Afrotropical secondary forests exhibit fast diversity and functional recovery, but slow compositional and carbon recovery after shifting cultivation

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Abstract

Question: Human disturbance is increasingly affecting forest dynamics across the tropics. Forests can recover via natural secondary succession to pre-disturbance levels of biodiversity, species
composition, and ecosystem carbon stocks. Central Africa will be subject to increasingly high shifting cultivation pressure in the next decades, but succession trajectories of these ecosystem properties are still poorly known for the Congo basin. We addressed two questions: (1) how does taxonomic and functional composition and diversity shift during secondary succession? (2) How fast do aboveground carbon stocks recover during secondary succession in tropical forests?

**Location:** Central Congo basin

**Methods:** We conducted an inventory of trees (DBH $\geq 10$ cm diameter), measured species traits and soil texture and carbon content in 18 plots, located along six secondary succession stages (i.e. from agricultural to old growth forest sites). We measured tree diameter, height for 20% of trees distributed across diameter classes, wood traits from all species, and leaf traits from species that contributed to 85% of the plot basal area.

**Results:** We showed that secondary forests recover relatively fast in terms of tree species diversity, alpha functional diversity, and fine root carbon, with near-old-growth forest values after six decades past disturbance, while floristic composition exhibited slower recovery. Secondary forests only partially shifted from acquisitive to a conservative life-history, with shifts in leaf traits being largely decoupled from wood traits. Only 43% of above-ground carbon recovered after 60 years of forest regrowth, potentially through a slow recovery of the large-sized tree stems that dominate carbon stocks of old-growth forests.

**Conclusions:** Our findings underline the capacity of Afrotropical forests to recover species and alpha functional diversity after clear-cutting through shifting cultivation. Simultaneously, old-growth forests harbors a particular floristic community and store a large quantity of carbon with much longer recovery trajectories, stressing the need for conservation of these forests in the Congo Basin.

**Keywords:** Carbon stocks, Central Africa, Congo Basin, Functional composition, Natural regeneration, Secondary succession, Species diversity and composition, shifting cultivation.

**Introduction**

Tropical forests are globally important for hosting biodiversity and storing substantial amounts of carbon (Beer *et al.*, 2010; Pan *et al.*, 2011; Slik *et al.*, 2015), but they are vulnerable to the effects of global changes. Increased land-use change, in particular, affects large areas of tropical biomes
and will become increasingly important in the future (Boit et al., 2016), especially by means of three main anthropogenic drivers of tropical forest loss, namely agriculture, resource extraction, and infrastructure building (Curtis et al., 2018; Tyukavina et al., 2018). In Central Africa, shifting cultivation has been identified as the main and single-most important driver of deforestation (Curtis et al., 2018; Tyukavina et al., 2018). The sustainability and impact of slash-and-burn agriculture on tropical forest carbon and biodiversity have been a subject of much debate (Moonen et al., 2016; Tyukavina et al., 2018). Shifting cultivation can induce major forest losses as a result of rapid expansion of rural complex area (1% year\(^{-1}\)), turning a forest landscape into a mosaic of roads, villages, agriculture and fallow fields, and secondary forest (Molinario et al. 2015). At the scale of the Congo basin, forest clearing for agriculture accounts for 84% of forest disturbances, and shifting cultivation contributes to almost two-thirds of the deforestation rate in the Democratic Republic of the Congo specifically (Tyukavina et al., 2018).

Traditional shifting cultivation is characterized by an alternation of crops and fallow periods. This causes a mosaic of naturally regenerating, secondary forests, leftover old-growth forests (OG), and agricultural fields. Chronosequence studies essentially rely on this mosaic of stands of different ages (Richards, 1955) as a space-for-time observational setup for forest recovery. During secondary succession, the forest evolves directionally towards a pre-disturbance, i.e. OG-like condition in terms of composition, structure, diversity, and biomass following a disturbance event (Chazdon, 2008; Derroire, 2016). The development trajectory of ecosystem characteristics, such as biomass stocks and net productivity, is ultimately the result of complex interactions of both abiotic and biotic factors, which drive the rate of recovery of forest succession. Many studies have focused on South American forest regrowth (Letcher and Chazdon, 2009; Lohbeck et al., 2013; Poorter et al., 2016; Rozendaal et al., 2019). Neotropical secondary forests potentially recover more than 80% of the above-ground biomass and 100% of the alpha diversity of the pre-disturbance status after 50 years (Martin, Newton and Bullock, 2013; Poorter et al., 2016; Rozendaal et al., 2019). The functional composition of Neotropical wet forests shifts from the dominance of species with an acquisitive strategy with, among others, a high specific leaf area (SLA), low leaf dry matter content (LDMC), low wood density (WD), in the early successional stages when light is less limiting, to the dominance of species with a conservative strategy exhibiting a low SLA, high LDMC and high WD (Poorter et al., 2004; Lohbeck et al., 2013; Boukili and Chazdon, 2017). However, studies have clearly shown that the Congo basin is
different from Neotropical forests in terms of structure, carbon stocks, and species composition (Lewis et al. 2009; Slik et al. 2015). Despite these differences, central African forest - the second largest contiguous block of tropical forest - is still widely understudied (Verbeeck, Boeckx and Steppe, 2011). Only a handful of studies have focused on the recovery potential of C stocks, biodiversity, and the environmental consequences of the traditional slash-and-burn agriculture for the central Congo basin forest (Mukul, Herbohn and Firn, 2016; Bauters et al., 2019; Moonen et al., 2019; Bauters et al., 2021). This lack of studies complicates the evaluation of forest recovery potential in the central Congo basin. Consequently, in the Democratic Republic of the Congo, policymakers lack important knowledge for the implementation of forest restoration policies. Especially in the light of a four-fold projected population expansion by 2100 on the African continent (Gerland et al., 2014), a good understanding of landscape-dynamics will become increasingly important for food security, forest management, biodiversity conservation planning and climate mitigation policies.

In this study, we aimed to quantify recovery rates of biodiversity and carbon storage of natural forest regeneration after shifting cultivation disturbance in the central Congo basin, Democratic Republic of the Congo in the African continent. Understanding how carbon stocks, plant community composition, and diversity of plant functional traits recover after slash-and-burn events in this region is important to improve knowledge on African forest resilience to anthropogenic disturbances and to complement information from other tropical regions. Therefore, we surveyed semi-deciduous lowland tropical forests in the central Congo Basin, along a chronosequence ranging from 5 to 60 years old secondary forests, also considering agriculture fields and old-growth forest as start and end points of succession, respectively. Specifically, we addressed two questions: (1) how does taxonomic and functional composition and diversity shift during secondary succession? In addition (2) how fast do carbon stocks recover during secondary succession in tropical forests? We expected fast increase of both taxonomic and functional diversity and carbon but slow recovery of floristic composition with forest age as have been shown for the Neotropics (Brown and Lugo, 1990; Chazdon et al., 2007; Rozendaal et al., 2019). Furthermore, we expected a shift from a tree community with acquisitive traits and high growth rate to a community with conservative traits, as has been shown for the Neotropics (Lohbeck et al., 2013). To test these hypotheses, we used different metrics of taxonomic and functional diversity, community taxonomic and functional composition and carbon stocks along succession.
Methods

Study area

The study was carried out in post-agriculture forests at different growth stages in and around the forest reserve of Yoko (00°17′ N; 25°18′ E; mean elevation 435 m a.s.l.), situated between 29 to 39 km Southeast of Kisangani, in the Democratic Republic of the Congo (Appendix S1). The OG vegetation in the region is classified as semi-deciduous tropical forest and is dominated by the mixed lowland tropical forest at our study site. Climate falls within the Af-type following the Köppen-Geiger classification (Peel, Finlayson and McMahon, 2007). Annual rainfall ranges from 1418 to 1915 mm with mean monthly temperatures varying from 23.7°C to 26.2°C. Throughout the year, the region is marked by a long and a short rainy season interrupted by two small dry seasons in December-January and June-August, respectively. Soils in the region are highly weathered ferralsols, being poor in nutrients (Van Ranst et al., 2010), with low pH and generally characterized by a sandy texture. Specifically, our plots were installed on sandy soils (Appendix S2).

Vegetation survey

The study was conducted in a set of 18 plots of 40 x 40 m (Appendix S1). We used small plot sizes because the shifting cultivation field sizes are also small, i.e. typically ca. 0.5 ha (Wilkie and Finn, 1990). The plots were arranged in triplicate along six successional stages: agricultural sites, 5, 12, 20, 60 years old secondary forest, and old-growth forest (hereafter 5yr, 12yr, 20yr, 60yr, OG). We conducted a tree inventory in 15 plots (from 5yr to OG, because agricultural sites had no trees) while the soil was characterized in all plots (i.e., including agricultural sites). We interviewed owners, farmers, and local experts to determine the time-since-disturbance of all plots. Thus, we visited all the plots with the landowners and local experts and selected each plot, if (1) we were able to find at least three plots of the same age and land-use history (i.e. cut only once), and (2) plots did not show signs of disturbances (i.e. trees harvested, etc.) since abandonment. The plots of different age classes were well spread over the landscape matrix in the study area except for an apparent spatial clustering of 12yr and OG plots due to the availability of these stages in the landscape (Appendix S2). Distance between plots and stages were variable, because for the younger plots, we used real and existing farmer’s owned plots. The minimum distance between
stages was 78 m (agriculture and 5yr) and maximum was 8373 m (5yr and 20yr). The minimum distance within plots of the same stages was 102 m (OG1 and OG2) and the maximum was 7155 m (20yr1 and 20 yr3). The forest inventory was conducted according to the RAINFOR protocol, an international standardized protocol for tropical forest inventories (Malhi et al., 2002). In short, all tree individuals with a diameter larger or equal to 10 cm at 1.3 m height parallel to the stem were identified at the species level and their diameter recorded. All species names were corrected according to the Taxonomic Name Resolution Service (TNRS, http://tnrs.iplantcollaborative.org/), which is incorporated in the function correctTaxo of the BIOMASS package in R (Réjou-Méchain et al., 2017; R Development Core Team, 2018). Tree height measurements were recorded at the plot level for 20% of individuals of each diameter class, using a hypsometer (Nikon Forestry Pro, Nikon, Japan). Within each diameter class, individual trees were selected randomly.

Functional trait measurements

We selected key leaf and wood traits for the functional community analyses. Specific leaf area (SLA) is a key leaf trait, which co-varies with leaf photosynthesis performance. Leaf dry matter content (LDMC) and leaf N content (LNC) (Wright et al., 2004; Reich, 2014) are related to primary production, carbon and nutrient cycling, and litter decomposition (Reich, 2014). Wood density (WD) is a key trait for growth rates and turnover rates of the wood (Chave et al., 2009). The leaf phosphorus content (LPC), leaf C:N and N:P ratios were included in this study to reflect the canopy stoichiometry. Leaf samples were collected from the most abundant species, i.e. the species that, together, make up a minimum of 85% of the standing basal area per plot (Pakeman and Quested, 2007; Lohbeck et al., 2013). For the selected species, samples were collected from at least three individuals (see Appendix S3) because of logistical limitations on the field. Fresh leaves needed to be sampled by tree climbers, which is highly time-consuming. Leaf traits were measured on mature and fully sunlit leaves collected following internationally accepted protocols (Pérez-Harguindeguy et al., 2013). Leaf fresh weight and leaf area were measured with no rehydration on the same day leaves were collected. The fresh weight of our samples was not affected because we were weighing samples directly after the fieldwork. The study sites were located at maximum 30 minutes of our field lab. Moreover, reliable measurement of some leaf traits such as SLA are still possible on highly dried leaves as those from herbarium (Perez et al., 2020). In the meantime, the samples were sun dried at the field lab and then, in less than 4 days.
after collection, leaves were oven-dried at 80°C for 48h, weighed again, and prepared for future chemical analyses. Leaf area was measured by the Easy Leaf Area (ELA) App for android (Easlon and Bloom, 2014). The ELA algorithm estimates the leaf area regardless the assessment of camera distance and focal length measuring (see Appendix S4 for more information). We then calculated SLA per tree individual by dividing the total leaf area by total leaf dry mass and determined LDMC by dividing leaf dry mass by leaf fresh mass. Leaf samples were further analyzed for leaf carbon content (LCC) and LNC using an elemental analyzer (ANCA-SL, SerCon, UK), interfaced with an isotope ratio mass spectrometer (IRMS) (20-22, SerCon, UK). Finally, LPC was obtained by dry-ashing 0.5 g of plant material for 5h at 550°C. Subsequently, the resulting ash was dissolved in 5 ml HCl (2 M) which was further diluted in a flask to 25 ml, and filtered over a P-free filter paper. The P was subsequently measured colorimetrically in the extract according to the ascorbic acid technique (John, 1970) on an autoanalyzer (AA3; Bran and Luebbe, Norderstedt, Germany). In this paper, all C, N, and P ratios that are reported are mass-based stoichiometric ratios and not molar ratios.

For WD determination, two wood cores were sampled from the opposite sides of individual trees at the point of DBH measurement and collected from all the surveyed species, outside of the permanent monitoring plots (Chave, 2006). The wood cores were first measured for green volume by the water displacement method (WDM) method, subsequently oven-dried for 48 h at 105°C, and weighed again to obtain the oven-dry mass. Subsequently, we calculated the wood density as the ratio of wood oven-dried mass by the mass of water displaced by the wood sample (Chave, 2006; Pérez-Harguindeguy et al., 2013).

Standing fine root stocks (diameter ≤ 2 mm) were sampled following an established protocol (Metcalfe et al., 2007), with three soil cores (diameter 12 cm and depth 30 cm) per plot. The roots were extracted manually in the field for 40 minutes per core, divided into a sampling period of 10 minutes intervals each to further assess the number of fine roots that were not collected (Marthews et al., 2014). Next, the root samples were oven-dried to constant mass at 80°C and kept for further analyses.

Soil property measurements
We collected the soil samples within each plot at five depth increments (0–10, 10–20, 20–30, 30–50, and 50–100 cm). The samples for bulk density were collected at three different places and dried to constant weight at 105°C. Composite soil samples for chemical analyses were collected at five different profile, dried at 40°C for 48 h, ground, and sieved over a 2 mm mesh for analyses. The soil samples for C were combusted at 1200°C and measured by a thermal conductivity detector in a CNS elemental analyzer (Vario Macro Cube, Elementar, Germany). The soil texture was analyzed by the pipet method (Burt, 2004). Organic matter was removed with H2O2 30% and soil particles dispersed by Na4P2O7. 10H2O.

**Taxonomic and functional composition and diversity**

We opted to calculate multiple diversity indices to get a deeper insight in the important aspects of diversity at play in the study system (Morris et al., 2014). Four alpha diversity metrics (see Appendix S5 for description of Shannon index, Pielou’s evenness and unique species) were calculated to assess the shift of species diversity across the chronosequence. The functional diversity is a multi-faceted ecological concept (Carmona et al., 2016), generally characterized in terms of three descriptors: functional richness (FRic), evenness (FEve), and divergence (FDiv) (Mason et al., 2005; Villéger et al., 2008; see Appendix S5 for further information). We calculated functional diversity indexes using the function dbFD of the ‘FD’ package in R (Laliberté, Legendre and Shipley, 2017). These functional diversity indexes were calculated at the plot level based on species key leaf and wood economic spectrum traits including SLA, WD, LPC, leaf carbon to nitrogen ratio (C:N), and leaf nitrogen to phosphorous ratio (N:P). We further calculated the community-weighted mean (CWM) for all measured functional traits per plot, weighed with the basal area per species per plot (Garnier et al., 2004). Species were further grouped according to their light requirement for growth into three functional groups according to the literature (Hawthorne, 1995) – pioneer species, non-pioneer light-demanding species, and shade tolerant species – and their relative abundance calculated per successional stage. Functional diversity (FD) and community weighed mean (CWM) were analyzed to assess the potential for complementarity and resilience and to get an idea of the functional response trajectory of the community, respectively.
Carbon stocks

We used 16 published diameter-height models (Brown, Gillespie and Lugo, 1989; Banin et al., 2012; Feldpausch et al., 2012) to parameterize a diameter-height relation, based on our DBH and height data (Appendix S6). The best-fitting model was selected at the plot level based on the Akaike information criterion (AIC) and was then used to estimate the tree height of all individual trees. Subsequently, above-ground biomass (AGB) was estimated for every individual of each tree species by a pan-tropical tree allometric relationship including height, DBH, and WD (Chave et al., 2014). The conversion of AGB to above-ground carbon (AGC) was conducted under the assumption that wood carbon content is 50% of wood biomass (Chave et al., 2005). The total number of stem per plot was calculated as the sum of all living trees with a diameter equal or larger than 10 cm at 1.3 m height. The maximum tree height at the plot-scale was estimated by averaging the height of the three highest trees in each plot (see Bauters et al., 2019).

The soil organic carbon (SOC) was determined for the five increment depths as the product of mass-based soil organic carbon content, bulk density, and thickness of the increment layer, and extrapolated at the plot-scale surface area (1 ha). Subsequently, SOC to a depth of 1 m was quantified for each plot by summing the SOC stocks per depth increment.

Data analyses

We used non-metric multidimensional scaling (NMDS) to visualize the difference in community composition between the successional stages. We used perMANOVA with Bray-Curtis dissimilarity by means of the adonis function in R package vegan (Oksanen et al., 2013; Anderson, 2017) to test whether species composition differed between successional stages. For this, we used the successional stages as explanatory variable and species abundances as a response matrix. Further, based on the Bray-Curtis dissimilarity index we estimated the relative recovery rate of species composition. From the dissimilarity index, we computed the similarity as 1 minus Bray-Curtis. Therefore, we performed pairwise floristic similarity comparisons between plots and computed an average floristic similarity per successional stage. The mean floristic similarity of the secondary forest to OG was used to estimate the relative recovery of species composition.
To test for variation in alpha diversity metrics, community trait means, AGC, and SOC through forest succession, we used ANOVA, followed by Tukey HSD post hoc tests when the difference was significant. When assumptions were not met, we applied a non-parametric Kruskal-Wallis test, and subsequently, the post-hoc Dunn’s test in case the Kruskal test was significant. A simple linear regression model was used to explore the link between the density of large-size stems (DBH ≥ 60 cm) to the plots AGC. All the statistical analyses were performed using the R programming language 3.6.1 (R Development core 2018).

Results

In total, we recorded 1178 stems belonging to 107 species, 89 genera, and 34 families (Appendix S7). The five successional stages exhibited largely divergent structural properties. Stem density decreased from younger fallow/forest plots to old-growth forest plots, while mean tree diameter, maximum tree height, and BA increased significantly (Appendix S2). We noticed a high variability in the density of stems for the 20 years successional stage. The density of large-sized trees (defined here as trees with DBH ≥ 60 cm) was highest in the old-growth forest (33.3 ± 3.6 stems ha⁻¹), followed by 60 years old forest (10.4 ± 13.3 stems ha⁻¹), while up to 20 years, the large tree density was lower than 3 stems ha⁻¹ (Appendix S2). Along the successional gradients, the relative proportion of DBH ≥ 60 cm trees increased from 0.3 % in 5 years old forest to 8.6 % in the old-growth forest (Appendix S2).

There were large differences in species composition among the five successional stages. The NMDS displays different vegetation communities separated along the first axis (Figure 1A). In general, the floristic composition recovered slowly along the secondary succession (Figure 1A, B). However, between 20 and 60 years there was an apparent rapid species turnover. Absolute recovery of rarefied species richness (Figure 1C, Appendix S8) and functional richness (Figure 1D) increased significantly with time since disturbance until 60 years (Figure 2). For all analyzed parameters of alpha taxonomical and functional diversity, we did not observe differences between 60 years and old-growth forests (Figure 1C, D, Appendix S9A, B).

Leaf traits responded differently to forest age. Leaf LPC, LDMC (Appendix S9C), and N:P (Figure 2A) did not change significantly along the successional stages. However, both LNC (Appendix S9C), SLA, and WD (Figure 2A) significantly increased [with high values in the late-
successional stages] while the C:N ratio (Figure 2A) decreased along successional gradients. The SLA community means in the early successional are, however, impacted by the low values observed for Musanga ceropioides (see Appendix S9). The classification of the species according to their light demand for growth and establishment showed an abrupt decrease for pioneer species, an increase of pioneer light-demanding species, and an abrupt increase of shade tolerant species after the 20 first years of succession (Figure 2B).

AGC and fine root carbon increased significantly with the age of the successional stages, while soil organic carbon stocks were not affected by the successional stage (Figure 3, Appendix S10). After 60 years, the secondary forest only recovered 43% AGC of the old-growth forest. Large-size trees contributed proportionally more to carbon storage in late-successional stages; they stored 24% and 72% of the AGC in the 60 years forest and the old-growth forest, respectively (Figure 3A). Large-size trees explain 81% of AGC variation across the plots (Figure 3B). Fine root carbon stock increased significantly along the succession gradient (Figure 3C).

Discussion

In this study, we have assessed recovery trajectories of structure, composition and carbon stocks of secondary forests in the central Congo basin. Our main findings were that: 1) successional changes in forest structure were marked by the slow recovery of trees of DBH ≥ 60 cm; 2) the taxonomic diversity and functional diversity and composition after six decades forest recovery were close to those of OG forest, contrary to the floristic composition; and 3) AGC showed a slow recovery rate, linked to slow regrowth dynamics of trees of DBH ≥ 60 cm. Here, we discuss our results in terms of recovery pattern of forest structure, taxonomic and functional diversity and composition and carbon stocks after shifting cultivation. We do acknowledge the limited number of sampling plots between and within stages. However, this is –to our knowledge- the first detailed chronosequence study (including trait information) in central African forests.

Shift in forest structure, taxonomic diversity and composition

The forest structural changes along the secondary succession were marked by the slow recovery of the density of large-sized trees (DBH ≥ 60 cm). Compared to South America (7.5 ± 5.3 trees ha⁻¹) and Southeast Asia (13.4 ± 6.7 trees ha⁻¹), the African tropical forest (15.8 ± 5.4 trees ha⁻¹)
exhibits a high density of large (DBH ≥ 70 cm) stems (Slik et al., 2013). The relative density of large-size trees in the tropical old-growth forest seems to be sensitive to the forest disturbance regimes and the biomass density of megafauna (Lewis et al., 2013; Terborgh et al., 2016), but, the mechanisms underlying temporal and spatial distribution patterns of large trees in the tropical forest are still under debate. Other structural metrics, such as tree height, plot-based basal area, mean tree diameter and stem density, recovered quickly with secondary succession in accordance with other tropical forest sites (Madeira et al., 2009; Mukul, Herbohn and Firn, 2016; Bauters et al., 2019). Along the successional gradients, species richness is expected to increase as a result of the recruitment of new species (Chazdon et al., 2007). The low values of species richness, Shannon, Pielou’s evenness and high floristic similarity of the early successional stages were related to the dominance of *Musanga cecropioides*. *Musanga cecropioides* is a fast-growing species that can grow up to 2 m in height per year (Coombe & Hadfield 1962) and attains 20 m height (Lejoly, Ndjele and Eerinck, 2012), with a lifespan of 15 to 20 years (Ross, 1954). The species recruits in large numbers and closes the canopy within 5 years after farm abandonment (Ross, 1954). This leads to a near monodominance early in the succession, lasting more or less two decades (Wilkie and Finn, 1990), which is characterized by low tree diversity (Connell and Lowman, 1989; Peh, Lewis and Lloyd, 2011). The subsequent increase in species diversity with stand age is associated with the decrease in its monodominance. Furthermore, the lack of difference in species diversity between the 60yr and the OG stages suggests that diversity recovers relatively fast (six decades), after an initial phase of monodominance early on in the succession.

Recent studies from the Neotropics and Southeast Asia underline also the rapid recovery rate of species diversity, as recorded in similar chronosequence studies. Hence, a rapid recovery rate of alpha taxonomic diversity seems to be consistent across the tropics, when considering trees with DBH ≥ 10 cm. For example, in the Neotropics, 80% of the species richness can recover after 20 years of succession (Ruiz, Fandino and Chazdon, 2005; Letcher and Chazdon, 2009; Lennox et al., 2018; Rozendaal et al., 2019) and a similar species richness, Shannon and Pielou’s evenness is found between old-growth forest and regrowth forest in Southeast Asia after a couple of decades of forest regeneration (Deng et al., 2018; Yirdaw et al., 2019).

Further, we showed a slower convergence of the floristic composition of the young regrowth forest to the OG community composition. Our results corroborate findings that have been reported for the Neotropics (Ruiz, Fandino and Chazdon, 2005; Rozendaal et al., 2019) and Southeast Asia.
The floristic composition recovers more slowly because of the slower establishment of long-lived secondary forest specialists (Letcher et al., 2015). This is reflected in the uniqueness of 34% of the species pool present in the 60yr old forest. Moreover, species belonging to all functional groups are present in the first stage of succession, although their dominance peaks at different stages along the succession. As a result, the floristic difference between the 60 years and OG is still rather large, as 50% of species present in the OG are absent in the 60 years. This floristic difference is likely due to a delayed arrival of rarer species of the old-growth forest (Chazdon et al., 2007), given that 40% of species present in the OG forest are absent in the other successional stages.

Recovery of functional diversity and composition

For the functional diversity, only the FRic increased significantly along the secondary succession, while the FEve and FDiv did not change. It has been demonstrated that the FRic is sensitive to the variation of species richness (Cornwell, Schwikl and Ackerly, 2006; Villéger, Mason and Mouillot, 2008; Mouchet et al., 2010). In contrast to the FRic, the FEve and FDiv may decrease, increase or remain stable with addition of new species in the community depending on the effect of the new incomes on the species relative abundance (Villéger, Mason and Mouillot, 2008). Altogether, the variation pattern of the functional diversity indexes suggests that though the functional space of the tree community is expanding along succession; i.e. that more extreme trait combinations are being exploited as the forest grows (Villéger et al., 2008; Lohbeck et al., 2012), the relative abundance of species with extreme traits remains rather stable (Magnago et al., 2014).

The increasing SLA and LNC along the successional gradients do not support the paradigm of resource conservation in maturing wet tropical forest (Lohbeck et al., 2013; Kearsley, 2015; Bauters et al., 2019), where net primary production is supposed to be limited by N- or P-availability (Lavorel and Garnier, 2002; Cernusak et al., 2013). The low values of community weighed mean of SLA and LNC in the early successional stages may be influenced by the high dominance of *Musanga ceropiodes*, which is acknowledged as a species that presents low SLA and nutrient content (Kearsley, 2015; Appendix S11). The high values of LNC in 60yr and OG suggest that in the central Congo basin, nitrogen might not be limiting for production in OG forest, which is in accordance with numerous studies (Davidson et al 2007, Hedin et al. 2009, Brookshire
et al. 2012, Winbourne et al. 2018, Bauters et al. 2019). Additionally, N rapidly recovers after disturbance in the central Congo basin due to the active biological nitrogen fixation in the early successional stages (Bauters et al., 2016) and deposition of high amounts of exogenous nitrogen in older forests (Bauters et al., 2018). Leaf phosphorous slightly increased from 5yr to 60yr and then decreased in OG, while canopy N:P initially decreased (5yr to 12yr) before it increased again, suggesting increasing P limitation for the most advanced successional stages (Sullivan et al. 2019).

Our results suggest a rapid recovery of the functional composition and diversity along succession except for the WD composition, which after 60 years of succession, did not yet reach the OG value. The shift of a community with low WD to a community with high WD is consistent with the community tradeoff between the resource acquisition in early successional stages and resource conservation strategy in the late-successional stages (Lohbeck et al., 2012). Wood density is a good indicator of tree growth rate, with fast-growing species exhibiting low wood densities (Ter Steege and Hammond, 2001), while species with denser woods invest more in wood architecture and mechanical support, leading to slower growth and longer life (Chave et al. 2009; Figure 3C).

Carbon stock recovery

Our data showed a constant increase in AGC with secondary succession. In accordance with our observations, a low recovery rate of AGC in the Congo basin has been noted recently, only at approximately 300 km of our study site (Bauters et al., 2019). At our study site, the 60 years second-growth forest had AGC that was only 43% of those of our OG plots, which corroborates the earlier observation of slow carbon stock recovery. We showed in the late-successional stages a large-sized trees store ca. 72% of AGC at these stages. The importance of large-sized trees in forest structure, carbon storage, and diversity is being increasingly investigated (Slik et al., 2013; Bastin et al., 2015; Clark et al., 2019). The Congo basin has a higher density of large-size trees, which give rise to a relatively higher carbon density in central African forests (Lewis et al. 2013). We also showed that the density of large-size trees exhibits a low recovery rate in the central Congo basin, being at 32% relative to OG after 60 years of succession. This slow recovery rate of AGC is not consistent with results from the Latin American tropical forest (Poorter et al., 2016), possibly because of the structural difference between these forests. Also, across the tropics, trees tend to decelerate their growth rate with DBH growth (Battipaglia et al., 2015; Groenendijk et al.,
Therefore, we advocate for more research on the dynamics of large size trees both in long-term and chronosequence studies to capture the pattern of carbon recovery in the Congo basin.

The SOC stocks did not differ between successional stages. Globally, land-use change has divergent and widely varying effects on SOC in the tropical region (Saynes et al., 2005; Marin-Spiotta and Sharma, 2013; Sayer et al., 2019). Sayer et al. (2019) showed that increasing litter input does not increase the capacity of tropical soils to sequester carbon. Some categories of land use have a stronger effect on SOC than others (Bauters et al., 2021). For instance, intensive agriculture and pasture can lower SOC to 25% in comparison to the OG after a decade (Rhoades, Ckert and Coleman, 2000; Anaya and Huber-sannwald, 2015), while this was not observed under shifting cultivation. The relative low impact of the shifting cultivation systems on the SOC might be explained by its intrinsic characteristics. Indeed, in shifting cultivation a certain number of trees (i.e. remnant trees) are typically retained in the farm and the parcels are recurrently subject to the fallow period (Pedroso-Junior, Adams and Murrieta, 2015). These two shifting cultivation characteristics, i.e. the capacity to maintain a certain number of trees in the system and fallow period, may favor the SOC conservation in the system.

Generally, fine root response to disturbances varies greatly with (1) season (Barbhuiya et al., 2012), (2) the type of vegetation and recovery trajectory (Du et al., 2019), and (3) interaction between vegetation development and soil properties (Brearley, 2011). We showed a rapid recovery of fine root carbon. On one hand, the low mass of fine roots found in the agricultural plots can be attributed to the effects of recent fire events and land preparation of the upper soil layer (Du et al., 2019). On the other hand, the relatively high mass and carbon of fine roots in the older successional stages is often explained by the intense competition for the limiting resources in the topsoil (Brearley, 2011; Barbhuiya et al., 2012; Powers and Peréz-Aviles, 2012).

Conclusion

We showed how forests in the central Congo basin naturally recover from shifting cultivation disturbances. Overall, the forest structure (stem density, plot basal area, and maximum tree height), species diversity, functional diversity, and composition, along with fine root carbon stocks recovered relatively fastly (only after 2 to 5 decades) after disturbance. In contrast, our data suggested a slow recovery rate of the floristic species composition, the density of large size trees, and the above-ground carbon pools. Hence, secondary forest shows potential to recover the full
characteristics of the old-growth forest but the duration of recovery varies with the trait that is assessed. Our results further stress that it is key in biodiversity conservation and climate mitigation policies to conserve the old-growth forest for their high carbon stocks and particular floristic composition. We suggest more research on the dynamics of large size trees in particular, given their strong impact on overall AGC during forest regrowth.

Acknowledgments
The Belgian Development Cooperation through VLIR-UOS project funding has supported this research. VLIR-UOS supports partnerships between universities and university colleges in Flanders (Belgium) and the South, looking for innovative responses to global and local challenges. We specifically thank, the Professor Van Oost for his critical reading of our manuscript.

Author contributions
M.A.I, M.B, P.B and K.V conceived the research idea; M.A.I collected data; M.A.I and M.B performed statistical analyses; M.A.I, with contributions from M.B, K.V and P.B, wrote the paper; all authors discussed the results and commented on the manuscript.

Data accessibility
Part of the data that support our finding are available via supplementary information. Raw data is available as DRYAD data set: https://doi.org/10.5061/dryad.0gb5km1t.

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and within stages and values within brackets are the standard deviation. C) Variation of species richness rarefied at 48 individuals between successional stages. D) Functional richness variation between successional stages. For C and D dots represent the stage mean of species and functional diversity and error bars represent the standard deviation, main P-values are related to the ANOVA and different letters indicate significant (P<0.05) differences between stages according to the Tukey HSD post hoc test.
Figure 2 Plant traits and functional group variation along the successional gradients. A) Shift in community weighed mean carbon to nitrogen ratio (C:N), nitrogen to phosphorous ratio (N:P), specific leaf area (SLA) and wood density (WD); B) Functional group variation along the succession: pioneer species, non-pioneer light demanding species, shade tolerant species. Dots and bars represent the stage-weighted mean of the traits and the mean relative basal area (BA %) of each functional group, respectively. Error bars represent the standard deviation, main P-values are related to the Kruskal-wallis test and different letters indicate statistical significant (P<0.05) differences between the stages and functional groups according to the post hoc Dunn’s test.
Figure 3 Carbon recovery along a secondary succession gradient. A) Recovery of above-ground carbon (AGC), the stacked bars are mean values of AGC per stem diameter classes (in cm; 10-20, 20-30, 30-40, 40-50, 50-60, ≥60). B) Relationship between AGC and large tree (DBH ≥60 cm) density. C) Fine root carbon (FRC), and D) Soil organic carbon (SOC) stocks to 1m depth. AG, 5yr, 12yr, 20yr, 60yr and OG represent the agriculture field, 5, 12, 20 and 60 years old secondary forest and old-growth forest, respectively. The bars are mean values for fine root carbon and soil organic carbon and the error bars represent the standard deviation. The SOC was calculated along a soil profile segmented into five soil layers (0-10 cm, 10-20 cm, 20-30 cm, 30-50 cm and 50-100 cm). Main P-values are related to Kruskal-wallis sub-figure A and ANOVA for the sub-figure C.
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