (Kaspari et al. 2008, Kagezi et al. 2016). This enhances soil carbon storage and underpins the significant carbon sink role of tropical forest soils (Pan et al. 2011).

Foliar nutrient concentrations varied among the dominant tree species (ranged: 25.6−31.6, 1.2−2.4, 9.9−20.2 and 362−462 mg g^{-1} for foliar N, P, K and C respectively) as well as their responses to fertilization. These variations are consistent with other tropical forests and may reflect differences in phenology; related to leaf life-span, photosynthetic capacity and investment in defence against herbivory (Santiago et al. 2004, 2005). As expected, the increase in foliar N in CMI and CYA (a legume) may have been driven by the increased N input and consistent with earlier findings of N limitation in this forest although not specific to these two-tree species (Chapter 2). In contrast, foliar N decreased with K additions resulting in an increased foliar C:N ratio in FUE. This may suggest a possible reallocation of N to other parts of the plant other than the leaves e.g. for wood biomass production as was found in some groups of trees with K additions at the same site (Chapter 2). Although the role of P in promoting stem growth was not pronounced at this site, P fertilization increased P content (33 %; Fig. 3.3h), ^{15}N natural abundance (10 %) and decreased N:P ratio (16 %; Fig. 3.3q) in CDU. The increased P may suggest a possible P limitation or a high demand of P among individuals of this species.

Inferences to ecosystem nutrient limitation have been made using stoichiometric ratios in literature and are, in some cases, consistent with results of ecosystem-scale fertilization experiments (Koerselman and Meuleman 1996, Sullivan et al. 2014). Shifts in N:P ratio is one of such tools in the assessment of nutrient limitation across ecosystems, families or among species. For example, a suggestion of N limitation is made when foliar N:P ratio < 14 or P-limitation when foliar N:P ratio > 16 (Koerselman and Meuleman 1996, Sullivan et al. 2014; Aerts & Chapin, 2000). Either N, P or both could be (co-) limiting plant growth when N:P ratios are between 14 and 16 (Koerselman and Meuleman 1996). Foliar N:P ratio (17.6 ± 1.1) in this Budongo forest experimental site is within the range of values reported for humid tropical forest (Santiago et al. 2005, Townsend et al. 2007, Sullivan et al. 2014, Mo et al. 2015) and varied among tree species as all other foliar nutrient contents (Table 1). suggest that P might be limiting in this forest ecosystem. Whereas N:P ratio of FUE (13.2 ± 0.8) and CDU (15.1 ± 1.0) are consistent with earlier findings that suggest N limitation among some trees of this forest, N:P ratios of CMI (27.8 ± 2.6; above range reported for tropical forests (Sullivan et al. 2014)) and CYA (16.1 ± 1.4) seem to suggest that P could also be limited at this site which is in sharp contrast to the results of our earlier study (Chapter 2). We advise that the diagnoses of nutrient limitation using these N:P ratios should be done with caution and that in agreement with Powers et al. (2015), ecosystem-scale fertilization experiments, although laborious and scarce, remain the “gold standard” of resolving nutrient limitations in ecosystem ecology

3.5 Conclusions

Our goal was to evaluate how nutrients control litter productivity and foliar chemistry using a factorial N- P- K fertilization experiment. Our data suggest that: (1) Although annual fine litter production was not significantly affected by nutrient fertilization in the short-term (3 years), an observed trend towards higher annual fine-litter production in the N addition plots may become stronger with continued nutrient additions. (2) Our prediction of multiple nutrients rather than a single nutrient regulation of litter production rates was supported. Specifically, following a prolonged dry season in the first year of the experiment, leaf litterfall reduced significantly with P and K additions. This reduction in litterfall highlights the critical role that both P and K have in regulating water use efficiency or drought-stress tolerance in plant and suggest that seasonality mediate the relative importance of soil nutrients as well as their limitation to ecosystem processes (3) As predicted, both leaf litter and foliar nutrient contents were affected by the elevated availability of all three nutrients in both positive and negative directions, and dependent on tree species in this diverse ecosystem. The long-term consequences of these nutrient perturbations on this ecosystem are yet to be known and therefore many of such fertilization experiments particularly for understudied African tropical forests are needed to reconcile spatial differences in ecosystem nutrient controls as well as in ecosystem response to future perturbations.

3.6 Appendix S3

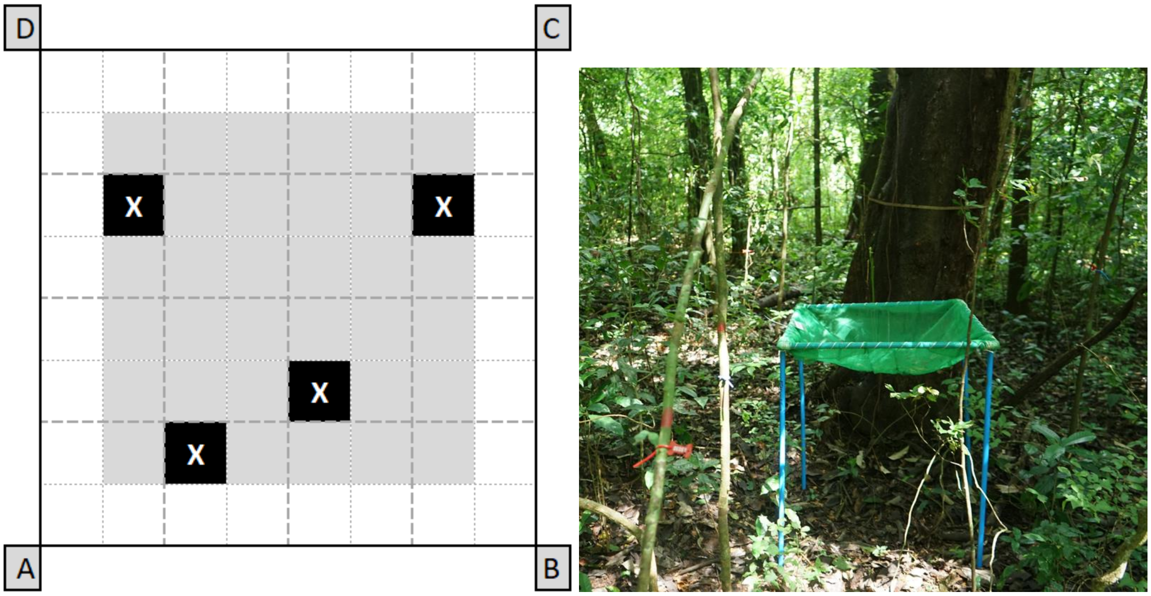


Figure S3.1: Layout of litter collector (X) locations (left) within the core measurement zone (30 m × 30 m in size - grey shaded) of each plot. Installed litter collector (right plate) in the forest plot made up of PVC frame and a mesh.

Figure S3.2: Litter sorted into categories prior to dying and weighing for the determination of litter biomass.

Figure S3.3 Sling-shot catapult system used to collect leaves from the top of trees. The big slingshot was used as a throw-line launcher, the line is equipped with a throw-weight and portable chain-saw for cutting down small tree branches from which sunlit leaves are sampled.

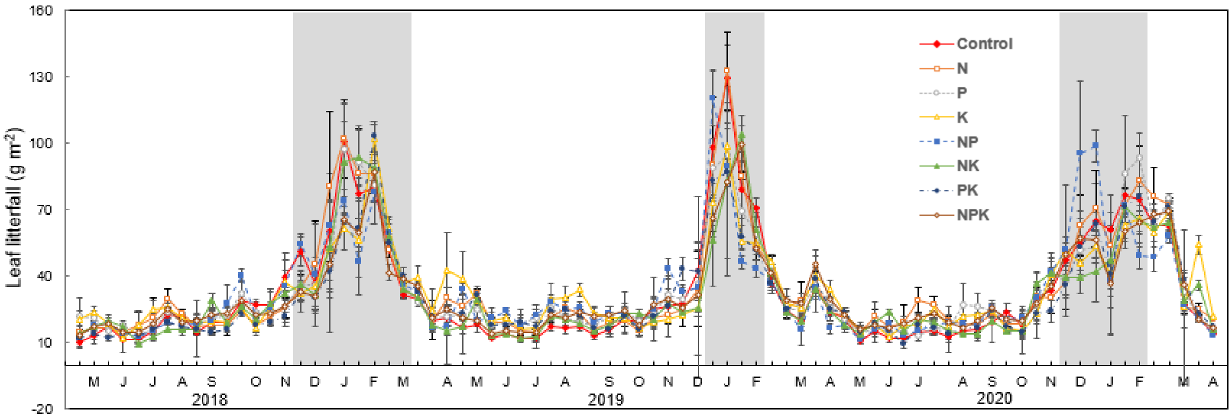


Figure S3.4: Temporal response pattern of bi-weekly leaf litterfall (mean ± SE, n = 4 plots) to N-P-K fertilization experiment in the Budongo Forest Reserve in Uganda. Grey portions represent the major dry seasons (rainfall < 100 mm).

Table S3.1: Leaf litter chemistry (mean ± SE, n = 4 plots) based on measurements of May 2018, September 2018 and September 2019.

Treatment	C mg g^{-1}	N mg g^{-1}	Al mg g^{-1}	Ca mg g^{-1}	Fe mg g^{-1}	K mg g^{-1}	Mg mg g^{-1}	P mg g^{-1}	^{15}N (‰)	C:N	N:P	C:P	N:K	C:K
Control	443 ± 4	28 ± 0	0.3 ± 0	34 ± 2	0.4 ± 0	9 ± 0.1	4 ± 0.2	1.5 ± 0.1	6.3 ± 0.2	16 ± 0.2	19 ± 0.6	306 ± 9	3 ± 0.1	49 ± 1
N	455 ± 7	25 ± 1	0.4 ± 0	29 ± 2	0.5 ± 0	9 ± 0.3	3 ± 0.2	1.2 ± 0.1	6.1 ± 0.4	18 ± 0.6	21 ± 1.1	392 ± 23	3 ± 0.1	55 ± 2
P	451 ± 8	26 ± 2	0.4 ± 0	26 ± 2	0.5 ± 0	8 ± 0.6	3 ± 0.4	1.3 ± 0.1	6.3 ± 0.3	18 ± 1.6	20 ± 1.1	358 ± 34	3 ± 0.2	56 ± 5
K	459 ±7	25 ± 2	0.4 ± 0	27 ± 4	0.4 ± 0	8 ± 0.5	3 ± 0.3	1.3 ± 0.1	6.7 ± 0.1	18 ± 1.2	19 ± 0.6	345 ± 25	3 ± 0.1	56 ± 5
NP	456 ± 9	27 ± 1	0.5 ± 0	30 ± 3	0.6 ± 0	9 ± 0.3	4 ± 0.1	1.4 ± 0.1	6.2 ± 0.5	17 ± 0.7	21 ± 1.4	348 ± 34	3 ± 0.1	52 ± 2
NK	436 ± 1	24 ± 1	0.4 ± 0	31 ± 2	0.4 ± 0	9 ± 0.7	3 ± 0.2	1.4 ± 0.1	6.5 ± 0.2	18 ± 0.6	18 ± 0.6	329 ±15	3 ± 0.2	52 ± 3
PK	441 ± 7	26 ± 1	0.5 ± 0	31 ± 4	0.7 ± 0.	9 ± 0.5	3 ± 0.2	1.4 ± 0.1	7.1 ± 0.2	17 ± 0.9	19 ± 1.5	330 ± 35	3 ± 0.1	49 ± 3
NPK	459 ± 4	25 ± 1	0.5 ± 0	25 ± 1	0.5 ± 0	8 ± 0.8	3 ± 0.1	1.3 ± 0.1	6.3 ± 0.2	19 ± 0.4	19 ± 0.9	359 ± 23	3 ± 0.2	58 ± 6

Table S3.2: Composite sunlit-leaf chemistry (mean ± SE, n = 4 plots) after 1.5 years of fertilization.

Treatment	C mg g^{-1}	N mg g^{-1}	Al mg g^{-1}	Ca mg g^{-1}	K mg g^{-1}	Mg mg g^{-1}	P mg g^{-1}	^{15}N (‰)	C:N	N:P	C:P	N:K	C:K
Control	420 ± 8	27 ± 2	0.3 ± 0.02	25 ± 2	12 ± 1	4 ± 0.2	1.7 ± 0.1	6.6 ± 0.4	17 ± 2	18 ± 1	293 ± 29	3 ± 0.2	43 ± 6
N	425 ± 5	29 ± 1	0.3 ± 0.05	24 ± 1	13 ± 1	3 ± 0.2	1.7 ± 0.1	7.8 ± 0.2	15 ± 1	19 ± 2	275 ± 11	2 ± 0.2	36 ± 2
P	416 ± 4	30 ± 1	0.3 ± 0.03	26 ± 2	14 ± 1	4 ± 0.2	1.8 ± 0.1	7.8 ± 0.3	15 ± 0	18 ± 0	264 ± 12	2 ± 0.1	34 ± 1
K	425 ± 5	29 ± 1	0.3 ± 0.02	24 ± 2	14 ± 1	4 ± 0.2	1.7 ± 0.1	7.3 ± 0.1	15 ± 0	18 ± 1	255 ± 11	2 ± 0.1	33 ± 1
NP	426 ± 4	29 ±1	0.3 ± 0.03	24 ± 2	13 ± 1	3 ± 0.2	1.7 ± 0.1	7.0 ± 0.1	16 ± 1	18 ± 1	280 ± 24	3 ± 0.1	40 ± 4
NK	423 ± 6	29 ± 1	0.3 ± 0.02	26 ± 4	13 ± 0	4 ± 0.3	1.7 ± 0.0	7.2 ± 0.3	16 ± 1	18 ± 1	270 ± 9	2 ± 0.2	36 ± 2
PK	424 ± 3	28 ± 1	0.2 ± 0.03	24 ± 2	13 ± 0	3 ± 0.2	1.9 ± 0.1	7.8 ± 0.1	16 ± 1	16 ± 1	249 ± 6	2 ± 0.1	34 ± 1
NPK	420 ± 6	33 ± 1	0.3 ± 0.03	28 ± 2	14 ± 1	4 ± 0.1	1.9 ± 0.2	8.2 ± 0.1	13 ± 0	20 ± 0	257 ± 7	3 ± 0.1	33 ± 2

Table S3.3: Foliar chemistry (mean ± SE, n = 4 plots) of different species after 1.5 years of fertilization.

Species/ Treatments	N mg g^{-1}	P mg g^{-1}	K Mg g^{-1}	Ca Mg g^{-1}	Mg mg g^{-1}	Al mg g^{-1}	C:N	N:P
Funtumia elastica								
Control	31 ± 0	2.4 ± 0.2	20 ± 2	17 ± 1	8 ± 1	0.3 ± 0.1	15 ± 0	13 ± 1
N	31 ± 1	2.1 ± 0.2	17 ± 1	19 ± 2	7 ± 1	0.4 ± 0.0	15 ± 1	15 ± 1
P	30 ± 1	2.2 ± 0.3	20 ± 1	16 ± 1	7 ± 0	0.3 ± 0.0	15 ± 0	14 ± 1
K	29 ± 1	2.1 ± 0.2	18 ± 2	22 ± 3	7 ± 1	0.3 ± 0.1	16 ± 0	14 ± 1
NP	31 ± 1	1.8 ± 0.1	18 ± 3	17± 1	7 ± 1	0.3 ± 0.1	15 ± 1	17 ± 1
NK	28 ± 1	1.9 ± 0.2	17 ± 2	23 ± 3	8 ± 1	0.3 ± 0.0	16 ± 0	16 ± 1
PK	29 ± 0	2.2 ± 0.6	14 ± 2	21 ± 2	6 ± 1	0.3 ± 0.1	16 ± 0	15 ± 3
NPK	31 ± 0	1.8 ± 0.1	17 ± 1	20 ± 1	7 ± 0	0.4 ± 0.0	15 ± 0	17 ± 1
Cynometra alexandri								
Control	26 ± 1	1.6 ± 0.1	10 ± 1	16 ± 2	2 ± 0	0.2 ± 0.0	18 ± 0	16 ± 1
N	25 ± 1	1.7 ± 0.2	11 ± 1	13 ± 1	2 ± 0	0.2 ± 0.0	18 ± 1	15 ± 1
P	25 ± 1	1.5 ± 0.2	9 ± 1	16 ± 3	1 ± 0	0.2 ± 0.0	18 ± 1	17 ± 2
K	27 ± 1	1.7 ± 0.2	9 ± 1	14 ± 2	1 ± 0	0.2 ± 0.0	17 ± 1	16 ± 1
NP	27 ± 1	1.7 ± 0.2	9 ± 1	13 ± 1	1 ± 0	0.1 ± 0.0	17 ± 1	16 ± 1
NK	26 ± 1	1.6 ± 0.1	10 ±1	13 ± 1	1 ± 0	0.3 ± 0.0	18 ± 1	16 ± 2
PK	25 ± 1	1.8 ± 0.1	11 ± 1	10 ± 2	2 ± 0	0.1 ± 0.0	19 ± 1	14 ± 1
NPK	26 ± 1	1.6 ± 0.1	10 ± 0	17 ± 4	2 ± 0	0.2 ± 0.0	18 ± 1	17 ± 1
Celtis durandii								
Control	28 ± 4	1.9 ± 0.3	10 ± 2	27 ± 3	3 ± 0	0.4 ± 0.1	16 ± 4	15 ± 1
N	31 ± 1	2.2 ± 0.3	13 ± 2	29 ± 1	3 ± 0	0.3 ± 0.1	12 ± 0	15 ± 2
P	32 ± 1	2.5 ± 0.1	15 ± 3	29 ± 4	4 ± 0	0.4 ± 0.2	12 ± 0	13 ± 1
K	31 ± 1	2.1 ± 0.2	13 ± 1	27 ± 2	4 ± 1	0.2 ± 0.0	12 ± 1	16 ± 2
NP	34 ± 1	3.0 ± 0.4	16 ± 1	33 ± 2	3 ± 0	0.3 ± 0.1	11 ± 0	12 ± 2
NK	32 ± 1	2.4 ± 0.2	16 ± 2	36 ± 5	3 ± 0	0.4 ± 0.1	12 ± 0	14 ± 1
PK	31 ± 1	2.5 ± 0.3	13 ± 2	28 ± 3	3 ± 0	0.3 ± 0.0	13 ± 0	13 ± 2
NPK	40 ± 6	3.6 ± 1.1	20 ± 5	26 ± 7	4 ± 0	0.3 ± 0.0	10 ± 1	12 ± 2
Celtis mildbraedii								
Control	32 ± 2	1.2 ± 0.2	11 ± 2	45 ± 6	3 ± 0	0.4 ± 0.1	11 ± 0	28 ± 3
N	33 ± 0	1.1 ± 0	13 ± 2	38 ± 3	2 ± 0	0.4 ± 0.1	12 ± 0	29 ± 1
P	30 ± 1	1.1 ± 0	9 ± 1	40 ± 5	3 ± 0	0.4 ± 0.1	13 ± 1	28 ± 0
K	30 ± 0	1.0 ± 0	11 ± 0	53 ± 0	4 ± 0	0.3 ± 0.0	12 ± 0	29 ± 0
NP	33 ± 0	1.2 ± 0.1	10 ± 1	42 ± 2	3 ± 0	0.3 ± 0.1	12 ± 0	27 ± 2
NK	34 ± 2	1.5 ± 0.2	11 ± 2	38 ± 7	3 ± 0	0.3 ± 0.0	12 ± 0	24 ± 2
PK	31 ± 1	1.3 ± 0	12 ± 1	40 ± 3	3 ± 0	0.3 ± 0.0	12 ± 0	24 ± 0
NPK	32 ± 1	1.2 ± 0	10 ± 1	33 ± 4	3 ± 0	0.4 ± 0.1	12 ± 1	27 ± 1

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CHAPTER 4

Fine root and soil nutrient dynamics in response to elevated nutrient inputs in humid semi-deciduous tropical forest

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Manuscript under preparation

Abstract:

Fine roots represent a small but physiologically important part of belowground plant biomass. How forest plants cope with infertile soils and how nutrient availability control fine root production in species-rich tropical forests remain an imperative subject in ecology. We conducted a large-scale, full factorial nutrient manipulation experiment (8 treatment × 4 replicates: 32 plots of 40 × 40 m each) in a humid tropical forest in Uganda. We added nitrogen (N), phosphorus (P), potassium (K), their combinations (NP, NK, PK, and NPK) and control at the rates of 12.5 g N m^{-2} yr^{-1}, 5 g P m^{-2} yr^{-1} and 5 g K m^{-2} yr^{-1}, divided into four equal applications. Our objective was to evaluate the responses of fine root production and plant-available soil nutrients to N, P and K fertilization thereby identifying the role of nutrients in limiting growth and microbial processes. We quantified fine root biomass (0−10 cm soil depth) at the end of the first and second years of the experiment by excavating soil monoliths (20 cm × 20 cm) at six random locations within each plot. Fine root production in the top 30 cm soil depth was estimated using the sequential coring technique in the second year of the experiment. It was determined that the addition of N reduced fine root biomass by 35% after the first year of the experiment whereas K addition was associated with reduced fine root production, suggestive of an alleviated ecosystem-scale N and K limitation. This rapid reduction in fine root biomass and production highlight that maintaining a large fine root network is an energy and resource-intensive process, therefore, trees will scale back their root network when they have adequate resources available. Additionally, nutrient additions resulted in a cascade of biochemical responses in soil nutrient availability. Specifically, (1) net N mineralization and nitrification rates were enhanced by the interaction effects of all three nutrients. This highlights the complementary roles of these nutrients in regulating soil processes related to N-cycling in this ecosystem. (2) Microbial biomass C increased with P additions but was dependent on the season. Lastly, P additions increased plant-available P by 80%. This large increase could indicate that the demand for P was not very high. Our data show that N and K regulate fine root growth in this ecosystem

Keywords: *Budongo forest, fine root biomass, highly weathered soils, Lixisols, microbial biomass, net mineralization, net nitrification, nutrient limitation, potassium*

4.1 Introduction

Tropical forests play a critical role in the Earth's biogeochemical cycles through their vast exchange of water, carbon and nutrients within the terrestrial biosphere. These forests store nearly 30% of the world's soil carbon (Jobbagy and Jackson 2000) and account for 30 to 50% of terrestrial productivity (Field et al. 1998). With increasing anthropogenic CO_2 emissions into the atmosphere, these forests are expected to heighten their potential in carbon sequestration and storage to mitigate global climate change (Phillips et al. 1998, Malhi et al. 2004, Chave et al. 2008, Lewis et al. 2009). However, how soil nutrients regulate or limit carbon assimilation across tropical forest biomes is poorly understood particularly for underrepresented tropical regions (Wieder et al. 2015).

Soil nutrient acquisition by plants and microbial processes in soils play a key role in the global carbon cycle and are central to the concept of nutrient limitations. While most nutrients, including P and K in forest soils, are derived through weathering of parent materials or atmospheric deposition, N is mainly fixed into the soil by free-living N fixing bacteria or leguminous plants and accumulate with time (Vitousek and Farrington 1997, Hedin et al. 2009, Hietz et al. 2011). Additionally, the biochemical capacities of soil microbes (fungi and bacteria) enable the breakdown of organic compounds, nutrient mineralization and nutrient mining in mineral soils (van der Heijden 2008, Trivedi et al. 2016). These soil microbes require nutrients for metabolism and growth and generally, whether nutrients are immobilized (obscured to plant uptake) or mineralized (made available to plants) can be inferred from the C:N ratio of their biomass. This makes the soil microbial community both a sink and a source of nutrients (during turnover) and therefore have direct feedback on soil carbon dynamics (van der Heijden 2008). Studies have shown that these biogeochemical soil processes, which vary both spatially and temporally, across tropical forest biomes can be nutrient or carbon limited (Cleveland et al. 2002, Tamatamah et al.

2005, Townsend et al. 2008, Griffiths et al. 2012, Nasto et al. 2017, Camenzind et al. 2018, Darcy et al. 2018, Dynarski and Houlton 2018). Examples include P limitation of organic matter decomposition (Cleveland et al. 2002), molybdenum limitation of asymbiotic nitrogen fixation (Barron et al. 2009, Dynarski and Houlton 2018) and nutrient limitation of microbial biomass (Homeier et al. 2012, Camenzind et al. 2018). Rainfall seasonality, elevation, substrate age, pH and forest composition and structure are all known controllers of these microbial processes and bioavailability of soil nutrients (Stark and Firestone 1995, Corre et al. 2007, Sotta et al. 2008, van Straaten et al. 2011, Tchiofo Lontsi et al. 2020).

It is widely recognized that a large proportion of tropical forests have highly weathered and mostly infertile soils. How forest plants cope with these infertile soils and how nutrient availability control fine root (≤ 2 mm diameter) production remains an important subject in these species-rich forests. This is because fine roots represent a small but functionally important part of belowground plant biomass (Santantonio et al. 1977). They have the closest contact with soil and are responsible for the acquisition of water and soil nutrients due to their large surface area to volume ratio. Their production and turnover can serve as an important pathway of organic carbon input into the soil (Matamala et al. 2003). According to allocation theory, plants maintain a large network and biomass of fine roots when soil nutrients are scarce (Bloom et al. 1985, Powers et al. 2005). Presupposing that, decrease in fine-root biomass is directly linked with increased availability of the scarcest nutrient (Nadelhoffer 2000, Hendricks et al. 2006). The production of fine roots can represent the balance between building new roots and maintaining metabolically older roots (Yavitt et al. 2011), both of which requires enormous energy investment by the trees. Therefore, nutrient availability can be one of the major controls on fine-root dynamics (Vogt et al. 1995). To this end, fine root response to increased nutrient availability can serve as a diagnostic indicator of ecosystem nutrient status. This was evident in the decreased fine root biomass and increased turnover in response to K fertilization (Yavitt et al. 2011); decreased fine-root biomass under N additions (Adamek et al. 2011); under N and K additions (Wright et al. 2011) and declines in seedling root: shoot ratios (Santiago et al. 2012).

There are only a handful of replicated ecosystem-scale nutrient manipulation experiments in the tropics that rigorously evaluate nutrient limitation of primary productivity, which happen to be geographically biased, such that our knowledge of nutrient cycles and their limitations is geographically selective. There is a serious underrepresentation of such studies in Africa, which is home to the world’s second-largest tropical forest basin beside the Amazon in South America. A recent meta-analysis involving 48 nutrient manipulation experiments in tropical forests were mostly conducted in the neotropics (32), SE Asia (8) and Hawaii (8), with no representation from Africa or Australia (Wright 2019). To understand the controls of nutrient availability on ecosystem carbon assimilation, we established a large-scale nutrient manipulation experiment in a moist semi-deciduous tropical forest in Uganda using a replicated full factorial experimental design. Additionally, our study site has sandy-rich soil with near-neutral pH, in contrast to most fertilization studies that have been conducted on clay-rich and mostly acidic soils (Davidson et al. 2004, Siddique et al. 2010, Wright et al. 2011, Alvarez-Clare et al. 2013, Du et al. 2020). Earlier studies at this site found N and K limitations of tree stem growth after only two years of fertilization (Chapter 2).

In this study, however, we evaluate the roles of N, P and K or their interactions on fine root biomass and productivity as well as changes in plant-available nutrient concentrations in the soil. Specifically, our first objective was to investigate whether or not N, P and K or their interactions (co-) limit fine-root biomass and productivity. Here we predict that fine root biomass will show a strong response to the elevated input of N and K which are limiting stem growth at the same site (Chapter 2). Furthermore, the addition of K will alter root biomass allocations (Wright et al. 2011, Doetterl et al. 2015) because K is critical for regulating sucrose exports from leaves to roots. Our second objective was to evaluate changes in plant-available soil nutrients (phosphorus, N cycling rates and microbial C-N) in response to elevated N, P and K inputs. Again, based on growth limitations by N and K availability in this site (Chapter 2 and 3), we predict that soil biochemical responses will differ between the nutrient addition treatments. This is because the forests' nutrient demands and cycles may have potentially been altered through increased inputs of nutrients and their secondary effects on microbial processes and soil chemistry (Sayer 2006). The addition of N will likely stimulate the already high net N cycling rates in the soils, increasing NO_3- leaching, which in turn will promote losses of base cations (e.g. K+). The addition of P will increase plant-available P since this soil has a relatively high pH (Table 1). This means added P would be less adsorbed onto iron and aluminium (hydro) oxides. The combined additions of either two or three of these nutrients will have positive effects on plant nutrient availability rather than their single nutrient addition because of their complementarity in supplying the stoichiometric nutrient requirements of trees and soil organisms.

4.2 Materials and methods

4.2.1 Study site description

We conducted this experiment in the Budongo Forest Reserve in northwestern Uganda (1°44'28.4"N, 31°32'11.0"E; mean elevation: ~ 1050 m). The Budongo forest is a humid, semi-deciduous tropical rainforest situated on an uplifted Shield, specifically on a Precambrian gneissic-basaltic basement complex (van Straaten 1976). Annual air temperature and precipitation average 22.8 ± 0.1 °C and 1670 ± 50 mm, respectively (2000–2019; Budongo Conservation Field Station).

The region experiences a bimodal rainfall pattern with the dry seasons (< 100 mm per month) occurring from December to February and in July. Annual nutrient depositions were 8.5 kg N ha^{-1} yr^{-1}, 0.03 kg P ha^{-1} yr^{-1} and 4.3 kg K ha^{-1} yr^{-1} (Chapter 2).

Soils at the site are well-drained, highly weathered and are classified as Lixisols (IUSS Working Group WRB, 2014; Veldkamp et al., 2020). For the top 0.1 m soil depth, the soil is relatively sandy in texture (55 ± 2 % sand, 27 ± 2% silt and 18 ± 1% clay), characterized by high soil base saturation (98.2 ± 0.2 %), high soil pH (6.4 ± 0.0; 1:2.5 H_2O), calcium-dominated cation exchange capacity (149.2 ± 8.3), which are likely derived from the weathering of geological parent material as well as ash deposition from either regional biomass burning or historic volcanic activity (Fabian et al. 2005, Boy and Wilcke 2008, Bauters et al. 2021). Total organic carbon averaged 4.02 ± 0.13 kg m^{-2}, total organic nitrogen averaged 0.42 ± 0.01 kg m^{-2}, Bray II phosphorus averaged 1.8 ± 0.2 g m^{-2} and a bulk density of 1.2 g cm^{-3}. The soil's $\delta^{15}N$ signature was 8.0 ± 0.0 ‰ whereas net mineralization and nitrification rates were 95 ± 8 and 114.3 ± 7.0 mg N m^{-2} day^{-1} respectively. Microbial biomass C (MBC) was 73 ± 4 g N m^{-2} and microbial biomass N (MBN) was 12.3 ± 0.7 g N m^{-2} .

Vegetation at the experimental site is species-rich and diverse (126 tree species; Shannon-diversity index H': 2.53 ± 0.04). Among trees ≥ 10 cm DBH, 6 % represented nitrogen-fixing trees in stem abundance (Table S2.1), which accounted for 16 % of the forest's basal area (Table 2.1). Leaf litterfall at this site averaged 8.5 ± 0.3 Mg ha^{-1} yr^{-1} (Table 3.3), LAI averaged 3.3 ± 0.0 m^2 m^{-2} (determined in April 2018 and November 2019) in the control plots. The six most dominant species of all trees ≥ 10 cm DBH at the experimental site are *Funtumia elastica (24 %), Celtis mildbraedii (15 %), Cynometra alexandri (6 %), Celtis zenkeri (6 %), Lasiodiscus mildbraedii (6 %), Celtis durandii (6 %)* (Table S2.1).

4.2.2 Experimental design

In 2018, we established a factorial NPK experiment with eight treatments (Fig. 1.4). These treatments had four replicates and were randomly assigned to 32 plots, 40 m × 40 m each and at least 40 m apart. Nitrogen was added as urea ($(NH_2)_2CO$) at a rate of 12.5 g N m^{-2} yr^{-1}, P as triple superphosphate ($Ca(H_2PO4)_2$) at a rate of 5 g P m^{-2} yr^{-1} and K as muriate of potash (KCl) at a rate of 5 g K m^{-} yr^{-1}. Fertilizers were mixed with soil directly adjacent to the plot and broadcasted by hand, walking forward and back and subsequently changing directions (north to south and east to west). We fertilized four times (beginning from 17th May 2018) in a year in equal doses during the wet season.

4.2.3 Soil sampling and analysis

Soil biochemical characteristics were measured in April 2018 prior to initial fertilization. Soil samples were taken from 10 random locations per plot at 0−0.1 m depth in all 32 plots. Soil organic carbon (SOC) and total N were analyzed using a CN elemental analyzer (VARIO EL Cube, Elementar Analysis Systems GmbH, Hanau, Germany). Exchangeable cations (Ca, Mg, K, Na, Al, Fe, Mn) were determined by percolating the soil samples with unbuffered 1M NH_4Cl and cation concentrations in percolate were analyzed using the inductively coupled plasma-atomic emission spectrometer (ICP-AES; iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH, Dreich, Germany). Soil extractable P was determined using the Bray II method (Bray and Kurtz 1945) and analyzed with ICP-AES. Soil pH was analyzed in 1:2.5 of soil-to-distilled water ratio. Soil texture for each plot was determined from a composite sample using the pipette method after iron oxide and organic matter removal (Kroetsch and Wang 2008). Soil bulk density (corrected for stone content) was measured from soil pits dug next to each plot using the core method (Blake and Hartge 1986).

To evaluate how elevated nutrient input affected levels of plant-available N and P, we measured changes in net rates of soil N cycling and resin-exchangeable P in all plots twice a year, once during the dry season (February) and once during the wet season (November). Soil net N mineralization and net nitrification rates were measured in situ using the "buried bag" incubation method (Hart et al. 1994). Here, we randomly select two grid points per plot, and took two intact soil cores from the top 0.05 m depth at each point. One of the paired soil cores is immediately extracted with a 0.5 mol L^{-1} of K_2SO4 solution (T0), while the other core is put in a plastic bag and placed back into the soil for a 7-days incubation (T1), it is also extracted in the same manner. Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations are then analyzed (SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany). Net N cycling (mineralization and nitrification) rates are the differences in mineral N concentrations between T1 and T0 samples. Since this method excludes N uptake by roots but includes microbial immobilization, it is used as an index of the amount of mineral N produced in the soil that is available for plant uptake (Schmidt et al. 2015, Wolf et al. 2011, Hart et al. 1994). Plant-available P was determined using the resin-extraction method, often used in tropical forests (e.g. Condit et al. 2013) from a pooled soil sample taken from nine grid points (0.05 m depth) per plot, this sampling was done twice a year (dry and wet seasons).

Microbial biomass C and N were determined from undisturbed soil core samples taken from two grid points and at 0.05 m depth) per plot using the chloroform ($CHCl_3$) fumigation-extraction method (Brookes et al. 1985). Similar to the N extractions, one of the paired soil cores is immediately extracted with a 0.5 mol L^{-1} of K_2SO4 solution (T0) whilst the other part is fumigated with $CHCl_3$ for 7-days and then extracted in the same manner. Organic C in the extracts was measured with a Total Organic Carbon Analyzer (TOC-VWP, Shimadzu Europa GmbH, Duisburg, Germany). Total N in the extracts was measured using continuous flow injection colorimetry (Method G-157-96; SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany). The microbial biomass C and N is the difference in extractable C and N between the fumigated and unfumigated samples, divided by a constant (kC = 0.45 and kN = 0.68) which represent mineralizable fractions of C and N. For all extracted soil samples, the gravimetric moisture contents (oven-drying for 24 hours at 105°C) from each soil core were determined to calculate the dry mass of extracted soil and express rates of soil N cycling and microbial biomass on dry soil mass basis (μg C/g soil/day) or extrapolated to area basis using the measured soil bulk density.

4.2.4 Root biomass and productivity measurement

To evaluate the effects of nutrient additions on below-ground woody biomass production, we quantified fine root biomass (0−10 cm soil depth where ~60 % of roots are found; Fig. 4.1) at the end of the first and second year of the experiment by excavating soil monoliths (20 cm × 20 cm × 10 cm depth) at six random locations within each plot. Roots from each soil monolith were separated into coarse (≥ 2 mm diameter) and fine roots (≤ 2 mm diameter) and oven-dried at 60°C for 24 hours. We also analyzed the spatial variability of root biomass against possible controlling factors such as distance to the nearest tree (≥ 10 cm DBH), tree density, and soil extractable N (nitrate and ammonium) concentrations.

Fine root production was estimated using the sequential coring technique in the top 30 cm of soil depth (0−10 cm and 10−30 cm; ~ 80 % of roots are found in the top 30 cm; Fig. 4.1), using a sharp root corer, similar to the method employed by Adamek et al. (2011). Although this technique is laborious, it is known to give the most reliable results (Hertel and Leuschner 2002). Root samples were taken every three months in the second year (i.e. 4 sampling times), handwashed over a 1-mm sieve, then dried and weighed. To evaluate the effect of nutrient addition on root productivity, we calculated fine root biomass production using the ‘minimum-maximum method (McClaugherty et al. 1982) and root turnover. The ‘minimum-maximum method refers to the minimum and maximum difference in fine root biomass during the one-year measurement period equated as root production irrespective of the other measured values. We calculated the turnover rate of fine root biomass (year^{-1}) by dividing the annual fine root production by the standing fine root biomass.

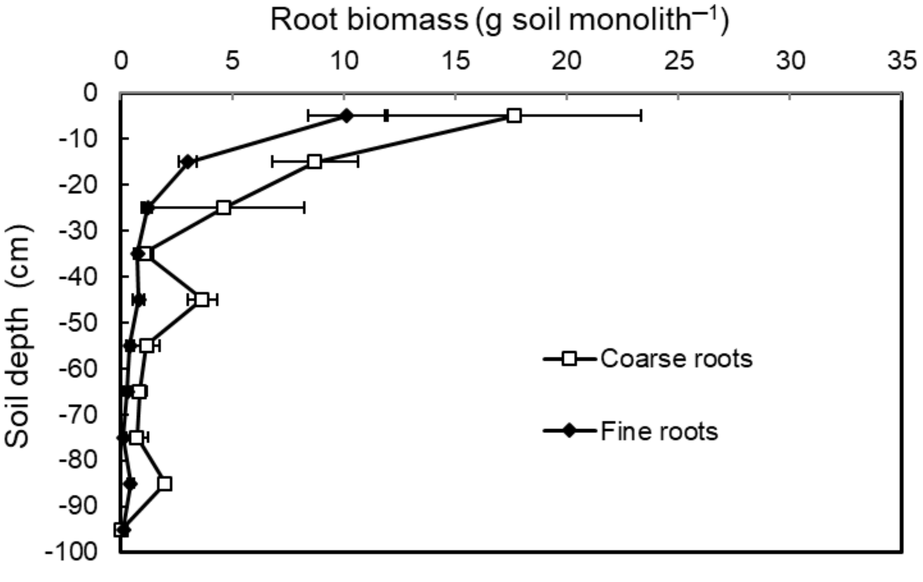

Figure 4.1: Distribution of root biomass (mean ± SE) with soil depth in the Budongo experimental site. About 80 % of roots are found in the top 30 cm soil depth.

4.2.4 Statistical analyses

We used linear mixed-effect (LME) models to test the effect of fertilization treatments and their interaction in the full factorial N × P × K experimental design on all repeated measurements (root biomass, net N mineralization and nitrification, microbial biomass C and N, and resin extractible P). The absence/presence of each of the main nutrients (Wright et al. 2011) and time were used as fixed effects, replicate plot was included in the LME model as random effects. The LME models were run in the ‘nlme’ package (lme function) in the R software and the significance of the fixed effect was evaluated using ANOVA (Crawley, 2009). If residual plots revealed non-normal distribution or non-homogenous variance, we log-transformed the data and then repeated the analyses. Baseline measurements (soil physical and biochemical characteristics, net N mineralization and nitrification, microbial biomass C and N, and resin extractible P) were tested separately for differences in pre-treatment plots that were later assigned to the eight nutrient addition treatments using one-way ANOVA. This was done to examine if inherent differences existed proir to fertilization. All parameters were first tested for normal distribution (Shapiro-Wilk's test) and equality of variance (Levene's test; Sokal and Rohlf 1981). Logarithmic or square root transformation was applied when these assumptions are violated. In all tests, statistical significance was set at $P \leq 0.05$. All data used in the analyses were plot means, obtained by averaging values of the two random sample locations within each plot. All statistical analyses were performed using the statistical package R version 3.6.2 (R Development Core Team, 2018).

4.3 Results

4.3.1 Fertilization effect on root biomass and productivity

After two years of nutrient additions, fine root biomass (<2 mm diameter) was significantly less in the N addition plots compared with non-N additions plots (main effect of N; $P = 0.0001$; Fig. 4.2a and b). A 36% reduction in fine root biomass associated with N additions occurred after the first year of nutrient addition (2019), which remained almost unchanged (35%) after the second year (2020). The additions of K led to decreased fine root production by 5 % ($P = 0.045$) in the top 0−10 cm soil depth and 7 % ($P = 0.033$) in the 0−30 cm soil depth, with no treatment effect observed in the 10−30 soil depth. The additions of P did not affect fine root biomass and productivity. Fine root turnover rates, which were unaffected by nutrient additions, averaged 1.3 ± 0.2 yr^{-1} for the top 0−10 cm soil depth, 1.5 ± 0.2 yr^{-1} for 10−30 cm soil depth and 1.0 ± 0.1 yr^{-1} for a combined 0−30 cm soil depth in the control plots. We found no relationship between root biomass and the density or size of the neighbouring trees (either in 1m or 2 m distance) to the measurement locations.

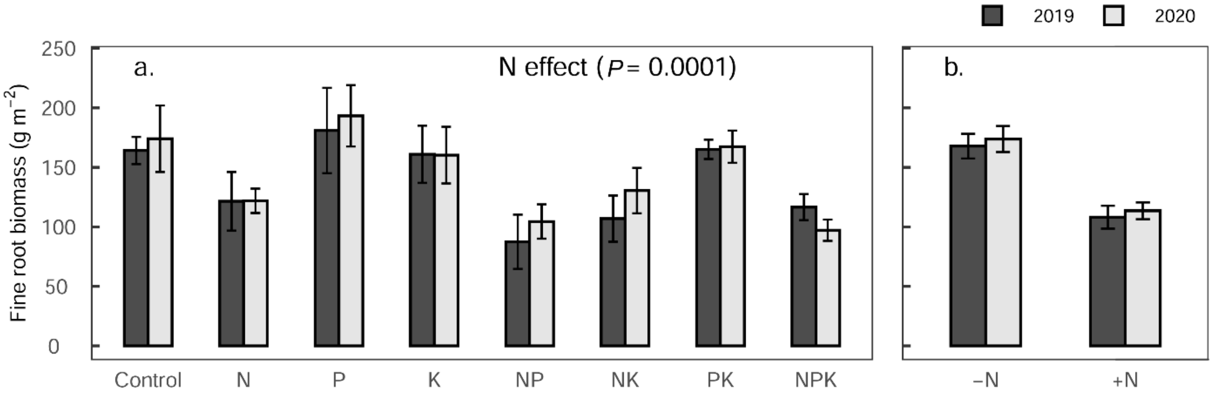

Figure 4.2: Effect of nutrient addition on root biomass (mean ± SE) in the top 10 cm soil depth of the Budongo forest experimental trial. Presented are fine root biomass (a; n = 4 plots), fine root biomass in +N vs -N addition plots (n = 16 plots) and coarse root biomass (c. n = 4 plots). Analysis was based on the full factorial NPK experimental design.

4.3.2 Fertilization effect on N cycling processes and P

Net N mineralization and nitrification rates were enhanced by the interaction effects of N × P × K interactions (*P* = 0.012 and 0.020, Fig. 4.3a and b). In February 2020, the additions of N alone increased net N mineralization (*P* = 0.035) and only marginally for net N nitrification (*P* = 0.052) rates, in contrast, we observed a negative N × P interactions effects on net N mineralization (*P* = 0.047) and net N nitrification rates (*P* = 0.046). There was, however, no clear time effect (*P* ≥ 0.068) nor seasonal effect (*P* = 0.559) from the LME analysis of the entire measurement period.

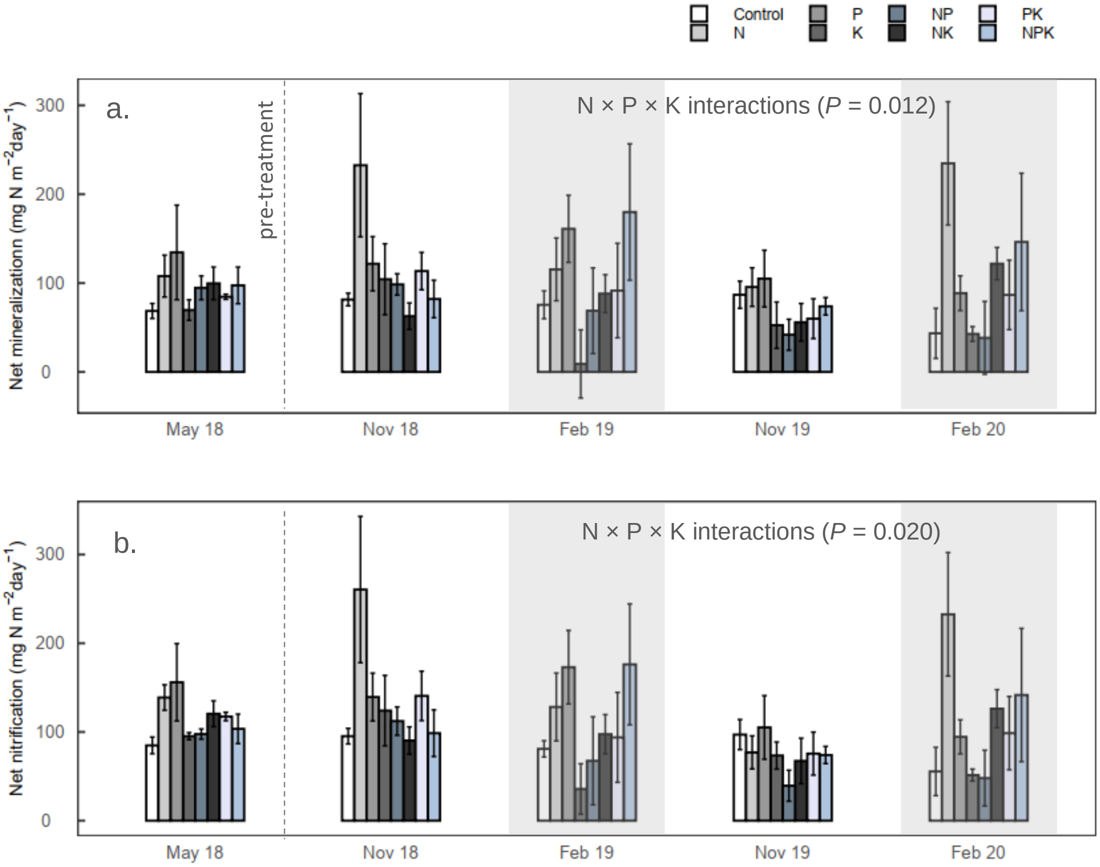

Figure 4.3: Response of N-cycling rates to nutrient additions (12.5 g N m^{-2} yr^{-1}, 5 g N m^{-2} yr^{-1} and 5 g N m^{-2} yr^{-1}). Presented are the mean values (± SE; n= 4 plots) for net N mineralization rate (a) and net N nitrification rate (b). The grey shading represents the dry season (< 100 mm rainfall per month). Analysis was based on a full factorial NPK experimental design.

Although NH_4^+ (ammonium) pools changed with time in the course of the experiment (time effect; $P<0.0001$, Fig. 4.4a), no detectable treatment effect was found. In contrast, N additions increased NO_3^- ($P = 0.037$, Fig. 4.4c and d) particularly in the dry seasons and as expected, decreased ammonium nitrate ratio (NH_4^+: NO_3^-) ($P = 0.047$) in the soil. The resin extracted P was significantly higher in P addition plots by 80 % ($P = 0.002$; based on full factorial NPK analysis) compared to P excluded plots and varied with the season (e.g. Table 4.1).

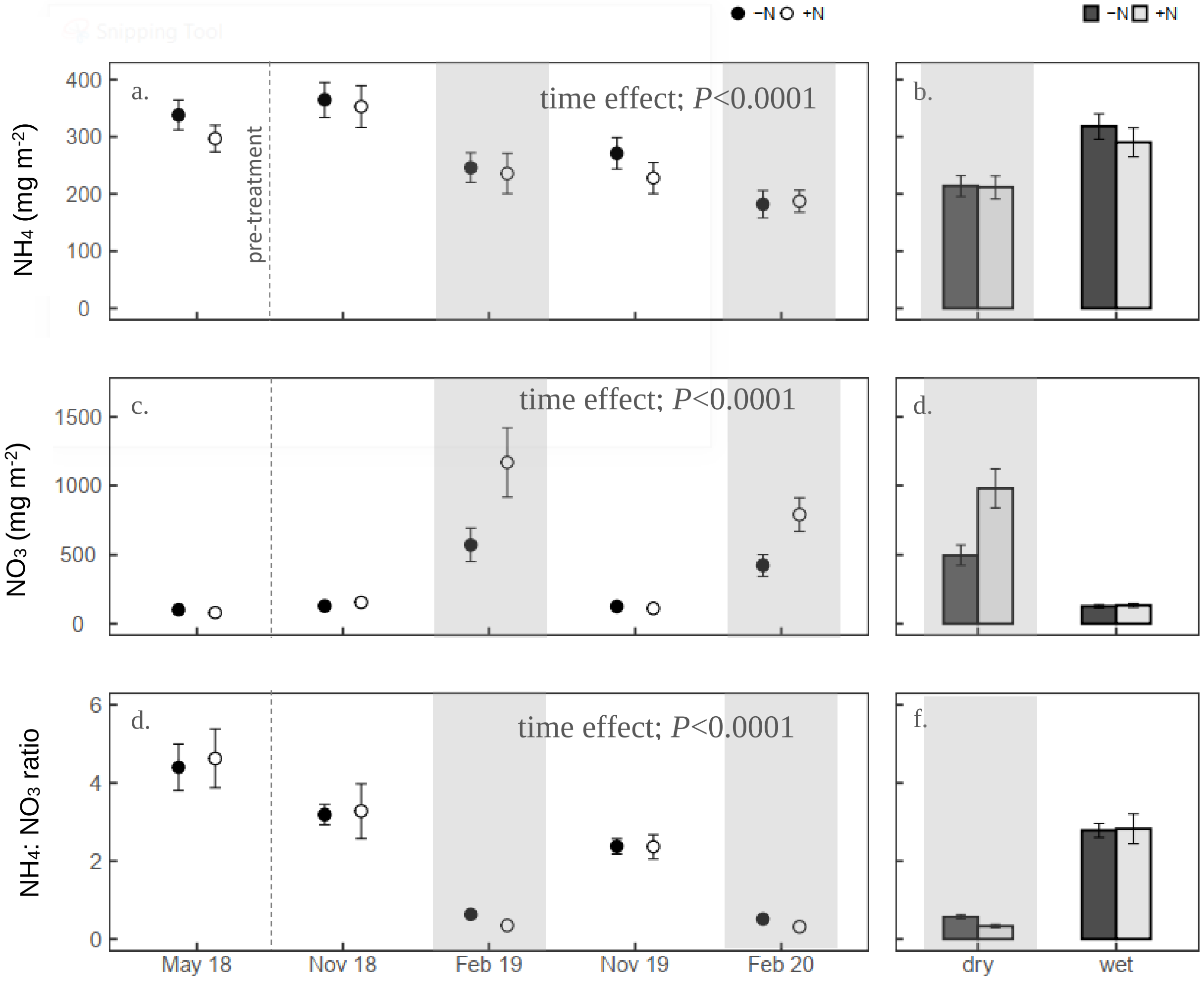

Figure 4.4: Effects of nitrogen additions (12.5 g N m^{-2} yr^{-1}) on nitrogen forms: ammonium concentration (a–b), nitrate concentration (c–d), ammonium to nitrate ratio (d–f). Presented are mean values (± SE; n = 16 plots) of +N addition and non-N addition plots. The grey shading represents the dry season (< 100 mm rainfall per month). Analysis was based on full factorial NPK experimental design.

Table 4.1: Net rates of soil-N transformations, microbial biomass C and N, and plant-available P in the semi-deciduous tropical forest in Budongo exposed to elevated N-P-K input, measured in the dry (February 2019 and 2020) and wet (November 2018 and 2020) seasons. Values presented are mean ± standard error, n = 4 plots.

Parameter	**Season**	**Control**	**N**	**P**	**K**	**NP**	**NK**	**PK**	**NPK**
Net N mineralization (mg m^{-2} day^{-1})	Wet	84 ± 11	164 ± 30	113 ± 22	79 ± 27	70 ± 11	59 ± 16	87 ± 16	78 ± 12
	dry	60 ± 18	175 ± 45	125 ± 27	26 ± 17	54 ± 44	105 ± 11	89 ± 19	163 ± 57
Net N nitrification (mg m^{-2} day^{-1})	Wet	96 ± 13	169 ± 32	122 ± 24	99 ± 25	76 ± 13	79 ± 20	108 ± 24	86 ± 17
	dry	68 ± 15	180 ± 47	134 ± 30	44 ± 11	58 ± 39	112 ± 13	96 ± 15	159 ± 55
Microbial biomass C (g m^{-2})	Wet	67 ± 10	64 ± 11	74 ± 20	66 ± 9	57 ± 9	70 ± 11	59 ± 6	60 ± 11
	dry	48 ± 20	54 ± 5	86 ± 15	47 ± 8	56 ± 6	51 ± 4	62 ± 10	43 ± 8
Microbial biomass N (g m^{-2})	Wet	13 ± 0.8	13 ± 2	16 ± 3	14 ± 1	12 ± 2	14 ± 2	12 ± 1	12 ± 2
	dry	11 ± 0.4	11 ± 1	14 ± 3	11 ± 2	10 ± 1	11 ± 2	12 ± 1	10 ± 2
Microbial biomass C:N	Wet	6 ± 0.6	5 ± 0.1	4 ± 0.3	5 ± 0.4	5 ± 0.2	5 ± 0.2	5 ± 0.1	5 ± 0
	dry	4 ± 0.1	5 ± 0.4	7 ± 2	4 ± 0.5	6 ± 1	5 ± 0.2	5 ± 1	5 ± 0.1
Resin extractible P (mg m^{-2})	Wet	88 ± 26	65 ± 15	193 ± 58	71 ± 13	92 ± 10	101 ± 10	132 ± 20	135± 17
	dry	22 ± 3	23 ± 3	60 ± 12	24 ± 2	47 ± 4	33 ± 4	47 ± 5	54 ± 4

Note: Net rates of soil-N transformations were determined by a 7-day field incubation (buried bag method)

4.3.3 Fertilization effect on soil microbial biomass C and N

Microbial biomass C, N and C:N ratio varied significantly with time over the experimental period (time effect; $P < 0.008$, Fig. 4.5). Nutrient addition did not affect microbial biomass C when analysed across the entire experimental period (measured in Nov. 2018, Feb. 2019, Nov. 2019 and Feb. 2020) although P additions increased microbial C by 28 % and microbial C:N ratio by 32% in February 2019 (dry season; $P = 0.03, 0.041$). In February 2020 (also a dry season), microbial biomass C:N ratio increased by 15 % with N additions ($P = 0.002$) but decreased with K additions (26 %) and N × K interactions ($P = 0.012, 0.010$). Microbial biomass N was enhanced by P × K × time interactions across the four response measurement periods together ($P = 0.018$).

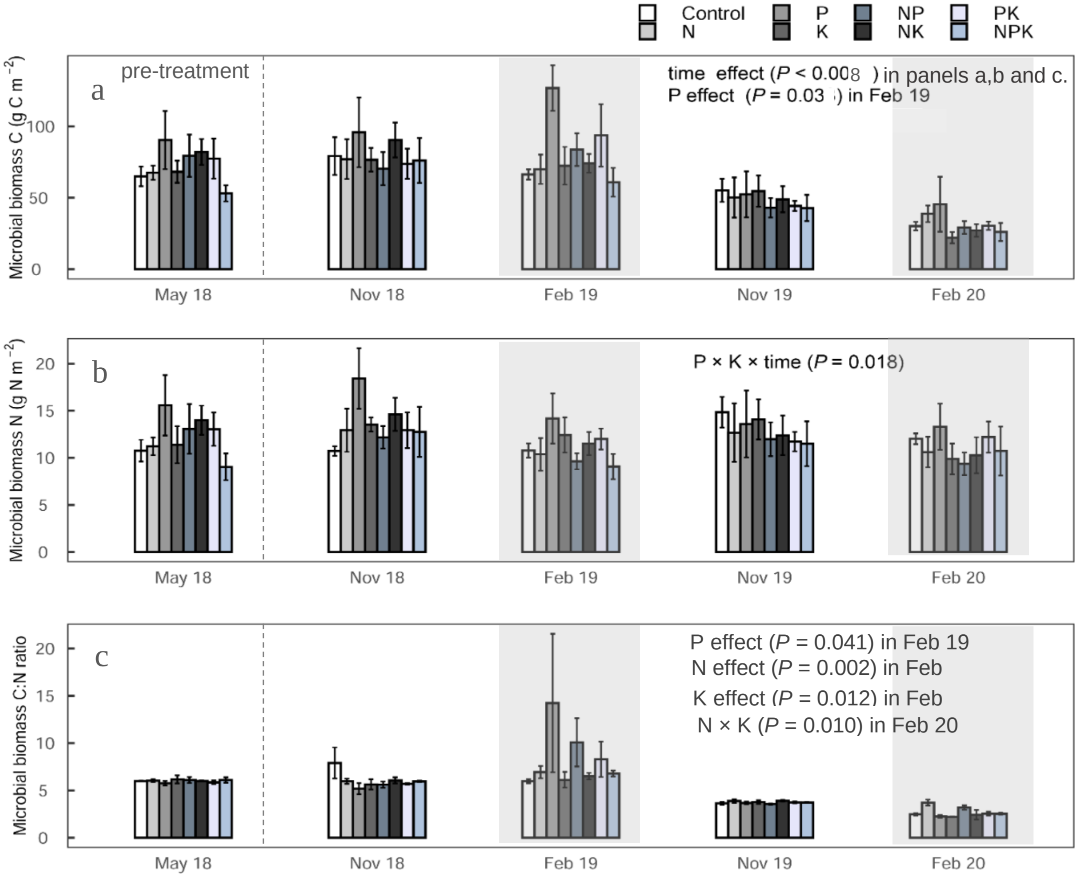

Figure 4.5: Response of microbial biomass to the additions of 12.5 g N m^{-2} yr^{-1}, g N m^{-2} yr^{-1} and g N m^{-2} yr^{-1} at the Budongo experimental site. Presented are the means (± SE; n = 4 plots) at each

measurement time point for Microbial biomass C (a), microbial biomass N (b), and microbial biomass C:N ratio (c). Analysis was based on a full factorial NPK experimental design.

4.4 Discussion

4.4.1 Root biomass and productivity

The fine root biomass (FRB) in the control plots of our site (169 ± 16 g m^{-2}; in the top 10 cm soil depth) was comparatively lower than those reported from a Panamanian humid lowland (Yavitt et al. 2011) and lower montane (Adamek et al. 2011) tropical forests. Similarly, the fine root production (FRP) of 170 ± 17 g m^{-2} yr^{-1} at this site was relatively lower (Adamek et al. 2011, Priess et al. 1999), possibly reflecting differences in stand age (Giardina and Ryan 2002) and soil fertility (Ryan et al. 1996) between these sites. In this forest site, for instance, the soils are Lixisols with near-neutral pH and a calcium dominated base saturation, this sharply contrasts the disproportionately high fine root production recorded in a Venezuelan forest underlain by very acidic Oxisol with low calcium availability (Priess et al. 1999).

The rapid reduction in fine roots in the N and K addition plots provides further evidence of an N and K limitation in this ecosystem, consistent with our results of the N and K limitation of stem growth (Chapter 2) for some trees in this forest. A similar trend was also observed in Panama, where N + P + K treatment led to reductions in FRB (Wurzburger and Wright 2015) and a 4 yr K additions led to less FRB (Yavitt et al. 2011). This reduction in FRB is consistent with the allocation theory (Bloom et al. 1985) and it is because maintaining a large fine root network is a high resource-intensive process, therefore, trees will scale back fine root network when they have adequate resources available (e.g. water and nutrients) (Bloom et al. 1985, Vogt et al. 1995, Nadelhoffer 2000, Powers et al. 2005). This is further supported by the absence of a relationship between root biomass and the density or size of the neighbouring trees thus suggesting that trees in this site extend their roots everywhere to find the needed resources regardless of tree stem location and that spatial distribution of FRB is driven by soil nitrogen availability (Fig 4.5).

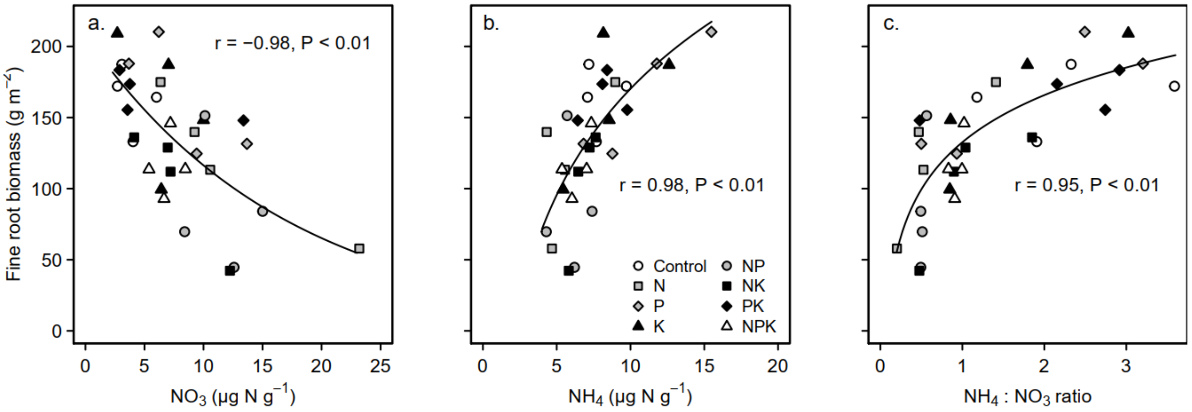

Figure 4.6: Relationship between fine root biomass after one year of fertilization and (a) soil nitrate concentrations, (b) soil ammonium concentrations and (c) NH_4^+:NO_3^- ratio.

The transformation of N (nitrification) into plant-accessible forms e.g. nitrate, is key to ecosystem productivity and is highly dependent on activities of microorganisms, such as archaea, bacteria and fungi. As is evident in Fig. 4.6, FRB was negatively correlated with soil nitrate and positively correlated with soil ammonium concentrations. This means that increases in nitrate (as a result of fertilization) resulted in decreases in FRB, whereas the opposite was evident for ammonium. This contrasting relationship can be explained thus the addition of urea fertilizer likely intensified soil nitrification rates, leading to lower NH_4^+ and higher NO_3^- concentrations in the soil (Olsson and Falkengren-Grerup 2003). Furthermore, increased nutrient (mostly N) additions may have promoted a diverse assemblage of nitrifying microorganisms, which then catalyses the process of nitrification by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), hence the increased NO_3^- concentration (Aarnio and Martikainen, 1992). In an old-growth lowland forest situated on Inceptisol in Panama, there was no immediate effect of N additions on nitrification until after 9 yrs. of chronic N additions, where increased NO_3^- were measured as leaching losses (Corre et al. 2010). Such differences in response to N additions by soil NO_3^- may be explained by differences in underlying substrate type and soil texture (Corre et al. 2010). While we cannot definitively say that the reductions in NH_4^+ were not responsible for the FRB reductions, it is more likely however that it was the corresponding increases in NO_3^- that reduced the FRB. This is because most terrestrial plants (including those in the tropics) prefer nitrate over ammonium (Kant, 2018). The relationship evident between FRB and the NH_4^+:NO_3^- ratio (Figure 4.5c), highlights how FRB decreased dramatically when the NO_3^- concentrations surpass NH_4^+ concentrations ($NH_4^+:NO_3^- < 1$).

4.4.2 Seasonality and effects of NPK additions on N-cycling

Rates of net N mineralization (1.5 ± 0.1 $\mu g\ g^{-1}$ DS day^{-1}) and net N nitrification (1.9 ± 0.1 $\mu g\ g^{-1}$ DS day^{-1}) in the control plots are rather lower than those reported for humid tropical forests dominantly underlain by Ultisols and Oxisols in the Brazilian Amazon Basin (Neill et al. 1997) and those reported from Panama (Santiago et al. 2005) but within range of values reported for nine forest sites in Costa Rica (Alvarez-Clare and Mack 2011). These variations in N transformation rates may be related to differences in plant community composition, litter quality and precipitation gradient (Santiago et al. 2005). The lack of a seasonal effect on net N mineralization and nitrification rates at this site is consistent with results of other tropical forests where net N mineralization and nitrification rates appeared to be aseasonal (Matson and Vitousek 1987, Neill et al. 1995). This is, however, in contrast to the rates of net N mineralization and net nitrification in some seasonal tropical forests, which decreases during the dry season compared to the wet season (Garcia Mendez et al. 1991).

Notwithstanding, the significant seasonal differences in NH_4^+ and NO_3^- concentrations, with higher NO_3^- concentrations occurring in the dry season, was consistent with other humid tropical forests (Neill et al. 1997), and is likely linked to the period of lower soil moisture prevalent in the dry season (Neill et al. 1995) or improved soil aeration during this time. This is because nitrification (i.e. transformation of NH_4^+ to NO_3^-) is an aerobic process that will normally decrease in the soils during the wet season (Breuer et al., 2002) on clayey soils. Considering that unlike other highly weathered soils such as Oxisols and Utisols, our site is largely sandy (~ 55% sandy), one would expect that a high porosity will ensure aerobic soil condition even in the wet season.

Overall, the addition of N alone did not enhance net N mineralization or net nitrification rates except in February 2020 (a dry season measurement campaign), instead, these N transformation rates were enhanced by the combined effects of N × P × K interaction. This suggests that the levels of plant-available N in the soil do not necessarily depend on N inputs in the soil. The demand for P and K by a possible increased diverse assemblage of microorganisms could explain the interactive roles of all three nutrients in enhancing N cycling rates. This may also explain why we did only find positive P × K × time interaction effects on microbial biomass N but not with N additions (Cleveland et al. 2002, Kaspari and Powers 2016, Camenzind et al. 2018).

4.4.3 Nutrient addition effects on microbial biomass C and N

Microbial biomass C and N at this site were affected by seasonality (Table 4.1). Higher microbial biomass C (67 ± 10 g N m^{-2}), microbial biomass N (48 ± 2 g N m^{-2}) and microbial biomass C:N ratio (6 ± 0.6) were recorded in the wet season (November 2018, 2019) compared to the dry season in the control plots. These are similar to values reported for lowland tropical forests but higher than those reported for montane tropical forests in Panama (Corre et al. 2010). The reduced microbial C:N ratio in the dry season reflects a reduction in microbial activities due to moisture stress (Stark and Firestone 1995).

The addition of N did not elicit any response from microbial biomass N or C even though tree growth in this forest site was found to be N limited in earlier studies (Chapter 2). It has often been reported that N additions have negative effects either on microbial biomass possibly due to soil acidity (LeBauer and Treseder 2008, Corre et al. 2010) or on organic matter decomposition due to the adverse effect of N additions on lignin-degrading fungi (Fog 1988). The measured pH (6.4) before nutrient addition at this site was not repeated throughout the experimental period hence we are unable to relate the lack of response to N addition to possible shifts in soil acidity. Furthermore, the low microbial biomass C:N ratio (< 10), which indicate that the microbial population in this forest site is bacteria-dominated (Anderson and Domsch 1980), is inconsistent with lignin-degrading fungi being negatively affected by N additions. This lack of N addition effect on microbial biomass N, in particular, may unlikely be due to the brevity of the experimental duration since unlike plant communities, changes in microbial communities or gross microbial growth occur much faster (Mori et al. 2019). On the other hand, the positive response of MBC to phosphorus addition is consistent with many reports that suggest that soil microbial activities in tropical forest ecosystems are limited by P and not by N (Cleveland et al. 2002, Homeier et al. 2012, Camenzind et al. 2018). Besides, the addition of P has been shown to stimulate the decomposition of organic matter (Cleveland et al. 2002, Ilstedt and Singh 2005, Kaspari et al. 2008). This is due to the high affinity that labile C has to bind with the sorption sites of mineral soils and thus P addition would replace organic matter bound to the sorption sites (Kaiser & Zech, 1996). The replaced organic matter C after P addition would be accessible to microbes, stimulate soil microbial activity by providing C (Fig. 4.5a).

Lastly, extracted P was 80 % higher in P addition plots compared to non-P addition plots. This was not surprising, considering that P at the site was already relatively high prior to nutrient additions despite being highly weathered soil (Newbery et al. 2002, Allen et al. 2015, Tchiofo Lontsi et al. 2019). A likely reason is that soils at the site have a near-neutral pH, meaning P is not fixed by either iron or aluminium hydrous oxides, and accordingly, P was likely already available in adequate amounts to support this ecosystem even before the experiment began. This is evident in the relatively minor role P had in promoting stem growth (Chapter 2) and belowground productivity (Fig. 4.2).

4.5 Conclusions

This 2-year nutrient addition experiment in an African semi-deciduous tropical forest provides insights into how elevated nutrient availability control belowground productivity and soil biogeochemical processes in a natural ecosystem. First, the addition of N and K reduced fine root biomass by 35% after the first year of the experiment which did not change after the second year, suggestive of an alleviated ecosystem-scale nitrogen limitation. This fast, dramatic reduction in fine root biomass in the N treatments highlights that maintaining a large fine root network is a resource-intensive process, and trees will scale back their root network when they have adequate resources available. Second, nutrient additions of N, P and K caused a cascade of biochemical responses in the soil nutrient availability. (1) Net N mineralization and nitrification rates were enhanced by the interactions effects of all three nutrients. This highlights the complementary roles of these nutrients in regulating soil processes related to N-cycling in this ecosystem. (2) Microbial biomass C increased with P additions but dependent on the season. Lastly, P additions increased plant-available P by 80 %. This large increase could indicate that the demand for P was not very high and underscore the largely non-limiting role of P in this ecosystem. Considering that most large-scale ecosystem experimental research has focused on the roles of N and P availability in limiting productivity, our data show that base cations such as K can be equally important in functional biochemical roles related to ecosystem carbon assimilation. More of such research is undoubtedly much needed particularly for the African tropical forest region, which is the least researched to better understand the mechanistic controls of nutrient availability on forest ecosystem productivity.

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CHAPTER 5

SYNTHESIS

5.1 Estimation of net primary productivity in the control plots

Globally, tropical forests remain a major carbon sink, repository of biodiversity and the most productive biome in the terrestrial biosphere (Myers et al. 2000, Pan et al. 2011). Understanding factors that threaten the productivity and carbon sink potential of tropical forests are of great importance in ecology (Brienen et al. 2015, Hubau et al. 2020, Rammig and Lapola I 2021). Moreover, the extent to which nutrient availability constrain NPP or different components of plant growth (root growth, stem growth, foliar quality and litter production) and which single or multiple nutrients (N, P, K, Ca or micronutrients) responsible for these limitations remain largely contentious. This dissertation provides important insights into understanding the identity and nature of ecosystem nutrient limitations by measuring ecosystem responses to nutrient additions in an understudied African tropical forest with implications on previous widely held hypotheses.

To compare the productivity of our experimental site with other tropical forests, NPP (Mg C ha^{-1} yr^{-1}) was calculated as the sum of annual aboveground wood biomass production (NPP_{stem}), fine litter production ($NPP_{fine\text{-}litter}$), fine root production ($NPP_{fine\text{-}root}$) and coarse root production ($NPP_{coarse\text{-}root:\ >\ 2\ mm}$) based on data presented in Chapter 1−3 of this dissertation. This NPP represents (i) the annual amount of carbon (new organic matter) retained by living plants at the end of the 2−3year measurement interval (e.g. NPP_{stem}), and (ii) the amount of organic matter both produced and lost during the same time interval by the plant (e.g. $NPP_{fine\text{-}litter}$) (Clark et al. 2001). Similar to most comprehensive NPP studies, NPP_{stem} and $NPP_{fine\text{-}litter}$ constitute aboveground NPP (ANPP) whereas $NPP_{fine\text{-}root}$ and $NPP_{coarse\text{-}root}$ constitute belowground NPP (BNPP) without root exudates, carbon allocated to symbionts or volatile organic compounds produced. The $NPP_{fine\text{-}root}$

and $NPP_{coarse\text{-}root}$ in this present study were estimated using the minimum-maximum method (sequential coring (McClaugherty et al. 1982)).

The total estimated NPP at our experimental site is at the upper end of values reported for other tropical forests (Table 5.1) because compared to these values, our NPP includes coarse roots. Most studies do not often estimate coarse root productivity because of the difficulty in measurement and high spatial variability, and often resort to extrapolations using ANPP. In this thesis, coarse root production was estimated but with a caveat, which is that the estimation was based on only two sampling locations per plot due to logistical constraints. There was a similar contribution of biomass production between ANPP (51 %) and BNPP (49 %) with a BNPP: ANPP ratio of 0.99 ± 0.17. However, a stronger linear relationship was found between NPP and BNPP (R^2= 0.82) than that between NPP and ANPP (R^2 = 0.25) suggesting that the response of BNPP to nutrient addition may exert a greater influence on the overall response of total NPP to nutrient addition. Aboveground net primary production at our study site averaged 8.4 ± 0.9 Mg C ha^{-1} yr^{-1}, which is higher than those reported in other humid tropical forests (Table 5.1). Fine litterfall (78 %) dominated ANPP similar to other tropical forests (Malhi et al. 2004, Adamek et al. 2009, Wright et al. 2011).

Table 5.1: Components of net primary productivity (NPP; mean ± SE; Mg C ha^{-1} yr^{-1}) in humid tropical forests.

Location	**ANPP**		**BNPP**		**NPP total**	**References**
	$NPP_{fine\text{-}litter}$	NPP_{wood}	$NPP_{fine\text{-}root}$	$NPP_{coarse\text{-}root}$		
Uganda	6.4 ± 0.4	2.2 ± 0.4	1.2 ± 0.1	6.9 ± 1.2	16.5 ± 1.6 (9.5 ± 0.9) *	This study
Brazil	2.94 ± 0.04	2.55 ± 0.06	3.89 ± 0.8	-	9.38 ± 0.8	Da Costa et al. 2013
Brazil	5.03 ± 0.07	3.02 ± 0.30	3.68 ± 0.52	-	11.73 ±0.6	Doughty et al. 2013
Malaysia	3.02 ± 0.36	3.74 ± 0.29	4.25 ± 0.84	-	11.01 ± 0.96	Katayama et al. 2013
Bolivia	6.20 ± 1.09	5.27 ± 0.31	4.03 ± 0.47	-	15.5 ± 1.23	Doughty et al. 2014
Peru	5.39 ± 0.35	2.41 ± 0.24	4.54 ± 0.71	-	12.34 ± 0.83	Malhi et al. 2014
Panama	4.41	2.03	-	-	-	Adamek et al. 2009
Panama	5.7	2.1	-	-	-	Wright et al. 2011
Indonesia	4.9 ± 0.70	5.2 ± 0.79	1.07 ± 0.23	1.7 ± 0.11	12.4 ± 1.2	Kotowska et al. 2015

*Excluding $NPP_{coarse\text{-}roots}$

5.2 Nutrient controls regulating net primary productivity

Globally, the response of NPP or its components to elevated nutrient inputs displays a variety of patterns in natural forest ecosystems. Although some of these patterns are consistent with theoretical biogeochemical models (Walker and Syers 1976), recent divergent reports underscore the complexities of tropical ecosystems and reflect both spatial and temporal variability in NPP in the tropics (Clark and Clark 2001). In this semi-deciduous tropical forest, we found significant responses of NPP_{total} and BNPP to N × K interaction effects ($P = 0.018$ and 0.019, respectively), but no response of ANPP to nutrient additions. This N × K interaction effects on NPP is consistent with the multiple nutrient co-limitation concepts as demonstrated throughout this dissertation (Chapter 2−4), where tree growth was dominantly controlled by N and K (Chapter 2), leaf litter production by P and K (Chapter 3), and root productivity by N and K availability (Chapter 4). Emerging evidence in previous studies shows that different components of NPP can be regulated by different and multiple nutrients even when NPP as a whole remain unaffected by elevated nutrient availability (Table 5.2). For instance, in a dry secondary tropical forest in Mexico, stem growth and litter production were enhanced by N and P additions (Campo et al. 2007). Consistent with our results, N and K additions also elicited increased stem growth response among a group of trees in Panama (Gigante) after 11 years of factorial N–P–K addition experiment (Wright et al. 2011). Compared to the latter, our observed response of different components to nutrient additions only represent a “snapshot” in time or at best a transient response. It, therefore, remains to be seen how this ecosystem will respond in the long term with sustained fertilizations. That said, information from both short and long term NMEs is critical to developing a comprehensive picture of the responses of tropical forests to nutrient amendments. The role of each added nutrient in regulating different components of NPP is discussed in detail:

5.2.1 Ecosystem response to nitrogen additions

It is commonly hypothesized that there is a higher bioavailability of N over plant demand in tropical forests (Vitousek 1984, Hedin et al. 2009). This claim was indirectly supported in the high abundance and diversity of N-fixing organisms in the tropics (Crews 1999, Hedin et al. 2009); rapid soil N cycling rates (Corre et al. 2010); high gaseous N losses (Koehler et al. 2009); high nitrate leaching (Schwendenmann and Veldkamp 2005) and high foliar and litter N:P ratios (McGroddy et al. 2004). However, based on a meta-analysis involving fifteen N addition experiments conducted in tropical forests, LeBauer and Treseder (2008) claimed that the N limitation of ANPP was strong in tropical montane forests as well as in temperate forests where the substrates are relatively younger (Walker and Syers 1976). Subsequently, this paradigm of N limitation in tropical montane forests gained support (Adamek et al. 2009, Wolf et al. 2011, Homeier et al. 2012) and even appeared to be more pronounced with elevation (Tanner et al. 1998, Graefe et al. 2010).

In this semi-deciduous forest located on an uplifted shield (mean elevation =1050 m a.s.l) underlain by highly weathered soils, N additions increased the stem growth of intermediate-sized trees (10−30 cm DBH) and *Lasiodiscus mildbraedii* (Chapter 2), increased foliar N content of *Celtis mildbraedii* (Chapter 3), drastically reduced fine root biomass in the first year of N additions (Chapter 4), and increased net N mineralization and nitrification rates in the dry period when reduced moisture may have constrained nutrient release through decomposition (Chapter 4). These stimulations by N additions underscore the important role of N availability in this forest ecosystem and can be interpreted as indications of N limitations to the aforementioned ecological processes (Tanner et al. 1998). Although a more recent meta-analysis involving 48 nutrient addition experiments (Wright 2019) suggest that both N and P are equally likely to limit plant function in tropical forests regardless of elevation (Table 5.2), the role of N in this forest gives credence to earlier studies that found N limitation in higher elevations (Adamek et al. 2009, Wolf et al. 2011, Homeier et al. 2012, Tanner et al. 1998, Graefe et al. 2010, Cleveland et al. 2011; McGroddy et al. 2004). The proposition that N availability is in excess of plant demand in tropical forests is hereby not supported.

It remains a paradox that N will be limiting in this site despite the abundance of N-fixing trees (6 % in stem abundance (Table S2.1), 16 % of the forest's basal area and 25 % of aboveground wood biomass (Table 2.1)) and the often favourable climate (wet and warm) present. Three mechanisms have been identified to prevent N fixation or reverse N limitation on NPP: (1) High energy cost of N fixation on N-fixing organisms, for instance, symbiotic N-fixers require 8-12 g of glucose to fix 1g of N excluding structural maintenances and constructions (Gutschick 1981, Vitousek and Howarth 1991). (2) N-fixing rate itself being limited by other nutrients (Wurzburger et al 2012), which may explain why the N-cycling rate at this site was only enhanced by the interactive effects of all three nutrients (Chapter 4). And (3) we speculate the possibility of ecological, physical or management constraints on N-fixing organisms (Vitousek and Howarth 1991).

5.2.2 Ecosystem responses to phosphorus additions

Plant-available P is typically low in most tropical forest soils since these soils often have an old and strongly weathered substrate that becomes depleted of rock-derived nutrients over time, and receive negligible atmospheric P inputs. Therefore, P limitation of primary production on strongly weathered tropical soils, particularly for N-fixing trees, is widely hypothesized (Walker and Syers 1976, Vitousek 1984, Hedin et al. 2009, Vitousek et al. 2010, Wright et al. 2011, Waring et al. 2019). In contrast to this hypothesis, there was no pronounced response in stem growth as a result of P additions at our experimental site.

The role of P to plant function in this ecosystem was rather restricted to promote drought tolerance (Chapter 3), mortality reduction among small trees (1−5 cm DBH; Chapter 2), increased foliar P of *Celtis durandii* (Chapter 3), increased plant-available P in the soil, and enhanced microbial biomass C content in the dry season (Chapter 4), with no detectable response from BNPP at all. Although these responses are considerably important to the ecosystem as a whole, it does not strongly suggest that NPP in this forest was constrained by P availability for three reasons: (1) Extractable P at this forest site was higher or comparable to other tropical forests where P was not found limiting (Allen et al. 2015, Newbery et al. 2002). (2) The near-neutral soil pH at this site (Chapter 1: Table 1.1) suggests that P is not fixed by hydrous oxides of Fe and Al hence not occluded from plant uptake. (3) The increased plant-available P in the soil with P addition suggests that the demand for P in this forest is not high and therefore sufficient P was available for plant uptake (Johnson et al. 2003). Therefore, in consonant with the recent review by Wright (2019), neither the prediction that P-limitation is widespread on old, highly weathered soils (Cárate-Tandalla et al. 2018, Turner et al. 2018, Vitousek et al. 2010, Walker and Syers 1976) nor a generalized P-limitation in N-fixing tree species (Waring et al. 2019) is supported.

5.2.3 Ecosystem responses to potassium additions

To date, very little is known about the mechanistic controls exerted by base cations on ecosystem processes and carbon assimilation particularly in natural tropical forests (Tripler et al. 2006, Baribault et al. 2012). Whereas the roles of N and P have received considerable biogeochemical research attention in tropical NME studies, the role of K in natural tropical forests has been largely overlooked. However, our findings suggest that K limitation (or that of other base cations) on ecosystem productivity and function could equally be widespread in this tropical forests. This assertion is based on the fact that, to our knowledge, our experiment is the second most comprehensive stand-level factorial NME to that of Wright et al. (2011) and the only one in

Africa to include K additions and consequently record rapid responses from root production, tree stem growth and leaf litter production (Table 5.2). The important role of K in this ecosystem can be explained by the biogeochemical view that, as a primarily rock-derived nutrient, K availability can deplete over time through leaching as the soil weather (Walker and Syers 1976, Veldkamp et al. 1990), thereby becoming less available for primary productivity.

Next, regardless of the geographical differences for the Budongo versus Panama tropical forests (i.e. in terms of montane vs lowland, secondary vs old growth, sandy vs clayey soils, near-neutral vs acidic soil), K increased stem growth of some trees in our site (Chapter 2) as it did in Panama (Wright et al 2011), reduced leaf litterfall particularly in the dry periods and enhanced leaf lifespan (Chapter 3; (Reich et al. 1992)), and reduced fine root production (Chapter 4). Although the reduction in leaf litterfall is related to K's stomatal regulatory role, the reduced fine root production in response to K is consistent with the allocation theory and other earlier studies (Yavitt et al. 2010, Wright et al 2011). These together are indicative of K limitation in this forest site although these limitations are mediated by climate and leaf habits for tree growth, and soil depth for root productivity. More NMEs are required to capture the broad range of factors that directly or indirectly control the responses of primary productivity to elevated nutrient availability and future environmental perturbations.

5.3 Reconsidering the definition of nutrient limitation

Based on direct assessments (Chapter 1) as has been demonstrated by this research, 'nutrient limitation' as a concept in modern biogeochemistry is said to be evident when the addition of nutrients leads to an increase in an ecosystem process (e.g. biomass) under consideration (Gibson 1971, Tanner et al. 1998, Perakis 2002, Danger et al. 2008, Vitousek et al. 2010). Going forward, I question the adequacy of this supposition as a determining indication of nutrient limitation. Must there always be an increase in the ecosystem process considered in response to increased nutrient availability? In some studies, the term nutrient limitation is used without a ‘formal’ definition and could lead to misapplication of the term among ecological disciplines. The varied responses of nutrient addition experiments in natural ecosystems have not always been consistent with this definition (i.e. nutrient addition leading to increases in NPP or ecosystem process) (Cleveland and Townsend 2006, Cleveland et al. 2006, Braun et al. 2010, Pedas et al. 2011, Mori et al. 2013, Fanin et al. 2015, Báez and Homeier 2018). Some studies report no response or lesser rate of a process (e.g. tree growth) after nutrient addition than in plots with no nutrient addition, these processes are not nutrient-limited. However, lesser leaf litterfall in response to P and K additions (Chapter 3) and reduced fine-root biomass or production to N and K additions (Chapter 4) does not mean trees in this forest are not limited by these nutrients. Trees would shed immature leaves only as a survival mechanism (e.g. against drought, disease or nutrient deficiency) because it is energy-costly to build new leaves (Gutschick 1981). If the availability of a nutrient will mitigate the deficiency and ensure the plant’s survival without shedding its leaves then it must have been limited by that nutrient. This translate into lesser litterfall and lesser NPP. Similar results have been reported elsewhere contrasting studies that observed increased growth responses to nutrient addition. This indicates that a definition based on the Liebigs annotated broken barrel may require modification to remain applicable to responses of tropical forests, which are increasingly being described as ‘a non-Liebig’s world’. Here, a modified definition build on that of Gibson (1971) and Vitousek et al. (2010) is submitted; nutrient limitation occurs when the potential rate in net primary production and quality of health is suppressed by inadequate bioavailability of a nutrient. This definition excludes the word ‘increase’ and it’s broad enough to accommodate the varied responses fundamentally driven by a limiting nutrient as an increased process.

Table 5.2: Effects of nutrient additions on tree growth in tropical forests

Location	**Elevation**	**Soil**	**Duration reported**	**Nutrient addition (kg ha^{-1} yr^{-1})**	**Responses to different components of NPP to nutrient addition**				**References**
					Tree/biomass growth	**Litter production**	**Tissue/soil nutrient content**	**Fine root production**	
Uganda	Lower Montane	Lixisol	2-3 yrs	N (125), P (50), K (50)	Increased with N & K	Decreased with P & K	Increased with N, P & K	Decreased with N & K	This study
Cameroon	Lowland	Arenosol	5 years	P (50)	Insignificant		Tissue P, soil P increased		Newbery et al. 2002
Agentina	lowland	Ultisols	5 years	N (125), P (50),	N+P limited				Villagra et al. 2013
Puerto Rico	Montane/ Lowland *	Acrisol/ Cambisol	6 years	N (50)	Insignificant				Cusack et al. 2011
Puerto Rico		Oxisols & Ultisols	4 years	N (125), P (50), K (50)	Insignificant	N+P+K limitation			Walker et al 1996; Li et al. 2006
Hawaii	Montane *	Cambisol/ Ferralsol	2 years	N (100), P (100)	N and P limitation				Vitousek & Farrington 1997
Costa Rica	Lowland	Andosol	3 years	N (100), P (47)	P limitation		Increased foliar N		Alvarez-Clare et al. 2013, 2015
Jamaica	Montane	Histosol	4 years	N (150), P (50)	N+P limitation				Tanner et al. 1990
Venezuela	Montane	Cambisol	4 years	N (225-150), P (75-50)	Increased with N+P	Increased with N+P	P conc. increased litter P		Tanner et al. 1992
Brazil	Sec. lowland	Ferralsol	2 years	N (100), P (50)	N+P limitation				Davidson et al. 2004
Brazil	Secondary	Oxisol	2 years	P	Insignificant				Markewitz et al 2012
Panama	Lowland	Cambisol/ Nitisol	11 years	N (125), P(50), K (50)	N+K limitation			Decreased with K	Wright et al. 2011; Yavitt et al. 2011
Panama	Lower Montane	Andosol	2 years	N (125)	N limitation			Decreased with N	Adamek et al. 2009, 2011
Mexico	Dry/Sec. lowland	lithic rendolls	3 years	N (220), P(75)	Increased with N & P	Increase with N+P			Campo et al 2007
Peru	Montane	Alluvial	4 years	N (125), P(50),	Insignificant		Insignificant		Fischer et al 2013
China	Montane	-	5 years	N (50), P (50)	Increased with P				Jiang et al 2018
Costa Rica	Secondary/ dry	Andic/Typic Haplustepts	3 years	N (150), P (45)	Increased with P	Insignificant		Increased with P	Waring et al 2019
Indonesia	Lowland	Acrisol	3 years	N (56), P (19)	Insignificant	Increased with N, P, N+P	N, P , N+P increased litter N & P	Insignificant	Mirmanto et al. 1999

* in contrast to typical tropical forests, the Puerto Rico and Hawaii lowland sites are dominated by one tree species.
Responses not studied are left blank

5.3 References

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DECLARATION

I, Raphael Manu, hereby declare that I have independently composed this dissertation entitled "Responses of net primary production and plant-available soil nutrients to elevated nutrient input in a tropical forest on highly weathered soil". I have accepted the assistance of third parties only in a scope that is scientifically justifiable and compliant with the legal statutes of the examinations and appropriately acknowledged all references and data sources that were used in the dissertation. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure.

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