Tree seedling recruitment dynamics in forest-savanna transitions

Trait responses to vegetation controls mediate differential seedling establishment success of tree functional types

Hamza Issifu

- In the absence of active fire suppression, restoration of dry forests will be successful with tree species found in savannas or in the transition between savannas and forests, but not with forest tree species. (*This thesis*)
- 2. If future climate is characterized by increased frequency of dry periods, resource conservation is a more beneficial strategy than resource acquisition. (*This thesis*)
- Root carbohydrate reserves is the most important trait for tree seedlings to persist in tropical savannas.
- 4. Emphasis on research novelty is more a weakness than a strength of contemporary science.
- 5. The future of science is like showbiz, as the ability to communicate scientific ideas is more rewarding than having them.
- If you can predict the next economic crisis, the best strategy is to be like a juvenile savanna tree: show less and store more.

Propositions belonging to the thesis entitled: "Tree seedling recruitment dynamics in forest-savanna transitions: *Trait responses to vegetation controls mediate differential seedling establishment success of tree functional types*".

Hamza Issifu

Wageningen, 27 May, 2020

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Thesis committee

Promotor

Prof. Dr F van Langevelde Professor of Wildlife Ecology and Conservation Wageningen University & Research

Co-promotors

Dr E Veenendaal Associate Professor, Plant Ecology and Nature Conservation Wageningen University & Research

Dr P Vergeer Associate Professor, Plant Ecology and Nature Conservation Wageningen University & Research

Other members

Prof. Dr L Poorter, Wageningen University & ResearchProf. Dr JHC Cornelissen, VU University AmsterdamProf. P De Frenne, Ghent University, BelgiumDr J Harbinson, Wageningen University & Research

This research was conducted under the auspices of the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC), the Netherlands

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Thesis

submitted in fulfilment of the requirements for the degree of doctor at Wageningen University by the authority of the Rector Magnificus, Prof. Dr A.P.J. Mol, in the presence of the Thesis Committee appointed by the Academic Board to be defended in public on Wednesday 27 May 2020 at 4:00 pm in the Aula.

Hamza Issifu

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208 pages.

PhD thesis, Wageningen University, Wageningen, NL (2020)

With references, with summaries in English and Dutch

ISBN: 978-94-6395-388-7

DOI: https://doi.org/10.18174/520417

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Tropical forests, savannas and forest-savanna transitions

Tropical forests and savannas make up between 15% to 20% of the earth's terrestrial surface (Grace et al. 2006) and account for over 60% of terrestrial productivity (Beer et al. 2010). Tropical forests and savannas represent distinct biomes both of which are crucial to the earth's land-atmosphere feedbacks, other ecosystem services (Bonan 2008, Furley et al. 1992) and support many socio-cultural and economic livelihoods (Grace et al. 2006).

Tropical forests refer to those tropical vegetation types dominated by woody species with a canopy cover sufficiently high to reduce (or eliminate) C₄ grasses (Torello-Raventos et al. 2013, Veenendaal et al. 2015). This definition includes a wide range of canopy cover types, but generally with a canopy area index \geq 0.7, tree height \geq 12 m and fractional herb and grass cover \leq 0.1 (Torello-Raventos et al. 2013, Veenendaal et al. 2015). At times, "grassy" are also distinguished, e.g. forests in South East Asia, (Torello-Raventos et al. 2013). The term woodland (sometimes, with a prefix, e.g. "tall" or "closed") is used to distinguish tropical vegetation formations with almost closed canopies but often comprising species that may not be regarded as true forest species, the latter being defined as more shade-tolerant and fire-sensitive (see for various views e.g. Torello-Raventos et al. 2013, Ratnam et al. 2011).

Tropical savannas, on the other hand, are vegetation types characterized by the coexistence of herbaceous vegetation with woody species (Torrelo-Raventos et al. 2013, Scholes & Archer 1997). The relative dominance of trees and grasses varies considerably across savanna types (Ratnam et al. 2011, Torrelo-Raventos et al. 2013, Veenendaal et al. 2015). Generally, canopy area index is < 0.7 (i.e. not closed-canopy) with fractional herb and grass cover > 0.1, although woody plant height may be as tall as forest vegetation (Torrelo-Raventos et al. 2013).

The interface of tropical forests and savannas constitutes an ecotone; the forest-savanna transition (Torrelo-Raventos et al. 2013, Veenendaal et al. 2015). Figure 1.1a shows this zone and adjacent vegetation types for West Africa and Ghana. The forest-savanna transition is a mosaic of forest patches in an otherwise large expanse of woodland (Torrelo-Raventos et al. 2013, Swaine et al. 1976, Cuni-Sanchez et al. 2016). Kent & Coker (1996) defined a vegetation mosaic as the existence in a specific area of different plant communities.

The forest-savanna landscape is dominated by the two contrasting vegetation types, forest versus savanna, which differ in structure and composition despite occurring in close proximity under similar climate (Armani et al. 2018, Azihou et al. 2013, Torrelo-Raventos et al. 2013, Veenendaal et al. 2015). Consequently, tree species that occur in the transition are often viewed as belonging either to forest or savanna tree functional types (Box 1.1). This dichotomy has been the basis for many studies seeking to explain patterns in the forest savanna-transition (see e.g. Hoffmann & Franco 2003, Hoffmann et al. 2004, Gignoux et al. 2009; 2016).

In this thesis, I make a distinction between forest tree species and humid savanna tree species that occur predominantly in the forest-savanna transition (hereafter, savanna-transition tree species). Savanna-transition species often occur both in humid savannas and in dry forests (particularly in secondary or degraded forests). Savanna-transition species are variously named in the literature; e.g. ubiquitous species (Armani et al. 2018), transition species (Ametsitsi et al. *in prep*. Boonman et al. 2019). In West Africa, transition forests (or woodlands) exist as mixtures of savanna-transition species and forest tree species (Ametsitsi et al. *in prep*., Armani et al. 2018, Asare 1962, Hopkins 1974, Swaine et al. 1976, Swaine 1992). Many of these dry forests are prone to

disturbance (fire) and their structures are likely maintained by the existence of different tree functional types (Box 1.1).



Figure 1.1. (a) Distribution of forest types in West Africa. Dry forest is shaded. Area to the north bordering the shaded region falls within the forest-savanna transition zone (Source: Swaine 1992). **(b)** Condition of forest reserves in the forest (and forest-savanna transition) zone of Ghana (Source: Hawthorne and Abu-Juam 1995). It shows forests bordering the forest-savanna transition are mostly degraded.

Growth and survival responses of savanna-transition and forest tree species to vegetation controls (environmental factors that influence vegetation development) or changes in these controls is important for understanding recruitment patterns or for predicting the movement of the transition subjected to land use or climate change.

In Ghana, the forest-savanna transition is bordered to the north by the northern Guinea savanna and to the south by the dry semi-deciduous forest (Figure 1.1a and b). Forest types regarded as dry forests include forest vegetation within the forest-savanna transition and the adjoining deciduous forest, occurring at approximately 1250 mm mean annual precipitation (Swaine 1992). Elsewhere in the tropics, dry forests may occur at lower rainfall on highly fertile soils at a much wider precipitation amplitude (500 mm to 2000 mm annual precipitation) (Holdridge 1976, Veenendaal et al. 2015). The condition of forest reserves in the drier forest types of Ghana had been described, already in 1995, as being "mostly degraded" or "very poor" (Figure 1.1b, Hawthorne & Abu-Juam 1995) as a result of deforestation fuelled largely by timber harvest and agriculture (Hawthorne & Abu-Juam 1995). Occasional ground fires (which spread from the adjacent forest-savanna transition) occur in the dry semi-deciduous forest (in the fire-zone subtype, Swaine et al. 1976), giving this dry forest subtype a unique mix of forest and savanna-transition tree species.

The forest-savanna transition in Ghana is subjected to many disturbances. Large quantities of charcoal (fuelwood) are supplied from the transition zone to meet the energy needs of the growing urban population (Mayaux et al. 2013). In Ghana, the contribution of fuelwood and timber harvest to deforestation in the transition comes, perhaps only second to land clearing for agricultural activities due to the fertile soils in the transition (Swaine 1992). Thus, the coupling of

increased land use pressures and climate change render these transition zones at present even more vulnerable to degradation disturbance (Malhi et al. 2013).

Determinants of vegetation distribution in forest-savanna transitions

An explanation for the nature of the forest-savanna transition has been a subject of long-standing debate. Fire has been historically seen as the main determinant of savanna formations in landscapes largely viewed capable of supporting forests (Stebbing 1935). The current view, first proposed by Morgan & Moss in their seminal 1965 paper, is that at broader scales, different vegetation types that occur within the forest-savanna transition are associated with distinct site characteristics driven by many factors including geology, variations in soil conditions, hydrology (water table and drainage) and disturbance history (Swaine 1992, Swaine et al. 1976, Oliveras & Malhi 2016, Swaine et al. 1976). Fire is considered the main disturbance factor, which may constrain forests and savannas as alternative stable states (Hirota et al. 2011, Staver et al. 2011). This view is disputed by others who propose soil moisture and nutrient contents as principal drivers of vegetation structure and distribution (Lloyd & Veenendaal 2016 versus Staal & Flores 2015, Veenendaal et al. 2015; 2018). The existence of transition forests, stability of different vegetation formations in some places and advance of forest over savannas in other places suggest complex dynamics, possibly involving many different factors, which still need to be investigated.

Factors which influence vegetation development (and distribution) have been summarised as "bottom-up" and "top-down" vegetation controls (Bond 2008). Bottom-up vegetation controls refer mainly to edaphic factors which influence vegetation structure, composition or juvenile recruitment. Evidence exists for the influences of soil depth, soil water retention capacity and nutrient

content on the distribution and structure of tropical vegetation (Keay 1960, Lloyd et al. 2008; 2015, San José & Farinas 1983, Veenendaal et al. 1996a; 2015; 2018). In the forest-savanna transition of Ghana, soil physical and chemical properties are associated with distinct vegetation formations with higher exchangeable bases in forest sites (Markham & Babbedge 1978, Swaine 1992). Evidence for such associations, particularly those linking seedling establishment and juvenile-stage species composition to soil properties is, however, very scanty (Ametsitsi et al. *in prep.*, Armani et al. 2018).

Water availability is an important bottom-up control sometimes overlooked because climate is similar for all vegetation types within forest-savanna transitions. Soil moisture differences between forest and adjacent savannas may, however, result from differences in microclimate or soil depth and texture (Lloyd et al. 2015, Ametsitsi et al. *in prep*). For example, higher silt content or organic matter in forest may translate to lower moisture stress (due to a higher water holding capacity) for seedlings in forest than savanna. Thus, drought sensitive (forest) species may have a higher chance of establishing in forest than savanna (Hoffmann et al. 2004, but see Cardoso et al. 2016). Seedlings in forest may, however, experience enhanced drought stress in dry forests due to light-limited growth and competition with larger trees for water (Veenendaal et al. 1996b). Where water table is high, e.g. along streams, plants have access to ground water even in the dry season, which could explain the thickening of vegetation along streams in parts of the transition (Janssen et al. 2018).

Top-down vegetation controls often highlighted include fire (Gignoux et al. 2009; 2016, Hoffmann et al. 2012, van Langevelde et al. 2003), herbivory (Higgins et al. 2000, Sankaran et al. 2005, van Langevelde et al. 2003), canopy cover and light (Charles-Dominique et al. 2018, Gignoux et al. 2004; 2016, Hoffmann &

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Franco 2003). The effects of fire and herbivory are sometimes considered analogous to the extent that both remove aboveground biomass of plants (Bond & Keeley 2005) but the exact effects of herbivory and fire may differ because fire occurs in the dry season while herbivory occurs, in large parts, in the wet season. Fire may also cause a more extensive shoot damage than herbivory (Pausas et al. 2016). The capacity to recover from sprout loss is a key trait for persisting both in pyrogenic and in herbivore dominated environments (Hoffmann et al. 2012, Murphy & Bowman 2012, Wigley et al. 2019, Boonman et al. 2019). Resprouting capacity may, thus, form a basis for the separation of the recruitment niches of tree functional types (Oliveras & Malhi 2016, Wigley et al. 2019).

There may, however, be complex interactions involving resources (light, soil moisture and nutrient levels). Thus, plant-plant interactions between overstorey species and tree seedlings may be complex, resulting both in competition (water, light) or facilitation (reduction of fires). The extent of competitive interactions between tree seedlings and herbaceous vegetation may be an important determinant of the balance between woody and herbaceous components (Sankaran et al. 2004). Herbaceous vegetation and tree seedlings may compete for belowground resources (Kulmatiski et al. 2010, February et al. 2013, Reginos et al. 2009, Tomlinson et al. 2019, van der Waal et al. 2009) or for both belowground resources and light (Barbosa et al. 2014, de Dois et al. 2014, Holl 1998, Vadigi & Ward 2013). Competition with grass may influence forest and savanna-transition tree species differently (Figure 1.2), because capacity to coexist with grass may have been a selection pressure for savanna tree species but not forest species. These interactions may in turn influence (e.g. via growth rates) the effects of fire on tree seedling establishment success (see conceptual model; Figure 1.2).

Woody canopy cover may influence many vegetation controls including both bottom-up and top-down (some of which have already been discussed). The extent of woody canopy cover correlates with microsite conditions; including relative humidity, temperature variability, soil properties, composition and biomass of herbaceous vegetation, the latter being linked to fire behaviour (Figure 1.2, Ametsitsi et al. *in prep*, Cardoso et al. 2018, Charles-Dominique et al. 2019). Woody canopy cover also correlates strongly with light levels (Charles-Dominique et al. 2018, Veenendaal et al. 2015), which may influence recruitment differently for shade bearers than light demanders (Box 1.1) (Agyeman et al. 1999, Veenendaal et al. 1996). For example, shade intolerance is often cited as the reason for the inability of savanna species to thrive in intact forest environments (Hoffmann et al. 2004, Hoffmann & Franco 2003, Ratnam et al. 2011).

Tree seedling recruitment and vegetation dynamics

Forest-savanna transitions are characterised by complex dynamics involving contractions and occasional expansion of forests (Janssen et al. 2018, Malhi et al. 2013, Mitchard & Flintrop 2013, Oliveras & Malhi 2016). There is a long history of forest expansion and contraction in response to variability or change in climate (Malhi et al. 2013, Oliveras & Malhi 2016, Oslisly et al. 2013). Climate models predict, despite great uncertainty, greater precipitation declines and dry season water deficits for some regions in West Africa including Ghana (James et al. 2013, Sheffield & Wood 2008). Precipitation declines that have already occurred were greater in the rainforest zone and along the coast with steady declines in transitions zone of Ghana (Owusu & Waylen 2009). Assessment of Ghanaian forests revealed less impact of a drying trend on dry forest species than wet forest species (Fauset et al. 2012). Thus, tree functional types will likely play an important role in climate responses of tropical trees. More frequently, forest

contraction is associated with deforestation by humans (Malhi et al. 2013, Janssen et al. 2018). Where tree removal (i.e. canopy disturbance at larger scales) has taken place, the resulting savanna vegetation may remain for prolonged periods of time with very limited possibility for forest tree recruitment (Janssen et al. 2018, Cardoso et al. 2016, Veenendaal et al. 2018). Thus, where savannas appear stable (e.g. Janssen et al. 2018, Cuni-Sanchez et al. 2016), bottlenecks (from some of the vegetation controls discussed earlier) to (forest) tree seedling recruitment may exist.

Forest encroachment in savanna may be associated with a change in fire regime (i.e. a decreased fire frequency) and other land use changes which are sometimes associated with rural depopulation (Malhi et al. 2013, Mitchard et al. 2009, Mitchard & Flintrop 2013). Recent expansion of forest across Africa could also be due to recent increases in precipitation following a drying trend in 1970s and 1980s (Jury et al. 2009). This may confirm the point made earlier about the role of climate variability in forest expansion versus contraction. Also, CO₂ fertilisation, another climate change factor, may offset the adverse effect of precipitation decline through enhanced water use efficiency of trees (Bond & Midgely 2012, Malhi et al. 2013). This could tip the balance between trees and grasses in favour of trees (Bain & Day 2019, Bond & Midgley 2000, Hoffmann et al. 2000, Kgope 2010). I do not explore CO₂ fertilization hypothesis in this thesis.

It is relevant to study constraints to tree seedling recruitment due to current and predicted changes in forest-savanna transitions. It is well-established that the different vegetation controls are strongly interlinked (see above and conceptual framework, Figure 1.2). The interactions among top-down and bottom-up controls may therefore likely explain bottlenecks to seedling recruitment in forest-savanna transitions. Although there are good theoretical frameworks for explaining how

tropical tree seedlings may respond to changes in many vegetation controls, empirical tests of the relative importance of these factors are scant. In this thesis, I test interactions involving many vegetation controls and also disentangle their effects where needed to enable determination of their relative importance as bottlenecks to seedling recruitment.



Figure 1.2. Conceptual model of the influence of key vegetation controls in forest-savanna transitions on tree seedling recruitment. Herbivory effect is also important but not included (directly) in the model. The model shows that woody cover increases from humid savanna to forest, resulting in variation in certain environmental factors. The effects of grass competition, fire and light limitation on tree seedling establishment success are known, but interactions among them and the different tree functional types (TFT) that exist in the forest-savanna transition are poorly quantified. The model indicates that tree functional type effect

may be important due to functional trait variability and trait responses among forest and forest-savanna-transition tree species. Red arrows with questions marks represent current knowledge gaps. Arabic numbers in parentheses indicate which research chapter in this thesis addresses the specific research gap identified.

The link between functional traits and seedling recruitment

Regeneration niches of tropical tree species are linked to seedling functional traits (Poorter 2007). Trait variation may thus, be important in explaining seedling recruitment into the various vegetation types in the forest-savanna transition. Following the functional equilibrium hypothesis (Brouwer 1963), plants invest in organs that capture resources in most limiting supply in their environments. Accordingly, trait syndromes vary between forest and savanna tree functional types (Boonman et al. 2019, Hoffmann et al. 2004, Gignoux et al. 2016). Viewed from the resource economics theory (Box 1.1), species which occur in more open and pyrogenic environments may be more conservative, rather than acquisitive, as a conservative strategy may be better for persistence, despite the cost to growth, in such environments. In other environments (e.g. under closing canopy of forest vegetation), the opposite strategy (i.e. resource acquisition) may be a better strategy to allow for rapid growth to escape the closing canopy. Therefore, forest species may generally be more acquisitive than savanna-transition species.

There are also differences to be expected within forest and savannatransition tree functional types due to species adaptation to more specialised habitats. Seedling traits and recruitment requirements may differ among forest species depending on their successional guild (Amissah et al. 2015, Swaine & Whitmore 1988, Veenendaal et al. 1996). Also, trait syndromes differ between wet and dry forest species (Markesteijn & Poorter 2009). Savanna-transition species may feature greater variability in traits (and hence have a wider recruitment requirements) because they occur both in forest and savanna-environments

(Armani et al. 2018). Different species may also attain similar establishment successes by deploying different traits (Tomlinson et al. 2012). For example, some species (mainly humid-savanna species) overcome drought and sprout loss, through fire or herbivory, by increasing allocation to root biomass and carbohydrate storage (O'Brien et al. 2015; Hoffmann et al. 2004, Boonman et al. 2019). Species from less humid environments rather increase allocation to root mass, survive drought by foraging more efficiently for deeper water using finer and deeper roots with a faster root extension to reach a declining water column (Tomlinson et al. 2012). Trait plasticity (Box 1.2) may characterize species responses to changes in environment (e.g. irradiance and drought) has been linked to some fitness measures including seedling survival and growth performance among Ghanaian forest tree species (Amissah et al. 2015).

While trait information for tropical tree species exist, only few studies have compared forest and savanna-transition tree species, particularly for West African forest-savanna transitions (see Boonmann et al. 2019, Gignoux et al. 2016). Studies that have looked beyond fire and soil moisture to explain trait variation and seedling recruitment among forest and savanna-transition tree functional types are even scantier. This thesis relates trait variation (and responses) to seedling establishment success of forest and savanna-transition tree species under influences of multiple stress and disturbance factors. I focus on specific traits, selected based on their significance (and in relation to the hypotheses tested here) as shown in previous studies (Table 1.1).

Rationale and research questions

This thesis aims to explain how seedling recruitment differs between savannatransition and forest tree functional types under various vegetation controls.

Seedling establishment success is defined here as survival and growth of tree seedlings past one wet and one dry season. Four specific research questions are addressed in four corresponding research chapters (Table 1.2) to attain the overall research aim.

Thesis outline

Chapter two: How does variation in woody cover (and associated factors) influence establishment success of forest and savanna-transition tree seedlings? (Table 1.2). This chapter explores the effects of woody canopy cover variation and the associated factors: fuel load, light intensity and soil resources on tree seedling survival and growth in the forest-savanna transition. We investigate, also, how root traits (biomass allocation and starch concentration) are related to survival in the different vegetation types. We tested hypotheses (Table 1.2) for this chapter using two congeneric tree species pairs of forest and savanna species in a field transplant experiment within the forest-savanna transition of Ghana.

Chapter three: What are the relative influences of grass competition during the wet season followed by fire and lack of precipitation during the dry season on tree seedling establishment success for savanna-transition and forest tree functional types? (Table 1.2). In this chapter, we tested hypotheses (Table 1.2) related to the direct and indirect effects of grass competition on establishment success for savanna-transition and forest tree functional types. This was achieved in a common garden experiment in the humid Guinea savanna of Ghana, which involved eight tree species, four each for forest and savanna-transition tree functional types.

Chapter four: What are the relative influences of fire and lack of precipitation during the dry season on tree seedling establishment success among forest and savanna-transition tree species? (Table 1.2). This chapter is based on

results of another common garden experiment in which we disentangled the effects of fire and lack of precipitation during the dry season on seedling performance (growth and survival), related to seedling traits. The aim was to separate the effects of fire and dry season (lack of moisture), which are naturally entangled, so as to determine their relative importance as constraints to tree seedling recruitment. We tested responses of four forest tree species versus two savannatransition types.

Chapter five: How do seedlings of savanna-transition and forest tree functional types differ in allocation traits and growth performance under contrasting regimes of soil moisture and defoliation? This chapter is based on results from a greenhouse experiment in which we tested hypotheses related to the question posed (Table 1.2) using 12 species, six each for savanna-transition and forest tree functional types.

In *chapter six*, I synthesize the results of chapters 2 to 5 and discusses how this thesis contributes to advancing our understanding on vegetation dynamics across the forest-savanna transition in the face of land use and climate change. **Table 1.1.** Traits analysed in the various research chapters in this thesis and their functional roles [based on references provided; Arabic numerals] for different species and tree functional types.

Trait (units)	Significance	Chapter			
Root dry weight (g)	Indication of stored resources (or of foraging for resources)	3			
	belowground. Related to survival [2, 11, 17]				
Root mass fraction	Indication of stored resources (or of foraging for resources)	2, 3, 4, 5			
(g g ⁻¹)	belowground. Better predictor of survival than root dry weight as it				
	is standardised to plant size. [1, 7, 11, 13, 17]				
Specific rooting depth	pecific rooting depth Indication of taproot thickness and belowground foraging efficiency				
(g m ⁻¹)	Related to growth and survival. [13]				
Root extension rate	Indication of foraging for deeper water resources. Associated with				
(cm day ⁻¹)	survival in a declining water column. [13]				
Root starch	Belowground storage carbohydrates. Allows for resprouting after	2			
concentration (%)	drought or fire topkill [4, 9, 17]				
Root collar/stem	Indication of growth. Thicker stems are associated with 2, 11	2, 3, 4,			
diameter (mm)		5			
Stem dry weight (g)	Indication of aboveground growth. Important for light competition	3, 5			
	or avoiding fire topkill [11]				
Stem mass fraction (g)	Stem dry weight standardised to plant size, Indication of	5			
	aboveground growth. [1, 7, 11]				
Plant height/Stem	Indication of aboveground growth. Important for light competition	2, 3, 4,			
length (cm)	ath (cm) or avoiding fire topkill [2, 15]				
Stem extension rate	Stem extension rate Faster height growth. Shade, fire or herbivory avoidance [3, 14]				
(cm day ⁻¹)	(cm day ⁻¹)				
Specific stem length Indication of etiolation. Shade avoidance or aboveground foragi		3, 5			
(cm g ⁻¹)	efficiency [1]				
Leaf dry weight (g)	Light capture: indication of photosynthetic capacity and growth	3, 5			
	[11]				
Leaf mass fraction (g	Light capture: standardised to plant size and better related to	3, 4, 5			
g ⁻¹)	photosynthesis and growth than leaf dry weight [1, 7, 11]				
Leaf area (cm ²)	Light capture: photosynthetic surface area and growth [2, 6, 7]	5			
Specific leaf area (g	Standardised to leaf size, better predictor of photosynthetic	5			
cm ⁻²)	capacity and growth [1, 7, 8, 10, 11, 16]				
Plant dry weight	Indication of overall fitness (growth and survival) in the	2, 3			
	environment [6]				
Relative growth rate	Photosynthetic capacity, a measure of fitness in the environment.	5			
(g g ⁻¹ day ⁻¹)	g ⁻¹ day ⁻¹) Fast growth. [1, 10, 15]				
Survival (proportion	Indication of fitness to the environment [1, 6, 10]	2, 3, 4,			
of %)		5			
Resprouting	Recovery after disturbance [3, 4]	2,3,4,5			
(proportion or %)					

References: Amissah et al. 2015¹, Gignoux et al. 2016²; Higgins et al 2000³, Hoffmann et al. 2004⁴, , Lopez-Iglesias et al. 2014⁶, Markesteijn & Poorter 2009⁷, Nicotra et al. 2010⁸, Obrien et al. 2014⁹, Poorter & Bongers 2006¹⁰, Poorter & Markesteijn 2008¹¹, Quentin et al. 2015¹², Tomlinson et al. 2012¹³, Tomlinson et al. 2019¹⁴, Veenendaal et al. 1996¹⁵, Wright et al. 2004¹⁶, Boonman et al. 2019¹⁷

Table 1.2. Overview of research questions and associated hypotheses for each research chapter

Research question	Hypotheses	Chapter
How does variation in woody	Forest tree species have lower survival than their savanna	
cover (and associated factors)	congeners in open woodland vegetation types due to less	
influence seedling	favourable conditions (fire intensity, soil properties and	
establishment success, related	microclimate). Savanna species are less competitive	
to traits, of forest and	under the low light conditions in the forest vegetation	
savanna-transition tree	type. Trade-offs in higher root allocation versus shoot	
seedlings?	allocation are expected to separate higher survival in	
	woodland versus under closed canopy forest.	
What are the relative	Grass competition decreases forest tree seedling	3
influences of grass competition	establishment success (growth and survival) more for	
during the wet season	forest than savanna transition tree functional types in	
followed by fire and lack of	humid savannas. It is also expected that grass	
precipitation during the dry	competition preceding dry season fire result in lower	
season on tree seedling	post-fire seedling survival for forest than savanna-	
establishment success among	transition tree species.	
forest and savanna-transition		
tree species?		
What are the relative	Fire and dry season (acting separately) cause greater	4
influences of fire and lack of	declines in seedling establishment success for forest than	
moisture during the dry	savanna-transition tree seedlings. It is also expected that	
season on tree seedling	the interaction of fire and lack of moisture in the dry	
establishment success among	season result in the greatest declines in establishment,	
forest and savanna-transition	but acting separately, fire has greater effect than lack of	
tree species?	moisture in the dry season regardless of tree functional	
	type. Differences in root allocation (root mass fraction	
	and starch storage) are expected to mediate differential	
	survival to fire and moisture limitation.	
How do seedlings of savanna-	Greater decline in growth performance for forest than	5
transition and forest tree	savanna-transition tree functional type under lower soil	
functional types differ in	moisture regime and defoliation. It is also expected that	
allocation traits and growth	seedling growth performance after defoliation is related	
performance under contrasting	positively to higher belowground resource allocation and	
regimes of soil moisture and	starch storage in roots.	
sprout loss?		

Box 1.1. Tree functional types, ecological guilds and plant functional traits

The term tree functional type (TFT, coined from plant functional type, PFT) refers to groups of tree species that have similar resource-use strategies and function in an ecosystem (Smith et al. 1997). Phylogeny, life-form characteristics including morphology, size and specific characteristics of various plant parts may form the basis for grouping species into functional types (Box, 1996). Forest and savanna tree species represent different TFTs due to adaptations to different sets of environmental conditions, which result in a separation in function. Other TFTs that can be defined within forest and savanna TFTs including, dry savanna, humid savanna, dry forest, moist forest (Boonman et al. 2019, Tomlinson et al. 2012). Savanna-transition TFT is defined in this thesis to include humid savanna species that also occur in dry (or transition) forests. Different tree functional types may belong to different guilds. The term guild, although sometimes used interchangeably with functional type, is a concept used within the context of competition to refer to resource sharing by different species (Blondel et al. 2003). For example, based on light requirements, tropical forest species are grouped into guilds of pioneers versus non-pioneers (Swaine & Whitmore 1988). Pioneer species establish and grow in relative high light environments while non-pioneers are capable of establishment and growth in forest shade due to differences in physiological light tolerances (Agyeman 2009, Poorter 1999, Veenendaal et al. 1996).

Plant functional traits are quantitative plant features that are measurable on an individual and related to plant fitness (Nicotra et al. 2010, Violle et al. 2007). There is substantial information on plant traits globally (Kattge et al., 2011) perhaps due to threats posed by global environmental change, as traits provide good indicators of the ecological strategies and environmental tolerance of species (Nicotra et al. 2010). According to the resource economics theory, plant functional traits may be organised along a spectrum, ranging from conservative to acquisitive strategies (Craine 2009, Grime 1977). Tree species may trade-off between investing carbon in structures that allow for faster resource capture and growth (i.e. acquisitive strategy). Alternatively, plants may store or invest carbon in structures that allow for recovery or survival under unfavourable conditions.

The seedling stage is an important barrier to tree recruitment. Thus traits that predict the success at the seedling stage will also predict adult stage tree distribution (Lopez-Iglesias et al. 2014; Poorter & Markesteijn 2008, van Langevelde et al. 2003). Plants often show great flexibility in adjusting their traits to changes in their environments through phenotypic plasticity (i.e. the ability of a particular genotype to produce a range of phenotypes depending on its environment, Nicotra et al. 2010). Flexibility in responses of many different functional traits (of leaves, stems and roots) has been found to be an important for performance of tree species under changing environments in many tropical tree species (Amissah et al. 2015, McLean et al. 2014).

2 Variation in vegetation cover and seedling performance of tree species in a forest-savanna-ecotone

Hamza Issifu, George K. D. Ametsitsi, Lana J. de Vries, Gloria D. Djagbletey, Stephen Adu-

Bredu, Philippine Vergeer, Frank van Langevelde, Elmar Veenendaal

Published in Journal of Tropical Ecology, 35, 74-82 (2019)

Abstract

Differential tree seedling recruitment across forest-savanna ecotones is poorly understood, but hypothesized to be influenced by vegetation cover and associated factors. In a 3-y-long field transplant experiment in the forest-savanna ecotone of Ghana, we assessed performance and root allocation of 864 seedlings for two forest (Khaya ivorensis and Terminalia superba) and two savanna (Khaya senegalensis and Terminalia macroptera) species in savanna woodland, closedwoodland and forest. Herbaceous vegetation biomass was significantly higher in savanna woodland (1.0 \pm 0.4 kg m⁻² vs 0.2 \pm 0.1 kg m⁻² in forest) and hence expected fire intensities, while some soil properties were improved in forest. Regardless, seedling survival declined significantly in the first-year dry-season for all species with huge declines for the forest species (50% vs 6% for Khaya and 16% vs 2% for Terminalia) by year 2. After 3 y, only savanna species survived in savanna woodland. However, best performance for savanna Khaya was in forest, but in savanna woodland for savanna Terminalia which also had the highest biomass fraction (0.8 ± 0.1 g g⁻¹ vs 0.6 ± 0.1 g g⁻¹ and 0.4 ± 0.1 g g⁻¹) and starch concentration ($27\% \pm 10\%$ vs $15\% \pm 7\%$ and $10\% \pm 4\%$) in roots relative to savanna and forest Khaya respectively. Our results demonstrate that tree cover variation has species-specific effects on tree seedling recruitment which is related to root storage functions.

Keywords: Biomass allocation; canopy cover; drought survival; fuel load; root starch; seedling traits; soil properties; tropical trees

Introduction

Forest-savanna ecotones characterized by a mosaic of forest patches within savanna environments represent a common feature of the landscape of West Africa (Hennenberg et al. 2005, McCook 1994). Across the tropics, observations of forest encroachment in savannas are on the rise (Bowman et al. 2001, Mitchard et al. 2009, Schwartz et al. 1996, Veenendaal et al. 2015), generally occurring at decadal timescales with rapid changes in vegetation cover and species composition (Cuni-Sanchez et al. 2016, Jeffery et al. 2014). Such vegetation transitions have important implications for ecosystem services and local livelihoods due to changes in composition, productivity, diversity and abundance of species (Mitchard et al. 2009, Poulter et al. 2014).

The process of forest advancement into savannas is still little understood, and the relative influences of fire (Higgins et al. 2007, Hoffmann et al. 2012a), edaphic and climatic factors (Bowman et al. 2015, Lloyd et al. 2015, Veenendaal et al. 2015, 2018) on the formation of closed-canopy vegetation have been highlighted in several studies. It is also recognized that vegetation (canopy) cover has important influences on fire behaviour and intensity, light and edaphic factors. As a result, tree seedling establishment success can be mediated by the extent of vegetation cover via fire suppression (Bowman 2000, Cardoso et al. 2016, Gignoux et al. 2009, Hoffmann et al. 2012a) or through amelioration of factors such as irradiance, soil moisture and soil fertility (Cuni-Sanchez et al. 2016, Ruggiero et al. 2002, Saiz et al. 2012, Veenendaal et al. 1996a, 1996b).

Forest species generally may lack the suite of traits that make savanna species successful in open pyrogenic savannas, while savanna species may be less successful in closed-canopy forests for the same reason and forest advancement in savanna may be facilitated in sites with higher woody canopy cover where low-

light conditions constrain performance of savanna species (Armani et al. 2018, Bowman 2000, Cardoso et al. 2016, Hoffmann et al. 2004, Ruggiero et al. 2002). However, there is little empirical data on whether canopy closure facilitates the establishment of forest species and to what extent this limits survival and growth performance of savanna species across forest-savanna ecotones.

In this study, we investigated influences of vegetation type (with a focus on canopy cover levels being the main distinguishing factor) and its associated factors on seedling survival, growth and traits (i.e. root mass fraction and root starch concentration) in a field transplant experiment that lasted three growing seasons and two dry/fire seasons. We used two congeneric species pairs of forest and savanna species that are common to the forest-savanna ecotone or to nearby semi-deciduous forest in West Africa to test the following hypotheses: (1) The forest tree species have lower survival than their savanna congeners in savanna due to relatively lower root mass fraction and root starch content needed to survive dry periods and to resprout after fire. (2) Higher vegetation (canopy) cover, being associated with a lower fuel load and higher soil nutrient status, benefits mainly forest tree seedlings as savanna species are less competitive in deep shade.

Materials and methods

Study site

The field transplant experiment was carried out in Kogyae Strict Nature Reserve (KSNR) located in the forest-savanna transition zone of Ghana (7°19 01.661 0 0N, 1°05 05.863 0 0W). Climatically, the area has a bimodal rainfall pattern with major peaks occurring in May–June and September–October (Figure 1), with a mean annual rainfall of 1200–1300 mm. Four vegetation types are distinguished in the area: transitional forest, savanna, riparian woodland and boval vegetation (vegetation on flat iron pans) (Wildlife Department 1994), but plot selection for

this study was done following the structural classification of Torello-Raventos et al. (2013) in woodland, closed woodland and forest vegetation patches. In the study site, tree cover has been stable or slowly increasing over the last 30 y (Janssen et al. 2018).

Species selection

We selected four tree species from two families and two genera. Each species pair in a genus comprised one forest and one savanna species (Table 1). Seeds of *Khaya ivorensis* and *Terminalia superba* were collected from a moist semideciduous forest (Bobiri Forest reserve, 6.678°N, 1.32°W), while those of *Khaya senegalensis* and *Terminalia macroptera* were collected within Kogyae Strict Nature Reserve itself. Seedlings were raised from seeds at the Forestry Research Institute of Ghana nursery in April 2012. At 3 months old, seedlings were transported to the Kogyae Strict Nature reserve and allowed 7 days to recover from any transportation shock before transplanting.

Table 1. Classification and biophysical limits of tree species used in the study. All speciesthrive within the Kogyae Strict Nature Reserve or in nearby semi-deciduous forest inGhana. Sources of information: Hawthorne (1995),

Species	Family	Functional	Guild	Distribution	Rainfall
		type			range
					(mm)
Khaya ivorensis	Meliaceae	Forest	Non-pioneer	Moist-dry	1600-2500
(Desr.) A.Juss			light demander	forest	
Khaya senegalensis	Meliaceae	Savanna	Moderately	Gallery forest	400-1750
(Desr) A.Juss			shade tolerant		
Terminalia superba	Combretaceae	Forest	Pioneer	Moist-dry	1000-1800
(Engl.&Diels)				forest	
Terminalia	Combretaceae	Savanna	Open savanna	Moist open	700-1500
macroptera (Guill. &				woodland	
Perr.)					

http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp.

Transplantation experiment

Thirty-six 10×10 -m plots were randomly established under the three vegetation types differing in canopy closure; 12 each for cover classes typical for woodland, closed woodland and forest canopies (following Torello-Raventos et al. 2013) in three sites (blocks) that were about 750 m apart. In each plot, six seedlings each of the four species (Table 1) were assigned and planted in random positions in rows (1.4 m within and between rows of seedlings). A total of 864 seedlings were planted (i.e. 6 seedlings × 4 plots × 3 cover classes × 3 blocks × 4 species). Seedlings were transplanted in September 2012 at the beginning of the second rainy season (Figure 1). No additional watering was done and no fire protection was given during the experimental period. Canopy cover of plots in the various vegetation patches was assessed using leaf area index (LAI) and canopy openness in October before the end of the rainy season (peak leaf cover). Additionally, we assessed absence/presence of C₄ grasses in the herb layer.

LAI and canopy openness were obtained by analysing hemispheric photos, taken at 1 m above the ground in each plot with a fish-eye lens mounted on a Nikon E4500 camera. Images were then analysed with Gap Light Analyser software (Veenendaal et al. 2015). Mean percentage canopy openness and (LAI) ranged between 18.5-25% (1.7-2.0) for forest plots, 32-45% (0.7-1.0) for closed woodland plots and 60-73% (0.1-0.25) for (savanna) woodland plots. The herb layer in plots with highest LAI (forest plots) consisted mainly of C₃ species, while canopy cover was mainly provided by forest trees. In the closed woodlands tree cover was provided by a mix of different species with tree crowns not touching and C₄ grasses were present, while cover in woodlands was provided by savanna trees (Torello-Raventos et al. 2013).

Data on seedling height and survival were taken for three seasons. Before the first dry season, three censuses were conducted at 1, 2 and 3 mo after transplantation, the third month being at the onset of the first dry season (December 2012) (Figure 1). Subsequent censuses were conducted only at the end of the consecutive growth seasons (December) of 2013 and 2014. The first dry season and its associated fires occurred 5 mo into the experiment (19 January and subsequent days in 2013). The plots also burnt in the second year (around 4 February 2014).

The experiment ended in December 2014 at the end of the third wet season (Figure 1). Fire intensity was not measured separately in this experiment, but after each fire event we observed that the forest plots generally had been lightly touched by fire, whereas all plots in closed-woodland and woodland cover types burnt more heavily in both dry-season fires that occurred within the period of this study. All surviving seedlings at the end of the experiment were carefully excavated. Seedling height, total plant dry weight and root mass fraction were determined. Immediately after harvest, samples were microwaved, in preparation for determination of root starch content, following a carbohydrate extraction protocol of Duranceau et al. (1999) adapted from Dubois et al. (1956). Root starch content was analysed for all species (except *Terminalia superba*, for which we had no adequate samples available).

Environmental factors

Soil moisture content of the top layer (0–60 mm) was determined with a theta probe (Delta-T Devices, Cambridge, UK). Five moisture measurements were made across all four plots of each vegetation type within a block (as all four plots laid fairly close to one another). This was done at the centre and at the outer corners of the plots. Measurements were done twice, at 7 wk (November 2012) and 13 wk
Chapter 2

(December 2012). We took five soil samples per vegetation type per block using a cylindrical auger at the centre and at the mid-distance to the four corners of the outer plots. Sampling was done at three depths (0–10 cm, 10–20 cm and 20–30 cm) and composites were formed from the replicates for each depth category and put in zip-lock plastic bags and later analysed for soil organic matter content (losson-ignition method; Ball 1964) and some biogeochemical properties. CEC, Mg, Ca, K analyses (Gilman 1979) were done using an Atomic Absorption spectrometer (VARIAN AA240FS, Varian Inc.). Total N and P were analysed according to Novozamsky et al. (1983) using the Segmented Flow Analyser (SKALAR SAN⁺⁺ System) and P-Olsen was determined according to Olsen et al. (1954).

Data on fuel load and fuel composition as a proxy for fire intensity were taken from three random 1-m² quadrats per plot and averaged for each plot per vegetation type. In each plot, cover abundances of grasses and herbs were estimated. Also, dry weights of total herbaceous vegetation (i.e. including herbs and grasses and litter were determined from sub-samples by cutting vegetation and collecting litter and weighing them after oven drying. Daily rainfall data from August 2012 to December 2015 recorded in Ejura, the nearest meteorological station (25 km away from experimental site), were obtained from the Ghana Meteorological Agency. There were gaps in the data for some months (October 2012, November 2013 and June 2015). Mean monthly rainfall for months with missing data were estimated using records from the last 15 days of the month before and the first 15 days of the month after the reference month. For example, mean rainfall for October 2012 was estimated as mean of rainfall values from 16 September to 15 November 2012.



Figure 1. Mean monthly precipitation for Ejura, Ghana (nearest meteorological station to Kogyae Strict Nature Reserve-KSNR) for the experimental period and beyond. Timing of all five censuses conducted are shown.

Statistical analyses

We used linear mixed-effects models (Zuur et al. 2009) to test for differences in soil moisture content of the top soil layer among vegetation types and measurement weeks (as fixed factors), including the interaction term of two fixed factors and a random block effect. Similarly, we tested fixed effects of vegetation type including a random block effect on organic matter content, litter mass, herbaceous vegetation biomass and cover abundances of herbaceous vegetation using linear mixed-effects models. Block was included as random factor in these analyses. Soil organic matter content was analysed for each soil depth separately. Also, cover abundance of grass and herbs were analysed separately. We checked for normality and homoscedasticity and applied natural log (ln), square root and arcsine transformations (Sokal& Rohlf 1995) on herbaceous vegetation biomass, litter mass and cover abundance proportions of grasses and herbs respectively. A multivariate analysis of variance (MANOVA) was used to test, for each soil layer, differences in soil chemical properties among vegetation types.

Survival data from each census (conducted at months 1, 2, 3, 15 and 27) were analysed separately to compare survival among species and vegetation types in generalized linear models (GLM) using binomial distribution with logit link function. Sidak correction was used for multiple comparisons.

Seedling heights recorded in years 1, 2 and 3, were tested for differences among years and vegetation types separately for each species in linear mixedeffects models. A random block effect was included in the models and Sidak correction was used for multiple comparisons. Also, for each species, a Kruskal– Wallis test (Sokal & Rohlf 1995) was used to determine if seedling height differed among years 1, 2 and 3. For *T. superba*, a Mann–Whitney U-test was used to compare height of years 1 and 2 as insufficient samples were available in year 3. Data on total seedling dry weight, root mass fraction (RMF) and root starch concentration were analysed in separate linear mixed-effects models for each species to determine fixed effects of vegetation type. All analyses were done on SPSS version 23.0.

Results

Soil properties

Soil moisture content (SMC) of the top soil layer (0–60 mm) after 7 wk differed significantly ($F_{2, 84} = 8.4$, P < 0.001) among vegetation types, higher in forest and closed-woodland (6.11% ± 1.71% and 6.25% ± 1.89% respectively) than savanna woodland at 4.25% ± 2.07%. We found that SMC had dropped to an average of 2.7% at 13 wk into the experiment (i.e. at the start of the dry season) ($F_{1, 84} = 66.6$, P < 0.001). During the experimental period, all vegetation types showed a similar decline in moisture content and at the end of the experiment SMC was still lower in savanna woodland and closed woodland (1.95% ± 1.53% and 2.69% ± 1.89% respectively) than forest at 4.74% ± 2.2%.

Soil organic matter in the top 10 cm was significantly higher in the forest compared with savanna woodland and closed-woodland sites ($F_{2, 6} = 19.6$, P = 0.002). Interestingly, no significant differences between vegetation types were found for soil layers below 10 cm (Table 2). Significant differences between different vegetation types were also found for soil pH, total nitrogen and CEC ($F_{2, 9} = 5.85$, P = 0.039; $F_{2, 9} = 17.4$, P = 0.003; $F_{2, 9} = 16.7$, P = 0.004 respectively), but again only in the upper 10 cm soil layer. Soil pH was lowest in forest and highest in woodland. Total nitrogen was higher in forest than in savanna woodland and closed-woodland. CEC was lowest in closed-woodland and similar between savanna woodland and forest. No significant vegetation type effect was found for levels of Ca, Mg, K, total P and P-Olsen (Table 2).

Herbaceous vegetation and litter

Total biomass of herbaceous vegetation (including grasses and herbs) differed among vegetation types ($F_{2, 31} = 29.8$, P < 0.001). Biomass of herbaceous vegetation was 0.23 ± 0.12 kg m⁻² in forest, lower than biomass in closedwoodland and savanna woodland which had similar biomass of 0.84 ± 0.25 kg m⁻² and 0.99 ± 0.35 kg m⁻² respectively. Similarly, litter mass differed significantly ($F_{2, 31} = 23.3$, P < 0.001) among vegetation types being higher in forest (0.21 ± 0.11 kg m⁻²) than closed-woodland (0.06 ± 0.05 kg m⁻²) and savanna woodland (0.03 ± 0.05 kg m⁻²). Overall, grasses were more abundant ($F_{2, 31} = 111$, P < 0.001) in savanna woodland (51.5% ± 8.8%) and closed-woodland (50.8% ± 8.7%) than forest at 15.4% ± 3.1%. Percentage cover of herbs was low overall (average of 3%) and did not differ significantly ($F_{2, 33} = 1.68$, P = 0.2) among vegetation types.

statistically teste	d separately fo	or differences	s among veg	etation types	; (Linear mix	ed-effects m	odels for org	anic matter o	content and
MANOVA for all c	hemical prope	rties). Statist	tical differenc	ces (P < 0.05) are shown	with letters	only for para	meters for w	hich cover
classes differed s	ignificantly.								
		0-10 cm layer		F	.0-20 cm laye	2	£	0-40 cm laye	
	Woodland	Closed- woodland	Forest	Woodland	Closed- woodland	Forest	Woodland	Closed- woodland	Forest
рн-н20	7.09 ± 0.48ª	6.26 ± 0.38ª ^b	6.18 ± 0.13 ^b	6.81 ± 0.07 ª	5.82 ± 0.41 ^b	6.05 ± 0.21 ^b	6.69 ± 0.23ª	5.79 ± 0.20 ^b	5.92 ± 0.37 ^b
P-Olsen (mg kg ⁻	2.71 ± 0.61	3.59 ± 0.74	3.53 ± 0.77	1.75 ± 0.40	2.30 ± 0.96	2.00 ± 0.51	1.55 ± 0.75	1.70 ± 0.38	2.28 ± 0.06
N-total(mg kg ⁻¹)	497 ± 69.2ª	389 ± 49.8ª	805 ± 130 ^b	322 ± 8.22	251 ± 43.0	499 ±233	221 ± 72.8	299 ± 194	272 ± 47.4
P-total (mg kg ⁻¹)	79.0 ± 9.35	73.5 ±7.56	79.0 ± 5.91	62.2 ± 10.6	49.0 ± 5.24	56.8 ±11.1	64.1 ± 15.4	63.0 ± 32.1	42.7 ± 7.17
CEC (cmol kg ⁻¹)	4.0/ ± 0.5/ª	0.22 ± 0.29 ^b	3.86 ± 1.52ª	1.9/ ± 1.55	2.81 ± 4.8/	$1./6 \pm 0.81$	2.25 ± 3.27	0.50 ± 0č.U	1.36 ± 1.81
K (cmol kg ⁻¹)	0.33 ± 0.05	1.45 ± 1.87	0.60 ± 0.41	0.29 ± 0.04	0.68 ± 0.43	0.37 ± 0.18	0.23 ± 0.07	3.50 ± 5.28	0.79 ± 1.05
Mg (cmol kg ⁻¹)	0.11 ± 0.14	0.44 ± 0.77	0.83 ± 0.34	,	0.61 ± 0.53	0.26 ± 0.28	,	0.50 ± 0.5	0.07 ± 0.1
Ca (cmol kg ⁻¹)	0.75 ± 1.30	0.35 ± 0.60	1.21 ± 0.39	0.53 ± 0.92	0.04 ± 0.08	0.29 ± 0.49	0.33 ± 0.57	0.53 ± 0.92	ı
Organic matter (%)	1.69 ± 0.09ª	1.42 ± 0.16ª	2.76 ± 0.45⁵	1.25 ± 0.23	1.09 ± 0.10	1.48 ± 0.44	1.19 ± 0.48	1.07 ± 0.16	1.14 ± 0.16

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Table 2. Mean ± SD of soil properties in Kogyae Strict Nature Reserve, Ghana, taken at three depths. Each soil layer (depth) was

Seedling survival

A few weeks (4–8 wk) into the experiment, both Terminalia species showed lower survival, relative to the Khaya species, in forest plots (Figure 2). Survival differences for *T. superba* versus *K. ivorensis* (P = 0.02) and *T. macroptera* versus *K. senegalensis* (P < 0.001) were revealed through pairwise comparisons (with Sidak correction). Generally, seedling survival remained high, particularly for the Khaya species and regardless of vegetation cover until 3 months (i.e. onset of the first dry season). By this census, survival had considerably declined for all species (Figure 2). We found a significant species × vegetation cover interaction effect (Table 3), but differences among species were mainly between and not within vegetation cover type.

At 15 months, and after the first dry-season fire, significant survival differences were found among species (Table 3). Overall, 50% of all savanna Khaya was still alive versus 6% for its forest congener. We found a similar pattern in genus Terminalia with 16% survival for the savanna type versus 2% for its forest congener. Pairwise comparisons showed that survival of the savanna Khaya was significantly higher than all other species in all vegetation types. Savanna Terminalia also had a significantly higher survival than both forest species in savanna woodland. Between the two forest species, survival in savanna woodland and closed-woodland was higher for forest Khaya than forest Terminalia. In the final census (27 months on) after the second dry-season fire and third wet season (Figure 2), 12% of the total number of planted seedlings were still alive. There was a significant interaction effect of vegetation type and species (Table 3). None of the forest species was alive in savanna woodland where 20% survival for the savanna Khaya and 13% for savanna Terminalia were observed. Remarkably, higher survival was observed in forest (55%) and closed-woodland (33%) for the

savanna Khaya as compared with 8% and 4% in the respective vegetation types for forest Khaya. The savanna Terminalia survived in very low numbers in closedwoodland (4%) and forest (1%). There were no seedlings of the forest Terminalia surviving in savanna woodland and only 1% survived in forest (Figure 2).



Figure 2. Proportion of surviving seedlings of *Khaya senegalensis* (filled-triangle), Khaya ivorensis (filled-circle), *Terminalia macroptera* (open triangle), *Terminalia superba* (open circle) at woodland cover (**a**), closed-woodland cover (**b**) and forest cover (**c**) in Kogyae Strict Nature Reserve, Ghana. Month corresponds to the month of transplantation, with month of transplanting = 0. Grey vertical lines indicate times when the dry season fires occurred. Error bars show ± 1 standard error of the mean.

Table 3. Binomial analysis (with logit link) of seedling survival of four tree species in three vegetation types in Kogyae Strict Nature Reserve, Ghana. Analyses were done separately for each census (month) and all factors included in the separate models are presented. Significant effects are indicated with asterisks and non-significant effects by "ns".

Time (mo)	Main effects and interactions	df	Wald Chi- Square	Ρ
1	Vegetation type	2	0.0001	1.0 ns
	Species	3	5.42	0.144 ns
	Vegetation type x Species	6	9.38	0.159 ns
2	Vegetation type	2	3.29	0.192 ns
	Species	3	68.6	< 0.001*
	Vegetation type x Species	6	19.0	0.004*
3	Vegetation type	2	4.67	0.097 ns
	Species	3	10.7	0.013*
	Vegetation type x Species	6	21.5	0.001*
15	Vegetation type	2	0.0003	1.0 ns
	Species	3	97.4	< 0.001*
	Vegetation type x Species	6	0.88	0.99 ns
27	Vegetation type	2	0.000012	1.0 ns
	Species	3	34.4	< 0.001*
	Vegetation type x Species	6	13.5	0.036*

Seedling growth

Seedling height was significantly lower in year 2 for all species relative to year 1 heights evidencing shoot loss (Table 4, Figure 3). We found that for the forest species in both genera, seedling height did not differ among vegetation cover types, but for both savanna species, differences between vegetation types were significant ($F_{2,107} = 5.32$, P = 0.006 for Khaya and $F_{2,33} = 3.27$, P < 0.001 for Terminalia). Savanna Khaya was taller in forest and closed-woodland than woodland while savanna Terminalia was taller in savanna woodland than closed-woodland and forest (Figure 3).

At the end of the third wet season, plant height was higher than recorded for year 2 for all species except forest Terminalia for which there were insufficient seedlings for comparison suggesting recovery from year 2 drought/fire. Also compared to year 1, both savanna species in year 3 were significantly taller, but forest Khaya in year 3 did not differ from year 1 height (Table 4), suggesting a higher cumulative shoot recovery of the savanna than forest species in this study.

Overall, plant dry weight of the savanna Khaya was 3.4 ± 1.9 g and did not differ significantly (F_{2, 80} = 0.20, P = 0.82) among vegetation types as was the case for its forest congener (F_{1, 5} = 0.22, P = 0.67) (Figure 4a). Seedlings of the savanna Terminalia did grow significantly larger in savanna woodland (16.0 ± 12.9 g; F_{2, 15} = 17.4, P < 0.001) compared to closed-woodland and forest where seedlings weighed on average 1.9 ± 0.5 g. Unfortunately, for the forest Terminalia, biomass could not be analysed because not enough seedlings survived at final harvest (Figure 4a).

Biomass proportion and starch concentration in roots

We found that root mass fraction significantly differed ($F_{2,77} = 4.88$, P = 0.01) among vegetation types for savanna Khaya, which was higher in savanna woodland at 0.71 ± 0.10 g g⁻¹ and lowest in the forest at 0.61 ± 0.11 g g⁻¹ (Figure 4b). Root mass fraction of the forest Khaya did not differ significantly ($F_{1, 5} = 2.01$, P = 0.22) between closed-woodland and forest where it survived till the end (Figure 4b). Also for the savanna Terminalia, root mass fraction did not differ significantly ($F_{1, 10} = 1.14$, P = 0.31) between savanna woodland and closed-woodlands where it survived till the end. Overall root mass fraction (regardless of vegetation type) differed significantly ($F_{3, 95} = 14.6$, P < 0.001) among species being highest in savanna Terminalia (mean = 0.79 ± 0.09 g g⁻¹) and lowest in

forest Khaya (mean = 0.44 \pm 0.07 g g⁻¹). Root mass fraction of savanna Khaya was intermediate (mean = 0.64 \pm 0.13 g g⁻¹) between the two other species.

We found that root starch concentration differed significantly ($F_{2, 27} = 19.1$, P < 0.001) among species and also among vegetation types ($F_{2, 27} = 3.48$, P = 0.045). Pairwise comparisons revealed highest root starch concentration for seedlings in savanna woodland ($21.6\% \pm 10.7\%$) and lowest in closed-woodland ($13.8\% \pm 9.8\%$). Among species, savanna Terminalia stored the most starch in their roots ($27.1\% \pm 9.6\%$) whereas forest Khaya stored the least ($9.6\% \pm 3.9\%$) while savanna Khaya had intermediate root starch storage ($14.9\% \pm 6.8\%$).



Figure 3. Mean seedling height of each species at three vegetation types for the three growing seasons in Kogyae Strict Nature Reserve, Ghana. In year 3, *Terminalia superba* was excluded due to too few numbers to allow for analysis. Also, there were insufficient

samples of *Khaya ivorensis* and *Terminalia macroptera* for woodland and forest respectively. Statistical comparisons (with Sidak adjustment) are done among vegetation types for each species. Different letters indicate significant differences (P < 0.05). Error bars are ± 1 SE of mean.



Figure 4. Mean seedling dry weight and root mass fraction for three species in three vegetation types in Kogyae Strict Nature Reserve, Ghana. Statistical comparision is among vegetation types, but not among species. Data not presented for *Terminalia superba* due to insufficient samples. Also, samples were insufficient for *Khaya ivorensis* and *Terminalia macroptera* in woodland and forest respectively. Different letters indicate significant differences (P < 0.05). Error bars are ±1 SE of mean.

Table 4. Pairwise comparisons (from Kruskal-Wallis test) of tree seedling height recorded in years 1, 2 and 3 at Kogyae Strict Nature Reserve, Ghana. Analyses were done for each species separately. For *Terminalia superba*, only years 1 and 2 are compared using Mann-Whitney-U test. Years for which median seedling height differed significantly (P < 0.05) are indicated with asterisk.

Species	Years compared pairwise	Test statistic	Standard error	P-value
Khaya senegalensis				
	2-1*	91.5	13.8	< 0.001
	2-3*	-139	15.3	< 0.001
	1-3*	-47.3	17.3	0.019
Khaya ivorensis	2-1*	136	9.70	< 0.001
	2-3*	-177	30.4	< 0.001
	1-3	-40.7	30.9	0.563
Terminalia macroptera	2-1*	109	10.4	< 0.001
	2-3*	-181	24.3	< 0.001
	1-3*	-71.4	25.3	0.014
Terminalia superba	2-1*	809	596	< 0.001

Discussion

Forest and savanna species occur predominantly in their respective non-pyrogenic and pyrogenic environments. Yet, widespread observations have been made of forest species encroaching savannas in many places across the globe (Bowman et al. 2001, Mitchard et al. 2009). Higher vegetation cover is hypothesized to increase establishment of forest species, but tests in forest-savanna ecotones produced mixed results (Bowman et al. 2001, Cardoso et al. 2016, Geiger et al. 2011, Gignoux et al. 2009, Hoffmann et al. 2004).

Generally, higher canopy cover suppresses pyrogenic fuel loads (Hennenberg et al. 2006) and aids tree seedling survival as fire in open savanna vegetation induces high seedling mortality (Cardoso et al. 2016, Gignoux et al. 2009, Hoffmann et al. 2004). Here, we assessed survival and growth of seedlings of two congeneric pairs of forest and savanna trees over a period of 3 y (two dry/fire seasons) allowing for assessments at different moments in time and beyond one season in both forest and savanna environments. We assessed flammable material as an indication of fire intensity, soil characteristics as well as biomass fraction and starch concentration in roots in relation to different vegetation types (differing in extent of canopy closure).

The forest plots typically had higher soil moisture, organic matter and N content compared with the savanna woodland plots, which is in line with other studies (Fensham et al. 2003, Kellman 1985, Markham & Babbedge 1979) and which may be caused by the presence of increased cover and litter input by trees (Fensham et al. 2003, Kellman 1985). Higher N content could also be the result of fixation by trees and the nitrification of N (leftover after uptake by vegetation) could explain the lower pH found in forest plots (Ste-Marie & Pare 1999). These differences between forest and savanna woodlands were found in top (10 cm) soil only. Additionally, several other soil parameters measured such as P, K, Mg, Ca and CEC were similar in both environments. Thus, no firm conclusions can perhaps be drawn on whether soils differ markedly between forest and savanna patches in this ecotone. Nonetheless, higher top soil moisture content, organic matter and N may affect tree seedling growth and survival.

Savanna woodland plots were characterized by higher biomass of herbaceous vegetation, while litter load was somewhat higher in closed-canopy forest cover (but only ~0.2 kg m⁻²). This is the result of the higher canopy cover in forest excluding grasses, consistent with findings in other studies (Hennenberg et al. 2006, Hoffmann et al. 2012b). Faster and more intense fires have been observed for savanna than forests (Hennenberg et al. 2006, Hoffmann et al.

2012b) as a consequence of this difference in type and biomass of herbaceous vegetation.

Seedling survival

Survival in the first few weeks for both species in the genus Terminalia was lower in forest (at LAI of \sim 1.7–2.0) than in savanna, while species in genus Khaya were not affected. Clearly, both Terminalia species prefer higher light levels, at least in the first weeks after germination. The forest Khaya is a non-pioneer light demander (Hawthorne 1995) while its savanna congener is known to tolerate moderate shade (Kwesiga & Grace 1986). Three months into the first dry season, these apparent species differences were no longer observed and mortality had reached about half for nearly all individuals. Prior to this census (census 3) rainfall had declined from 37 mm in November to 3 mm in December (Figure 1).

Thus, the high mortality of the initial establishment phase may suggest a similar response to dry season drought for all species and regardless of vegetation cover. It was in the second dry season that the reported differences between forest and savanna species (Hoffmann et al. 2004) became evident in both genera tested in this study. At this stage, seedlings had gone through the first dry and fire seasons and subsequent recovery. And thus fire- and drought survival traits became more important explaining the greater survival of the savanna species.

At the end, the savanna Khaya had the most survivors in all vegetation types because it is both drought/fire tolerant and also moderately shade tolerant. None of the two forest species survived in woodlands where the highest biomass of flammable material was recorded. Except for the forest Khaya that had few surviving seedlings in closed-woodland, the forest species generally survived in forest, although in very low numbers. This suggests that the long dry seasons and associated fire events in this ecotone limits their colonization possibilities (Cardoso et al. 2016). Survival in savanna Khaya was similar to that of savanna Terminalia in savanna woodland, but markedly contrasting in closed-canopy forest and closed woodland with intermediate canopy cover (although to a lesser extent), with canopy closure favouring savanna Khaya and more open environments favouring savanna Terminalia. Again, this is consistent with the natural distributions of the two species.

Seedling growth, biomass proportions and starch concentration in roots

Within the first growing season, seedlings tended to grow taller (significantly for two species) in woodlands than forest. Perhaps this represents an increased growth response to the increasing light availability (Veenendaal et al. 1996b) associated with increasing canopy openness from forest to savanna. By the second year, seedling height had greatly reduced for all species indicating shoot loss resulting from drought and/or fire of the first dry season. The difference in height between years 2 and 1 which was greater for the two forest species than their savanna congeners may be an indication of a greater adverse effect of the dry season on the forest species. At the end of year 3, seedlings were a lot taller (relative to year 1 heights) for both savanna species. By contrast, y 3 seedlings of the surviving forest tree *K. ivorensis* (mortality of forest Terminalia was 99% at this stage) were not taller than they were in year 1 suggesting a higher cumulative recovery and resprouting capacity for the savanna species than their forest congeners. This is consistent with our prediction and also reported in several other studies (Fensham et al. 2003, Gignoux et al. 2016, Okali & Dodoo 1973).

Vegetation cover type did not have profound effects on shoot loss and subsequent regrowth over the 3-y period except on savanna Khaya in year 2. This is inconsistent with our expectation because flammable material differed among

vegetation types and should have influenced extent of stem die-back (top-kill) (Higgins et al. 2007). Perhaps this finding suggests that drought effect on stem die-back was stronger than the ameliorating influence of canopy cover. The fact that seedlings in closed-canopy forest also experience drought stress (Veenendaal et al. 1996c) lends support to this assertion. This may explain why patterns of forest development as well as mature trees of *Khaya senegalensis* appear to closely follow branching patterns of streams. It may also explain the overall rather slow development of forest vegetation on savanna patches in Kogyae Strict Nature Reserve and elsewhere in the transition (Armani et al. 2018, Janssen et al. 2018).

At the end of the experiment, the savanna species outperformed the forest species in terms of attained biomass at harvest, allocation to roots and root starch concentration. This was consistent with our expectation as species from drier pyrogenic environments have been reported to have higher root mass fraction and carbohydrate reserves for overcoming drought and fire (Cardoso et al. 2016, Hoffmann et al. 2004, O'Brien et al. 2014, Tomlinson et al. 2012).

Overall, our results suggest that the possibilities for establishment of moist semideciduous forest species in the forest-savanna ecotone are particularly limited by the dry season and its associated pyrogenic environment. More interestingly, we demonstrate that savanna species also differ in their tolerance to canopy cover and open pyrogenic environments specifically related to root storage functions, thus contributing to a better understanding of differences in tree seedling recruitment between species across the forest-savanna ecotones.

Acknowledgements

Financial support was provided by NUFFIC and Wageningen University for various stages of data gathering. We are grateful to the Ghana Wildlife Division (GWD) of the Forestry Commission for granting us permission to conduct this research in

Kogyae Strict Nature Reserve (KSNR). We thank staff of GWD at Dome Camp of KSNR for their assistance during fieldwork. Nuni Ferawati and José A. Medina-Vega assisted with field data collection of the second year. We thank David Kleijn and two anonymous reviewers for giving critical feedback that greatly improved this paper. We are grateful to Frans Moller and Jan van Walsem for providing assistance with laboratory analysis of plant and soil samples.

Variation in vegetation cover and seedling performance of tree species in a forest-savanna ecotone

3

Exploring the effects of grass competition on postfire survival among forest and savanna-transition tree seedlings

Hamza Issifu, Philippine Vergeer, George K.D. Ametsitsi, Jelle Klijn, Paolo Satorelli, Millicent Tanson, Hypolite Bayor, Vincent Logah, Frank van Langevelde, Elmar Veenendaal



Abstract

Competition with grasses may affect growth and post-fire survival of tree seedlings in tropical savannas. Different tree functional types (e.g. forest and savannatransition species) that co-occur in the forest-savanna transition may have different responses to grass competition which may result in differential establishment success. We performed a common garden experiment in the humid Guinea savanna of Ghana, which involved eight forest and savanna-transition tree species in factorial combinations with wet season grass treatment and dry season fire. Savanna-transition species are common to both forest and savannas in the forest-savanna transition. We tested direct effects of competition on seedling growth and survival before the dry season fire and indirect effects on post-fire survival. We found that grass competition suppressed tree seedling growth but not survival. Except for seedling height and stem basal diameter, the effect of grass competition on growth performance was similar for both tree functional types. Grass competition decreased root starch content due to reduction in root mass of forest tree seedlings. Consequently, post-fire survival (which averaged only 6%) was four times lower for seedlings in prior grass competition among forest species. Post-fire survival of the savanna-transition species was 91% and found to be related to pre-fire seedling size and root starch reserves. Our results show that wet season grass competition suppresses growth performance similarly for savanna-transition and forest tree species, and while it does not affect post-fire survival of savanna-transition seedlings, it may render forest seedlings more susceptible to fire-induced mortality.

Keywords: Tropical tree species, Ghana, savanna, forest, seedling traits, growth, survival, dry season, fire

Introduction

The forest-savanna transition is often a mosaic of forest patches in a large expanse of humid savanna (McCook 1994) characterised by different tree functional types including species that occur in forest, savanna or in the transition between forest and savanna (Armani et al. 2018, Veenendaal et al. 2015). Fire is generally considered a significant bottleneck for the establishment and subsequent survival and growth of forest tree seedlings in savanna (Gignoux et al. 2009, Hoffmann et al. 2004) thereby maintaining the typical mosaic pattern of the forest-savanna transition (Hopkins 1974, Swaine et al. 1976).

Competition with grasses may also play an important role in limiting establishment of forest tree seedlings in savannas. Models of tropical savannas often assume a negative effect of trees on grasses (e.g. Van Langevelde et al. 2003), but these ignore the seedling stage of trees (Van Langevelde et al. 2014). Suppression of tree seedlings due to grass competition is often reported for savanna tree seedlings (Barbosa et al. 2014, February et al. 2013, Tomlinson et al. 2019, Vadigi & Ward 2013, van der Waal et al. 2009). Grasses may compete with tree seedlings particularly for water (Anthelme & Michalet 2009, Kulmatiski et al. 2010, February et al. 2013) or soil nutrients (Tomlinson et al. 2019, van der Waal et al. 2009) or for both soil resources and light (Barbosa et al. 2014, de Dois et al. 2014, Holl 1998, Vadigi & Ward 2013). Some studies have reported facilitation of tree seedlings by grasses via amelioration of microclimate under dry conditions (Anthelme & Michalet 2009, de Dois et al. 2014).

Due to differences in the amounts of stored reserves, larger plants survive drought and fire better than smaller ones (Cardoso et al. 2016, Hoffmann et al. 2012). Suppression of tree seedling growth in the wet season due to grass competition may, therefore, decrease the chances of dry season fire survival.

Additionally, by slowing down seedling growth rates, grass competition may also prolong how long it takes for seedlings to reach a "threshold of fire resistance" (Hoffmann et al. 2012) thereby affecting the dynamics between fire frequency and seedling recruitment into bigger size classes. Thus, competition with grasses may affect growth and post-fire survival of tree seedlings in tropical savannas. Different tree functional types may have different responses to grass competition. Differences in responses of tree functional types will provide a better understanding of the role that grasses (aside fuelling fire) may have in shaping tree recruitment in savannas. Interactions of forest tree species with grasses may differ from tree species found in savannas or in the transition between savanna and forest due to differences in resource use strategies (and hence trait syndromes) (Boonman et al. 2019) and also because the forest environment is naturally less grassy. Studies testing the effects of grass competition on forest species are important because increased forest degradation means greater invasion by herbaceous vegetation (Hoffmann & Haridasan 2008) which may (together with fire) provide an effective bottleneck for the restoration of forest cover (Ratnam et al. 2011). Additionally, grass competition, in addition to fire, may limit encroachment of forest species in savannas. However, only few studies have explored tree-grass interactions that involve forest tree species (e.g. Holl 1998, Sun & Dickson 1996).

In this study, we ask: 1) are survival and growth responses to grass competition different for forest than savanna-transition tree functional types? 2) How does wet season grass competition influence seedling survival after dry season fire for forest and savanna-transition tree functional types? We hypothesise that: 1) grass competition in the wet season reduces tree seedling growth and survival, with larger effect on the forest than savanna-transition tree functional

types, and 2) wet season growth suppression of seedlings decreases the survival chances to dry season fire more for forest than savanna-transition tree functional types.

Materials and methods

Study site and species

The study was performed at the experimental garden on the Nyankpala Campus of the University for Development Studies, Ghana (9°24'52.0"N, 0°58'43.6"W). The site is within the humid part of the Guinea savanna vegetation zone with an annual rainfall of ~ 1100 mm yr⁻¹. The dry season is from November to March and followed by an April to October wet season. Mean annual temperature is 28°C. The experimental garden is a former agricultural land with high herbaceous cover dominated by the two grass species Pennisetum pedicellatum and Andropogon *pseudapricus*. The soil in the garden is sandy loam in texture with medium to coarse stones. Soils of the general study location are described as Plinthustalf, sandy over-clays skeletal phase and classified by FAO as Plinthic Lixisols (FAO 2001, Serno & van de Weg 1985). Eight tree species were used in this study, classified a priori into species typical for forests (four species) or forest savannatransition (four species) (Table 1). Of the latter group, Afzelia africana is a transition/non-selective species (Ametsitsi et al. in prep.) occurring both in savanna and in dry tropical forest. Seeds of the humid savanna-transition species were collected in the transition zone at Kogyae Strict Nature Reserve (7°19' 1.66" N, 1°05' 5.863" W) whereas those of the forest species were collected from the semi-deciduous forest near Abofour (7°19' 1.66"N, 1°05' 5.863"W), between December 2017 and February 2018. Seedlings were raised in a temporary nursery at the experimental site and transplanted into the experiment at \sim 8 weeks old in early April 2018.

Table 1. Functional classification of tree species used in the common garden experimentin the Guinea savanna of Ghana. Sources of species information: Hawthorne 2005; Hall &Swaine 1976; Orwa et al. 2009.

Species	Family	Guild	Functional type
<i>Khaya senegalensis</i> (Desr.) A. Juss	Meliacaea	-	Savanna
<i>Khaya anthotheca</i> (Welw.) C.DC <i>.</i>	Meliacaea	Non-pioneer light demander	Forest
Khaya ivorensis (Desr.) A. Juss	Meliacaea	Non-pioneer light demander	Forest
<i>Terminalia superba</i> (Engl. & Diels)	Combretacae	Pioneer	Forest
Terminalia ivorensis A.Chev.	Combratacae	Pioneer	Forest
Pterocarpus erinaceous Poir.	Fabaceae	-	Savanna
Afzelia africana Sm. ex Pers.	Cesalpinaceae	Non-pioneer light demander	Transitional
<i>Daniella oliveri</i> (Rolfe) Hutch. & Dalziel	Cesalpinaceae	-	Savanna

Study approach

The experiment consisted of two phases: a grass/no-grass competition treatment in the wet season, followed by a fire/no fire treatment in the dry season in a full factorial design (Figure 1). Twelve 48 m² plots were established at the experimental site in early April, just before the onset of the wet season of 2018. All herbaceous vegetation was removed in six randomly chosen plots. Vegetation in the other six plots was left intact (i.e. grass competition plots). If necessary, tufts of *Pennisetum pedicellatum* and *Andropogon pseudapricus* were transplanted in the grass plots to ensure a homogeneous sward of herbaceous vegetation throughout. In each plot, four seedlings each of the eight species, except *Daniella oliveri* (Table 1) were transplanted at a spacing of 0.7 m x 1.0 m within and between rows (respectively) of seedlings at the onset of the rainy season (April, 2018) with *D. oliveri* only present in three control and five competition plots due to the low seedling availability. We assigned seedlings at random to planting positions. In total, 360 seedlings were transplanted (12 plots × 7 species × 4 seedlings + 3 seedlings x 8 plots for *D. oliveri*). All plots received natural precipitation over the seven months of the wet season (Figure 1). No-grass plots were kept free of grass by periodic weeding until October 2018 when the rains ended. At this point, we randomly selected six plots (three each for grass and nograss) for destructive harvest to measure the pre-fire plant performance. The remaining six plots were reserved for the fire/no fire treatment.

The fire experiment

The percentage grass cover was estimated in three 1 m² quadrats randomly located per plot with standing grass biomass and the maximum height of the grass determined at the end of the rainy season. Then all six plots (three grass and three no-grass plots) were prepared for burning with pre-prepared grass fuel to ensure uniformity in fuel (type, moisture content and load) for plots to burn. Fuel used consisted of *Pennisetum pedicellatum* and *Andropogon pseudapricus*, harvested from the adjoining savanna and air-dried for two weeks.

We made fuel beds by covering each plot (entirely) with grass at a fuel load of 1 kg of grass m⁻². All plots were burnt separately between 15 hrs and 17 hrs in November 2018. Fire temperature in each plot was measured using a thermocouple thermometer (Hanna instruments, Singapore), with the probe cable buried while the full length of the probe was sticking out allowing for the most accurate fire temperature close to the seedling to be determined (Dayamba et al. 2010). After this, the plots were left untouched until being re-watered on February 1, 2019 to mimic early start of the wet season after four months of dry season. From then on each seedling received 1.4 L per day of water for three weeks after which plants were harvested.

Measurements on plants during the experiment

Three months after the start of the experiment, we measured seedling height and stem basal diameter for all seedlings in all plots. Census of live or dead was conducted for seedlings in all 12 plots at the end of the wet season, before the fire/no-fire treatment (November 2018). We defined dead plants as seedlings with dry (dead) aboveground parts (stem and leaves). We determined changes in seedling height and stem basal diameter using data from July 2018 and November 2018 for all 12 plots. Post-fire and/or dry season survival (henceforth post-fire survival) was assessed in each plot (total of 6 plots) by counting the number of resprouting seedlings for each species.

Plant biomass and trait measurements

We determined seedling start (dry) mass from five random seedlings (from the seedling pool) per species prior to transplantation. Pre-fire harvest (in November 2018) was done just before the fire treatment in November by carefully excavating all seedlings in six random plots (three grass and three no-grass). Seedlings were separated into leaf, stem and root parts before oven drying (at 70 °C) to constant weight. Various plant traits (Table 2) were then derived based on data at this harvest. Leaf mass fraction (LMF) was calculated as leaf dry weight divided by total plant dry weight; stem mass fractions (SMF) as stem dry weight divided by total plant dry weight; root mass fraction (RMF) as root dry weight divided by total plant dry weight; specific stem length (SSL) as stem length divided by total plant dry weight; specific rooting depth (SRD) as rooting depth divided by total root dry weight. We also calculated average seedling mass gain as the difference between start plant mass and mass at end of wet season harvest. RGR was calculated using In-transformed final plant mass and mean In-transformed initial mass and the

growth period (d = 32 weeks) in the following equation (adapted from Hoffmann & Poorter 2002):

$$RGR = \frac{lnMass_{final} - lnMass_{final}}{d}$$

We did the final harvest in March 2019. This was after 28 weeks of the post-fire regrowth phase. Prior to excavation, we watered all seedlings for four weeks (to ascertain mortality). Live seedlings were counted as those with (at least) live belowground buds. After oven drying, we determined the total mass of new shoot as new stem mass plus new leaf mass. Total plant mass was determined as mass of new shoot plus mass of root.

Trait	Abbr.	Relevance
	(units)	
Leaf dry weight	LDW (g)	Light capture/growth
Stem dry weight	SDW (g)	Height growth/light capture/avoiding fire top-kill
Root dry weight	RDW (g)	Belowground reserves/fire or drought survival
Leaf mass fraction	LMF (g g ⁻¹)	Investment in light capture, photosynthesis and growth
Stem mass fraction	SMF (g g⁻¹)	Investment in light capture and aboveground growth
Root mass fraction	RMF (g g ⁻¹)	Storage of reserves and/or belowground foraging
Plant height gain	PH (cm)	Indication of aboveground growth. Important for light competition or avoiding fire top-kill
Stem length	SL (cm)	Light capture/aboveground growth
Specific stem length	SSL (cm g ⁻¹)	Light foraging and growth, avoiding fire top-kill
Rooting depth	RD (cm)	Foraging for deeper soil moisture
Specific rooting depth	SRD (cm g ⁻¹)	Investment in deep water foraging
Relative growth rate	RGR (g g ⁻¹ week ⁻¹)	Photosynthetic capacity, biomass accumulation
Plant mass gain	PDW (g)	Biomass accumulation, growth
Stem basal diameter	SBD (mm)	Thicker stem/root, protection of tissues from fire, indication of root size or plant growth

Table 2. Functional traits analysed in the study and their relevance.

Roots of plants excavated in both harvests (November 2018 and March 2019) were analysed for root starch concentration and starch content (i.e. total root starch reserves). We used the carbohydrate extraction protocol of Duranceau et al. (1999) which is adapted from Dubois et al. (1956). Samples available for the

November 2018 harvest were sufficient: 6 seedlings x 2 grass treatments x 7 species (three savanna-transition tree species and four forest tree species). At the March 2019 harvest, only three savanna-transition tree species were available for root starch analysis due to mortality of particularly the forest species.

Environmental measurements

We obtained data on precipitation, temperature and relative humidity for the whole experimental period (Figure 1) from the Agro-meteorological station of Savanna Agricultural Research Institute of Ghana (within 1 km of the study site). We measured soil moisture (vmc %) within the top 10 cm of the soil using a theta probe (Spectrum technologies Inc.) in mid-August (peak wet season of 2018) and then in November (one week into the dry season). Measurement in August was done at five random positions in all 12 plots (six each for grass and no grass) while the November measurement was done at every seedling position but in six plots (three each for grass and no-grass). After the fire treatment, we sampled soil for chemical analysis. Sampling was done within the top 10 cm soil layer in nine plots, three each for fire + grass, fire + no-grass and no-fire + no-grass. We made composite samples from 4 locations within each plot.

Light availability to seedlings was measured using a PAR quantum sensor (Skye Instruments, Llandyndrod Wells, UK) by measuring light reaching the top canopy leaves of randomly selected seedlings in the grass and no grass plots. Light measurements were done under overcast conditions between 11:30 hrs and 12 hrs. Monthly summaries of temperature, relative humidity and rainfall before and after the experimental fire are presented in Figure 1 and temperature, relative humidity and wind speed on the day of burning were 30°C, 66% and 0.88 kph respectively.



Figure 1. Weather variables, monthly rainfall, relative humidity and temperature during the experimental period in Nyankpala within the Guinea savanna of Ghana. Red vertical line indicate month (in the dry season) of experimental burn. Weather data are from agrometeorological station of the Savanna Agricultural Research Institute of Ghana, within 1 km of the experimental site.

Statistical analysis

We analysed all data in *R* (R Development Core Team 2017) and used *ggplot2* (Wickham, 2009) for data visualization except Figure 1. Soil moisture content (MC) in grass and no-grass plots was tested for differences for August and November measurements separately using linear mixed-effects models (LMMs). This was achieved with the *lmer* function in *lme4* package (Bates et al. 2015) together with *lmerTest* package (Kuznetsova et al. 2019). In the LMMs, plot was included as a random factor. We tested light intensity for differences between grass and no-grass plots using an LMM including plot and seedling position as random factors.

Chapter 3

We tested growth-related traits (i.e. RGR, gains in total plant mass, height and stem diameter), root starch concentration, total root starch content and seedling morphology traits (LMF, SMF, RMF, RD, SRD and SSL) determined at the end of the competition phase for fixed effects of tree functional type (TFT) × grass treatment. Plot was included as a random effect. Root starch (content and concentration) and SRD were In-transformed prior to analyses to meet assumptions of normality and residual variances. Root dry weight was included as a covariate in the models for root starch. We estimated the effect size of competition for growth-related traits using Hedges' g (Hedges & Olkin 1985). In separate models (for each TFT) we tested fixed effects of species × grass treatment including random plot effect. We separated significant species effects (or interaction effects) using Tukey's post hoc contrasts achieved in the *emmeans* package (Lenth et al. 2019).

We analysed seedling survival in a generalized linear mixed model (GLMM) with the binary data using binomial error (with logit link function). For this, we used the *glmer* function in *lme4* package. We analysed survival for fixed effects of TFT × grass treatment and then for species × grass treatment separately for each TFT, including random plot effect in each separate model. Post-fire seedling survival (also binary data) was analysed in GLMMs to test competition and TFT effects, including random plot effect following the same procedure as described for survival due to grass competition. Within the forest TFT, we analysed survival of *K. anthotheca* and *K. ivorensis* together (as there were no survivors of the two Terminalia species) for fixed effect of species × grass treatment, including random plot effect in an LMM that included random plot effect. Also among the savanna-transition species, we assessed the relationship of post-fire

survival to pre-fire seedling size (plant height) in a GLMM and evaluated changes in root starch concentrations in a LM (after In-transformation) for fixed effects of harvest (i.e. pre-fire or post-fire), species and grass treatment. Correlations among traits were performed separately for seedlings in grass and no-grass treatments using Pearson's product-moment.

Results

Environmental variables

Total rainfall received within the wet season was 1053.8 mm, which peaked in August (Figure 1). Soil moisture content (vmc %) averaged from 26.4 ± 2.6 % in August to 12.4 ± 2.2 % in November. In both measurement months, soil moisture content was higher in the grass treatment than no-grass (Table 3). Grass dominated the herbaceous layer in the grass plots (> 75 % vs 5-12 % cover for herbs). Mass of herbaceous vegetation averaged 0.76 kg m⁻² \pm 0.15 kg m⁻², with a maximum height of 2.26 m \pm 0.29 m. Light intensity recorded in grass plots averaged 10.9 % \pm 5.0 %, which was lower (F _{1.33} = 467, p < 0.001) than intensity recorded in the no-grass plots at 90.5 $\% \pm 5.9 \%$ of full light levels. The amount of light reaching the seedling crowns varied between 75-100% of above canopy height, and related ($\beta = -0.25$, t = -2.80, df = 14, p = 0.015) to seedling height in the no-grass treatment. In the grass treatment, the amount varied between 10% and 27%, but was not related ($\beta = -0.015$, t = -0.15, df = 16, p = 0.886) to seedling height (Figure SI). Height of the fuel bed made in the fire experiment averaged 0.23 m \pm 0.1 m, resulting in a fuel bulk density of 1.1 kg m⁻³. Mean moisture content of grass (calculated on a dry weight basis) used as fuel for the experimental burn was 3.0 ± 1.1 % and mean fire temperature recorded was 535°C ± 157°C.

Table 3: Results of ANOVA (Type III, with Satterthwaite's method) from linear mixedeffects models on differences in soil moisture content between grass and no-grass plots in August (peak wet season) and November (start of dry season) in a common garden in the Guinea savanna of Ghana.

	Mean (VMC % ± sd)		F-value	df	p-value
	Grass	No grass			
August MC	28.2 ± 2.73	24.5 ± 2.81	10.1	1	0.009
November MC	13.2 ± 2.57	11.6 ± 1.84	17.3	1	0.015

Influence of competition on pre-fire survival and growth-related parameters

Seedling survival proportion at the end of the wet season averaged 0.93 ± 0.14 irrespective of TFT or grass treatment (Tables 4 & 5). However, relative growth rate (RGR) was lower for seedlings in the grass treatment (at 0.03 \pm 0.03 g g⁻¹ week⁻¹) than no-grass (at 0.06 \pm 0.05 g g⁻¹ week⁻¹) irrespective of TFT (Table 5). The effect size of grass treatment on RGR was similar for both TFTs (mean q = -0.89). Plant mass gain was lower for seedlings in the grass treatment (1.14 ± 1.70) g) than no-grass (6.20 \pm 6.70 g) irrespective of TFT (Table 5). Grass treatment had a larger effect on mass gain than it did on RGR, but effect sizes were again similar for both TFTs (g = -1.03 for forest and -1.02 for savanna-transition). Height gain was irrespective of TFT, lower for seedlings in the grass treatment (at 1.80 $cm \pm 5.46$ cm) than no-grass (at 9.9 cm \pm 10.7 cm). Also irrespective of grass treatment, forest seedlings grew taller $(8.0 \text{ cm} \pm 10.9 \text{ cm})$ than savanna-transition seedlings at 3.2 cm \pm 6.4 cm (Table 5). Effect size estimated for plant height gain was larger for savanna-transition than forest TFT (g = -1.26 vs g = -0.91). We found an interaction effect of TFT × grass treatment on increment in stem basal diameter (SBD) (Table 5). In no-grass, SBD was higher for forest TFT (at 4.2 \pm 3.1 mm) than savanna-transition (at 2.7 \pm 2.5 mm), but SBD did not differ

between TFTs in grass treatment. Thus, effect of grass treatment on SBD was larger for forest TFT (g = -1.56) than savanna-transition TFT (g = -0.95).

We assessed species differences within each TFT separately. Seedling survival within the forest TFT did not differ between grass treatments or among species (Tables 4 & 5). We found an interaction effect of species \times grass treatment on RGR (Table 5), with slower RGR in grass treatment for both T. superba and T. ivorensis while RGR was similar in both grass treatments for K. anthotheca and K. *ivorensis* (Figure 2a). There was also an interaction effect of species \times grass treatment on plant mass gain (Table 5), with higher plant mass gain for both Terminalia species than K. ivorensis. Here, T. ivorensis had a bigger mass gain than K. anthotheca in the no-grass treatment, but we did not observe species differences in the grass treatment (Figure 2d). We found interaction effects of species \times grass treatment on both gains in plant height and SBD (Table 5). Irrespective of grass treatment, increments in seedling height and SBD were higher for both T. ivorensis and T. superba than for both K. ivorensis and K. anthotheca. We observed these species differences (for both height and SBD) in the no-grass treatment (Figure 2b & 2c). SBD was higher only for *T. ivorensis* than the two Khaya species in the grass treatment.

Within the savanna-transition TFT, survival was similar for seedlings in both grass treatments and also did not differ among species (Tables 4 & 5). There was an interaction effect of species × grass on RGR (Table 5), with faster RGR observed in the no-grass than grass treatment for *K. senegalensis* and *P. erinaceus* whereas *A. africana* and *D. oliveri* maintained similar RGR in both grass treatments (Figure 2e). We also found an interaction of species × grass treatment on plant mass gain (Table 5). Mass gain differed in the no-grass treatment between *K. senegalensis* and *P. erinaceus* (on one hand with higher mass gain) and *D. oliveri* and *A. africana*

on the other (Figure 2h). Plant height gain was lower in grass (-0.2 ± 3.6 cm) than no-grass (6.7 ± 6.8 cm) irrespective of species. Also irrespective of grass, both *K. senegalensis* and *P. erinaceus* grew taller than *A. africana* but not *D. oliveri* (Table 5, Figure 2f). There was an interaction effect of species × grass on SBD gain (Table 5). SBD gain was similar among species in the grass treatment, but SBD gain was higher for *K. senegalensis* than all other savanna-transition species in the no-grass treatment (Figure 2g).

Table 4. Seedling survival proportion (\pm sd) at the end of the wet season (October 2018) and end of dry season (March 2019) in a common garden in the Guinea Savanna of Ghana. Statistical comparisions (binomial model with logit link) for Tree functional types (TFT) × grass treatments and species (TFT) × grass treatments are conducted for each census separately and significant (p < 0.05) effects are indicated by different letters. Same or no letters indicate non-significant results. *D. oliveri* is left out due to too few replications in grass plots at final harvest.

	Wet season	ason census Dry season(post-fire)		t-fire) census
TFT(species)	Grass	No-Grass	Grass	No-Grass
Forest TFT	0.91 ± 0.16	0.90 ± 0.16	$0.02 \pm 0.08^{\circ}$	0.08 ± 0.16^{a}
Khaya anthotheca	0.96	0.83	0.08	0.25
Khaya ivorensis	0.83	0.88	0.00	0.08
Terminalia ivorensis	0.96	1.00	0.00	0.00
Terminalia superba	0.88	0.88	0.00	0.00
Savanna TFT	0.96 ± 0.13	0.95 ± 0.10	0.89 ± 0.18^{b}	0.92 ± 0.18^{b}
Khaya senegalensis	1.00	0.96	0.83	0.83
Afzelia africana	0.96	0.92	0.92	0.92
Daniella oliveri	0.88	0.92	-	-
Pterocarpus erinaceus	0.96	1.00	0.92	1.00



Figure 2. Plant growth in the wet season as influenced by grass treatments among seedlings of forest (green shaded in species list) and savanna-transition tree species in a common garden experiment in the Guinea savanna of Ghana. Means across species for each grass treatment are presented and compared within each TFT (*p < 0.001; **p \geq 0.001< 0.01; *p < 0.05>0.01; ns = non-significant). Letters compare species (box and whiskers) across grass treatments within TFT only. Different letters are significant at p < 0.05 from Tukey pairwise comparisons following linear mixed-effects models.

Influence of grass treatment on pre-fire seedling morphology traits and root starch

At the TFT level, leaf mass fraction (LMF) averaged 0.19 \pm 0.11 g g⁻¹ for seedlings in grass treatment, which was lower than LMF in the no-grass treatment at 0.28 \pm 0.14 g g⁻¹ (Table 6). There was a trend of higher (although at borderline
significance) LMF for forest $(0.34 \pm 0.12 \text{ g g}^{-1})$ than savanna-transition $(0.23 \pm 0.15 \text{ g g}^{-1})$ in the no-grass treatment, but no apparent TFT difference in the grass treatment (Table 6). Forest seedlings had higher stem mass fraction (SMF) at 0.43 \pm 0.10 g g⁻¹ than savanna-transition seedlings at 0.19 \pm 0.09 g g⁻¹ (Table 6) irrespective of grass treatment savanna-transition seedlings had higher RMF (at 0.62 \pm 0.18) than forest (at 0.29 \pm 0.10) seedlings irrespective of grass treatment (at 0.47 \pm 0.21 g g⁻¹ for no-grass and 0.43 \pm 0.22 g g⁻¹ for grass treatment).

We found that specific stem length (SSL) was higher for savanna-transition (at 37.1 \pm 24.0 cm g⁻¹) than forest TFT (at 28.2 \pm 19.8 cm g⁻¹) irrespective of grass treatment. SSL was also higher (F _{1, 4} = 27.0, p = 0.006) in the grass treatment at 44.3 cm g⁻¹ \pm 22.1 cm g⁻¹ than in no-grass at 21.2 cm g⁻¹ \pm 15.8 cm g⁻¹ (Table 6). Savanna-transition seedlings rooted more deeply (at 21.3 \pm 6.7 cm) than forest seedlings (at 16.2 \pm 6.1 cm) irrespective of grass treatment (Table 6). However, specific rooting depth (SRD) was higher for the forest than savanna TFT irrespective of grass (Table 6). Also irrespective of TFT, SRD was higher in the grass treatment (at 29.8 \pm 29.2 cm g⁻¹) than in the no-grass treatment at 16.8 \pm 21.2 cm g⁻¹.

Within the forest TFT, we found an interaction effect of species × grass treatment on LMF (Table 6). Both *K. anthotheca* and *K. ivorensis* had higher LMF than both *T. superba* and *T. ivorensis* in the grass treatment, but not in the nograss treatment (Figure 3a). There was a similar interaction effect on SMF (Table 6), with both Terminalia species, but not the two Khaya species, having higher SMF in the grass treatment than in the no-grass treatment (Figure 3b). RMF was similar between grass treatments and among species within this TFT (Table 6, Figure 3c). We found an effect of species × grass treatment on SSL (Table 6). SSL was similar among the two Khaya species irrespective of grass treatment, but SSL

was also higher for both Terminalia species in the grass than no-grass treatment (Figure 4a). Seedlings rooted more deeply in the no-grass compared to grass treatment irrespective of species. Also, both Terminalia species rooted deeper than both Khaya species irrespective of grass treatment (Table 6, Figure 4c). We found an interaction effect of species × grass on SRD (Table 6). The two Terminalia species had higher SRD in the grass than no-grass treatment whereas the two Khaya species maintained similar SRD in both grass treatments (Figure 4b).



Figure 3. Plant biomass allocation: leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF) for seedlings of forest (shaded green in species legend) and savanna-transition tree species in grass and no-grass plots at the end of the wet season in a common garden experiment in the Guinea savanna of Ghana. Means (across species) for each grass treatment are compared within each TFT (*p < 0.001; **p \ge 0.001< 0.01; *p< 0.05>0.01; ns = non-significant). Letters compare species (box and whiskers) across grass

treatments within TFT only. Different letters are significant at p < 0.05 from Tukey pairwise comparisons following linear mixed-effects models.

Within the savanna-transition TFT, there was an effect of species \times grass interaction on LMF (Table 6). *K. senegalensis* had the highest LMF and was also the only species with a lower LMF in grass than no-grass treatment (Figure 3d). We found a similar interaction effect for SMF (Table 6), with higher SMF in grass than no-grass treatment for *K. senegalensis*. SMF was similar in both grass treatments for all other species (Figure 3e). Seedlings in grass had higher RMF than seedlings in no-grass treatment irrespective of species. Also, *K. senegalensis* had the lowest RMF regardless of grass treatment (Table 6, Figure 3f). We found an effect of species \times grass interaction on SSL (Table 6). *D. oliveri* and *P. erinaceus* had lower SSL in the no-grass than grass treatment while all other species maintained similar SSL irrespective of grass treatment (Figure 4d). Within this TFT, RD was similar among species and between grass treatments (Table 6, Figure 4f). There was, however, a species \times grass than no-grass treatment interaction effect on SRD (Table 6). SRD was higher in grass than no-grass treatment only for *K. senegalensis* (Figure 4e).

At the TFT level, root starch concentration [RS] was lower (F _{1, 83} = 101, p < 0.001) for forest (at 3.50 \pm 3.0%) than savanna-transition (at 17.5 \pm 8.7%) irrespective of grass treatment and also regardless of root dry weight (Figure 5a). Total root starch content (RSC) however, increased with root dry weight (β = 0.31, t = 7.8, p < 0.001). There was also an apparent interaction effect (at borderline) of TFT × grass treatment (F _{1, 83} = 3.9, p = 0.051) as RSC of savanna-transition seedlings were similar between grass treatments whereas RSC was lower in grass treatment than no-grass for the forest TFT (Figure 5b). Within the forest TFT, [RS] was similar among species irrespective of grass treatments. Within the savanna-

transition TFT, [RS] differed among species (F $_{3, 34} = 13.2$, p < 0.001) irrespective of grass treatment. [RS] for *K. senegalensis was* 9.5% ± 5.2%, which was lower than [RS] for all others (which were similar) at 23 ± 6% for *A. africana*, 20 ± 13% for *D. oliveri* and 19 ± 4% for *P. erinaceus*.

Trait correlations

Correlations among traits (growth-related and morphology) were positive (ranging in strength from medium to strong) and largely significant (p < 0.05) among forest (Table S1a) than savanna-transition (Table S1b) TFTs. Grass treatment decreased the extent (strength) of correlations in both TFTs. Exceptions were correlations between stem length and stem dry weight and also between root dry weight and total plant dry weight (in both TFTs), which were stronger in grass than no-grass treatment. RGR correlated positively and significantly with RD in forest but not savanna-transition in both grass and no-grass treatments while SRD negatively correlated with RGR only in no-grass for both TFTs. This RGR and RD (or SRD) relationship influenced correlations of several other growth traits to RD and SRD (Table S1a and S1b).



Figure 4. Foraging efficiency; specific stem length (SSL) specific rooting depth (SRD) and rooting depth (RD) among seedlings of forest (shaded green in species list) and savanna-transition tree species in grass and no-grass plots at the end of the wet season in a common garden experiment in the Guinea savanna of Ghana. Means across species for each grass treatment are presented and compared within each TFT (*p < 0.001; **p \ge 0.001< 0.01; *p < 0.05>0.01; ns = non-significant). Letters compare species (box and whiskers) across grass treatments within TFT only. Different letters are significant at p < 0.05 from Tukey pairwise comparisons following linear mixed-effects models.

Table 5. ANOVA Tables based on Satterthwaite's method from GLMM on seedling survival proportions and LMM on growth-related parameters for all species and TFTs, only forest TFT and only savanna-transition TFT.

Source of	Surviv	a		RGR			Plant	mass		Plant	height	
variation												
	ш	df	p-value	ш	df	p-value	L	df	p-value	ш	df	p-value
TFT	0.91		0.341	2.15	1,148	0.145	0.38	1,148	0.539	28.8	1, 307	< 0.001
Grass	0.28		0.600	16.1	1, 4	0.014	8.71	1, 4	0.041	21.3	1, 10	< 0.001
TFT × Grass	0.33	н	0.564	0.09	1,148	0.770	0.12	1,148	0.725	1.53	1, 307	0.217
Forest TFT												
Species	0.47	ε	0.707	44.2	3, 70	< 0.001	7.05	3, 70	< 0.001	30.1	3, 154	< 0.001
Grass	0.07	ч	0.800	14.6	1, 40	0.017	7.24	1, 4	0.051	7.67	1, 10	0.019
Species × Grass	0.56	m	0.644	6.46	3, 70	< 0.001	6.26	3, 70	< 0.001	4.92	3, 154	0.002
ST TFT												
Species	0.80	4	0.527	51.3	3, 63	< 0.001	3.21	3, 63	0.029	4.75	3, 140	0.003
Grass	0.006		0.937	14.4	1, 40	0.017	6.49	1, 4	0.063	50.2	1, 140	< 0.001
Species × Grass	,	'	'	6.70	3, 63	< 0.001	4.50	3, 62	0.006	1.91	3, 140	0.130

Exploring the effects of grass competition on post-fire survival among forest and savanna-transition tree seedlings

Source of variation		LMF			SMF			RMF			SSL			ßD			SRD	
	L	df	d d	L	df	<u></u> ч	ш	df	4	ш	df	<u>-</u> д	ш	df	4	ш	df	<u></u> 4
			value			value			value			value			value			value
TFT	15.0	1,	v	286	1,	v	197	1,	v	8.93	1,	0.003	25.5	1,	v	51.7	1,	v
		151	0.001		148	0.001		151	0.001		143			142	0.001		143	0.001
Grass	20.1	1,	v	11.4	1, 4	0.025	2.20	1,	0.140	27.0	1, 4	0.006	2.74	1, 4	0.173	21.6	1, 4	0.009
		151	0.001					151										
TFT ×	3.70	1,	0.056	3.60	1,	0.059	0.04	1,	0.849	1.80	1,	0.182	0.39	1,	0.529	0.005	1,	0.944
Grass		151			148			151			143			142			143	
Forest TFT																		
Species	3.82	3, 73	0.013	6.20	3, 70	V	1.79	3, 73	0.013	1.05	3, 65	0.377	9.62	3, 65	v d	3.11	3, 65	0.032
						TOU.U									100.0			
Grass	31.0	1, 73	V	22.5	1, 4	0.008	2.26	1, 73	V	23.2	1, 4	0.008	8.82	1, 4	0.041	11.5	1, 4	0.023
			0.001						0.001									
Species ×	5.12	3, 73	0.002	3.19	3, 70	0.028	2.14	3, 73	0.002	5.75	3, 65	0.001	2.56	3, 65	0.062	4.85	3, 65	0.004
Grass																		
ST TFT																		
Species	45.1	4, 62	v	7.03	4, 63	v	39.7	4, 65	v	9.44	4, 62	v	1.27	4, 62	0.291	4.00	3, 63	0.005
			0.001			0.001			0.001			0.001						
Grass	4.74	1, 5	0.086	0.10	1, 5	0.76	4.56	1, 65	0.036	20.2	1, 4	0.009	2.10	1, 4	0.215	5.84	1, 4	0.073
Species x	5.28	3, 62	0.002	4.98	3, 63	0.003	0.77	3, 65	0.513	4.51	3, 62	0.006	0.97	3, 62	0.410	4.56	3, 63	0.007
Grass																		

separately at TFT level (i.e. for all species together) and then at the species level for each TFT (i.e. species compared only within forest Table 6. ANOVA tables based on Satterthwaite's method from separate LMMs for seedling morphology traits. Analyses were done TFT and only with savanna-transition TFT).

Post-fire and dry season survival, regrowth and root starch

All plants were top-killed by the fire and/or dry season. Therefore we assessed TFT/species resprouting capacity as influenced by grass competition. At the TFT level, post-fire survival was lower (F = 62.2, p < 0.001) for the forest TFT (at 0.06 \pm 0.12) than savanna-transition (at 0.91 \pm 0.18) irrespective of grass treatment (Table 4). At the species level, both *T. ivorensis* and *T. superba* failed (completely) to survive from the dry season and fire (Table 4) within the forest TFT. Post-fire survival for *K. ivorensis* and *K. anthotheca* averaged 0.14 \pm 0.18, and there were no species or grass treatment effects. Notwithstanding the lack of statistical significance, we observed a four-fold decline in post-fire survival for forest seedlings in prior grass treatment compared to the no-grass treatment across all species. Within the savanna-transition TFT, seedlings largely survived the dry season fire irrespective of prior grass treatment or species (Table 4).

We analysed post-fire regrowth only for savanna-transition species as recovery was too low for the forest TFT at this stage. Across all three savanna-transition species, new shoot mass (post-fire) averaged 0.16 g \pm 0.4 g. We observed that root mass constituted 97% \pm 6.7% of total plant mass across species (Figure S2b), which was lower for seedlings in previous grass treatment (at 1.48 g \pm 1.07 g) than no-grass treatment (at 5.71 g \pm 3.61 g). There was also an interaction effect of species × grass (F _{2, 59} = 8.22, p < 0.001). Total plant mass differed between pre-fire grass treatments for *K. senegalensis* and *P. erinaceus*, but not *A. africana* (Figure S2a).

Across all three savanna-transition species analysed for post-fire survival, the chances of a seedling surviving fire increased ($\beta = 0.27$, z = 2.03, p = 0.043) with seedling height (Figure 6a). Total root starch reserves (RSC) increased with root mass ($\beta = 0.122$, t = 2.08, p = 0.046) for seedlings analysed post-fire. We

found an interaction effect of species × grass treatment (F $_{2, 25} = 6.87$, p = 0.004) on post-fire RSC. *K. senegalensis* seedlings had lower post-fire RSC in (previous) grass treatment than no-grass treatment whereas post-fire RSC for *A. africana* and *P. erinaceus* were similar between previous grass treatments (Figure 6b).

We assessed the effect of fire on root starch concentration [RS]. We found an effect of species × harvest time (i.e. pre-fire vs post-fire) interaction (F _{2, 59} = 12.1, p < 0.001) on [RS] irrespective of prior grass treatment. [RS] was lower among post-fire than pre-fire seedlings of *K. senegalensis* (2.5% ± 5% vs 10% ± 2%) but not of *A. africana* (20% ± 10% vs 23% ± 6.5%) or *P. erinaceus* (17% ± 6% vs 19% ± 4%).

Discussion

In this study, we first tested whether direct competition effect on wet season tree seedling performance was larger for the forest tree functional types than for the savanna-transition tree functional types. We then determined whether any growth reduction subsequently resulted in greater decrease in probability of surviving dry season fire among seedlings of forest than savanna-transition tree functional types. Overall, we found differences in seedling performance in the face of grass competition and in post-fire survival of seedlings, which were related to trait differences between the two tree functional types.

Grass competition directly suppresses tree seedling growth performance but not survival

We predicted lower growth for tree seedlings in grass plots, with forest tree seedlings being more affected than savanna-transition seedlings. This prediction was based on the fact that forests are naturally less grassy than humid savannas (Issifu et al. 2019; Ratnam et al. 2011). Indeed, we found overall significant

suppression of all growth-related parameters for both TFTs, consistent with other studies reporting on suppressed tree seedling growth by grass competition (Barbosa et al. 2014, Reginos et al. 2009, Scholes & Archer 1997, Sankaran et al. 2004, Tomlinson et al. 2019).



Figure 5: (a) Root starch concentration and (b) root starch content (total reserves in roots) of seedlings of forest and savanna-transition tree species in grass and no-grass plots at the end of the wet season in a common garden experiment in the humid Guinea-savanna of Ghana. *Data points*: triangles are savanna-transition seedlings, circles are forest seedlings. *Regression lines*: red line is savanna transition in in grass (ST: G), green line is

savanna-transition in control (ST: nG), blue line is forest in grass (F:G), grey line is forest in control. Black line in (a) is the mean across treatments. Grey-shaded area is 95% CI. *Forest species*: ka = *K. anthotheca*, ki = *K. ivorensis*, ti = *T. ivorensis*, ts = *T. superba. Savanna-transition species*: aa = *A. africana*, ks = *K. senegalensis*, pe = *Pterocarpus erinaceus*, do = *D. oliveri*.



Figure 6: (a) Dry season survival probability as predicted by wet season plant height (in a GLMM) (b) Total root starch reserves as predicted by root dry weight post-burn in previous grass vs no-grass plots for three savanna species in a common garden in the Guinea savanna of Ghana. Grey-shaded area is 95 % confidence interval for species average (black regression line).

However, not all species responded similarly. *Terminalia superba* and *T. ivorensis* (both forest species) and *Khaya senegalensis* and *Pterocarpus erinaceus* (both savanna-transition species) responded more strongly to grass competition. Interestingly, species that were not adversely affected by competition, *Khaya ivorensis*, *Khaya anthotheca* (forest) and *Afzelia africana*, *Daniella oliveri* (savanna-transition) were slow growing even in the absence of grass competition (Figure 2). Response of this latter group of species is in line with a conservative resource-use strategy (see, e.g. Boonman et al. 2019) and consistent with the idea that grass competition effect is greater on faster-growing species (Tomlinson et al. 2019). Grass competition thus, merely prevents fast-growing species from attaining their growth potential, but in terms of growth, these species perform similar to slow-growing species under competition (Figure 2).

Differences in tree growth, seedling growth rate in particular, is mostly attributed to competition for soil moisture (Kulmatiski et al. 2010, February et al. 2013), soil nutrients (Reginos et al. 2009, Tomlinson et al. 2019, van der Waal et al. 2009), a combination of moisture and nutrients or light (Barbosa et al. 2014, de Dois et al. 2014, Holl 1998, Vadigi & Ward 2013). In our plots, topsoil moisture content was not regarded as a limiting growth factor as moisture content was generally high, and even slightly higher in grass plots, and expectedly was probably sufficient for ample water uptake (Veenendaal et al. 1996). Light intensity was however much lower in grass plots suggesting that light competition may present a potential mechanism for tree seedling suppression in productive humid savannas. We found relative light levels of 11% in the grass treatment. Although some forest tree species (the shade bearers in this study) may still maintain considerable growth or even attain maximum growth (Agyeman et al. 1999, Veenendaal et al. 1996b) under these light conditions, such relatively low light

levels may pose growth limitation to the forest pioneers (Veenendaal et al. 1996) and the savanna-transition species (Gignoux et al. 2016, Ratnam et al. 2011). Competition for soil moisture, or more likely nutrients, may have occurred, but would still be minimal due to the low light intensity. In shaded environments, seedlings tend not to respond strongly to limitations in soil moisture and nutrients (Hoffmann et al. 2004, Tomlinson et al. 2019, Vadigi & Ward 2013).

Effect of grass competition on seedling performance is similar for forest and savanna-transition tree functional types

In contrast to prediction, we did not find evidence for greater competition effect for the forest than savanna-transition species, particularly on RGR and plant mass gain. Competition effect on plant height was instead, higher for the savannatransition than forest species. We found this because the savanna-transition species invested much less in height growth, particularly when in competition with grass. The forest species (particularly the Terminalia species) on the other hand, generally grew tall with their height in competition being comparable with height of the savanna-transition seedlings without competition (Figure 2b and 2f). The difference in investment in height growth reflects different growth strategies for the forest than savanna species (Hoffmann & Franco 2003, Issifu et al. 2019, Boonman et al. 2019). Among forest species, stem basal diameter correlated very strongly to root mass regardless of grass treatment. Among savanna-transition seedlings in grass treatment, however, there was no correlation between stem basal diameter and root dry weight in competition (Table S1). Therefore, root mass was unrelated to plant size for savanna-transition species (i.e. they had disproportionately large roots), unlike the forest species. Overall, differences in species responses to competition appear to reflect strategies for faster growing and highly competitive pioneer species (Terminalia superba and T. ivorensis) and the slow-growing, more resource-conserving and less competitive shade bearers (*Khaya anthotheca and K. ivorensis*) (Hawthorne et al. 1995).

Also, we did not find evidence for direct competition effect on seedling survival for either TFT. This contrasts with some reports on direct competition effect on savanna tree seedling survival (e.g. Migley & bond 2001 van der Waal et al. 2009, Ward & Elser 2011), but consistent with other studies that also did not find this effect (Barbosa et al. 2014, Vadiqi & Ward 2013). A plausible explanation for this would be that soil moisture was high in our plots as opposed to studies in semi-arid savannas (e.g. Migley & bond 2001, van der Waal et al. 2009, but see also Barbosa et al. 2014). Also, our plots were not limiting in nutrients, and the levels of nutrients found were also similar in both grass treatments (Table S2). Only light intensity differed between grass treatments, but the forest pioneers and savanna species which should be affected by lower light could survive the intensities recorded in grass plots, albeit with reduced growth. These findings imply that grass competition alone does not directly preclude the establishment of tree seedlings irrespective of functional type. This finding may (partly) explain why forest species are capable of establishing in some humid savannas under fire exclusion (Murphy & Bowman 2012 and references therein).

Seedling trait responses to grass competition suggest an important role for light competition in humid savanna

We observed adjustments in some biomass allocation and foraging traits in response to grass competition. Seedlings in grass plots (irrespective of TFT) had higher specific stem length (thinner stems), which is often associated with shade avoidance (Schmitt et al. 1999). Seedlings also had higher specific rooting depth, which is needed for foraging for deeper water (Tomlinson et al. 2012). However, seedlings in the grass treatment did not root more deeply compared to those in

the no-grass treatment. Thus, there was no evidence for the nearly elusive root niche separation for water uptake between roots of tree seedlings and grasses (Kulmatiski et al. 2010, Sankaran et al. 2004, Walter 1971). Irrespective of grass treatment, savanna-transition seedlings rooted more deeply than forest seedlings, reflecting the need for species from dryer environments to forage more for deeper water compared to those from more moist environments (Tilman 1988).

We observed that patterns of larger investment in foraging traits were mainly by Terminalia superba and T. ivorensis. These species increased investment in aboveground traits in grass treatments to avoid shading, which is typical of forest pioneers (Veenendaal et al. 1996). They had higher rooting depth, perhaps, only because they were bigger (Table S1), which also explains why specific rooting depth was lower in the no grass treatment (as thicker roots tend to yield lower specific rooting depth values). Among Savanna-transition species, specific stem length was higher for open-woodland species (P. erinaceus and D. oliveri) than closed-woodland types (K. senegalensis and A. africana), which suggests differences in shade tolerances even within this TFT (Issifu et al. 2019). Only K. senegalensis increased specific rooting depth in competition, again suggesting thinner roots in grass compared to no-grass treatment. Savanna-transition species increased root mass fraction in grass plots, but forest species did not. When in competition with grass, forest pioneers instead, invested more in stems than leaves or roots in competition. Biomass allocation patterns of K. senegalensis was similar to the forest pioneers. These patterns again reveal strategies for being competitive for light and investment in growth structures versus investing in survival structures (Wright et al. 2010).

Wet season grass competition does not decrease the chances of post-fire survival for savanna-transition tree seedlings, but may be important for forest tree seedlings via influences on root allocation and starch storage

Forest species were strongly affected by fire, illustrated by a 15-fold lower survival after fire compared to the savanna-transition species (Table 4). This finding is in line with other studies across forest-savanna boundaries (Gignoux et al. 2009; 2016, Hoffmann et al. 2004; 2012, Issifu et al. 2019) and reflects sensitivity of forest seedlings to fire, particularly at the early life stages. We, however, acknowledge that the effect attributed here to fire may not represent a fire-alone effect due to the coupling of fire with the dry season.

Among savanna-transition species, the probability of a seedling surviving fire was related to seedling height. Such a relationship among savanna juveniles is often attributed to the benefit of growing above the flame height (Higgins et al. 2000, Hoffmann et al. 2012). However, all seedlings in our experiment were topkilled, perhaps because they were still young (under one year) and also due to the high fire temperature they were subjected to in our study. Therefore, it may be safe to assume that other traits which correlated strongly with seedling height (e.g. root mass and hence starch concentration) could explain higher survival of taller individuals. This explanation is in line with other studies which also found higher survival to be dependent on root allocation and carbohydrate storage (Hoffmann et al. 2004, Issifu et al. 2019). This also explains the difference in survival between TFTs. The savanna-transition species survived while all seedlings of both forest pioneers died and the few forest seedlings that survived the fire were from the Khaya species (particularly K. anthotheca). This is interesting because it shows that being more conservative (as in savanna tree seedlings) ensures a higher survival, in line with the growth-survival hypothesis (Wright et al. 2010).

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Root starch concentration was not affected by grass treatment, but root dry weight decreased in the grass-present plots. This suggests that allocation to root storage was fixed (and unrelated to root mass) (Figure 5a). However, total root reserves was lower in the grass treatment due to a decrease in root mass. Reduction in root mass (due to competition) was higher among the forest TFT than the savanna-transition TFT (Figure 5b) possibly linked to the latter foraging more aboveground to avoid being shaded by grass. Consequently forest (but not savanna-transition) seedlings that had been in competition had a tendency to survive much less (Table 4). A lower fire intensity (than used in this study) may reveal the potential adverse effect of wet season grass competition on post-fire survival of forest species, which was not found (statistically) in this study. This needs to be explored in further studies manipulating different fire intensities. All species (only savanna-transition) which survived the fire failed to regain their prefire sizes. This adverse competition effect was mainly evident in root mass, and therefore in the total root starch reserves (Figure 6b). This could influence the sizes of regrowth in subsequent seasons, and may contribute to savanna-transition species persisting only as small plants in pyrogenic humid savannas (Freeman et al. 2017, Hoffmann et al. 2009).

In conclusion, our results show that grass competition (in the wet season) suppresses tree seedling growth for both forest and savanna-transition tree species. Savanna-transition tree species survive after fire regardless of prior grass competition, while forest tree species (which are already more sensitive to fire) may be more susceptible to fire-induced mortality due to reduction in total starch reserves in roots. This may explain current patterns in juvenile tree densities of forest and savanna-transition tree species in humid savannas. Our findings also

provide insights into the possible conditions under which forest species can establish in humid savannas.

Acknowledgements

Wageningen University and Research with support from the Academy Ecology Fund of the KNAW made field work possible. We are grateful to Adnan Sumaila, Atampuri Amidu and Abdallah Tajudeen for assisting in field data collection and to Jan van Walsem for laboratory analysis of plant samples.

Appendices

Fire	Grass	рН	N%	С%	P(av.)	К	Ca	Mg	Na
Burnt	Grass	7.08 (0.1)	0.08 (0.02)	0.84 (0.18)	3.21 (0.1)	0.24 (0.08)	2.13 (0.4)	1.27 (0.1)	0.01 (0.001)
	No-	7.07	0.07	0.71	3.33	0.31	2.55	0.65	0.01
	grass	(0.1)	(0.00)	(0.06)	(0.5)	(0.08)	(0.8)	(0.3)	(0.001)
Unburnt	No-	7.00	0.07	0.66	3.26	0.18	2.60	0.73	0.01
	grass	(0.1)	(0.01)	(0.16)	(0.4)	(0.08)	(1.4)	(0.4)	(0.001)

Table S2: Soil chemical properties in burnt and unburnt plots in a common garden in a humid Guinea savanna. P (av.) is Available P (mg/kg).



Figure S1. Light intensity reaching seedling crowns in relation to plant height in grass and no-grass plots in a common garden in the Guinea savanna of Ghana.

Table S1a	a. Pearson's c	orrelations amo	ng growth trai	its for forest	c species ir	າ grass (G)	and in no g	Jrass (NG) a	it pre-bur	n harvest	(i.e. end of
wet seasor	n). R-values i	n bold are signii	ficant at p < 0	.05. Detaile	d trait info	ormation ar	e presente	d in Table 2			
	RGR	SI	RDW	SDW	μDW	MOA	RMF	SMF	LMF	RD	SRD

RD	G																	
Ñ	NG																H	
ð	G														1		0.34	
4	NG														1			0.26
ц.	G												1			0.21		0.23
LM	NG												1		0.27			0.41
	(1)													0.74		0.19		0.04
SMF	9										-		'	.46		.31		38.
	N										1	14	'	56 0	54 -	•	- 68	•
RMF	G								1		•	6	•	5	0		9.0	
	NG								1		•	0.4	•	0.5	0.0		0.7	_
MQ	9								0.13			0.16	0.04		0.45			0.54
4	NG							1		0.25		0.06	0.32		0.59			0.46
M	в						1	0.80		0.09		0.52	0.50		0.35			0.47
Г	NG						1	0.92		0.39		0.20	0.59		0.52			0.46
N	G					1	0.50	0.91	0.12		0.17			0.22	0.35			0.54
SDV	NG					1	0.83	96.0		0.21	0.05		0.17		0.56			0.44
	(5					.89	0.73	.97	0.34			0.17		.09	0.55			0.41
RDW	,c (.97 (.75 (.94 (-	.08		.02	- 60.	0	.62 (ľ	.40
	<				84 1	.76 G	.63 6	.83 G	- 28	0		12 6	0	60	35 0		ľ	.46 G
SD	9			1	0 26	0 06	72 0	89 0	0	1	•	0.0		0	63 0		'	49 0
	NG			1	0.0	0.0	0	r.0	•	0.1	•	0.0	8 0.1		0.0		•	ò
SL	G		1	0.34	0.52	0.71	0.09	0.51	0.10		0.38		÷-		0.42		-0.26	
	NG		1	0.88	0.86	0.87	0.77	0.87	-0.28		-0.06		0.34		0.61		-0.61	
	(1		0.43	0.38	0.55	0.42	0.38	0.50	0.42		0.27		0.06		0.61		0.09	
RGR	÷		8	3	3	5 6	0	8.	42 6		10 -		5		2		- 09	
	NG		0.6	0.6	0.6	0.6	0.8	0.7	Ģ		-0-		0.5		0.5		Ģ	
		RGR	SL	SD	RDW	SDW	LDW	PDW	RMF		SMF		LMF		RD		SRD	

Table S1b. Pearson's correlations among growth traits for savanna-transition species in grass (G) and no grass (NG) at pre-burn harvest (i.e. end of the wet season). R-values in bold are significant at p < 0.05. Detailed trait information are presented in Table 2.

Q	G																
SR	NG															1	
q	G														1	0.34	
<u>~</u>	NG														1	-0.09	
Ľ,	9												1		0.10		92.0
2	NG												1		0.11		070
ш	9										1		0.50		-0.09		07.0
δ	NG										1		0.09		-0.22	-0.01	
ц.	9								1			0.87		0.86	-0.01	0.42	
RM	NG								1			0.48		0.92	-0.01	0.46	
	5							1	0.18		-0.12		-0.18		0.04		000
PDW	- 9/								0.26		.08				.44		22
								.63		44.	.20 C		.57 0		.20 0		
ΠDW	0						1	80 0		59 0	01 0		.66 0		.31 0	•	
	N						47 1	53 0	•	36 0	54 0.		0 20		0 SC	•	, ,
SDW	9					1	57 0.	38 0.	- 29	o	0.01		9		8 0.1	•	9
	NG					3 1	3.0.5	4 0.8	з- ⁻ 0.		7'0	8	0.1	9	11 0.2		č
RDW	9				1	8 0.2	5 0.4	6.0	0.4		-	0.3	-	0.3	-0.0	-0.2	
	NG				1	0.78	0.46	0.85	0.12		-0.0-		0.1		0.47	•	Ċ
SD	9			1	0.18	0.60	-0.20	0.35	0.10		0.04		-0.20		0.07	-0.21	
	NG			1	0.81	0.82	0.64	0.88	-0.20		0.12		0.18		0.36		990
_	9		1	0.41	0.31	0.77	0.45	0.53	-0.12		0.33		-0.02		0.08	-0.44	
S	NG		1	0.59	0.65	0.62	0.25	0.59	-0.02		0.10		-0.02		0.32	-0.48	
ж К	ß	1	-0.08	0.16	0.17	-0.05	0.05	0.13	0.29		-0.37		-0.13		-0.27	-0.03	
RG	NG	1	0.60	0.68	0.68	0.54	0.43	0.66	-0.02		-0.27		0.14		0.25	-0.56	
		RGR	SL	SD	RDW	SDW	LDW	PDW	RMF		SMF		LMF		RD	SRD	

Exploring the effects of grass competition on post-fire survival among forest and savanna-transition tree seedlings

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Figure S2. (a) Total plant mass at final harvest, **(b)** root mass fraction among seedlings of three savanna-transition tree species grown with and without grass in the Guinea savanna of Ghana. Letters compare species across grass treatments. Different letters are significant at p < 0.05 from Tukey pairwise comparisons following linear mixed-effects models.

Exploring the effects of grass competition on post-fire survival among forest and savanna-transition tree seedlings

Disentangling the effects of Giventangling the effect fire and dry season on seedling establishment success of forest and seedling establishment savanna-transition tree species

Hamza Issifu, Frank van Langevelde, Vincent Logah, George K.D. Ametsitsi, Jelle Klijn, Paolo Satorelli, Millicent Tanson, Hypolite Bayor, Philippine Vergeer, Elmar Veenendaal

Abstract

Fire is often adduced to explain differential tree species recruitment in humid savannas. However, fire generally occurs during the dry season and hence the effects of the two on recruitment are entangled and their separate and interactive effects on seedling establishment success for different tree functional types (TFTs) are poorly understood. We studied seedling establishment success in a common garden experiment for seven tropical tree species selected from moist forest to savanna-transition in the humid savanna of Ghana. Transplantation of the seedlings was done in the wet season and followed in the dry season by factorial combinations of irrigation (vs no-irrigation) and fire (vs no-fire). Survival in the dry season was lower for burnt than unburnt seedlings of the forest species, whereas there was no such difference for the savanna-transition species despite all burnt seedlings having been top-killed. Irrigation increased survival of savannatransition but not forest tree seedlings and fire effect on survival was 5 times larger than no-irrigation. The largest adverse effect was observed for the combined fire and no-dry season irrigation treatment which was 4 times as large on survival and twice as large on growth for the forest than the savanna-transition tree functional type. Seedling size at onset of the dry season related significantly to starch in roots at final harvest for savanna transition and not for forest seedlings. Savannatransition tree seedlings survived at much smaller sizes, possibly linked to this early investment in stored reserves than forest tree seedlings. Our results demonstrate that fire is a stronger bottleneck than lack of moisture in the dry season for forest tree seedlings.

Keywords: Savanna-forest transitions, forest and savanna tree species, fire effects, tropical trees, tree functional types, seedling growth and survival, biomass allocation, root starch

Introduction

Predicting the movement of the forest-savanna ecotone requires insights into changes in environmental factors that influence tree recruitment as well as into the responses of different tree functional types (TFTs) to these changes. Instances of forest advance into humid savannas observed across many forest-savanna boundaries have often been attributed to changes in fire regimes (Mitchard & Flintrop 2013 and references therein) but not much attention given to changes in soil moisture regimes. However, as fire and lack of soil moisture during the dry season co-occur their separate effects on seedling establishment success in humid savannas are less clear.

The dry season constitutes an important constraint to forest tree seedling establishment in savannas (Gignoux et al. 2009) and soil moisture could vary substantially in the dry season even between adjacent forest and savannas via influences of microclimate and soil properties including texture and depth (Ametsitsi et al. *in press*, Issifu et al. 2019). Despite great variability in drought tolerance among forest tree species, they are generally less drought tolerant (and also less fire-tolerant) than savanna tree species as a result of trait variation (e.g. biomass allocation and carbohydrate storage in roots) between these TFTs (Hoffmann et al. 2004, Issifu et al. 2019) which perhaps explains the generally lower abundance of juveniles of forest (vs savanna) tree species in humid savannas (Armani et al. 2019, Azihou et al. 2013, Gignoux et al. 2009).

Fire occurs in the dry season (irrespective of distinctions between early versus late season fires) when microclimate and fuel characteristics are suitable to sustain fires of various intensities (Hoffmann et al. 2012, Prior et al. 2010, Sow et al. 2013, Veenendaal et al. 2018). Consequently in pyrogenic environments, fire effects and those of the dry season are naturally entangled in field experiments on

seedling recruitment (e.g. Cardoso et al. 2016, Gignoux et al. 2009; 2016, Issifu et al. 2019, Lawes et al. 2011). Decoupling influences of lack of soil moisture during the dry season from fire on tree seedling establishment success (defined here as survival and growth past the first wet and dry seasons) is relevant because yearly variations in soil moisture regimes (e.g. wetter versus drier wet season or longer versus shorter dry seasons) could make it difficult to determine which factor(s) are responsible for tree seedling mortality in the generally regarded pyrogenic humid savannas. Such insights are needed for understanding the forestsavanna ecotone dynamics under different global change scenarios.

Here, we asked which of the factors constrain survival and growth of forest and savanna-transition tree seedlings; fire, dry season or their interaction. To address this question, we tested two hypotheses: (1) Both fire and dry season (acting separately) cause greater declines in establishment success among forest than savanna-transition tree seedlings, linked to differences in root biomass allocation and carbohydrate reserves. (2) Fire has greater influence than dry season on seedling establishment success regardless of TFT, but the interaction of fire and dry season is the most important constraint.

Materials and methods

Study site

The study was conducted at the experimental site of the University for Development Studies, Nyankpala Campus (9° 25'41" N and 0° 5' W) in the Guinea savanna ecological zone of Ghana. The area has a unimodal rainfall with April to October wet season and a November to March dry season. Average annual precipitation is 1100 mm and temperature is 28°C. The soil in the experimental garden is sandy loam in texture with medium to coarse stones. Soils in the study

site belong to the Nyankpala Series, described as shallow and taxonomically classified as Plinthustalf, sandy over-clayey skeletal phase (Serno & van de Weg 1985) or Plinthic Lixisols (FAO 2001).

Study approach and species

We conducted a common garden experiment using seven tree species selected from a wide range of habitat affinity from moist semi-deciduous forest to the savanna-transition vegetation (Table 1). The forest tree species were collected from moist semi-deciduous forest near Abofour (7°7'60" N and 1°45'0" W) in the Ashanti region of Ghana, while the savanna-transition tree species were collected from Kogyae Strict Nature Reserve (7°14'N and 1°05'W) also in the Ashanti region. *Khaya senegalensis* and *Afzelia africana* are both common to savanna and transition environments (Armani et al. 2018). Therefore we put both species into the savanna-transition TFT and the forest species into the forest TFT. Forest species in this study occur in variously categorised, but typical forest environments with *Albizia zygia* being the "driest" in the group while *Khaya ivorensis* is the "wettest" based on lower bounds of their tolerance ranges for dry period and rainfall (Hawthorne 1995). Seedlings were raised from seeds and transplanted into the experiment after 5-8 weeks.

Experimental treatments

Site preparation was done in April 2018 with ploughing of the experimental garden to homogenise soil. In June 2018, we established 12 plots (blocks), each of about 37 m² and planted 24 seedlings per plot, four per species assigned to random seedling positions in rows. Spacing between and within rows of seedlings was 1 m \times 0.7 m. In October 2018 (i.e. end of the wet season), we randomly assigned combinations of irrigation (irrigation vs no-irrigation) and fire (burn vs no-burn) treatments to plots.

Table 1. Species and tree functional types (TFTs) used in this study and a description of their environmental tolerances. Species origin; MSD = moist semi-deciduous, DSD = dry semi-deciduous, T = transitional species, S-T = savanna-transitional. Biophysical limits are based on known species distribution, not seed collection for this study. Sources of species information: Hawthorne 2005; Hall & Swaine 1976; De Bie et al. 1998.

Species	Family	TFT	Origin	Rainfall range
Khaya senegalensis	Meliaceae	ST	ST	650 mm – 1800 mm
Afzelia Africana	Fabaceae	Π	ST	> 900 mm
Albizia zygia	Fabaceae	F	DSD-MSD	2700 mm av. (wide range)
Terminalia ivorensis	Combretacea	F	DSD-MSD	760-1500 mm
Khaya anthotheca	Meliaceae	F	MSD	1200-1800 mm
Terminalia superba	Combretacea	F	MSD	1000-3500 mm
Khaya ivorensis	Meliaceae	F	MSD	1600-2500 mm

There were three plots assigned, in a randomised block design, to each of four treatment combinations viz. no-irrigation + fire, no-irrigation - fire, irrigation + fire and irrigation - fire, respectively representing: a dry season with fire (the natural situation in fire-frequent savannas), dry season without fire, fire with extended wet season, and extended wet season without fire. There were 12 seedlings per species (3 plots × 4 seedlings) in each treatment combination except *A. zygia* and *T. ivorensis* due to lack of seedlings. Final replications are shown in Table 3. Irrigation in the dry season involved giving, by means of hand watering, 33.6 L of water per plot per day from November 2018 to mid-March 2019, equivalent to 3860 mm of rainfall over the period. This amount of rainfall (although not corrected for the evaporation of the dry season) was slightly higher than the annual rainfall for the wettest forest species (Table 1). The fire treatment involved burning seedlings with pre-prepared grass fuel.

Fuel preparation and fire application

Burning took place four months after transplantation and involved burning seedlings with dried grass, composed mainly of *Pennisetum pedicellatum* and *Andropogon pseudapricus*, which are the dominant grass species in the study location. We made grass fuel beds in each of the six plots to burn by placing 0.93 kg of dry grass in an area of 0.93 m² on each row of seedlings, based on the fuel mass of ~1 kg m⁻² reported for humid savannas of West Africa (Hennenberg et al. 2006, Issifu et al. 2019). Average fuel bed height was 0.20 m yielding a fuel bulk density of 4.7 kg m⁻³.

Moisture content of grass (fuel) was determined on a dry weight basis from 12 samples as (fresh weight – dry weight / dry weight) × 100). Burning was done on the afternoon of November 19, with all rows within each plot torched simultaneously. Fire temperature was recorded for each burn (i.e. once measurement per plot) using an HI 935005 K-thermocouple thermometer (Hanna Instruments, Singapore). Probe cable was buried with the full length of the probe sticking out of the ground close to seedling base to allow for the most accurate determination of fire temperature (Dayamba et al. 2010).

Plant and biomass measurements

Prior to transplanting, start plant mass was determined for five random seedlings per species after oven-drying. We measured seedling height and stem basal diameter just before implementing the fire treatment in November, following which we monitored post-burn (re)growth until March 2019 when we did final census of live or dead seedlings in all plots and then excavated all live ones. Excavation was done as practically as possible to recover as much root biomass as possible. We considered a seedling dead only if in addition to top-kill also the main root system near the surface had dried up. This definition was suitable because whereas fire usually results in top-kill, a live belowground part often results in regrowth among resprouters. We measured final plant height and estimated total plant dry weight for all plants and regrowth (new shoot) dry weight for burnt plants after ovendrying. We then calculated gains in plant mass as difference in mass between transplantation (species average) and final harvest (for each individual).

Environmental factors

Weather data for the experimental period (and few months before) were obtained from the Nyankpala Weather Station of the Savanna Agricultural Research Institute of Ghana-SARI (9-25-41" N and 0-58' 42"W), situated within 1 km of our experimental garden.

We measured soil moisture content (vmc %) at the end of the wet season (i.e. October 2018) using a theta probe (Spectrum Technologies Inc.) at five locations (plot centre and corners) within each of the 12 plots. This was to determine soil moisture content of our plots at onset of the dry season and to test if soil moisture content differed among plots assigned to receive either dry season (no-irrigation) or irrigation treatment. Also, at final harvest in March 2019, we measured soil moisture content gravimetrically at three depths (5-10 cm, 25-30 cm and 45-50 cm) and at three seedling positions, selected diagonally across plot (middle and two plot corners) for all 12 plots. We also sampled soil at two depths (10 cm and 20 cm) in three holes per plot for six plots (three each for burnt and unburnt) for analysis for chemical properties (Table S1). We estimated soil matric potential at onset and end of the dry season based on a drying cycle water release curve for soils in the experimental garden (using the filter paper technique, data not shown).



Figure 1. Mean temperature and relative humidity (on the secondary axis) and total rainfall and evaporation (on the primary axis) for the experimental period in Nyankpala in the Guinea savanna, Ghana. Red arrow indicate moment of fire event, black arrows indicate start of the experiment and final harvest. Data are from Nyankpala weather station of SARI, within 1 km of our experimental site. Data on total evaporation between March and August were not available.

Statistical analysis

We analysed all data in R (R Core Team 2017) and used ggplot2 package (Wickham 2009) for all figures (except figure 1). We tested soil moisture content (SMC) at the onset of the dry season for differences between plots to receive irrigation or no irrigation in linear mixed effects models (LMM) including plot as random factor. For this we used the *Imer* function in *Ime4* package (Bates et al. 2015) and generated ANOVA tables with statistical significance based on Satterthwaite's method using the *ImerTest* package (Kuznetova et al. 2014). SMC at final harvest was analysed for fixed effects of irrigation × soil depth also in an LMM including random factors of soil pit within plot. Tukey pairwise comparisons for significant irrigation × soil depth was done using the *emmeans* package (Lenth et al. 2019).

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We analysed seedling survival with binomial error (logit link function) in generalised linear mixed models (GLMM) using the *glmer* function in *lme4* package. First, we tested whether survival differed between TFTs in a model that included fixed effects of TFT × irrigation × fire and random plot effect. As we also expected variation within TFT, we performed separate analyses for each TFT for fixed effects of species × irrigation × fire and random plot effect. Tukey pairwise contrasts were performed for significant species or interaction terms.

For seedling growth, we took a subset of the data (due to high post-fire mortality in some species) to include only species with sufficient replicates in the combined fire and dry season treatment to allow for testing interactions. We then performed a linear mixed effects model (LMM) separately for plant mass gain and seedling height at final harvest with species × fire × irrigation as fixed effects and including random plot effect.

For both seedling survival and growth performance, we estimated effect size (Hedges' g) for each treatment using the irrigation without fire treatment as the control treatment to estimate both magnitude (value of g) and direction (whether g was positive or negative) of effect for the other treatments.

We compared plant dry weight between irrigated and non-irrigated plants and assessed biomass allocation traits in separate LMMs. Plant mass was analysed both at the TFT and species levels but allocation traits were analysed only at the TFT level. Plant mass, SMF and RMF were LN-transformed to stabilise residual variances. We tested root starch concentration for TFT × irrigation (for unburnt seedlings) and TFT × fire (for irrigated seedlings) in the separate analyses due to insufficient replicates in some treatments at final harvest. We compared total root starch content (total reserves in roots) between TFTs, we performed an LMM including root dry weight as covariate and plot as random effect.

We assessed relationships (within each TFT) between pre-dry season stem diameter and dry season survival in separate GLMMs for fixed effect of fire × dry season, plot as random factor and stem basal diameter as covariate. Prior to this we had assessed correlations of stem diameter to other pre-dry season plant size variables (i.e. height and pre-transplantation plant mass). We also assessed relationship of pre-dry season basal stem diameter to final root starch content to explain influences of pre-dry season size on dry season survival.

Results

Environmental factors

Rainfall of the general study location peaked in August and ended in October (Figure 1) with a total of 959.4 mm received within the experimental period (June 2018 to March 2019). Temperature, relative humidity and wind speed on the moment of burning were 30.7 °C, 70% and 0.92 kph respectively. Mean moisture content of grass used in the experimental burn was 3.0% and mean fire temperature recorded was 519 °C. Soil moisture content in October (onset of the dry season) averaged 15.3% and did not differ significantly (F $_{1,10}$ = 1.70, p = 0.22) between plots to receive irrigation (at 15.7%) or no irrigation (at 14.9%). This equates to a matric potential of approximately -0.004 MPa to -0.006 MPa (i.e. no water stress for seedlings at this point). Overall, soil moisture content at the end of the experiment was lower in non-irrigated than irrigated plots, but there was also a significant irrigation \times soil depth interaction effect (F _{2,68} = 20.3, p < 0.001). Soil moisture content was progressively (and significantly) higher with depth such that the difference between irrigated and non-irrigated plots was largest (and significant only) in the topmost (5-10 cm) layer (Figure 2), with matric potentials of -0.3 MPa for irrigated and -433 MPa for non-irrigated seedlings. Irrigated and non-irrigated seedlings in the 25 cm-30 cm layer were under similar

moisture stress (-0.20 MPa and -0.27 MPa), while at 45-50 cm soil layer matric potentials were -0.11 MPa and -0.42 MPa respectively.

Soils were slightly acidic to neutral and except for exchangeable Mg which was slightly higher in some burnt plots, soils in the experimental garden had high levels of available P and exchangeable bases which were similar in plots. There was also no effect of burning on many of the soil chemical parameters we measured (Table S1).

Seedling survival responses to fire and dry season

Survival proportion (across treatments) was 0.52 ± 0.63 for the forest TFT which was significantly lower than the savanna-transition TFT at 0.87 ± 0.79 (Table 2), and the TFT × fire interaction was significant (Table 3). Pairwise comparisons revealed that survival was lower for burnt (0.26 ± 0.76) than unburnt ($0.79 \pm$ 0.75) seedlings of forest whereas no difference existed between burnt (at $0.85 \pm$ 0.75) and unburnt (at 0.90 ± 0.83) savanna-transition seedlings. Irrigation effect was not significant (Table 3).

Within the forest TFT, we observed the largest difference among species (across treatments) between *A. zygia* (at 0.75 \pm 0.31) and *T. superba* (at 0.42 \pm 0.36) (Table 2) but species effect was not significant (Table 3). Also, there was no effect of species × fire interaction and there was no irrigation effect within this TFT (Table 3). Within the savanna-transition TFT, survival (across treatments) was 0.96 \pm 0.04 for *K. senegalensis* and 0.79 \pm 0.14 for *A. africana* (Table 2) but species effect was not significant (Table 3). Across both species, survival was higher among irrigated (0.96 \pm 0.91) than non-irrigated (0.79 \pm 0.74) seedlings and there was no effect of fire (Table 3).

Effect size estimates (Hedge's g) for the experimental treatments (Table 2) showed that across all species, the no-irrigation *with* fire treatment had the largest

(adverse) effect on seedling survival. Compared between TFTs, this effect was ~ 4 times larger for the forest (mean d = -3.70) than the savanna-transition (mean d = -0.99) tree species. Within the savanna-transition TFT, the effect was ~ 1.5 times larger for *A. africana* than *K. senegalensis*. Within the forest TFT, the effect was largest for *T. superba* as all seedlings died in this treatment. Differences in effect size of the combined fire and no-irrigation treatment among forest species for which *g* could be estimated were small (Table 2) suggesting similar responses across species. Also across all forest species, effect size on survival for fire *with* irrigation treatment (i.e. fire alone) was 5 times larger than no-irrigation *without* fire treatment (i.e. irrigation alone). Among savanna-transition tree seedlings on the other hand, fire had no effect (all burnt seedlings resprouted) whereas effect of irrigation was medium to large (-0.83 on average) for both species *A. africana* and *K. senegalensis*.

Seedling (re)growth performance as influenced by fire and dry season

Growth performance data analysed for the interactive effects of fire and irrigation included three species viz. *K. anthotheca, A. africana* and *K. senegalensis*. We found significant effects of irrigation × species ($F_{2,86} = 7.54$, p < 0.001) and fire × species ($F_{2,86} = 8.78$, p < 0.001) interactions on plant mass gain at final harvest (Figure 3b). Pairwise comparisons revealed lower mass gain for burnt than unburnt seedlings irrespective of irrigation for *K. anthotheca* and *K. senegalensis* but not *A. africana*. Also regardless of fire treatment, mass gain was higher for irrigated than non-irrigated seedlings of *K. anthotheca* and *K. senegalensis*, but not *A. africana*. Unburnt seedlings were taller ($F_{1,10} = 91.8$, p < 0.001) than burnt seedlings. Also, irrigated seedlings were taller ($F_{1,10} = 7.00$, p = 0.027) than nonirrigated seedlings irrespective of species. There were no significant interaction effects on plant height (Figure 3a).
Table 2. Statistics on seedling survival (from GLMM) and plant mass (from LMM) at final harvest. For each parameter, comparisons are done between tree functional types (TFT) and then among species for each TFT separately. GLMM on survival included fixed effects of fire, irrigation and TFT (or species). LMM on plant mass is for unburnt seedlings, testing fixed effects of irrigation and TFT (or species). For savanna-transition TFT, the three way interaction term was removed. Effect sizes associated with survival and plant mass are presented in Table 2 and Table 4 respectively.

Source of variation	:	Surv	ival		Plant m	ass
	F	df	p-value	F	Df	p-value
TFT	21.4		< 0.001	3.07	1,104	0.082
Fire	34.3		< 0.001			
Irrigation	1.75		0.186	3.24	1,4	0.152
TFT × Fire	7.76		0.003			
TFT × irrigation	1.72		0.191	5.90	1,104	0.016
Fire × irrigation	0.09		0.759			
TFT \times Fire \times irrigation	0.24		0.624			
Forest TFT						
Species	0.31		0.875	11.2	3, 57	< 0.001
Fire	22.8		< 0.001			
Irrigation	0.20		0.653	7.94	1,3	0.064
Species × Fire	0.98		0.418			
Species \times irrigation	1.04		0.388	2.0	3, 57	0.126
Fire × irrigation	1.49		0.222			
Species \times Fire \times irrigation	0.37		0.828			
S-T TFT						
Species	2.37		0.125	26.2	1,36	< 0.001
Fire	0.43		0.513			
Irrigation	4.05		0.045	0.42	1,4	0.553
Species × Fire	0.11		0.736			
Fire \times irrigation	0.20		0.658	1.30	1,36	0.262

Table 3. Survival proportions and effect size estimates (Hedge's g) for the various experimental treatment combinations for seven tree species in a common garden experiment. Effect size was estimated for each treatment combination relative to irrigation without fire which represented the control treatment (with best possible outcome for seedling survival). Effect size could not be calculated for *A. zygia* and *T. ivorensis* due to too few survivors are the end.

Species	Treatment	Survival prop	Ν	Hedge's g
K senegalensis	Dry season + fire	0.92	12	-0.83
	Dry season - fire	0.92	12	-0.83
	Fire + Irrigation	1.00	12	-0.00
	Irrigation - fire	1.00	12	
A. africana	Dry season + fire	0.58	12	-1.15
	Dry season - fire	0.75	12	-0.82
	Fire + Irrigation	0.92	12	-0.00
	Irrigation - fire	0.92	12	
A. zygia	Dry season + fire	0.25	4	-
	Dry season - fire	1.00	4	-
	Fire + Irrigation	0.75	8	-
	Irrigation - fire	1.00	8	
K. anthotheca	Dry season + fire	0.42	12	-3.46
	Dry season - fire	0.67	12	-1.10
	Fire + Irrigation	0.25	12	-3.27
	Irrigation - fire	0.92	12	
K. ivorensis	Dry season + fire	0.25	12	-3.27
	Dry season - fire	0.92	12	-0.00
	Fire + Irrigation	0.14	12	-3.81
	Irrigation - fire	0.92	12	
T. ivorensis	Dry season + fire	0.12	7	-4.33
	Dry season – fire	0.62	8	-0.85
	Fire + Irrigation	0.50	4	-2.00
	Irrigation - fire	0.75	4	
T. superba	Dry season + fire	0.00	12	-
	Dry season – fire	0.58	12	-0.22
	Fire + Irrigation	0.17	12	-1.73
	Irrigation - fire	0.92	12	



Figure 3. (a) Plant height and **(b)** mass gain at final harvest (after dry season/fire) for *K. anthotheca* (forest species) and *Afzelia africana* and *K. senegalensis* (both savanna-transition species) in fire and irrigation treatment combinations. Different letters indicate significant differences among irrigation and fire treatments (Tukey pairwise comparisons at 0.05 level of significance).

Seedling size, morphology and root starch reserves at final harvest

There was a significant TFT × irrigation interaction effect on plant mass at final harvest (Table 3, Figure 4d). Pairwise comparisons revealed that among nonirrigated seedlings, savanna-transition TFT was bigger (at 18.5 ± 13.1 g) than forest TFT (at 13.2 ± 13.6 g) but TFTs did not differ among irrigated seedlings (30.0 ± 25.5 g and 23.2 ± 14.7 g for savanna-transition and forest TFT respectively). Within the savanna-transition TFT, *K. senegalensis* seedlings were bigger than *A. africana* and there was no significant irrigation effect (Table 3, Figure 4d). Within the forest TFT, plant mass differed among species. *Terminalia superba* was the biggest while all other species attained similar mass irrespective of irrigation (Table 3, Figure 4d).

An assessment of biomass allocation patterns among unburnt seedlings revealed that stem mass fraction differed between TFTs, which was higher for the forest than savanna-transition TFT. Also, stem mass fraction was higher for non-

irrigated than irrigated seedlings (Table 5, Figure 4a). We found significant effects of TFT \times irrigation on both leaf mass fraction (Table 5, Figure 4b) and root mass fraction (Table 5, Figure 4c). Savanna-transition species maintained similar leaf mass fraction under both non-irrigated (0.27 ± 0.11) and irrigated (0.25 ± 0.11) treatments across both species, but leaf mass fraction of forest species was lower for non-irrigated $(0.20 \pm 0.14 \text{ vs } 0.33 \pm 0.09 \text{ for irrigated seedlings})$. As a result, leaf mass fraction was higher for forest seedlings in irrigation, but higher for savanna-transition seedlings in the no-irrigation treatment (Figure 4b). Root mass fraction of the savanna-transition TFT averaged 0.58 \pm 0.16 g g⁻¹ for irrigated seedlings, significantly higher than forest (at 0.41 \pm 0.18 g g⁻¹), but there was no TFT difference in the no-irrigation treatment (Figure 4c). Within the forest TFT, Stem mass fraction was, irrespective of irrigation, higher for T. superba and lowest for A. zygia. Leaf mass fraction was highest for K. ivorensis and lowest for T. superba. Also, irrespective of species, leaf mass fraction was lower among nonirrigated than irrigated seedlings. Root mass fraction was highest for A. zygia (Figure 4, Table 5). Within the savanna-transition TFT, there was a significant effect of species \times irrigation on stem mass fraction; which was higher for K. senegalensis than A. africana for irrigated but not for non-irrigated seedlings. Both leaf mass fraction and root mass fraction were also higher for K. senegalensis than A. africana irrespective of irrigation (Figure 4, Table 5).

Root starch concentration was lower for forest ($10.0 \pm 5.9\%$) than savannatransition ($18.7 \pm 8.1\%$) TFTs but did not differ between non-irrigated (at $12.8 \pm$ 9.6%) and irrigated seedlings (at $13.7 \pm 6.4\%$) (Table 5). Tested separately for each TFT, root starch concentration did not differ among species or irrigation treatments (Table 5).



Figure 4. Biomass allocation to stem (a), leaf (b), roots (c) and total plant dry weight (d) at final harvest among irrigated and non-irrigated seedlings of six tropical tree species in a common garden experiment in the humid Guinea savanna of Ghana. *Khaya ivorensis*, *Khaya anthotheca, Albizia zygia, Terminalia superba* are forest species while *Afzelia africana* and *Khaya senegalensis* are savanna-transition species. Different letters indicate significant differences among species across irrigation treatments (Tukey pairwise comparisons at 0.05 level of significance).

In the separate TFT × fire analysis, root starch was lower (F $_{1, 43} = 43.2$, p < 0.001) for burnt (6.04 ± 4.0%) than unburnt (14.6 ± 6.5%) seedlings and there was no interaction effect. Within the forest TFT, root starch concentration differed (F $_{1, 19} = 12.3$, p < 0.001) among species, highest for *A. zygia* (12.0 ± 6.0%), intermediate for *K. anthotheca* (9.2 ± 5.5%) and lowest for *T. superba* (6.8 ± 3.5%). Root starch concentration was also lower (F $_{1, 19} = 53.1$, p < 0.001) for burnt (4.8 ± 3.1%) than unburnt (12.0 ± 4.6%) forest seedlings irrespective of species and there was no species × fire interaction effect. Within the savannatransition TFT, root starch was also lower (F $_{1, 18} = 24.5$, p < 0.001) for burnt than unburnt seedlings but with no species differences for either burnt (at 8.55 ± 5.3%)

for *K. senegalensis* vs $5.33 \pm 2.8\%$ for *A. africana*) or unburnt seedlings (at 18.0 \pm 7.5% for *K. senegalensis* vs 19.6 \pm 7.1% for *A. africana*).

Table 4. Effect sizes (Hedges' g) for the various treatment combinations for gains in plant mass and plant height among seven tropical tree species in a common garden experiment in the humid Guinea savanna of Ghana. Effect size was estimated for each treatment combination relative to irrigation without fire which represented the control treatment (with best possible outcome for seedling growth).

Species	Treatment	Plant mass	Plant height
K senegalensis	Dry season + fire	-2.14	-1.86
	Dry season - fire	-0.90	-0.49
	Fire + irrigation	-1.33	-1.39
A. africana	Dry season + fire	-0.62	-1.42
	Dry season - fire	-0.35	0.17
	Fire + irrigation	-0.59	-1.14
A. zygia	Dry season + fire	-1.82	-4.72
	Dry season - fire	0.38	0.86
	Fire + irrigation	-1.82	-4.81
K. anthotheca	Dry season + fire	-1.88	-0.42
	Dry season - fire	-1.05	-0.39
	Fire + irrigation	-1.64	-2.95
K. ivorensis	Dry season + fire	-2.54	-4.41
	Ds - fire	-1.98	-1.18
	Fire + irrigation	-1.79	-3.13
T. ivorensis	Dry season + fire	-3.42	-5.16
	Ds - fire	-2.82	-4.36
	Fire + irrigation	-2.35	-4.70
T. superba	Dry season + fire	-2.19	-5.14
	Dry season - fire	-0.82	-0.84
	Fire + irrigation	-2.15	-5.83

Root starch content (total reserves in roots) increased ($\beta = 0.10$, t = 5.33, p < 0.001) with root mass, but there was also an effect (F 1, 54 = 7.41, p = 0.008) of TFT × irrigation interaction (Figure 5b). The difference between TFTs was significant only among non-irrigated seedlings. Among burnt seedlings, we found also that root starch content increased ($\beta = 0.10$, t = 6.07, p < 0.001) with root mass and was lower (F 1, 41 = 23.3, p < 0.001) for burnt than unburnt seedlings, but the difference between TFTs was not significant (F _{1, 41} = 3.47, p = 0.069) (Figure 5a) and there was no TFT × fire interaction effect.



Figure 5. Total root starch reserves for (a) burnt seedlings and (b) unburnt seedlings of forest and savanna-transition tree species in a common garden experiment. Root starch was tested for effect of burning (a) and effect of irrigation (b) in separate analyses which included only species with sufficient replicates at final harvest.

Relationship of pre-dry season seedling size to dry season survival

Stem basal diameter at onset of the dry season was related to plant height, although the relationship was slightly stronger for forest (β = 2.8, t = 14.4, p < 0.001) than savanna-transition (β = 2.2, t = 8.2, p < 0.001) TFTs (Figure S1a). Neither stem diameter nor height was related significantly to plant mass at

transplantation; neither for forest ($\beta = -0.11$, t = -0.08, p = 0.93) nor savannatransition ($\beta = -0.69$, t = -0.55, p = 0.58) (Figure S1b). Total root starch reserves (at final harvest) was related ($\beta = 0.25$, t = 3.20, p = 0.004) to pre-burn basal stem diameter for savanna-transition but not for the forest seedlings (Figure 7). We found that survival probability for savanna-transition seedlings (across treatments) increased ($\beta = 0.84$, z = 2.84, p = 0.004) with stem basal diameter, with survival guaranteed (on average) at ~ 10 mm (Figure 6b). By contrast, the overall relationship between stem basal diameter and survival was not significant ($\beta = 0.02$, z = 0.28, p = 0.78) for the forest seedlings. For this TFT, survival was lowest in the fire + irrigation combination and highest in the no-fire + irrigation treatment regardless of stem diameter (Figure 6a).



Figure 6. Predicted survival probability as a function of pre-burn stem basal diameter (proxy for seedling size) under various experimental treatments for **(a)** forest and **(b)** savanna-transition tree functional types in a common garden experiment in the humid Guinea savanna of Ghana.



Figure 7. Relationship of pre-fire stem basal diameter to root starch content of unburnt seedlings at final harvest for forest and savanna-transition tree species. Root starch data include both irrigated and non-irrigated seedlings (not statistically different and so not distinguished here).

Discussion

In this study, we decoupled dry season and fire effects on survival and growth of forest and savanna-transition tree seedlings. Overall, our findings show that fire, dry season and their interactive effects influence tropical tree seedling establishment success but their relative importance differ for forest and savannatransition tree functional types.

We expected poorer seedling survival for the forest than the savannatransition species under the 5-month long dry season. We found that survival did not differ between forest and savanna-transition species irrespective of irrigation treatment in the absence of fire (79% vs 90% for forest and savanna-transition respectively). Also fast growing forest species such as *Terminalia superba* and *T. ivorensis* avoided drought stress by shedding leaves.

₹ q	ANOVA table from LMN seedlings (only for unb
	ANOVA table from L seedlings (only for u

Source of variation	[RS]				MF			ΨF		R	MF	
	L	df	p-value	L	df	p-value	Ŀ	Df	p-value	ш	df	p-value
TFT	15.4	1, 59	< 0.001	49.7	1, 105	< 0.001	0.16	1, 102	0.691	57.0	1, 105	< 0.001
Irrigation	3.67	1, 59	0.060	7.25	1, 105	0.008	0.97	1, 4	0.382	0.85	1, 4	0.411
TFT × irrigation	1.90	1, 59	0.172	0.014	1, 105	0.906	14.2	1, 102	< 0.001	4.62	1, 105	0.033
Forest TFT												
Species	2.17	3, 29	0.113	42.6	3, 57	< 0.001	7.97	3, 55	< 0.001	24.3	3, 57	< 0.001
Irrigation	4.36	1, 4	0.110	11.3	1, 2	0.059	3.66	1, 4	0.047	5.07	1, 4	0.093
Species × irrigation	2.0	3, 29	0.135	0.34	3, 57	0.795	1.14	3, 55	0.343	1.87	3, 57	0.144
Savanna-transition TI	F											
Species	2.30	1, 15	0.151	7.92	1, 37	0.008	12.6	1, 36	0.001	37.5	1, 39	< 0.001
Irrigation	0.21	1, 4	0.674	4.00	1, 37	0.053	0.68	1, 4	0.450	0.35	1, 39	0.558
Species × irrigation	3.87	1, 15	0.067	8.24	1, 37	0.007	0.24	1, 36	0.626	0.002	1, 39	0.968

Disentangling the effects of fire and dry season on seedling establishment success of forest and savanna-transition tree species

Chapter 4

Our results were not consistent with expectation based on known differences in drought tolerances between forest and savanna species (Gignoux et al. 2009; Hoffmann et al. 2004, Hoffmann and Franco, 2003). We attribute this finding to the large sizes attained by forest seedlings by the onset of the dry season as some seedlings reached stem basal diameter of 16 mm in the four-month growth period. Rainfall was high in this humid savanna site (Figure 1) such that at the end of the dry season soil moisture content did not differ between irrigation treatments beyond 10 cm (Figure 2). Our irrigation treatment did little to alleviate moisture stress in the dry season, but it did have an effect on soil matric potentials, which decreased with depth (regardless of moisture treatment). Therefore, seedlings which had roots in the 25 cm-30 cm soil layer and beyond (which was nearly all species, Figure S2) were not in drought stress. Additional reasons may include the absence of competing grasses which is linked to growth suppression (Barbosa et al. 2014, Chapter 3 of this thesis) and also, plots were not nutrient limited (Table S1). Therefore, seedlings were big enough to withstand the 5 month long dry season due to the specific experimental conditions in this study. Large mortality attributed to dry season effects have been reported for forest seedlings in other studies (Cardoso et al. 2016, Gignoux et al. 2009, Prior et al. 2007).

Consistent with prediction, fire decreased survival of forest seedlings but not savanna-transition seedlings (Gignoux et al. 2009, 2016, Hoffmann et al. 2004, Chapter 3 of this thesis). Starch concentration in roots, often linked to resprout capacity, is reportedly higher for savanna than forest species (Hoffmann et al. 2004, Issifu et al. 2019, Wigely et al. 2019). We found that seedling size at the onset of the dry season did not predict root starch at final harvest for forest species unlike the savanna-transition species (Figure 7). However, plants with bigger roots at final harvest also had more starch stored in roots even for forest

species (Figure 5). Thus, as an important addition, our finding suggests that savanna-transition species invest in starch storage much earlier and thus have more starch reserves than forest species. Also, savanna-transition seedlings had more starch reserves at smaller sizes than forest seedlings. Therefore, a basal diameter of 10 mm guaranteed survival (across treatments) for savanna-transition seedlings whereas forest seedlings did not attain complete survival (not even for the largest individuals) at sizes attained in this study (Figure 6). This may explain why survival of forest species does not necessarily increase with age (Gignoux et al. 2009) and why savanna seedlings require several times less biomass, than required by forest seedlings, to survive fire (see also Gignoux et al. 2016).

Due to the lack of capacity to regrow from belowground buds, forest species need to avoid aboveground tissue loss (top-kill). However, compared to savanna species, forest species reach the "threshold of fire resistance", which is the size sufficient to avoid top-kill (Hoffmann et al. 2012b), at much bigger sizes. Therefore, while the forest species in this study were big enough to survive the dry season (in the absence of fire), they were largely killed by fire (all seedlings including those of savanna-transition were top-killed). This finding suggests that forest species can survive humid savanna dry season at sizes smaller than required to survive fire. Fire is thus a stronger constraint to forest seedlings than lack of moisture during the dry season where soil conditions are conducive to their establishment. This may justify why the high mortality of forest seedlings observed in dry season fire experiments is often regarded as a fire effect (Cardoso et al. 2016; Gignoux et al. 2009; 2016, Issifu et al. 2019, Lawes et al. 2011). We observed the largest effect on seedling performance in the combination treatment of dry season with fire. This effect which represents the natural occurrence of fire and dry season drought, was stronger than the separate effects of fire and

irrigation. This may perhaps reflect the influence of soil moisture on survival and regrowth of resprouting plants (Pausas et al. 2016, Pratt et al. 2014) The interactive effect was also stronger for forest than savanna-transition species with the implication being that encroachment of forest species in savannas that are moist enough is only possible under a diminished fire regime (Mitchard & Flintrop 2013, Aubreville 1949, Louppe 1995, Veenendaal et al. 2018). Otherwise, savanna-transition (rather than forest species) may initiate forest encroachment in savannas (Hennenberg et al. 2005).

Seedling growth in the dry season was limited, possibly due to unfavourable microclimate (Figure 1) as irrigation in a common garden could only increase soil moisture without ameliorating microclimate. However, higher resprouting among irrigated burnt seedlings yielded an overall significant effect of irrigation on plant mass and height (Figure 3). Also, higher mass gain among unburnt irrigated than non-irrigated seedlings suggest that seedlings still grew regardless of any constraints imposed by microclimate. Differences in resprout and subsequent regrowth between TFTs is largely attributable to differences in root starch reserves (Figure 5) also reported in other studies (Issifu et al. 2019, Wigley et al. 2019). Root starch reserves were lower for burnt than unburnt seedlings (Figure 5) because stored reserves were re-invested in regrowth among burnt seedlings (Tomlinson et al. 2012) which also explains why starch reserves (post-fire) were lower for savanna-transition than forest (Figure 5a) since regrowth was also higher for savanna-transition seedlings.

Some forest species decreased their leaf mass in the no-irrigation treatment which increased their root mass fraction possibly to minimise drought stress under this treatment (Figures 4b and 4c). Biomass allocation to aboveground plant parts (stem and leaves) and roots as found in this study were consistent with allocation

patterns for resource acquiring versus resource conserving traits with such differences playing an important role in in TFT responses to fire and irrigation (Tomlinson et al. 2012, Boonman et al. 2019, Issifu et al 2019, Cardoso et al. 2018).

Overall, our study showed that irrigation during the dry season had a positive effect on savanna-transition seedlings but not on forest seedlings (which may avoid mortality through drought stress, by shedding leaves, but also then may not grow). Fire negatively influenced forest tree seedlings only. Overall, the combined effect of fire and lack of moisture during the dry season had the strongest effect on forest species, which thus face the greatest recruitment bottleneck in humid savanna.

Acknowledgements

Wageningen University and Research with support from the Academy Ecology Fund of the KNAW made field work possible. We are grateful to Adnan Sumaila, Atampuri Amidu and Abdallah Tajudeen for assisting in field data collection and to Jan van Walsem for laboratory analysis of plant samples.

Appendices

Table S1. Soil chemical properties in burnt and unburnt plots in a common garden in a humid Guinea savanna. P (av.) is Available P (mg/kg).

Fire	Layer	pН	N%	С%	P(av.)	к	Са	Mg	Na
Burnt	10 cm	6.67 (0.14)	0.07 (0.01)	0.61 (0.14)	3.64 (0.30)	0.29 (0.02)	2.53 (0.6)	0.6 (0.35)	0.01 (0.001)
	20 cm	6.78 (0.05)	0.09 (0.02)	0.47 (0.03)	4.24 (0.87)	0.17 (0.02)	1.80 (0.4)	1.0* (0.20)	0.012 (0.001)
Unburnt	10 cm	6.67 (0.06)	0.15 (0.15)	0.57 (0.09)	4.47 (0.42)	0.21 (0.05)	1.77 (0.5)	0.83 (0.10)	0.011 (0.0008)
	20 cm	6.62 (0.09)	0.09 (0.06)	1.96 (2.4)	4.70 (1.3)	0.33 (0.17)	1.85 (0.40)	0.35 (0.05)	0.011 (0.0003)



Figure S1. (a) Relationship of pre-fire plant height to stem basal diameter and **(b)** relationship of pre-fire plant height to seedling mass at transplantation for forest and savanna-transition tree species grown in common garden experiment in the humid Guinea savanna of Ghana.



Figure S2. Rooting depth at final harvest for non-irrigated and irrigated seedlings. Species names: ki = K. *ivorensis*, ts = T. *superba*, ka = K. *anthotheca*, az = A. *zygia* (which are all forest species) and aa = A. *africana* and ks = K. *senegalensis* (which are both savanna-transiton species).

5

Growth performance and allocation traits of forest and savanna-transition tree seedlings under soil moisture limitation and defoliation

Hamza Issifu, Frank van Langevelde, Vincent Logah, George K.D. Ametsitsi, Jaap Hamelink, Jelle Klijn, Maaike Pyck, Gerard Okwonko, Millicent Tanson, Hypolite Bayor, Philippine Vergeer, Elmar Veenendaal

Abstract

The transition between tropical forests and savannas is characterised by frequent disturbances due to intense drought, the presence of herbivores and the occurrence of fire. Seedling growth and allocation responses to these disturbances, which may increase with changes in land use and climate, may differ between tree functional types. We performed a greenhouse experiment to compare growth performance and allocation traits of forest and savanna-transition tree functional types after defoliation under the constraints of moisture limitation. Savannatransition species are common to both forest and savannas in the forest-savanna transition. Seedlings of both tree functional types started off with similar relative growth rates, but forest species grew faster in the end. Low soil moisture decreased seedling growth performance with slightly larger effect for savannatransition than forest tree seedlings. Defoliation at the early growth phase decreased growth performance in older seedlings, with a larger effect under low soil moisture. Forest and savanna-transition tree species differed in foraging and allocation traits, with patterns establishing at the early growth phase. Regrowth performance, following defoliation was lower under low soil moisture availability and not different between the tree functional types. Our findings reveal adverse synergistic effects of defoliation and soil moisture limitation on tree seedling growth performance irrespective of tree functional type, with implications for tropical tree seedling recovery from disturbance in a drier climate as predicted for the future.

Keywords: Tree functional types, tropical tree seedlings, biomass allocation, root starch reserves, forest-savanna transition

Introduction

Multiple disturbance factors including fire, herbivory or drought may cause aboveground biomass loss among juvenile trees in open vegetation types, particularly in tropical savannas and forest-savanna transitions (Hempson et al. 2015, Sankaran et al. 2019, van Langevelde et al. 2003). Following substantial shoot loss, plants compensate by increasing resource allocation to shoot growth (Ballina-Gomez 2010). Post-disturbance recovery (survival and regrowth) depend on carbon accumulation and the allocation of resources prior to the disturbance event (Boonman et al. 2019, Leishman et al. 2000, Poorter et al. 2012, Tomlinson et al. 2012). Factors that govern plant growth (and carbon gain) are thus considered to be important for tree species responses to disturbance events across tropical forest-savanna transitions.

Precipitation is a key determinant of seedling growth among tropical tree species (Gentry 1991, Bunker & Carson 2005). Lower soil moisture regimes may decrease post-disturbance recovery by decreasing seedling growth (Gignoux et al. 2016, Lawes et al. 2011, Zeppel et al. 2015). Consistent with the functional equilibrium hypothesis (Brouwer 1963), forest species differ from savannatransition species (which occur both in humid savanna and transition forests) in allocation patterns. Forest species generally invest more in plant organs for light capture to aid a more rapid growth (Amissah et al 2015) while savanna-transition tree species invest more in organs for belowground resource capture and storage of reserves (Boonman et al. 2019, Gignoux et al. 2016, Hoffmann et al. 2004, Issifu et al. 2019, Wigley et al. 2019). These general patterns offer alternative ways of resource allocation that maximize fitness under different environmental conditions, and form a basis for niche separation (Freschet et al. 2015, Gignoux et al. 2016).

Chapter 5

Precipitation amount affects distribution of forest species (Amissah et al. 2014), which also have lower resprouting capacity (Chapters 3 & 4 of this thesis). Thus, interactions of defoliation (e.g. via fire, herbivory or trampling) and lower soil moisture are expected to have greater effects on (re)growth performance of forest than savanna-transition species. Effects of such interactions are more likely to intensify under future global change scenarios as precipitation patterns are predicted to change across much of the tropics (Malhi et al. 2013, Zeppel et al. 2015) including a steady decline in rainfall in the forest zone of Ghana (Owusu & Waylen 2009). Tree species are sometimes found to increase belowground biomass allocation in response to precipitation decline (Nicotra et al. 2002, Poorter et al. 2012), which should reduce the adverse effect of lower moisture on postdisturbance regrowth. How traits for foraging, resource allocation and storage in roots change with soil moisture limitation and how any such changes are associated with regrowth following defoliation among forest and savannatransition tree species is still poorly understood. The responses of different tree species in the forest-savanna transition, i.e. forest and savanna-transition tree species, to these factors will determine vegetation dynamics across this transition. Here, savanna-transition tree species are common to both forest and savannas in the forest-savanna transition.

We performed a greenhouse experiment to compare growth performance and allocation traits of forest and savanna-transition tree functional types after defoliation under constraints of moisture limitation. We predicted the following: (1) greater decline in growth performance for forest than savanna-transition tree seedlings under both soil moisture limitation and defoliation, and (2) seedling regrowth performance after defoliation is positively associated with higher belowground resource allocation and starch storage in roots.

Materials and methods

Species selection

We selected tree species typical of humid savanna to moist semi-deciduous forest in Ghana. These species may co-occur in the forest-savanna transition or in adjacent forest types. Based on the distributions and habitat affinity described for each, we grouped the species as belonging either to forest or savanna-transition tree functional type (TFT) (Table 1 and references in legend). Taxonomic information of the studied species is provided in Table 1. Seed collection was done for the savanna-transition tree species in Kogyae Strict Nature Reserve (7°19' 1.66'' N, 1°05' 5.863'' W). The forest species were collected from Bobiri (6°40'41''N and 1°19'12''W) and Abofour (7°7'60''N and 1°45'0''W), which are both semideciduous forests in the Ashanti region of Ghana. Seedlings were raised from seeds and used in the experiment at about four weeks old.

Greenhouse experiment

Plants were grown in the greenhouse of the University for Development Studies, Nyankpala Campus, Ghana (9°24'55"N; 0°58'56"W). We used two batchrepetitions of the experiment to accommodate all 12 species tested. This was due to limitation in greenhouse space and number of species for which seeds could be obtained in a particular season. The first batch of the experiment was conducted between July 2016 and December 2016. The second batch was between March 2017 and December 2018. Light level in the greenhouse (measured with a quantum sensor, Skye instruments, Llandindrod Wells, UK) was ~12% (averaged across measurements in both batches). Plants were grown in 15 cm wide x 50 cm deep poly pots filled (up to 45 cm of pot depth) with topsoil harvested in the nearby garden in the Guinea savanna. **Table 1.** List of studied species. All species occur in the forest savanna-transition or in adjacent semi deciduous forest. Species marked with ++ are species present in both batch repetitions of the greenhouse experiment; # was formerly known as *Annogeissus leiocarpus* (DC.) Guill. & Perr) (Hochst. exHutch. & Dalziel) Gere & Boatwr. TFT refers to tree functional type: savanna-transition (ST) or forest (as categorised in this study). For vegetation type affinity, S = savanna, FST = forest-savanna transition, DSD = dry semi-deciduous, MSD = moist semi-deciduous.

Species	Family	TFT	Vegetation-
			type affinity
Khaya senegalensis ⁺⁺	Meliaceae	ST	S/FST
Pterocarpus erinaceus	Fabaceae	ST	S/FST
Afzelia africana	Fabaceae	ST	S/FST
Daniella oliveri	Caesalpiniaceae	ST	S/FST
Terminalia glaucescens	Combretacea	ST	S/FST
Terminalia schimperi [#]	Combretacea	ST	S/FST
Terminalia superba++	Combretacea	Forest	MSD
Terminalia ivorensis	Combretacea	Forest	DSD - MSD
Khaya ivorensis	Meliaceae	Forest	MSD
Afzelia bella	Fabaceae	Forest	MSD
Albizia zygia	Fabaceae	Forest	DSD - MSD
Daniella ogea	Caesalpiniaceae	Forest	MSD

Sources of species information: Hall & Swaine (1976), Hawthorne (1995), Orwa et al. (2009).

The experiment included moisture (low versus control) and defoliation (defoliated seedlings versus non-defoliated control) treatments, which were assigned in a fully cross-factored, three-factor design in three blocks. Batch 1 had six species while batch 2 had eight, with two species common to both batches (Table 1). For each species, there were nine seedlings per species per moisture × defoliation combination. In each batch, additional 12 seedlings (six each for low and control moisture treatments) were included for intermediate harvest which was done at week 8 (seedlings were 12 weeks old). Seedlings in the control moisture regime received 40 ml day⁻¹ of water while those in the low moisture treatment twice (when pots were getting too dry) in the course of the experiment to prevent mortality as we

wanted to measure traits of live seedlings. We monitored changes in pot moisture content as described below.

The defoliation treatment immediately followed the intermediate harvest at week 8. We removed shoots by cutting the stem just above the first leaf-bearing node. All leaves and cotyledons (if present) were removed. Rather than cutting at similar height on the stem for all seedlings, the approach we used was to give each species an equal chance at recovery.

Soil moisture and nutrient content

Top soil was sampled in triplicate after thorough mixing during the potting stage. Samples were later bulked and analysed for some soil chemical properties. Soils for both batches came from the same site.

During intermediate and final harvests, soil moisture (VMC %) was determined at the top, middle and bottom layers of each pot using a soil moisture meter (TDR 150, Spectrum Technologies, Inc. II, USA). In batch 2, we also monitored moisture content (MC) in between harvests in 12 dedicated pots (2 pots \times 2 moisture treatments \times 3 blocks) for soil moisture determination (this was to follow pot moisture changes, but data are not presented here).

Plant biomass measurements

At transplanting, we sampled five seedlings per species from the seedling pool for determination of start plant mass (i.e. week 0 harvest) after oven-drying. We planned two other harvests: at 12 weeks (intermediate harvest) and after 20 weeks (final harvest). At intermediate harvest, we randomly selected 12 seedlings (six seedlings × two soil moisture treatments) across all three blocks for plant biomass measurement. We measured plant height and then separated seedlings into root, stem and leaves. Three "sun-leaves" per seedling were scanned with a

flatbed scanner (in batch 2 all leaves were scanned) for the determination of leaf area. Roots were washed out of soil after soaking in water to soften and total root length was recorded as rooting depth.

At final harvest (at the end of the experiment), all seedlings were harvested for determination of dry weight of various plant organs following the same protocol as described for the intermediate harvest. Roots were microwaved within six hours of harvest and later analysed for root starch concentration following the extraction protocol of Duranceau et al. (1999) adapted from Dubois et al. (1956) at the laboratory of Plant Ecology and Nature Conservation in Wageningen University, the Netherlands.

Analyses of seedling functional traits

We assessed seedling morphology traits related to growth, biomass allocation and foraging for resources using data from either the intermediate or final harvest (and for some traits, e.g. relative growth rate, in combination with week 0 data). Leaf scans were analysed for leaf area (LA) using ImageJ and total leaf surface area was determined as LA × leaf dry weight (LDW, this was not necessary in batch 2 as all leaves were scanned). We determined specific leaf area (SLA) as LA/LDW; root extension rate (RER) using rooting depth (RD) as $RD_{wk12} - RD_{wk0}/12$ weeks (i.e. growth duration); specific rooting depth (SRD) as RD/RDW; and rooting depth per leaf area (RDLA) as RD/LA.

Using root dry weight (RDW), leaf dry weight (LDW), stem dry weight (SDW) and plant dry weight (PDW), biomass allocation traits were determined: RMF as RDW/PDW; LMF as LDW/PDW; and SMF as SDW/PDW. We calculated RGR (adapted from Hoffmann and Poorter 2002) as follows:

$$RGR = \frac{ln \underline{Mass_{final} - ln \underline{Mass_{initial}}}}{d} eqn. 1$$

RD, RER, SRD and RDLA were analysed using data from intermediate harvest only as estimates from older seedlings could be skewed due to the possibility of pot limitation on root growth (Tomlinson et al. 2012).

Table 2. Functional traits quantified in this study. Numbers in parentheses after relevance indicate when (which stage of the greenhouse experiment) trait was measured: 1, 2 and 3 indicate first (week 0), second (intermediate) and final harvests respectively.

Trait	Abbrv. (units)	Trait type and Relevance
Relative growth rate	RGR (g g ⁻¹ week ⁻¹)	Biomass accumulation. Fitness measure (2&3)
Leaf dry weight	LDW (g)	Light capture /growth (1,2&3)
Stem dry weight	SDW (g)	Light capture/growth (1,2&3)
Shoot dry weight	ShootDW (g)	Light capture and growth (3)
Root dry weight	RDW (g)	Belowground resource capture/growth (1,2&3)
Plant dry weight	PDW (g)	Biomass accumulation/fitness measure (1,2&3)
Root to shoot ratio	R:S (g g ⁻¹)	Biomass allocation related to resource capture (2)
Root mass fraction	RMF (g g ⁻¹)	Investment in belowground resource capture,
		storage in roots (2&3)
Root starch	[RS] (%)	Allocation of resources to storage in roots. Related
concentration		to recovery from disturbance (3)
Total root starch	TRS (mg)	Total resources stored in roots. Related to recovery
reserves		from disturbance (3)
Root extension rate	RER (cm day-1)	Indication of foraging for deeper water (2)
Stem mass fraction	SMF (g g ⁻¹)	Stem/height investment for light capture (2&3)
Specific stem length	SSL (cm g ⁻¹)	Indication of etiolation. Shade avoidance or
		aboveground foraging efficiency (2)
Leaf mass fraction	LMF (g g ⁻¹)	Light capture/growth (2&3)
Rooting depth	RD (cm)	Belowground foraging, drought avoidance (2)
Specific rooting depth	SRD (m g ⁻¹)	Investment in deeper rooting, drought avoidance (2)
Rooting depth per leaf	RDPLA (m cm ⁻²)	Foraging for deeper water while reducing transpiring
area		surface area (2)
Leaf area	LA (cm ²)	Light capture and growth (2&3)
Specific leaf area	SLA (cm ² g ⁻¹)	Light capture (2&3)

Data analysis

We performed all data analyses using R (R Development Core Team, 2017). We tested seedling relative growth rate (RGR) and seedling mass at intermediate harvest for fixed effects of tree functional type (TFT) and soil moisture treatment

and their interaction using linear mixed model (LMM) in the *lme4* package (Bates et al. 2015). We included (experimental) batch and block as random factors. We tested RGR and mass gain for fixed effects of TFT \times soil moisture \times defoliation also using LMM. Again block and batch were added as random factors. We calculated effect size (using Hedge's g) of the defoliation treatment on mass gain separately for forest and savanna-transition TFTs and also separately for seedlings in low and control soil moisture treatments. Similarly, effect size of soil moisture limitation was estimated for the TFTs for defoliated and control seedlings separately. Then we calculated overall effect size for the defoliation treatment (as mean *g* under the control moisture regime) and the soil moisture treatment (as mean *g* for control seedlings).

We analysed selected seedling traits related to foraging, biomass allocation and root starch storage for differences between TFTs and soil moisture treatment (as fixed effects). The separate models (for each trait) included block and batch as random factors. Foraging traits were analysed only for seedlings at intermediate harvest as it was impractical to do this for older seedlings due to pot limitation, on rooting depth particularly. Leaf area, specific leaf area and rooting depth per leaf area were analysed using data from one experimental batch only (i.e. three species each within each TFT). For some traits (e.g. SRD, LA and RDLA) we applied Intransformation to meet assumptions of normality and homoscedasticity of residuals.

To assess trait associations as influenced by soil moisture, TFT and defoliation, we performed principal component analysis (PCA) based on 11 root, leaf and whole-plant morphology traits (related to biomass allocation) and starch storage in roots at final harvest using the *vegan* package with function *rda()* (Oksanen et al. 2019). We performed the PCA separately for defoliated and control

seedlings. Data were scaled (mean = 0, sd =1) for all variables prior to analysis. Scores of the first two principal components were tested (each one separately with Bonferroni correction) for differences between TFTs and soil moisture treatments in LMMs (which included block as random factor).

Finally, we analysed the allocation traits between the different treatments and TFTs. We tested root starch concentration of the control seedlings for fixed effects of TFT and soil moisture (and their interaction) using LMM. We used data from both batch repetitions and included only species with sufficient replicates in all treatment combinations. The model included block and batch as random factors. We also tested for differences in total root starch reserves using a LMM, only for the first batch (which included both defoliated and control seedlings) to test for interactions between soil moisture, defoliation and TFT. Block was added as random factor.

Results

Pot moisture distribution

At intermediate harvest, our control and low moisture treatments differed significantly (F _{1, 280} = 59.9, p < 0.001) at 14.6 \pm 3.1 (~ -0.006 MPa –indicating no moisture stress) and 12.7 \pm 2.6% (~ -0.014 MPa- indicating moderate moisture stress) respectively. Also, moisture content (MC) differed significantly (F _{2, 280} = 131.3, p < 0.001) among different pot layers, with MC being progressively lower with depth (Figure S1).

At final harvest, pot moisture content in the low moisture treatment was 8.6 $\% \pm 2.3\%$ (~-0.42 MPa- indicating moisture stress) while moisture in the control treatment was 16.0% \pm 7.0% (~ -0.004 MPa- indicating no moisture stress). The difference in moisture content was significant (F_{1, 473} = 332, p < 0.001) but there was also a significant moisture treatment x defoliation interaction (F_{1, 492} = 9.61,

p = 0.0020). Under control moisture conditions, non-defoliated seedlings had lower soil moisture than defoliated ones, but there was no difference between defoliation treatments under low soil moisture treatment (Figure S2).

Initial (pre-defoliation) seedling growth, foraging efficiency and biomass allocation

Relative growth rate (RGR) for 12 week-old seedlings did not differ between forest (at 0.035 \pm 0.02 g g⁻¹ week⁻¹) and savanna-transition (at 0.039 \pm 0.02 g g⁻¹ week⁻¹) tree functional types, irrespective of soil moisture regime. Irrespective of tree functional type, RGR was higher for seedlings in control (0.040 \pm 0.02 g g⁻¹ week⁻¹) than low (0.030 \pm 0.02 g g⁻¹ week⁻¹) soil moisture regimes (Table 3). Seedlings at 12 weeks were, thus, bigger in the control (1.0 \pm 0.66 g) than the low moisture treatment (0.75 \pm 0.56 g) irrespective of tree functional type (Table 3).

We found differences between tree functional types and moisture treatments for some foraging traits. Savanna-transition seedlings had higher specific stem length (SSL; Figure 1a, Table 4) and stem extension rate (SER; Figure 1b, Table 4) than forest seedlings irrespective of soil moisture treatment. Savanna-transition seedlings were, therefore, taller than the forest species ($21 \pm 11 \text{ cm}$ versus $13 \pm 4.7 \text{ cm}$) at 12 weeks (i.e. at intermediate harvest). Seedlings in low moisture regime tended to have lower specific rooting depth (SRD), but effects of both soil moisture treatment and tree functional type on SRD were not significant (Figure 1c, Table 4). Irrespective of soil moisture treatment, root extension rate (RER) was higher for savanna-transition than forest TFTs. There was no effect of soil moisture treatment on RER (Figure 1d, Table 4). Thus, savanna-transition seedlings rooted deeper than forest seedlings (($29.1 \pm 12 \text{ cm}$ versus $25.1 \pm 10 \text{ cm}$) at 12 weeks.

Leaf area (LA) was higher for forest than savanna-transition species, irrespective of soil moisture treatment. Also irrespective of tree functional type, LA was higher for seedlings in the control than low moisture treatments (Figure 2a, Table 4). Specific leaf area (SLA) was, however, similar for seedlings for both tree functional types and moisture treatments (Figure 2b, Table 4). At the whole-plant level, root to shoot ratio was higher for savanna-transition than forest seedlings, irrespective of soil moisture treatment (Figure 2c, Table 4). Rooting depth per leaf area (RDLA) was higher for savanna-transition than forest species. RDLA was higher for seedlings in low than control moisture treatment irrespective of tree functional type (Figure 2d, Table 4).

Table 3. ANOVA Table based on Satterthwaite's method from LMM for relative growth rate	e
(RGR) and seedling mass at intermediate harvest.	

Parameter	Source of variation	df	F	p-value
RGR _{wk12}	TFT	1, 151	0.06	0.800
	Moisture	1, 151	6.60	0.011
	TFT × Moisture	1, 151	0.65	0.421
Seedling	TFT	1, 151	7.36	0.007
mass _{wk12}	Moisture	1, 151	6.64	0.011
	TFT * Moisture	1, 151	0.41	0.523



Figure 1. Traits related to below and aboveground foraging efficiency at the initial seedling establishment phase for forest and savanna-transition tree species under defoliation and soil moisture regimes in a greenhouse experiment. Error bars are ± 1 SE of mean. Different letters indicate significant differences (from Tukey pairwise comparisons at 0.05 level of significance).



Figure 2. Mean **(a)** leaf area **(b)** specific leaf area and **(c)** ratio of rooting depth to leaf area based on data for six tree species (three each for forest and savanna-transition species) and **(d)** root to shoot ratio based on 12 species (6 each for forest and savanna-

transition species) in a greenhouse experiment. Error bars are ± 1 SE of mean. Different letters indicate significant differences (from Tukey pairwise comparisons at 0.05 level of significance).

Influence of soil moisture and defoliation on trait associations in older seedlings

The principal component analysis (PCA) for control seedlings at final harvest showed that the first two principal components (PC1 and PC2) explained together 75% of the total variance in the data. PC1 correlated negatively to relative growth rate, total seedling mass gain, shoot mass, leaf mass and stem mass (Figure 3a). Thus, PC1 represents a growth axis: separating fast growing (and thus, bigger) seedlings from slow growing (and smaller) ones. Seedlings in low moisture had higher scores on this axis ($F_{1, 104} = 11.1$, p = 0.001) than those in the control moisture treatment irrespective of tree functional type. Also, forest and savanna-transition seedlings differed along this axis irrespective of soil moisture treatment ($F_{1, 104} = 92.2$, p < 0.001) (Figure 4a). PC2 correlated negatively to LMF and leaf dry weight. Thus, this second axis represents trade-offs in below-ground versus aboveground biomass allocation and starch storage in roots. Savanna-transition seedlings had higher ($F_{1, 104} = 92.2$, p < 0.001) scores than forest seedlings along this axis, but there was no effect of soil moisture treatment (Figure 4b).

Among defoliated seedlings, the first two principal components explained 73% of the total variance. PC1 correlated positively to LMF, leaf mass, plant mass gain, root mass, stem mass and RGR (Figure 3b). PC1 thus represents for the defoliated seedlings the "regrowth axis". We found that PC1 scores were lower (F_{1} , $_{88} = 15.8$, p < 0.001) for seedlings in the low moisture than control soil moisture treatment, but tree functional types did not differ along this axis irrespective of

moisture treatment (Figure 4c). PC2 correlated positively to root starch concentration, RMF and root starch reserves, and negatively to SMF (Figure 3b). This axis, thus, separated seedlings based on higher biomass allocation and starch storage in roots versus higher investment in stem regrowth following defoliation. Axis scores on PC2 were higher ($F_{1, 90} = 19.1$, p < 0.001) for forest than savanna-transition seedlings, but not between soil moisture treatments (Figure 4d).

Table 4. ANOVA table (based on Satterthwaite's method) from LMM on initial biomass allocation and foraging traits at intermediate harvest. Data for leaf area (LA), specific leaf area (SLA) and rooting depth per leaf area (RDLA) come from experimental batch 1. The rest of the traits were estimated based on data from both experimental batches. TFT = tree functional type

Trait	Source of	df	F	p-value
	variation			
SRD	TFT	1, 152	1.25	0.266
	Moisture	1, 152	3.19	0.076
	TFT * Moisture	1, 152	0.05	0.828
RER	TFT	1, 149	7.06	0.008
	Moisture	1, 149	2.06	0.136
	TFT × Moisture	1, 149	0.08	0.994
SSL	TFT	1, 146	38.2	0.002
	Moisture	1, 146	0.26	0.148
	TFT * Moisture	1, 146	1.92	0.682
SER	TFT	1, 150	50.2	< 0.001
	Moisture	1, 150	2.05	0.154
	TFT * Moisture	1, 150	0.38	0.717
LA	TFT	1,66	21.3	< 0.001
	Moisture	1,66	4.11	0.050
	TFT * Moisture	1,66	0.12	0.731
SLA	TFT	1, 67	0.19	0.663
	Moisture	1,67	0.62	0.435
	TFT * Moisture	1,67	0.15	0.700
RDLA	TFT	1,68	25.7	< 0.001
	Moisture	1,68	6.02	0.017
	TFT * Moisture	1,68	0.04	0.846
RS ratio	TFT	1, 151	4.96	0.027
	Moisture	1, 151	0.97	0.330
	TFT × Moisture	1, 151	0.05	0.817



Figure 3. Principal component analyses of 11 root, leaf and whole plant traits related to biomass allocation and starch storage in roots for **(a)** control and **(b)** defoliated forest (circles) and savanna-transition (triangles) tree seedlings. Seedlings in high moisture (HM, which is control) and low moisture (LM) are given red and blue labels respectively. Variables included are root starch concentration (RStarchconcentration), root starch content (RStarchreserves), relative growth rate (RGR), root mass (RM), stem mass (SM), leaf mass (LeafM), shoot mass (ShootM), plant mass gain (PMgain), root mass fraction, stem mass fraction, leaf mass fraction (LMF).



Figure 4. Principal component scores of the first two axes (72% variance explained) as influenced by soil moisture and tree functional type for control seedlings (**a**) and (**b**) and defoliated seedlings (**c**) and (**d**). Different letters indicate significant differences (from Tukey pairwise comparisons at 0.05 level of significance).

Seedling (re)growth performance as influenced by defoliation, soil moisture and tree functional type

Beyond associations revealed by the PCA, we explored further for the effects of defoliation, soil moisture and tree functional type (and their interactions) on seedling growth performance. We observed that seedling relative growth rate (RGR) was higher for forest ($0.11 \pm 0.07 \text{ g g}^{-1}$ week⁻¹) than savanna-transition ($0.09 \pm 0.06 \text{ g g}^{-1}$ week⁻¹) tree functional types irrespective of soil moisture or defoliation treatments (Figure 5, Table 5). RGR was also affected by the defoliation × soil moisture interaction. Irrespective of TFT, RGR was six times lower for defoliated than control seedlings under the low soil moisture treatment, but the difference was not significant under the control moisture treatment ($0.01 \pm 0.05 \times 0.15 \pm 0.07$ for defoliated vs control seedlings) (Figure 5, Table 5).

We found a significant three-way interaction effect of TFT × soil moisture × defoliation on seedling mass gain (Table 5). Mass gain was higher for forest (at 7.4 \pm 8.0 g) than savanna-transition (at 5.9 \pm 4.7 g) seedlings for the control, but not for the low soil moisture treatment (1.4 \pm 1.9 g for forest and 1.4 \pm 1.3 for savanna-transition TFT). Among defoliated seedlings, mass gain was similar for both tree functional types in both soil moisture treatments (Figures 5c and 5d).

Hedge's effect size (g) indicated an overall large effect of defoliation on seedling mass gain. Effect size of defoliation was similar for both forest (g = -1.36) and savanna-transition (g = -1.40) TFTs under the control moisture treatment. In the low moisture treatment, the effect of defoliation was smaller for the forest (g= -0.80) than savanna-transition (g = -1.18) TFT. Effect size of lower soil moisture treatment on seedling mass gain (among control seedlings) was larger, overall, but smaller for the forest (g = -1.55) than savanna-transition (g = -1.87) TFT. Among defoliated seedlings, the effect of lower soil moisture on seedling mass gain was smaller for the forest (g = -0.83) than the savanna-transition (g = -1.25) TFT. Overall effect of lower soil moisture (g = 1.71) represented a larger effect than defoliation effect (g = 1.38) on seedling mass gain.

Irrespective of defoliation or soil moisture treatment, shoot mass was higher for forest than savanna-transition TFT ($3.4 \pm 6.7 \text{ g vs } 2.0 \pm 2.5 \text{ g}$) while root mass was higher for the savanna-transition than forest TFT ($1.8 \pm 2.0 \text{ g vs } 1.4 \pm 2.0 \text{ g}$). Within each TFT, both root mass and shoot mass were lower at final harvest for defoliated seedlings and also for seedlings in the low soil moisture treatment relative to controls (Figure 6, Table 5). Among defoliated seedlings, shoot mass (which indicates extent of shoot compensation) was lower under low than the control soil moisture treatments, i.e. three times (for forest seedlings) and four times (for savanna-transition seedlings).


Figure 5. Seedling relative growth rate for forest (a) and savanna-transition seedlings (b) and mass gain for forest (a) and savanna-transition seedlings (b) under defoliation and soil moisture regimes in a greenhouse experiment. Error bars are ± 1 SD of mean. Tukey pairwise comparisons are done among tree functional types, defoliation and soil moisture and different letters indicate significant differences (at 0.05 level of significance).

Root allocation and starch storage as influenced by soil moisture and defoliation

Root mass fraction (RMF) at final harvest was higher for savanna-transition (0.50 \pm 0.18 g g⁻¹) than forest TFT (0.35 \pm 0.15 g g⁻¹) irrespective of defoliation or soil moisture treatments. There was a defoliation × soil moisture interaction effect, without a significant three-way interaction (Table 6). Irrespective of TFT, RMF was higher for seedlings in the control (0.33 \pm 0.16 g g⁻¹) than low (0.40 \pm 0.19 g g⁻¹) soil moisture treatments only for non-defoliated seedlings (Table 6, Figure 7).

Root starch concentration was lower for forest (9.0 \pm 11%) than savannatransition (16.5 \pm 12.7%) TFTs irrespective of soil moisture treatment (Table 7a). Root starch concentration did not differ between the soil moisture treatments, and neither was there a TFT × soil moisture interaction effect (Figure 8a, Table 7a). Irrespective of moisture treatment, total root starch reserves were lower for the forest (18.8 \pm 38.4 mg) than savanna-transition (32.2 \pm 42.0 mg) TFTs. Also, irrespective of TFT, root starch reserves were lower for seedlings in low (6.5 \pm 6.0 mg) than control (41.4 \pm 49.7 mg) soil moisture regimes (Figure 8b, Table 7a).

Table 5. ANOVA Table based on Satterthwaite's method from LMM testing fixed effects of treatments (including block and batch as random factors) on growth parameters at final harvest in a greenhouse experiment.

Parameter	Source of variation	df	F	p-value
RGR	TFT	1, 491	21.0	< 0.001
	Moisture	1, 491	145	< 0.001
	Defoliation	1, 491	186	< 0.001
	TFT Moisture	1, 491	0.25	0.617
	Moisture × Defoliation	1, 491	4.83	0.028
	TFT × Defoliation	1, 491	2.13	0.145
	TFT \times Moisture \times Defoliation	1, 491	0.30	0.583
Seedling	TFT	1, 491	6.06	0.014
mass gain	Moisture	1, 491	226	< 0.001
	Defoliation	1, 491	153	< 0.001
	TFT × Moisture	1, 491	4.38	0.037
	Moisture × Defoliation	1, 491	70.6	< 0.001
	TFT × Defoliation	1, 491	7.53	0.006
	TFT × Moisture × Defoliation	1, 491	6.01	0.015
Shoot mass	TFT	1, 471	22.7	< 0.001
	Moisture	1, 471	153	< 0.001
	Defoliation	1, 471	236	< 0.001
	TFT × Moisture	1, 471	0.36	0.550
	Moisture × Defoliation	1, 471	1.24	0.267
	TFT × Defoliation	1, 471	2.16	0.142
	TFT \times Moisture \times Defoliation	1, 471	0.23	0.627
Root mass	TFT	1, 486	18.0	< 0.001
	Moisture	1, 486	370	< 0.001
	Defoliation	1, 486	159	< 0.001
	TFT × Moisture	1, 486	0.01	0.907
	Moisture × Defoliation	1, 486	10.8	0.001
	TFT × Defoliation	1, 486	0.85	0.355
	TFT × Moisture × Defoliation	1, 486	0.74	0.315

For the first experimental batch starch data for both defoliated and nondefoliated seedlings, we tested for possible interactions involving defoliation, soil moisture and tree functional type. Not only did we find a TFT effect (as reported above), we also found that root starch concentration was lower for defoliated (2.2 \pm 2.6 %) than control (6.7 \pm 5.7 %) seedlings irrespective of TFT or soil moisture treatment (Figure 9, Table 7b). For total root starch reserves, there was an effect of soil moisture treatment in addition to effects of both TFT and defoliation, but there were no interaction effects (Figure 9, Table 7b).



Figure 6. Shoot and root mass at final harvest for forest and savanna-transition tree species under defoliation and soil moisture regimes in a greenhose experiment. Error bars are ± 1 SD of mean. Tukey pairwise comparisons are done among tree functional types, defoliation and soil moisture and different letters indicate significant differences (at 0.05 level of significance).

Table 6: ANOVA table (based on Satterthwaite's method) from LMM on biomassallocation at final harvest. Data are from both batches of the greenhouse experiment.

Trait	Source of variation	df	F	p-value	
R:S ratio	TFT	1,237	51.6	< 0.001	
	Moisture TFT × Moisture	1, 237 1, 237	5.24 0.02	0.022 0.880	
LMF	TFT	1, 243	45.3	< 0.001	
	Moisture	1, 243	0.94	0.333	
	TFT × Moisture	1, 243	0.51	0.477	
SMF	TFT	1, 243	6.38	0.012	
	Moisture	1, 243	4.31	0.038	
	TFT × Moisture	1, 243	0.60	0.439	
RMF	TFT	1, 242	79.5	< 0.001	
	Moisture	1, 242	6.46	0.011	
	TFT × Moisture	1, 242	0.16	0.687	



Figure 7. Root mass fraction of **(a)** forest TFT, **(b)** savanna-transition TFT for 12 tree species of forest and savanna-transition origins under defoliation and soil moisture regimes in a greenhouse experiment. Error bars are ± 1 SD of mean. Tukey pairwise comparisons are done among tree functional types, defoliation and soil moisture and different letters indicate significant differences (at 0.05 level of significance).

Table 7a. ANOVA table (based on Satterthwaite's method) from LMM on root starch concentration and total root starch reserves at week at final harvest. Data from both batches of the greenhouse experiment was used in this analysis.

Parameter	Source of variation	df	F	p-value	
Root starch	TFT	1, 153	25.4	< 0.001	
concentration	Moisture	1, 153	0.15	0.696	
	TFT × Moisture	1, 153	0.43	0.514	
Root starch	t starch TFT		21.4	< 0.001	
content	Moisture	1, 153	33.8	< 0.001	
	TFT × Moisture	1, 153	0.06	0.805	

Table 7b. Vertical root distribution, SRL and root starch reserves for non-defoliated seedlings at final harvest. Forest and savanna-transition TFTs were analysed separately in LMM, including block as random factor.

Parameter Source of variation		df	F	p-value			
Savanna-transition TFT							
Root starch	Moisture	1,86	0.43	0.515			
concentration	Defoliated	1,86	42.0	< 0.001			
	Defoliated × Moisture	1,86	3.68	0.059			
Root starch	Moisture	1,86	6.16	0.015			
content	Defoliated	1,86	69.8	< 0.001			
	Defoliated × Moisture	1,86	0.88	0.349			
Forest TFT							
Root starch	Moisture	1, 98	0.43	0.947			
concentration	Defoliated	1, 98	24.4	< 0.001			
	Defoliated × Moisture	1, 98	0.20	0.658			
Root starch	Moisture	1, 98	16.2	< 0.001			
content	Defoliated	1, 98	92.1	< 0.001			
	Defoliated × Moisture	1, 98	1.33	0.251			

Seedling growth performance and allocation traits of forest and savanna-transition tree seedlings under defoliation and soil moisture limitation



Figure 8. (a) Root starch concentration, **(b)** root starch reserves based on seedlings from 12 tree species of forest and savanna-transition origins under defoliation and soil moisture regimes in greenhouse experiment. Only species with sufficient replications were included in both root starch analyses. Error bars are ± 1 SD of mean.



Figure 9. Root starch concentration (**a**) and (**b**) and total starch reserves (**c**) and (**d**) under defoliation and soil moisture treatments for forest and savanna-transition tree functional types (data are from experimental batch 1 only: 3 forest and 3 savanna-transition species). Error bars are ± 1 SD of mean. Tukey pairwise comparisons are done

among tree functional types, defoliation and soil moisture and different letters indicate significant differences (at 0.05 level of significance).

Discussion

We tested two hypotheses related to seedling growth performance, morphology traits and starch storage in roots in response to defoliation and soil moisture limitation for forest and savanna-transition tree functional types in a greenhouse experiment. We found adverse synergistic effects of defoliation and soil moisture limitation on tree seedling growth performance. Regrowth following defoliation was associated with rapid growth, greatly influenced by soil moisture regime. We discuss implications for seedling growth performance under precipitation declines and defoliation disturbance factors. We further discuss a new question arising from this study, on whether specific disturbance type may matter more for differences in regrowth capabilities inherent to TFTs.

Allocation patterns differ between tree functional types and establish early regardless of moisture treatment

At the early seedling establishment phase (among 12 week old seedlings), savanna-transition species invested more (relative to forest species) in height growth with thinner stems (higher specific stem length) and higher rate of stem extension regardless of soil moisture regime. These trait differences suggest a stronger response of the savanna-transition (than forest) tree functional type to the light environment (~12% of full light) in the greenhouse (Gignoux et al. 2016). Such light levels should not limit growth for many of the forest species in this study (Agyeman et al. 1999, Amissah et al. 2015, Veenendaal et al. 1996). Beyond the initial acclimation response as observed for the savanna-transition tree seedlings,

we did not expect strong growth limitation to result from the greenhouse light conditions.

We observed a general pattern of higher aboveground versus belowground foraging and biomass allocation, consistent with expected differences between the tree functional types (following the functional equilibrium hypothesis, Brouwer 1963). Savanna-transition tree seedlings had higher rooting depth, root to shoot ratio, rooting depth per leaf area, and lower leaf area than forest seedlings. These traits indicate greater allocation to belowground foraging and storage, typical of species from moisture-limited or disturbance prone environments. Forest seedlings increased photosynthetic surface area to maximize light capture for their inherent faster growth rates (Gignoux et al. 2016, Boonman et al. 2019). Leaf area was lower under low moisture regime as seedlings attempted to trade-off leaf mass for root mass to enhance survival (Poorter et al. 2012). Also, irrespective of tree functional type, seedlings in low moisture regime had higher values of rooting depth per leaf area, due to the negative effect of low moisture on leaf area. This may represent an attempt to reduce transpiring surface area relative to water uptake, which has potential benefits in a drier environment (Lopez-Iglesias et al. 2014). Savanna-transition species had this tendency, but the difference between tree functional types was not significant due to high variability.

Higher rooting depth observed for savanna-transition seedlings at intermediate harvest was supported by faster root extension. We did not find specific rooting depth to differ between tree functional types, possibly because roots had thickened by week 12, when we did the intermediate harvest (Tomlinson et al. 2012). Both root extension rate and specific rooting depth are associated with semi-arid savanna species which have the need to chase after a declining water column (Tomlinson et al. 2012). Therefore, the savanna-transition tree

functional type in this study may combine many traits characteristic of humid, mesic or even semi-arid savannas because this group of species are supposed to have a wide ecological amplitude (Armani et al. 2018).

At final harvest, we found higher biomass allocation and carbohydrate storage in roots for savanna-transition seedlings versus higher allocation to shoot for forest tree seedlings. Biomass allocation patterns were similar as found for seedlings at 12 weeks. Higher allocation and storage in roots is typical of species from humid savannas (or more disturbed environments) because it enhances recovery after disturbance (Boonman et al. 2019, Issifu et al. 2019, Wigley et al. 2019). Lower soil moisture regime decreased biomass allocation to roots without changing tree functional type allocation patterns to roots. There was also no effect of lower soil moisture on root starch concentration even though growth decreased under low moisture, which may suggest fixed allocation to root storage. However, there may still be an effect of moisture on recovery capacity via reductions in total amount of stored reserves. For example we found lower total root starch content for seedlings in lower moisture relative to control because root dry weight was also lower in the low moisture treatment.

Forest seedlings grow faster but not at the initial growth phase

Forest and savanna-transition tree seedlings had similar growth rates at 12 weeks, but forest seedlings grew faster at the end (after ~28 weeks). The tree functional types started off similarly possibly because initial maternal investments in seed reserves were also similar for the species selected (this has not been explored in this study, but see Leishman et al. 2000, Tomlinson et al. 2019). Also, regardless of tree functional type, plants at the initial stages of development need to build tissues that are important for carbon gain. Leaves (which are the primary photosynthetic tissues) and stems (structural biomass needed for light

competition) are needed to be constructed first (through rapid growth) before any resource use (or economics) strategies take hold. Thus, the trade-offs in biomass allocation that separate forest from savanna tree species (discussed above for the initial growth phase) were perhaps not yet strong enough to cause differences in growth allocation between tree functional types at the initial establishment phase (Hoffmann & Franco 2003). Thus, general patterns of resource conservation (among savanna-transition tree seedlings) versus resource acquisition (among forest tree seedlings) had been strongly established by final harvest, possibly explaining the observed difference in growth rate between the tree functional types among older seedlings (Gignoux et al. 2016).

Seedling growth performance was lower under low moisture treatment relative to the control treatment. This adverse effect of low soil moisture was similar for both forest and savanna-transition tree functional types, which was in contrast with our expectation that soil moisture limitation should have a stronger negative effect on forest than savanna-transition tree seedlings due to differences in the climate of the species origin (Table 1). Species that constituted the savanna-transition tree functional type in this study may be quite limited in their distribution by soil moisture. For example, *Khaya senegalensis, A. africana, Daniella olivera* are associated with moist savanna woodlands and when they occur in drier savanna types, they are associated with gallery forests or found along water courses (Orwa et al. 2009). Thus, humid savanna species subjected to moisture stress may respond strongly with conservative strategies and in the end being affected similarly as (dry) forest species.

Chapter 5

Low soil moisture and defoliation produce adverse synergistic effects on (re)growth performance irrespective of tree functional type

We found that defoliation and low soil moisture synergistically decreased growth performance (both seedling relative growth rate and mass gain) irrespective of tree functional type. Thus, the extent of compensation for shoot loss was lower under low soil moisture regime for both forest and savanna-transition tree seedlings, which may be explained in two (not mutually exclusive) ways. First, we found that low soil moisture caused a reduction in mass gain and likely decreased amount of stored resources prior to defoliation (starch content in roots was not measured at this stage, but this is plausible based on positive correlations of root mass with starch content, e.g. as reported in Chapter 4 of this thesis). Thus, resources available for recovery were likely lower compared to seedlings in the control treatment (although seedlings may also increase storage reserves under moisture limitation, O'Brien et al. 2014). Second, seedlings in the low moisture treatment which resprouted, had lower post-resprout growth rate possibly due to smaller photosynthetic surface area (and unit leaf rate) in addition to moisture stress which could affect stomatal conductance (Pratt et al. 2014).

Defoliation (as applied in this study) represents a more moderate disturbance type. For example, the low soil moisture treatment had a stronger adverse effect on growth than the effect of defoliation, which differs from the effects found for fire (Chapters 3 and 4 of this thesis). This was possibly because defoliation above the first node (see methods) allowed for epicormic resprouting, instead of basal and belowground resprouting that dominate post-fire resprouting (Lawes et al. 2013). The higher sensitivity of forest species (compared to savanna species) to stronger disturbance types (e.g. high intensity fires, Chapters 3 and 4 of this thesis) could be due to lower capacity to resprout from belowground buds.

This needs to be tested empirically, but such an idea may explain the importance of aboveground bud protection for post-disturbance recovery of forest seedlings. These findings imply that herbivores and fire (as disturbance factors) may differ in extent of influences on tropical tree seedling growth performance (Pausas et al. 2016, but see Zeppel et al. 2015).

We found (from the principal component analysis on allocation and regrowth performance traits among defoliated seedlings) that regrowth performance parameters (plant mass gain and shoot mass) were more associated with plant size parameters and influenced by soil moisture, but not tree functional type. Findings were not consistent with expectation that seedlings with higher biomass allocation to roots and carbohydrate storage should have a higher regrowth performance following defoliation (see also Hoffmann et al. 2009). Stored resources may have a stronger influence on whether or not seedlings resprout after defoliation (Moreira et al. 2012), which may also be determined by disturbance type (and its intensity). Once seedlings survive defoliation, regrowth allocation (after a brief period of compensating for shoot loss, Moreira et al. 2012) may then depend on species resources use strategy. We found in this study that post-defoliation allocation (as measured at final harvest) followed similar patterns as non-defoliated seedlings, with forest species investing more in shoots while savanna species continued to invest more in roots.

In conclusion, our findings indicate that declines in precipitation amounts may decrease growth performance and post-disturbance recovery among both forest and savanna-transition tree seedlings with implications for tree recruitment in forest-savanna transitions.

Acknowledgement

Wageningen University and Research with support from the Academy Ecology Fund of the KNAW made field work possible. We are grateful to Yinye Kwaku and Emmanuel Kodua for assisting with seed collection and to Jan van Walsem for laboratory analysis of plant samples. Thank you to all former students of the Department of Forestry and Forest Resources Management, UDS, who assisted in the greenhouse.

Appendices

Table S2: Some soil chemical properties of soil used in the pot experiment. AP = Available P (mg/kg). K, Ca, Mg, Na are Exchangeable Cations (cmol+/kg soil).

	рН	N%	С%	AP	К	Ca	Mg	Na
Batch 1								
Mean	-	0.52	1.58	-	0.10	3.40	1.78	0.002
Batch 2								
Mean	7.26	0.09	0.93	4.34	0.25	2.57	0.87	0.0096
SD	0.14	0.01	0.06	0.60	0.05	0.74	0.27	0.0048



Figure S1. Pot moisture content at intermediate harvest under moisture treatments in batch 2 of the greenhouse experiment. Soil moisture was measured prior to applying the defoliation treatment



Figure S2. Pot moisture content at final harvest under moisture treatments for defoliated and non-defoliated seedlings in batch 2 of the greenhouse experiment.



Introduction

In this thesis, I aim to explain how seedling recruitment differs between tropical savanna-transition and forest tree functional types under constraints imposed by various vegetation controls across the forest-savanna transition. In the previous chapters, I presented results from semi-manipulative field transplant, common garden and greenhouse experiments to address the following specific research questions (in four corresponding chapters):

- [1] How does variation in woody cover (and associated factors) influence seedling establishment success, related to traits, of forest and savannatransition tree species?
- [2] What are the relative influences of grass competition during the wet season followed by fire and lack of precipitation during the dry season on tree seedling establishment success among forest and savanna-transition tree species?
- [3] What are the relative influences of fire and lack of moisture during the dry season on tree seedling establishment success, related to traits, among forest and savanna-transition tree species?
- [4]To what extent does soil moisture regime influence growth performance, allocation traits and recovery from defoliation among forest and savannatransition tree functional types?

In this last synthesis chapter, I integrate and discuss results presented in the different chapters as well as raise some general issues. I highlight how this thesis may contribute to the understanding of the transition between savanna and forests and restoration of dry forests. Finally, I propose directions for future research.

Synthesis

Nature and dynamics of tropical forest-savanna transitions

The forest-savanna transition in West Africa is a mosaic of forest patches in a matrix of humid savanna with transition vegetation occurring in close proximity and under similar climatic conditions differing in composition and vegetation structure (Ametsitsi et al. *in prep.*, Swaine et al. 1976, Cuni-Sanchez et al. 2016, Hennenberg et al. 2006). The relative importance of various factors (such as fire and soil resources) thought to govern the distribution of the different vegetation formations has been a question of long-standing debate (Fairhead & Leach 1996, Veenendaal et al. 2015). It is increasingly being accepted that different vegetation formations are associated with soil factors, but shaped locally by top-down controls including fire and herbivory (Oliveras & Malhi 2016, Veenendaal et al. 2015; 2018).

The seedling stage of trees is an important bottleneck for tree species distribution due to influences of the regeneration phase on plant success (Poorter 2007, van Langevelde et al. 2011). Current trends of forest retreat and (occasional) advance across the forest-savanna transition (Janssen et al. 2018, Mitchard & Flintrop 2013) suggest an important role for seedling recruitment into the different vegetation types in response to changes in the transition. Fire is also suggested as a key constraint to forest seedling establishment success in humid savannas (Cardoso et al. 2016, Gignoux et al. 2009). However, the close relation between soil fertility and soil water retention and vegetation structure in the tropics suggests that other factors, such as soil resources, may be just as important (see e.g. Veenendaal et al. 2015, Lloyd et al. 2015 and Staal & Flores 2015 versus Lloyd & Veenendaal 2016).

In this thesis, I propose that woody cover variability (and its influences on herbaceous vegetation) across the transition cause differential tree functional type seedling recruitment through both bottom-up and top-down controls (Figure 1.2).

Figure 1.2 identifies current research gaps, which form a basis for this thesis. The constraints on tree seedling recruitment are relevant to study as the seedlings develop into adult trees determining the constraints for new tree seedlings. Seedlings of different tree species may respond differently to bottom up and top down constraints, and adult trees of different species may trigger different constraints on seedlings. From the conceptual model (Figure 1.2), fire may indeed be an important constraint to seedling establishment success in savanna woodlands, but different responses to fire can be expected for seedlings belonging to different tree functional types due to trait variability. Such differences in tree functional type responses may explain the observed variation in species types dominating various vegetation types in the transition (Armani et al. 2018), including the existence of transitional vegetation types in the fire zone of African dry forests (Swaine 1992, Cuni-Sanchez et al. 2016, Hopkins 1974). Using the conceptual model (Figure 1.2), I propose that other factors may be important on their own or in interaction with fire. The relative influences of these different factors, interactions among them and with tree functional type, determine vegetation changes across the forest-savanna transition and future stand composition under land use and climatic change. Further explanation of these influences will following in the next sections.

Tree seedling functional types and species selection for experiments in this thesis

I set out with the broad hypothesis that trait variation (including performance traits) in response to certain vegetation controls between forest and savanna-transition tree species, explain species recruitment and the mosaic vegetation pattern observed across the forest-savanna transition. Emphasis was placed on contrasting trait responses of forest and savanna-transition tree functional types

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that co-occur in the forest-savanna transition or in semi-deciduous forests. Savanna-transition species are important in the transition due to their role in maintaining vegetation types that are intermediate between closed forest and savanna woodland (Ametsitsi et al. *in prep.*, Cuni-Sanchez et al. 2016, Hennenberg et al. 2006, Swaine 1992).

While particular seedling root trait syndromes may vary strongly along resource gradients, they are not exclusively assigned to distinct tree types (Boonman et al. 2019), suggesting a broad range of variation. Noting that the number of species within tree functional types in our experiments was by necessity limited, we undoubtedly did not capture the full breadth of trait variation across functional types. This is potentially an important limitation of the experiments reported in this thesis. Nevertheless, in nearly all chapters, general patterns emerged for separating forest tree species from savanna-transition tree species, consistent with studies which looked for similar patterns (e.g. Boonman et al. 2019, Gignoux et al. 2016). I analysed species-specific patterns to demonstrate within tree functional type variability. Therefore, despite limitation in species numbers, I was able to demonstrate patterns and responses of the selected species which helped to answer questions posed in this thesis. Below, I provide a synthesis of the answers.

Influence of canopy cover variation on tree functional type establishment success

Different vegetation types (woodland, closed woodland and forest) that co-occur within the forest-savanna transition vary in the extent of woody canopy cover (crown area index, leaf area index; **Chapter 2**, Torrelo-Raventos et al. 2013, Veenendaal et al. 2015) and herbaceous biomass and its composition (**Chapter 2**, Cardoso et al. 2018, Charles-Dominic et al. 2018, Veenendaal et al. 2015). Thus,

Chapter 6

fire intensities tend to be higher in savanna woodland than in vegetation types with more closed canopy (Ametsitsi et al. *in prep.*, Laris 2011, Laris et al. 2015, Veenendaal et al. 2018). Therefore, I expected woodland vegetation type to select for fire tolerant savanna-transition tree species over fire-sensitive forest species (**Chapter 2**). Consistent with this expectation, and in line with other studies, savanna species survived and grew in fire-prone woodland (**Chapter 2**, Gignoux et al. 2009, Hoffmann et al. 2004) – with survival linked to a resource conservation root trait syndrome. I explored the hypothesis on fire effect further with more species in the common garden experiments (**Chapters 3 and 4**), where I could better control and disentangle effects of a number of drivers. The effect of fire on seedling growth and mortality was tested in combination with other factors (which I will discuss later in this chapter). I found that in all experiments where seedlings of forest and savanna-transition species were subjected to a fire treatment (**Chapters 3 and 4**), savanna-transition tree species were more likely to survive than forest species.

Forest species are more competitive under denser woody canopy cover because they are generally more shade-tolerant compared to savanna species, and also because they require more soil resources – which are more abundant in forest – for their intrinsic faster growth rates. The moist semi-deciduous species selected in the study hardly survived. As in many field studies, many factors likely contributed to mortality of the seedlings, but we determined that lack of moisture during the dry season was important, and that not even closed canopy of the forest vegetation ameliorated the adverse effect of a prolonged dry period contrary to expectation based on net house pot studies (see e.g. Amissah et al. 2015). Poor survival may be attributed to increased competition for water between seedlings and mature trees in closed canopy vegetation, which reduces seedling growth

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(Veenendaal et al. 1996a,b). In my field study area, a strong link between recruitment success and drought tolerance in forest trees has been demonstrated (Cardoso et al. 2016) – forested patches depleting water resources to greater extents than the surrounding savanna (Ametsitsi et al. *in prep*.). However, forest species could survive the dry season if sufficient soil moisture conditions prevailed (**Chapter 4**). Considering that forest patches populated by forest-type species coexist with savanna vegetation (e.g. Armani et al. 2018, Swaine 1992), recruitment of forest species in transitional vegetation is not impossible, but is for forest species only possible linked to years of higher rainfall and short dry seasons. Further work is needed to explore this possibility.

Light limitation is likely an important bottleneck to the recruitment of shadeintolerant species and could be the basis for differential recruitment of tree functional types into various vegetation types in the forest-savanna mosaic. I compared a typical savanna woodland species (Terminalia macroptera) with the savanna-transition species Khaya senegalensis (originally also categorised as a savanna species) in Chapter 2. Seedlings of the first species were, in the course of three years, excluded from closed canopy forest, while the more shade-tolerant K. senegalensis (Kwesiga & Grace 1986) survived well under the closed canopy forest. This finding was not consistent with the idea that shade excludes savanna species from closed-canopy forests, and was perhaps due to variability in speciesspecific traits that may exist even for species within the same tree functional type. Khaya senegalensis is variously categorised; as a savanna species (Chapter 2 of this thesis, Okali & Dodoo 1973, Keay 1960), ubiquitous or transition species (Armani et al. 2018, Boonman et al. 2019) or even as a forest species (Kwesiga & Grace 1986), suggesting that it has more intermediate traits or trait plasticity. This species, thus, represents an example of the existence of "many shades of green"

within the forest-savanna-transition trait spectrum. Interestingly, it is the only representative of the forest tree genus Khaya (African Mahoganies) that has made it into the savanna biome.

Woody canopy cover may thus govern tree recruitment patterns of different tree functional types in the forest-savanna transition through its influences on bottom-up and top-down controls. Although soil resources and microclimate are known to have an important influence on seedling establishment success (as discussed earlier), the link between soil resources or microclimate and establishment success of seedlings in different vegetation types was not very clearly established in the field (Chapter 2). Admittedly this link was not investigated in detail in Chapter 2, but also such a link is very difficult to establish for juvenile trees under field conditions (see Armani et al. 2018). One reason could be the many interacting factors (e.g. interactions between soil nutrients and light levels or drought). It appears that a stronger effect of dry season moisture stress on seedling establishment success masked the influences of other factors (including soil resources and light) in Chapter 2. It may also be due to low representation of dry forest species, which are better adapted to the local field conditions of the transition. I investigated the link between soil resources and seedling establishment success in more manipulative experiments which I discuss later on in this chapter.

Relative effects of tree seedling-grass competition, soil resources and fire on tree functional type establishment success

Field transplants (e.g. **Chapter 2**, Cardoso et al. 2016, Gignoux et al. 2009; 2016, Lawes et al. 2011) provide general patterns on recruitment niches (as discussed earlier in this chapter) of different tree functional types within the transition. As is often the case with many field studies, field transplants fail to fully disentangle

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effects of the factors which correlate to influence seedling establishment success. For example, light limitation, relative effects of grass competition in the wet season, lack of precipitation in the dry season and fire as well as interactions involving combinations of these factors could not be properly separated in **Chapter 2**. Therefore, I focused on tree functional type responses to more specific factors (and their interactions) separately; grass competition and fire (**Chapter 3**), fire and dry season irrigation (**Chapter 4**) and soil moisture regime and defoliation (**Chapter 5**).

Apart from being fuel for dry season fire, which is seen as an important cause of seedling mortality, herbaceous vegetation also directly affects tree seedling performance through competition for soil resources or light in the wet season (**Chapter 3**, Migley & Bond 2001, van der Waal et al. 2009, Ward & Elser, 2011, Barbosa et al. 2014, February et al. 2013, Tomlinson et al. 2019). Depending on the strength of such competitive interactions, grass competition may be a direct constraint to tree seedling establishment success. Tree seedling-grass competition is mainly for soil moisture and nutrients (Tomlinson et al. 2019), but the forest-savanna transition is not limited in either factor (Swaine 1992). Therefore, direct effect of grass competition on tree seedling survival is expected to be less important (**Chapter 3**) unlike in drier savannas where grass competition may directly decrease seedling survival (Migley & Bond 2001, van der Waal et al. 2009, Ward & Elser 2011).

I expected grass competition to have greater adverse effect on growth performance of forest than savanna-transition tree seedlings due to possible evolutionary differences, mainly the development of adaptations to co-existence (e.g. root niche partitioning, Walter 1971, but see Kulmatiski et al. 2010) between the two tree functional types (Oliveras & Malhi 2016). However, I found that the

negative effect of grass competition on tree seedling growth performance was similar for both forest and savanna-transition tree seedlings (**Chapter 3**). A possible reason for this finding is that across tree functional types, competition effect was greater for faster growing species, but overall growth was similar between tree functional types (**Chapter 3**). Interestingly, while growth suppression was similar for both tree functional types, grass competition decreased root mass more (leading to a greater reduction in the amount of starch stored in roots) resulting in lower post-fire survival for forest than savanna-transition seedlings (**Chapter 3**, in consistence with other studies reporting on the role of carbohydrate storage on survival differences between forest and savanna species in drier savannas, e.g. Wigley et al. 2019).

Models on tree grass interactions focus a lot more on the suppressing effect of adult trees on grasses than on the reverse interactions at the seedling stage (van Langevelde et al. 2003; 2014, Charles-Dominic et al. 2018). This is especially true for forest-savanna mosaics where soil moisture or nutrients are not considered limiting (Swaine 1992, but see Veenendaal et al. 1996a). This thesis provides evidence (**chapter 3**) that wet season tree seedling-grass interactions are important and that the influences may not be via soil resources per se, but light limitation may also be important in tall grass woodlands that characterise West African forest-savanna transitions (**Chapter 2**, Hennenberg et al. 2006). Seedling trait responses may provide indications of which factors likely drive competitive interactions. For example, taller, thinner stems and a higher allocation to shoot (**Chapter 3**, Schmitt et al. 1999) may indicate shade avoidance by tree seedlings in the midst of tall grasses. These findings have important ecological implications. First, grass competition alone (in the absence of fire) is not sufficient to prevent seedling establishment in humid savanna regardless of tree functional

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type, but recruiting individuals may persist as very small seedlings. Second, via reductions in growth (and root carbohydrate reserves), tree seedlings, particularly of forest species, may be rendered more susceptible to fire-induced mortality, suggesting that variability in grass cover could influence patchy recruitment as pockets of seedlings are more likely to establish where grass cover is less.

many experiments (including the afore-discussed competition In experiment), fire effects are naturally entangled with lack of moisture in the dry season. Thus, questions arise on whether seedlings can survive the lack of moisture in the dry season in the absence of fire. I attempted to disentangle these effects using a dry season fire and irrigation experiment (**Chapter 4**). I found in a common garden experiment that in the absence of fire, forest tree seedlings survived a 5-month long dry season just as well as savanna-transition tree species. This finding was not consistent with the expectation that a lack of moisture in the dry season should have a stronger adverse effect on forest tree seedling establishment success (Gignoux et al. 2009, Lawes et al. 2011). Mortality in the dry season could result from moisture stress, but at certain sizes, seedlings either have enough carbohydrate reserves (Cardoso et al. 2016) or have roots in deeper soil layers and can, therefore, avoid moisture stress despite the absence of precipitation in the dry season (Chapter 4). Findings in Chapter 4 may imply that the dry season itself (in the absence of fire) may not be a barrier to forest seedling establishment in humid savannas particularly if there is no root competition with mature trees, which may explain forest encroachment in savannas in the absence of fire. However, the effect of dry season in humid savannas has to be interpreted carefully, since soils may remain moist over relatively long periods despite the absence of precipitation. I found also that dry season irrigation (continued access to moisture by tree seedlings) did not reduce the sensitivity of forest species to

fire, possibly because post-fire resprout capacity depends on the amount of carbohydrates stored in roots, which is lower for forest than savanna-transition tree species (**Chapter 4**, Gignoux et al. 2016, Boonman et al. 2019, Hoffmann et al. 2004).

Aside rainfall distribution, total rainfall amount received in the wet season and stored in the soil profile is important for tropical seedlings performance (Veenendaal et al. 1996b, Le Roux & Bariac 1998). Decreased precipitation in the wet season may result from climate variability (Fauset et al. 2012) and it is also one of possible scenarios of climate change. Rainfall shows a decline of ca. 10 -15% over the last 40 years for the transition zone of Ghana (Owusu & Waylen 2009). Sub-optimal precipitation levels may influence seedling establishment and growth of forest and savanna-transition species differently. Lower growth performance in the wet season may be linked to lower dry season survival and tolerance to defoliation since the effect of defoliation may depend on seedling size (Bond & Midgley 2003). I assessed the effect of lower soil moisture regime and its interaction with defoliation on (re)growth performance emphasising the link between allocation traits (as influenced by moisture) and regrowth capacity of defoliated seedlings (**Chapter 5**).

In Chapter 5, I already reported that at the seedling stage, savannatransition trees may just be as sensitive as forest species to moisture limitation (**Chapter 5**) and the adverse effect of defoliation on seedling growth performance was greater under lower soil moisture regime irrespective of tree functional type (**Chapter 4**). The implication is that in drier years, disturbance factors such as fire or herbivory will likely have greater negative effects on seedling growth performance of all tree functional types. Regrowth performance was not linked to allocation traits (which differed predictably between forest and savanna-transition

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tree seedlings) but determined by growth performance. This contradicts expectations that higher allocation to roots should enhance post-disturbance regrowth performance (**Chapters 2** and **3**). Possibly we found these results because disturbance type (defoliation versus fire) may not produce similar regrowth responses (Pausas et al. 2015, but see Bond & Keeley 2005, Zeppel et al. 2015). This needs further research.

Influence of seedling trait variation on establishment success under various constraints

Tree seedling traits (morphological, physiological or phonological) are good predictors of environmental tolerances and the recruitment niches of tropical tree species (Amissah et al. 2015, Boonman et al. 2019, Poorter 2007, Tomlinson et al. 2012; 2019). Therefore, as a final overarching research objective, I assessed the link between seedling traits and seedling establishment success under key environmental constraints investigated in this thesis, namely moisture limitation, light limitation, competing herbaceous vegetation, fire and defoliation. Emphasis was placed on seedling morphology traits related to biomass allocation and foraging efficiency, and also starch storage in roots. For each experiment, specific traits were selected based on the functional role they were expected to play under the constraints imposed, as described in previous studies (See Table 1.1 and references therein).

Root starch concentration and total root starch reserves are higher for savanna-transition than forest tree species (**Chapters 2, 3, 4, 5,** Boonman et al. 2019). These traits are associated with higher post-fire survival and explain the success of savanna-transition over forest tree seedlings (**Chapters 2, 3, 4**), except in the defoliation experiment (**Chapter 5**, reasons for this have been discussed in the previous section). This is in line with many other studies in which

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root carbohydrate reserves were linked to post-disturbance survival (Cardoso et al. 2016, Hoffmann et al. 2004, O'Brien et al. 2014; 2015, Wigley et al. 2019). Thus, carbohydrate storage in roots mediates resprout capacity, making root starch storage a key trait for persisting in pyrogenic or herbivore-dominated savanna woodlands (Wigley et al. 2019). The importance of root starch reserves in mediating recovery between tree functional types may depend on the type (or extent) of disturbance. From the point of view of defoliation, fire and herbivory effects have been considered analogous (Bond & Keeley 2005), which may suggest that re-sprouting success should not be dependent on disturbance type (Zeppel et al. 2015). However, defoliated seedlings (depending on extent of damage) may have a higher recovery success than burnt seedlings (see Pausas et al. 2015). This may explain the difference observed between savanna-transition and forest seedling responses in the fire experiments (Chapter 3 and 4) and the defoliation experiment (Chapter 5). Under little to moderate disturbance (e.g. where substantial residual stem remains as in our experiment in **Chapter 5**) higher postdisturbance growth allocation may not be less beneficial than a conservative strategy of higher resource storage in roots (**Chapter 5**). This leads to the tantalising but yet untested idea that a conservative strategy may be more beneficial under severe disturbance, such as savanna fires (Chapters 2, 3 and 4), and less beneficial under little to moderate disturbance e.g. browsing herbivores. Further experiments and field observations will be needed here.

Re-sprout capacity is crucial for plants to thrive in a disturbance-prone environment such as the forest-savanna transition, which is why I emphasized the role of root starch in this discussion. It may, however, be more practical to discuss trait syndromes and how they relate to tree functional type recruitment success in general. Following the functional equilibrium hypothesis (Brouwer 1963), savanna-

transition species may be expected to invest more in plant organs for the capture (and storage) of resources belowground for resprouting whereas forest species invest in aboveground parts for the capture of light (see for a review of this notion Boonman et al. 2019). Indeed, it is possible to separate savanna-transition and forest species based on combined traits into higher belowground versus aboveground allocating species (**Chapter 5**, Boonman et al. 2019, Hoffmann & Franco 2003). The generally acquisitive nature (which is in trade-off with a conservative strategy) of forest species is thus likely related to their poorer survival under the constraints explored in this thesis (**Chapters 2, 3** and **4**).

Implications of differential tree functional type responses for forest-savanna dynamics and dry-forest restoration

From the foregoing, the point has been established now that the composition and structure of the different vegetation types (which constitute the forest-savanna mosaic) reflect filtering effects, on tree functional type, by top-down and bottom-up constraints (described in **Chapter 1**). Therefore, any changes in these vegetation controls may alter tree functional type recruitment patterns across the forest-savanna transition. Currently, the forest-savanna transition including adjacent dry forest in Ghana, are heavily degraded (**Figure 1.1b**, **Chapter 1** and see Janssen et al. 2018). Much of the deciduous forest zone has become prone to fires due to increased fragmentation and consequent large edge effects (see **Figure 6.1**, Dwomoh et al. 2019). The large-scale canopy disturbance (even in protected areas) makes influences of top-down controls (mainly fire) very strong, preventing recovery (through natural regeneration) of forest species. Forest and savanna vegetation are, therefore, relatively stable across the forest-savanna transition of Ghana (Janssen et al. 2018).

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Forest advance over savannas in many parts of Africa (Mitchard & Flintrop 2013) may suggest that vegetation controls that constrain establishment of forest species are being relaxed (examples have been discussed in the previous section). This thesis has shown that forest tree seedlings respond very strongly to fire (Chapters 3 and 4; also detailed discussed in detail in previous sections of this chapter). Fire exclusion experiments suggest that forest species can establish in humid savannas in the absence of fire (Laris 2011, Laris and Wardell 2006). However, since the natural distribution of vegetation types within the transition is associated with soil factors (Veenendaal et al. 2015), it is likely that forest species may only (re)establish where soil factors are favourable. My experiment (**Chapter** 5) on the role of lack of precipitation in the dry season did not show a negative dry season effect on forest seedling survival (details discussed in the previous section). However, soil moisture deficit has been previously shown to be related to greater mortality of forest tree seedlings in the forest savanna transition of Ghana (Cardoso et al. 2016). It is a matter of debate if soil nutrients limit the success (growth and survival) of forest species in savanna (e.g. Bond 2010, Ricardo et al. 2011, Lloyd & Veenendaal 2016). The exact role of soil nutrients on seedling establishment success, however, has not been tested in any consistent manner in this thesis and needs further investigation.

Mostly, in addition to favourable soil factors, a relaxed top-down control; e.g. fire is an additional requirement for forest seedling establishment, but the relative importance of such a (top-down) control may depend on tree functional type. For example, some dry forest (e.g. pioneer) species can establish in woodlands in the forest-savanna transition under prevailing fire regimes (Cardoso et al 2016). Also, an admixture of more fire tolerant and more fire resistant tree

species may co-occur depending the degree of canopy closure (Ametsitsi et al. *in prep*; Torrelo-Raventos et al. 2013, Veenendaal et al. 2015, Swaine 1992).



Figure 6.1. Active fire detections in 2016 from MODIS for the forest zone of Ghana. Active fires points (fires Km⁻² year⁻¹) presented do not include non-vegetation fires. Source: Dwomoh et al. (2019).

From a global change perspective, a steady reduction in precipitation is taking place for the transition zone of Ghana (Owusu & Waylen 2009), which may be linked to increased fire occurrences. Other elements of climate change (e.g. warming and CO₂ fertilisation; discussed in **Chapter 1**) may be important in determining the balance between tree-dominated versus grass-dominated vegetation types in the transition, with CO₂ increases likely to reduce the competitive advantage of grasses over trees (due to the C₄ pathway). The resulting interactions between these factors on tropical vegetation are at present not clear. I did not explore effects of these global change factors in this thesis, but it may be speculated that based on fire responses (**Chapters 3** and **4**), the forest species

used in this thesis may be more vulnerable to any increase in fire frequency or intensity due to climate change. Human land use change itself remains an important driver of change in the forest-savanna transition of Ghana. While human rural depopulation may be the cause of forest expansion over savannas in some parts of Africa (as earlier discussed), there is no evidence of emigration from the transition zone in densely populated Ghana. Instead, pressure on land is actually increasing due to population increase and rising demand for resources from the transition zone (Afikorah-Danquah S 1997).



Figure 6.2. A simplified model for managing tree seedling establishment in forest-savanna transitions. This model is modified from Figure 1.2 to show how key vegetation controls may influence recruitment success outside of closed canopy areas. The model shows that weed removal may eliminate need for fire protection. Optimal growth conditions (from availability of soil resources; water and nutrients) may also decrease fire effect on seedling survival due to linkages between plant size (determined by growth) and post-disturbance survival. Also the need for weed control and fire protection may be less for transitional

than forest tree species making the choice of species type a crucial decision for success in reforestation.

The findings in this thesis have important implications for dry forest restoration. By manipulating top-down controls (e.g. fire or weeds) through management, it may be possible to restore dry forests (see conceptual model in **Figure 6.2**). Weed removal is beneficial to the growth performance of seedlings of tropical tree species (**Chapter 4**, Hoffmann & Haridasan 2008 (**Figure 6.2**), but may be unfeasible outside a commercial forestry setting. Active fire protection may be needed and feasible but may strongly depend on tree functional type. Forest species may require many folds the sizes required by savanna-transition species to resist fire-induced mortality (**Chapters 3** and **4**, Gignoux et al. 2016). The obvious choice for species for recovery of dry forest based on trait differences is therefore with savanna-transition species.

Outlook

Insights into tree recruitment across tropical forest-savanna transitions are needed for understanding current patterns in vegetation distribution, future changes under various scenarios of climate and land use change and for vegetation management decisions. The responses of forest and savanna-transition tree species to top-down and bottom-up vegetation controls largely determine the recruitment success of species across the forest-savanna transition. In this thesis, I assessed seedling responses (of growth, survival and morphology traits) to some important vegetation controls. From the results presented and discussed for the various research chapters, savanna-transition tree species are more tolerant than forest species to many of the constraints assessed.

The mosaic appearance of the transition (which is likely to expand under increased disturbance) reflects a filtering of tree functional type into the different

vegetation formations. However, trait variation (e.g. in shade tolerance) among forest and savanna-transition tree species may explain the mixing of tree functional types in transition vegetation types. The effects of various vegetation controls assessed in this thesis may be intensified by changes in land use and climate, making future dynamics in the transition complex.

In the forest-savanna transition in Ghana, there have been large scale shifts from forest to more savanna-like vegetation. This reflects the effects of certain disturbance regimes, likely put in place by human land use change, which constrain the recruitment of tree species. It is clear from this study that forest species are worst affected by fire (the dominant disturbance factor) hence fire suppression is needed to attain recovery of some degree of forest cover, as shown in fire exclusion trials (Aubreville 1949, Louppe 1995, Veenendaal et al. 2018). Savanna-transition tree species, which are capable of forming forest-physiognomies (transition forests, Ametsitsi et al. in prep., Swaine 1992) provide a degree of some resilience. This speculation is based on the higher establishment found for savanna-transition tree seedlings under the constraints imposed in this thesis. There, however, is also evidence for the existence of these forests in the fire prone transition (and the dry semi-deciduous forest) zone (Torrelo-Raventus et al. 2013, Swaine et al 1976). Resilience from transition forests may be influenced by the scale of deforestation as this determines the extent to which vegetation controls (mainly top-down, e.g. fire) constrain seedling recruitment. This explains why pocket of (transition) forests remain only in protected areas across the transition (and including the adjacent semi-deciduous forest).

Responses of savanna-transition species in my experiments also suggest possibilities for restoration of degraded dry forests. One practical challenge is that many savanna-transition tree species fall within the timber category of "lesser-

known" or "lesser-used" species which had traditionally been given much less priority in forestry practice due to their lower economic value (Oteng-Amoako 2006). Recently, however, species such as *Khaya senegalensis*, *Daniella oliveri* and *Afzelia africana* are key timber species in construction while *Pterocarpus erinaceus* is now being exploited for export (Dumenu & Bandoh 2016). Additionally, species aforementioned are preferred for use as charcoal and thus very useful in woodlots. Therefore, reforesting the transition zone with its characteristic species pool is important not only for ecological reasons but for economic ones as well. The need for protection from fire and weeds (as shown in **Figure 6.2**) may make logistical demands high, but possible when commercial forestry interests are involved.

General conclusions and future research needs

I showed that canopy cover variation across the forest-savanna transition selects for different tree functional types, with survival in savanna woodlands, with high grass biomass, being contingent upon higher root allocation and root starch storage, while shade tolerance played a role in survival in the forest. To conclude that differential tree species establishment represents niche partitioning (as results suggest) may be overstretching the results of this study due to the limited number of species pairs used. An important research need, therefore, will be to expand the number of species pairs so as to obtain the full range of trait variation in response to the different constraints in the various vegetation types. This thesis showed that competitive interactions between tree seedlings and grass results in direct growth suppression of tree seedlings regardless of tree functional type. Competition may also indirectly (via root mass and starch reserves) decrease post-fire survival for forest, but not savanna-transition tree seedlings. This latter notion needs further
testing since a strong pattern existed but the effect was not found. Seedling trait responses suggested light limitation may drive competitive interaction between tree seedlings and grasses in tall grass savannas, but the relative effects of soil moisture and nutrients need to be separated in additional carefully manipulated common garden experiments and follow-up field tests.

Generally, forest species are more sensitive to fire than savanna-transition tree species. The dry season soil moisture deficit may not have been properly simulated in this study, but my results suggest that under field conditions in the absence of competition with larger trees, forest species seedlings can survive the dry season in the savanna as long as they are big enough for their roots to reach a declining water column. This may explain the presence of some forest species in the forest-savanna transition and even in savanna vegetation.

Finally, this thesis showed adverse synergistic effects of defoliation and soil moisture limitation on tree seedling growth performance irrespective of tree functional type. At the seedling stage, savanna-transition trees may just be as sensitive as forest species to moisture limitation. Different tree functional types invest differently in above- and belowground foraging and allocation traits, but we did not find evidence that increased conservation improved regrowth performance following defoliation. As a further step, it is important to investigate the relationships between disturbance type (e.g. fire versus defoliation) and seedling allocation traits on regrowth performance more thoroughly. Nevertheless, I predict an important role for transition species in the recovery (and resilience) of transition forest in the forest-savanna transition of West Africa.

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Summary

Vegetation transition has important implications for the carbon cycle, landatmosphere feedbacks and the livelihoods of millions of people, but it is still a poorly understood phenomenon. The retreat, advance and stability of forests have all been observed across the forest-savanna boundaries of Africa, suggesting a complex interplay between tree recruitment and the dynamics in vegetation controls (e.g. canopy cover, fire, herbivory, precipitation and soil resources). However, it has yet to be demonstrated how known vegetation controls interact to influence recruitment of different tree functional types, leading to the observed variation in patterns of tree recruitment and mosaic appearance of the forestsavanna transition. Differential tree functional type responses to vegetation controls may also be important for predicting future vegetation dynamics and useful, also, for reforestation of degraded dry forests. By combining common garden and greenhouse experiments, I explain how savanna-transition and forest tree species differ in their responses to a number of vegetation controls across a West African forest-savanna transition.

In the first research chapter of this thesis, I showed (from a three-year long field transplant experiment) that woody canopy cover variation was associated with variations in microsite related to the degree of tree cover. I found differences in seedling establishment success among tree species and functional types. Survival in savanna woodland was contingent upon higher biomass allocation and starch storage in roots while shade tolerance played a role in adjacent forest. There were also important species-specific differences within tree functional types which indicated the possible existence of "many shades of green" and may explain the observed variation in vegetation composition within the forest-savanna transition.

Summary

In the second research chapter, I showed (from a common garden experiment in the humid savanna of Ghana) that high grass biomass, characteristic of humid savannas, had adverse effects on wet season tree seedling growth performance for both forest and savanna-transition tree functional types. Thus, grass competition alone (in the absence of fire) may not prevent establishment success of forest or savanna-transition tree species in humid savannas, possibly explaining instances of forest species slowly encroaching humid savannas in firefree periods. Due to the relationship between seedling growth performance (and hence seedling size) and recovery from disturbance (e.g. fire), I explored the possibility of wet season growth suppression (via grass competition) influencing post-fire seedling survival in the same research chapter. Competition with grass decreased root mass (and therefore, total starch reserves in roots) for forest species but not savanna-transition tree species, and is therefore expected to induce differential post-fire survival response between the tree functional types. Although patterns indicated such a process, this could not be proven. This chapter therefore reveals possible interaction of wet season grass competition and dry season fire survival which needs further testing.

In the third research chapter, I demonstrated (using a common garden experiment in the humid savanna of Ghana) that the establishment of forest and savanna-transition tree species in humid savannas is most constrained by the combination of the dry season and fire. I attempted to disentangle the influences of these important vegetation drivers (which effects could not be separated in the previous chapters) to show that fire alone, largely explained failure of forest species, but not savanna-transition species, to establish in humid savanna. Irrigation increased survival of savanna-transition species, but not forest species.

The findings here explain why forest advance is often associated with fire suppression and why transition species dominate pyrogenic humid savannas.

In the fourth and final research chapter, I explored (in a greenhouse experiment) the interactive effects of low soil moisture regime and defoliation on (re)growth performance of forest and savanna-transition tree seedlings. Forest seedlings grew faster than savanna-transition tree species only at a later growth stage, but lower soil moisture and defoliation synergistically decreased growth performance for both tree functional types. Allocation patterns and root starch reserves differed between forest and savanna-transition tree functional types consistent with expectation, but the difference in traits was unrelated to regrowth of defoliated seedlings. Instead, regrowth performance was lower under a lower soil moisture regime. This chapter highlights the potential negative effect of lower precipitation on post-disturbance recovery of tropical tree species, which is relevant in the forest-savanna transition subjected to changes in both climate and land use change.

In the synthesis, I provided an outlook for changes in the forest-savanna transition and the potential for the restoration of dry forest with its characteristic species pool.

Samenvatting

Samenvatting

Vegetatieovergangen hebben belangrijke implicaties voor de koolstofcyclus, broeikaseffect en het levensonderhoud van miljoenen mensen, maar het is nog steeds een slecht begrepen fenomeen. De terugtrekking, uitbreiding, en stabiliteit van bossen zijn allemaal waargenomen in de bos-savannegrenzen van Afrika, wat een complex samenspel suggereert tussen de vestiging van bomen en de dynamiek in mechanismen die vegetatie controleren (e.g. kroonbedekking, vuur, vraatschade, nutriëntenbeschikbaarheid). Het is echter nog niet aangetoond hoe bekende vegetatiecontroles op elkaar inwerken om de vestiging van verschillende soorten boomtypes te beïnvloeden, wat leidt tot de waargenomen variatie in patronen van boomvestiging en de mozaïek van de overgang van bos naar savanne. Verschillende reacties van boomtypen op vegetatiecontroles kunnen ook belangrijk zijn voor het voorspellen van toekomstige vegetatiedynamiek en kunnen ook nuttig zijn voor herbebossing van aangetaste droge bossen. Door zogenoemde common garden-, en kasexperimenten te combineren, leg ik uit hoe savanne-overgang en bosboomsoorten verschillen in hun reacties op een aantal vegetatiecontroles in een West-Afrikaanse bos-savanne-overgang.

In het eerste onderzoekshoofdstuk van dit proefschrift laat ik zien (met een driejarig transplantatie-experiment) dat de variatie in de kroonbedekking van bossen geassocieerd was met variaties in microhabitat gerelateerd aan de mate van boombedekking. Ik vond verschillen in de mate van succes van vestiging van zaailingen tussen boomsoorten en functionele typen. Overleving in savanne was afhankelijk van hogere biomassa en opslag van zetmeel in wortels, terwijl schaduwtolerantie een rol speelde in aangrenzend bos. Er waren ook belangrijke soortspecifieke verschillen binnen functionele boomtypen die het mogelijke bestaan van "vele tinten groen" aanduiden en mogelijk de waargenomen variatie

in vegetatiesamenstelling binnen de overgangsgebieden van bos naar savanne verklaren.

In het tweede onderzoekshoofdstuk laat ik zien (in een commongardenexperiment in de vochtige savanne van Ghana) dat de karakteristieke hoge grasbiomassa van vochtige savannes nadelige effecten had op de groeiprestaties van boomzaailingen van functionele typen van zowel bos- als savannetransitieboomsoorttypen in natte seizoenen. Zo kan grasconcurrentie alleen (in afwezigheid van brand) het vestigingssucces van bosof savannetransitieboomsoorttypen in vochtige savannes niet verhinderen, wat mogelijk een verklaring kan zijn voor het feit dat bossoorttypen zich langzaam in de vochtige savannes kunnen vestigen gedurende brandvrije periodes. Vanwege de relatie tussen de groeiprestaties van zaailingen (en dus de grootte van zaailingen) en herstel na verstoring (bijv. brand), heb ik in hetzelfde hoofdstuk de mogelijkheid onderzocht van groeionderdrukking in het natte seizoen (via grascompetitie). Concurrentie met gras verminderde de wortelmassa (en dus de totale zetmeelreserves in wortels) voor bosboomsoorttypen, maar niet voor savannetransitieboomsoorttypen, en daarom wordt verwacht dat ze een verschillende overlevingsreactie zullen laten zien. Hoewel een dergelijk patroon leek te bestaan, kon dit niet worden bewezen. Dit hoofdstuk laat daarom een mogelijke interactie zien tussen grascompetitie in het natte seizoen en brandoverleving in het droge seizoen die verder moet worden getest.

In het derde onderzoekshoofdstuk heb ik aangetoond (met behulp van een common garden-experiment in de vochtige savanne van Ghana) dat de vestiging van bos- en savanne-transitieboomsoorttypen in vochtige savannes het meest wordt beperkt door de combinatie van het droge seizoen met vuur. Ik heb geprobeerd de invloeden van deze belangrijke drijfveren van vegetatieverandering

Samenvatting

(die in de voorgaande hoofdstukken niet van elkaar konden worden gescheiden) te ontwarren om aan te tonen dat vuur in vochtige savannes grotendeels het niet kunnen vestigen van bosboomsoorttype kon verklaren, maar niet voor savanneovergangssoorttypen. Irrigatie verhoogde de overleving van savannetransitieboomsoorttypen, maar niet van bosboomsoorttypen. Dit resultaat kan verklaren waarom bosopkomst vaak wordt geassocieerd met brandbestrijding en waarom overgangssoorten brandgevoelige, vochtige savannes domineren.

In het vierde en laatste hoofdstuk van het onderzoek onderzocht ik (in een kasexperiment) de interactieve effecten van een laag bodemvochtregime en ontbladering ор de (her-)groeiprestaties van bosen savannetransitieboomzaailingen. Boszaailingen groeiden pas in een latere groeifase sneller savanne-overgangsboomsoorten, maar lagere bodemvochtigheid dan en ontbladering samen verminderden de groeiprestaties synergetisch voor beide functionele typen. Biomassa-opslagpatronen en wortelzetmeelreserves verschilden, zoals verwacht, tussen de bos- en savanne-transitieboomsoorttypen, maar het verschil in die eigenschappen kon de hergroei van ontbladerde zaailingen niet verklaren. In plaats daarvan waren de hergroeiprestaties lager bij een lager bodemvochtregime. Dit hoofdstuk schijnt licht op het potentiële negatieve effect van lagere neerslag op het herstel na verstoring van tropische boomsoorten, wat relevant is in de overgang van bos naar savanne die onderhevig is aan veranderingen in zowel klimaat- als landgebruiksverandering.

In de synthese heb ik vooruitzichten gegeven voor veranderingen in de overgang van bos naar savanne en het potentieel voor het herstel van droog bos met zijn karakteristieke soortengemeenschap.

Acknowledgments

Looking back on my entire PhD journey, I am reminded of an African proverb in Chinua Achebe's "*Things Fall Apart*" that "Those whose palm-kernels were cracked for them by a benevolent spirit should not forget to be humble". I interpret "benevolent spirit" as all of the support I got from so many people and institutions which made the completion of my PhD possible and in all humility, I wish to acknowledge these contributions.

My father, Chief D.M. Issifu, supported my education. My dear mother encouraged me to carry on with this PhD with the promise of lending support to the care of my children anytime I had to leave home. Unfortunately, mum did not live to see the end of my PhD journey. I dedicate this thesis to her memory.

I am grateful to Wageningen University for funding my PhD under the WU Sandwich PhD fellowship. It was several weeks of formulating ideas and putting together a preproposal which was finally awarded. I still remember the words of Elmar over the telephone: "Hi Hamza! Are you standing or sitting?" This was how Elmar announced that our proposal for a WU PhD fellowship was awarded. Without you, Elmar, none of these would be possible (at least not in their present forms) and for all the efforts you put in to make my PhD a reality, I am grateful to you. Thanks, Rose (Mrs Dr Veenendaal) for making me feel at home. Thanks for the food and drinks and the stories you shared, and more importantly, your pep talks about family and life in general.

Moving from the original ideas in the preproposal to the conduct of various experiments, to writing the various research chapters in this thesis took several meetings, both physical and virtual. For all of your time and efforts invested, I am grateful to you, my supervisors; Frank, Elmar and Philippine and to David (Chair holder, PEN). You took keen interest in my experiments and painstakingly read

through the draft manuscripts. The feedback you provided greatly improved this thesis. Thanks, Elmar for pushing me really hard to bring out the best in me and for providing me with an extensive network of (future) collaborators. Thanks, Frank and Philippine for my knowledge in statistics, and thanks Philippine for insisting on R. Thanks also to David and to all of you, my supervisors, for helping me navigate the elaborate administrative procedures throughout my PhD journey.

I am grateful to the Academy Ecology Fund of the Netherlands Academy of Arts and Sciences (KNAW) for awarding me two grants which helped in the completion of field work. I thank NUFFIC for their financial contribution to an experiment I set up in Ghana for my MSc thesis which continued as part of my PhD research.

For making the office the best possible work environment and for helping me through administrative procedures, I thank you, Petra. Thank you Gerda, for patiently guiding me through project financial accounting. Jan, Frans and Hennie deserve special mention. From the coffee table to the lab, you were an important part of my PhD life. Special thanks to Jan, for "endlessly" analysing my root samples without complain. To all staff of PEN, your various contributions to my work during lab meetings and also unofficially at the coffee table were important to me, thank you.

To my colleagues at PEN (both former and present); George, Lisette, Natalie, Marinka, Thijs, Peng, Wei, Robert, Maaike, Davide, Jose, Sina, Runa, Eline, Maartje, Zakaria, Miguel, Aaron, Maarten, Chen, Pengyao and Zulin, it was fantastic interacting with you all. Thanks for your inputs into my work during lab meetings and PhDs/Postdocs meetings. I take with me fond memories of our game nights and the stories we shared at the coffee table. George, your organisational skills are unmatched. Thanks for your tremendous support during field work and

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for all the materials and tips you shared. Special thanks to Jose for my beautiful thesis cover and to Thijs for editing my Dutch summary and for the tips and pieces of advice you provided. My friends; Erlinda, Marjoleijn, Luuk, Taufic, Cor, Isabella and Raldi you have made my long stay in Wageningen and the Netherlands very memorable. Thanks, Lana and Mark, for the many dinners and game nights.

Special thanks to the elders of the Ghanaian community in Wageningen (Sikaman); Uncle Samson and Auntie Monica (and their supportive partners) and to the entire Sikaman. Special thank you to Brain, Fulera, Confidence and Nancy for the many dinners and "talking points".

Throughout my PhD research, I collaborated with many people in Ghana. Hypolite helped with managing and co-supervising students on my PhD project. Thank you Vincent and the SOFIA team for everything I learnt from you, for my soil data, and for providing me with a network. Steven and Gloria of the Forestry Research Institute of Ghana, thank you. Thanks to the many former MSc students of Wageningen University who worked with me in Ghana; Jaap, Gerard, Maaike, Lana, Jelle, Paolo and Millicent. Thanks for the many days in the field/lab and many nights on data entry. Thank you Nuni Ferawati and José A. Medina-Vega for helping at Kogyae Strict Nature Reserve. My former BSc thesis students (at the UDS), thanks for your various contributions to the experiments in Ghana. Taju, ATM, James, Adnan, Paul, Afa Nuhu, Afa Baba, thanks for your help during field work. Richard, thanks for being exceptionally hardworking.

The Wildlife Division of the Forestry Commission of Ghana gave permission for field work in Kogyae Strict Nature Reserve (KSNR) and Mole National Park. Staff at the Dome Camp of KSNR thank you for assisting in field work: thanks to Shieq, Ajaku, Sarpong, Sina, Alhaji, Baswa, Captain, and others for your incredible support during field work. Special thanks to Bla Shieq for always volunteering to

Acknowledgements

cook for us and to Ajaku and Emmanuel for your tremendous help in seed collection. Ali and Jaward, thanks for the assistance you provided to me and my visitors at the Mole National Park.

I received tremendous support and encouragement from Deans of Faculty of Natural Resources and Environment and HoDs of the department of Forestry and Forest Resources Management at the UDS. Thanks, Prof. Oppong and Prof. Alhassan, for encouraging and supporting my PhD application and for hosting my visitors. Thanks, William, Bernard and Rikiatu and to other colleagues in the department: Kwame, Damian and Latif. I am grateful to the entire FNRE and the UDS for this opportunity. UDS International Relations and Advancement provided support documents for my visitors, thank you.

I enjoyed tremendous family support in this journey. Nazia, my dear wife, several times in the past five years, I have had to leave you and the children, sometimes for many months. In my absence, you were the mother and father, and the driver, and the maintenance woman. Thank you for enduring those lonely nights and for all the times you stayed awake to bring down those fevers on your own. Thank you for your sacrifices in supporting our dream. Thank you Abdala for always showing the way. I have become what I am today because you provided a mirroring opportunity. Thank you, big brother Ibrahim, several times you hosted me (and some of my visitors) in Accra and facilitated our movements to and from the Airport. I remember our regular visits to the Netherlands Embassy in Accra. Thank you for the role you played in this journey. Sule, Hamisu, JB, uncle Abudu, you stepped in to perform some of my obligations while I was away, thank you.

I belonged to the graduate school, PE&RC run by some incredibly brilliant people. Thanks Claudius and Lennart for the information and pieces of advice you offered and for your role in resolving (the few) sticky situations in this journey.

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I realise I cannot thank people enough and I may have left out some very important names in this acknowledgements. Thanks to anyone, not mentioned here, who has played a role in my PhD journey. *Asa ne kushun*!

About the Author

Hamza Issifu was born on the 7th of June 1983 in Makango, a small farming and fishing community fringing the Volta Lake in northern Ghana. At 8 years old, his family moved across and settled at the other side of the Volta Lake in Yeji where Hamza had his basic education. Hamza's initial interests were in fisheries but



he also spent a lot of his off-school hours in the savanna fetching garden sticks for his annual vegetable and corn backyard garden. As a result, Hamza developed an early interest in savanna vegetation. Hamza offered courses in general agriculture at Salaga Senior Secondary School and went on to study for a BSc in Agriculture Technology (with options in Renewable Natural Resources) at the University for Development Studies (UDS) in Tamale, Ghana. After his mandatory national service as a Teaching Assistant with the department of Forestry and Forest Resources Management at the UDS, Hamza was employed as a Research Assistant in the same department in 2009.

In 2011, Hamza won a scholarship from the Netherlands Fellowship Programme (NFP) to study for MSc Forest and Nature Conservation (Ecology specialisation) at Wageningen University in The Netherlands. He returned to work at the UDS as an Assistant Lecturer, where he taught ecology to undergraduates. In 2015, Hamza won a Wageningen University sandwich PhD fellowship to conduct PhD research with the Plant Ecology and Nature Conservation group (PEN) and the Resource Ecology group (now Wildlife Ecology and Conservation group) of Wageningen University.

Hamza has been married to Nazia since 2010 and now has three children: Bassapor-Enya, Matuwe and Tuntumba.

Selected publications

- Issifu, H., Ametsitsi, G. K. D., de Vries, L. J., Djagbletey, G. D., Adu-Bredu, S., Vergeer, P., van Langevelde, F. and Veenendaal, E. (2019). Variation in vegetation cover and seedling performance of tree species in a forestsavanna ecotone. *Journal of Tropical Ecology*, 35:74-82.
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Affiliations of co-authors

Frank van Langevelde

Wildlife Ecology and Conservation group, Wageningen University, the Netherlands

School of Life Sciences, Westville Campus, University of KwaZulu-Natal, Durban 4000, South Africa

Elmar Veenendaal

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

Philippine Vergeer

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

Vincent Logah

Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Hypolite Bayor

Department of Horticulture, University for Development Studies, Tamale, Ghana

Steven Adu-Bredu

CSIR-Forestry Research Institute of Ghana, Kumasi, Ghana

Gloria Djagbletey

CSIR-Forestry Research Institute of Ghana, Kumasi, Ghana

George KD Ametsitsi

CSIR-Forestry Research Institute of Ghana, Kumasi, Ghana

Lana J. de Vries

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

Jaap Hamelink

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

Maaike Pyck

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

Okonkwo Gerard Emmanuel

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

Paolo Sartorelli

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

Millicent Tanson

Plant Ecology and Nature Conservation group, Wageningen University, the Netherlands

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (5.6 ECTS)

- A review of the responses of tree functional types to vegetation controls in forest savanna transitions

Writing of Project proposal (4.5 ECTS)

- Many shades of green: disentangling tree seedling functional type responses to drought, fire and soil factors in Ghana

Post-graduate courses (5.2 ECTS)

- Training of PhDs in vegetation of forest islands and soil hydrology; Mole National Park, Ghana (2016)
- Linear models; PE&RC/SENSE (2017)
- Generalise linear models; PE&RC/SENSE (2017)
- Mixed models; PE&RC/SENSE (2017)
- Introduction to R for statistical analysis; PE&RC/WIMEK (2019)

Competence strengthening / skills courses (2.2 ECTS)

- Brain training; WGS (2015)
- Information literacy including Endnote introduction; WGS (2015)
- Capacity building in scientific writing and research planning; Science of Forest Islands in West Africa, KNUST, Ghana (2015)
- Information on multi-function software (Mendeley) and ethical issues in research; Faculty of Natural Resources and Environment and UDS Library, Tamale, Ghana (2016)

Scientific integrity / ethics in science activity (1.5 ECTS)

- Ethics and philosophy in life science; WGS (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.8 ECTS)

- PE&RC First year weekend (2015)
- PE&RC Day (2016)
- PE&RC Last year weekend (2019)

Discussion groups / local seminars / other scientific meetings (5.4 ECTS)

- Ecological theory and applications discussion group (2015-2016)
- Wageningen fire network meeting (2016)
- Annual interdisciplinary conference; oral presentation; UDS, Tamale, Ghana (2018)
- 44th New Phytologist symposium; poster presentation; Accra Ghana (2019)

International symposia, workshops and conferences (8.8 ECTS)

- Netherlands annual ecology meeting; poster presentation; Lunteren, the Netherlands (2016)
- British ecological society annual meeting; poster presentation; Belfast, UK (2019)

Lecturing / supervision of practicals / tutorials (9.6 ECTS)

- Basic ecology (2015-2019)

Supervision of MSc students (12 ECTS)

- Jaap Hamelink: synergistic effects of drought and fire on tree seedlings of the forest-savanna transition zone (2017)
- Maaike Pyck: belowground responses to drought and fire in the forestsavanna transitions, Ghana (2018)
- Lana J. de Vries: nutrient and polyacrylamide hydrogel addition improve establishment of dry tropical forest tree seedlings on the savanna in Ghana (2017)
- Okonkwo Gerard Emmanuel: above and belowground seedling traits determine drought survival of tree species of the forest-savanna transition zone (2017)
- Paolo Sartorelli: an examination of two experiments with tropical tree seedlings in Ghana (2019)

This PhD was funded by Wageningen University Sandwich PhD fellowship. Research presented in this thesis was, at various stages, supported financially by NUFFIC and Academy Ecology Funds of the KNAW.

Wageningen University also financed the printing of this thesis.

Thesis cover designed by

Jose van Paassen, Plant Ecology and Nature Conservation Group, Wageningen

University & Research

Thesis printed by

ProefschriftMaken, the Netherlands
