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Effects of fertilizer application on cacao pod development, pod nutrient content and yield

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ABSTRACT

Fertilizer application in cacao production systems in West Africa yields highly variable results, ranging from no effect to doubling of the yield. Mechanisms underlying yield responses to increased nutrient availability are still largely unkown. In this study, we assessed how fertilizer application affects pod development and production of cacao trees in a full sun cocoa system in Côte d'Ivoire and a shaded cocoa system in Ghana. We monitored pod growth over time and the number of pods produced simultaneously on the trees in the minor and major harvest seasons. Furthermore, we measured nutrient concentrations in cherelles and beans and husks of developing pods in the major harvest season to estimate the total nutrients allocated to developing pods per tree. Lastly, we performed detailed yield measurements (number of pods, location in the tree (canopy or trunk), pod size and bean content) in 2020 for the plot in Côte d'Ivoire.

Our results showed that, in the major harvest season, pods on fertilized trees grew wider (average: 15.4 cm) than pods on unfertilized trees (average: 11.9 cm). A higher pod growth rate resulted in a larger final pod size; larger pods took longer to mature. In the major harvest season, more cherelles on fertilized trees than on unfertilized trees reached maturity despite having an equal or lower nutrient content. Competition for assimilates rather than nutrients seems to induce cherelle wilt. In pods past wilting stage, fertilizer application slightly influenced nutrient dynamics of developing pods but not the final nutrient content in ripe pods. Lastly, increased nutrient availaibility did not change the absolute number of pods a tree produced annually. However, fertilizer application did increase the estimated annual dry bean yields from 2260 kg ha^{-1} to 2930 kg ha^{-1} by increasing the number of pods that developed during the major harvest season, when pods were heavier and the bean weight within the pods was relatively higher.

1. Introduction

Cacao is a global commodity and an important cash crop which provides income for millions of smallholder farmers. Worldwide, cacao production increased by 25% over the past 10 years (FAOSTAT, 2019). This increase is not due to higher yields but rather a result of expansion of the area of land on which cacao is grown, which is known to contribute to deforestation and forest degradation in West Africa (Gockowski et al., 2013; Wessel and Quist-Wessel, 2015). Côte d'Ivoire and Ghana are the two main producers of cacao and are responsible for over 60% of the global production (Fountain and Huetz-Adams, 2018; ICCO, 2017). In both countries, the average annual yield has remained practically unchanged in the past 10 years, fluctuating between 450 and 550 kg ha^{-1} (FAOSTAT, 2019). With potential yield estimates over 5000 kg ha^{-1} (Zuidema et al., 2005) and on-farm yields that reach 2125 kg ha^{-1} (Abdulai et al., 2020), yield gaps are large and argued to be the result of cultivation in climatically suboptimal zones (Asante et al., 2021), aged plantations (Wessel and Quist-Wessel, 2015), poor farm management practices (Aneani and Ofori-Frimpong, 2013) combined with high disease pressure and low application of fertilizer (Abdulai et al., 2020; Wessel and Quist-Wessel, 2015).

Fertilizer is frequently recommended as a central strategy to improve cacao yields (Abdulai et al., 2020; Kongor et al., 2018). However, most cacao farmers are smallholders with limited access to inputs, who use little or no fertilizer. Additionally, many smallholders seem unconvinced of the need and effect of fertilizer on cacao bean yield (Kenfack

Abbreviations: DAP, Days After Pollination.

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Essougong et al., 2020). This is most likely the result of the highly variable effects of fertilizer application; ranging from a doubling of the yield to no effect (Dossa et al., 2018), in combination with the lack of clear fertilizer recommendations with a solid scientific base. Nevertheless, continuous cropping of cacao without inputs results in a negative nutrient balance, which eventually leads to soil degradation and depletion and severe yield decline in older plantations (Abdulai et al., 2020; Aneani and Ofori-Frimpong, 2013; Kongor et al., 2019; Snoeck et al., 2010). It is essential to first enhance the understanding of how nutrient supply affects cacao pod production, to eventually be able to improve advice for nutrient management.

Cacao is a cauliflorous tree with large pods with a woody husk, containing 20–60 beans in a viscous pulp that take about 140 - 180 days to mature and ripen (Doaré et al., 2020; McKelvie, 1956). Pods are produced year-round, but in Côte d'Ivoire and Ghana there are two peaks, one around May and one around November, the minor and major harvest season respectively. Cacao trees tend to develop larger sink demand (fruits) than source supply. Immature pods up to 75 days old ("cherelles"; ± 10 cm length) can be aborted, commonly referred to as cherelle wilt, to adjust the total sink demand to the available resources (Humphries, 1943; Valle et al., 1990). Peaks of cherelle wilt are associated with high fruit set and often coincide with heavy leaf flushing, indicating that internal competition for photosynthates or mineral nutrients between reproductive and vegetative tissues prompts cherelle wilt (Alvim, 1954; Hurd and Cunningham, 1961). This is further corroborated by the observation that the mineral nutrient content in cacao leaves decreases during the minor and major cacao harvest (Verliere, 1981).

The effect of a mineral nutrient deficiency on fruit yield is either direct; as a result of competition for nutrients, or indirect; when the applied nutrients affect the levels of photosynthates or phytohormones which regulate yield (Engels et al., 2012). Deficiencies in N and P can lead to stunted leaf expansion, lower photosynthesis rates, reduced or delayed flowering, flower and fruit drops and reduced fruit size caused by reduced vegetative growth (Engels et al., 2012; Hawkesford et al., 2012). K deficient plants have reduced assimilate transport and are more susceptible to abiotic and biotic stresses which can cause loss of yield (Hawkesford et al., 2012). In mango and avocado, fertilizer (N) induced vegetative growth resulting in higher assimilate availability has the strongest positive effect on yield (as reviewed by Bally (2009) and Lahav and Kadman (1980)). In cacao, most available information on the effect of fertilizer on cacao yield is based on field level experiments, reporting effects on total yield only (Ahenkorah et al., 1974; Appiah et al., 2000; Dossa et al., 2018; Murray, 1958). The few more detailed articles reported that fertilizer application reduced cherelle wilt but did not increase pod number (Asomaning et al., 1971), increased fruit size but not dry bean yield (Noordiana et al., 2007) and increased bean numbers without reporting final yields (Lachenaud, 1995). All in all, the mechanisms underlying cacao yield responses to nutrient availability are still largely unknown.

In this study, we assess how fertilizer application affects pod development and pod production of cacao trees in plantations in Côte d'Ivoire and Ghana. We address the following research questions: (1) Does fertilizer application affect pod growth? (2) Does fertilizer application affect pod development through changes in nutrient concentrations and total content of N, P and K in the husk and bean tissues in different stages of pod development? (3) Is there a difference in the number of developing pods and total nutrients allocated to those pods that fertilized and unfertilized trees sustain? (4) Does fertilizer application increase dry bean yield by increasing pod numbers, pod size or bean content?

We hypothesize that low assimilate availability (caused by low nutrient availability) might delay pod filling and thus pod growth. For unfertilized trees, we expect a reduced nutrient concentration and content for cherelles. For pods past the cherelle stage, we expect a lower nutrient concentration and content in the husk due to high internal competition for nutrients. We expect a generally high nutrient content in the beans, as they are seeds, to ensure a higher survival chance of the seedlings. We hypothesize that the pod load and total pod nutrient content of fertilized trees will increase after the wilting stage, as unfertilized trees will show more cherelle wilt. In terms of effects on yield, we expect that fertilized trees will produce more and larger ripe pods, and that the bean weight to pod weight ratio will be higher for larger pods because of a smaller surface (husk) to volume ratio.

2. Materials and methods

2.1. Study sites

In this study, samples were collected from two cocoa plantations, one site in Côte d'Ivoire and one in Ghana. The site in Côte d'Ivoire was located in Divo in the Lôh-Djiboua region at the Centre National de Recherche Agronomique (CNRA) research station (5.769814 N, 5.236746 W), where the annual averages of the daily minimum and maximum temperatures are 21.7 – 31.6°C respectively, with an average rainfall of 1200 mm per year (calculated from weather station data in Divo, from 1971 to 2019). In Ghana, the study site was located at the Cacao Research Institute of Ghana (CRIG) in Tafo (6.232956 N, −0.342423 W), where the minimum and maximum daily temperatures are 22.3 to 31.5 on average annually, with an average rainfall of 1500 mm per year (from 2000 to 2017, weather station in Kumasi). In both locations, the dry season (<100 mm precipitation per month) lasts from December until March, and the two cacao harvest seasons are in May (minor harvest) and October-November (major harvest).

The study site in Côte d'Ivoire was a 12-year old, unshaded cacao plantation with trees planted 2.5 × 3.0 m apart. In April 2019, the field was subjected to weed slashing, a sanitation pruning and a cleaning harvest to remove all old and diseased pods. Afterwards, the field was maintained with regular weed slashing, removal of parasitic plants, and application of insecticide upon the first signs of infestation (of Heteroptera). This plantation was previously used for a fertilizer experiment, testing the effect of Triple SuperPhosphate (TSP; 46% P_2O_5) application at planting in 2009, and had not received fertilizer since. In Ghana, field work was conducted in a 22-year old, shaded, plantation with a tree spacing of 2.5 × 2.5 m. The parcel had been unused and unfertilized for at least 7 years before CRIG started using it in 2017 for a fertilizer experiment. At the onset of the experiment, a cleaning harvest was done and parasitic plants were removed. Both parcels consisted of genetically diverse cacao populations which, like common planting material for farmers fields, existed of genetically diverse hybrids (Wessel and Quist-Wessel, 2015).

These two unsimilar plots, differing in shade, planting material, tree age and plantation management were chosen to represent some of the wide variation of cocoa production strategies of West African farmers. Assessing fertilizer effects in these two contrasting plots aided the identification of more general effects of increased nutrient availability on pod development and production.

2.2. Experimental design

For the experiment in Côte d'Ivoire, we used eight plots from the original fertilizer experiment. Each plot consisted of 30 trees that were evenly distributed across the plot. Four of these plots had served as control plots in the past, and had never received fertilizer. These plots continued to serve as unfertilized control in our experiment. The other four plots all received 300 g TSP per tree at planting, and served as fertilized plots after we started re-fertilization 10 years later in 2019. All trees in the fertilized plots received 210 g calcium nitrate (15.4% N + 25.9% CaO), 75 g of TSP (45% P_2O_5) and 150 g of sulphate of potash (SOP; 50% K_2O + 17% S) per tree per year divided over three applications (in April, June and October) in 2019 and 2020. Unplanted borders around each of the plots allowed isolated fertilizer application. In each of the plots, 9 of the 30 trees were randomly selected, marked and used for the collection of pod development data: the pod counts and detailed yield records. Trees that were not selected were used for the harvest of pod samples for nutrient analysis (see data collection) so that sampling would not interfere with yield recordings.

For the experiment in Ghana, we used six plots from the current CRIG fertilizer experiment. Each plot consisted of 36 trees (16 centre trees and 20 fertilized outer trees) with a row of unfertilized border trees separating the plots. Each of the applied fertilizer treatments had three repetitions. For our experiment we selected the three plots that did not receive fertilizer in the current fertilizer trial as our unfertilized controls. We used three other plots, that received 340 gram of calcium nitrate (15.4% N + 25.9% CaO), 370 g of TSP (45% P_2O_5), and 135 g of muriate of potassium (MOP; 60% K_2O), per tree per year since 2017 as our fertilized plots. In May, the trees received half the application of calcium nitrate and the full amount of TSP and MOP, and in September the second half of the calcium nitrate. Nine out of the 16 centre trees were randomly selected for pod counts, the 20 fertilized outer trees were used to collect the pod samples for nutrient analysis (see data collection).

2.2.1. Data collection pod growth

Pod growth on selected trees was monitored for hand-pollinated stem pods on both fertilized and unfertilized trees in Côte d'Ivoire. In November 2019 (for minor harvest 2020) and May 2020 (for major harvest 2020), we hand-pollinated two flowers on the stems of each selected tree (if available) with pollen of neighbouring trees, for three subsequent weeks (six flowers per tree in total). Pollinated flowers were marked, and pod development was recorded on a weekly basis by measuring pod length and width of each pod.

2.2.2. Nutrient concentration and content of developing pods

Both in Côte d'Ivoire and Ghana, developing pods were collected from unselected trees in the following five developmental stages (Table 1 & Fig. 1): (1) small cherelles, with a width from 1 to 4 cm, for which husk and beans could not be separated; (2) large cherelles with gelatinous beans, and pod width between 4 and 6 cm (Fig. 1A); (3) filling pods with mostly gelatinous beans, but showing signs of developing cotyledons as pink structures (Fig. 1B); (4) unripe pods with completely filled dark purple beans in solid pulp (Fig. 1C); and (5) ripe pods with completely filled beans, with pulp in a layer around the beans (Fig. 1D). From each plot, per ripening stage, three pods from the stem and three pods from the canopy (if available), were harvested in September 2019. The fresh weight (in mg) of the husk and the beans (or in total) was determined for each sampled pod. A composite sample, containing an equal subsample of each of the three pods per plot, per developmental stage was taken, weighed and dried at 70°C until stable dry weight. Sample dry weight was measured to determine dry matter content of husks and beans (in%). We multiplied the dry matter content (in%) with the fresh weight (in mg) of the husks and beans to calculate the husk and bean dry matter (in mg) per sampled pod. Dried samples were ground (to 1 mm particle size) and digested (H2SO4/H2O2/Se) for determination of the total N and P concentration with a segmented-flow system (Skalar San++ System) using the Berthelot and molybdenum blue reactions respectively. The K, Ca and Mg concentration were determined using a fast sequential atomic absorption spectrometer (Varian AA240FS). We then calculated the nutrient content of the husk and beans per pod by multiplying the nutrient concentration with the corresponding husk or bean dry matter of the pods in that sample.

2.2.3. Pod counts

Both in Côte d'Ivoire and Ghana, all pods developing simultaneously were counted for each selected tree. Pods were counted per tree, per developmental stage (Table 1), per location in the tree. Pod counts were performed during or just before the minor harvest of two years (May 2019 in Côte d'Ivoire; May 2020 in Ghana; September 2019 and 2020 and May 2021 in Côte d'Ivoire and Ghana).

2.2.4. Yield recording

In Côte d'Ivoire, all ripe pods of the selected trees were harvested and counted (separately for stem and canopy) per tree on a 3-weekly basis from May 2019 until the end of 2020. In Ghana, it was not possible to harvest ripe pods from the selected trees, to not interfere with the ongoing fertilizer experiment. Annual pod production in Ghana ranged from 0 to 72 pods per tree, with an average of 11 pods produced per tree per year (personal communication with the soil sciences department of CRIG).

Additionally, per minor and major harvest season, one of the 3-weekly harvests was selected for detailed measurements (minor: May 2019 and May 2021; major: September 2019, November 2020). For the harvested pods of all trees in all plots, we measured the length, width and fresh weight. In 2019, we afterwards performed a stratified selection, first we used the weight measurements of all pods (of all plots) to determine three weight classes (equally divided over the total range in pod weight of that harvest), for which we then randomly selected three healthy stem pods and three healthy canopy pods per weight class per plot. For these selected pods, we measured fresh bean weight and husk and bean dry matter content after drying a subsample of beans and husk at 70 °C until stable dry weight. In November 2020 and May 2021, we similarly measured pod length, width, fresh weight, but also disease occurrence and fresh bean weight for all harvested pods from all plots.

2.3. Data analysis

All statistical analyses were conducted in R 4.0.2 (R Core Team, 2020). To analyse if fertilizer application had an effect on pod development rate, a non-linear mixed-effects models was fitted to the repeated length and width measurements using the nlme package (Pinheiro et al., 2013). We used the Richard's function (eq 1) (Richards, 1959) as it has been shown to be best suited for modelling cacao pod growth (ten Hoopen et al., 2012):

$$L = \frac{Lmax}{(1 + e^{b-ct})^{1/d}} \quad (1)$$

where L_{max} indicates the final pod width/length (thus the upper asymptote), b represents the growth rate of the pod, c influences the

Table 1
Characteristics of the cocoa pod groups sampled for the nutrient analysis of developing pods[1] and for the pod counts on the trees[2].

Pod stage	Pod width	Bean stage	Pod location	Nutrient analysis[1]	Counting groups[2]
Small cherelle	1.0–4.0	Gelatinous endosperm	Canopy Stem	Total	Small cherelle
Large cherelle	4.0–6.0	Gelatinous endosperm	Canopy Stem	Total and husk & beans	Large cherelle
Filling pods	6.0+	Embryo developing in beans (pink colour)	Canopy Stem	husk & beans	Filling/Filled
Filled pods	6.0+	Endosperm gone entirely. Mature beans filled with embryo (dark purple)	Canopy Stem	husk & beans	
Ripe pods	6.0+	Beans loose in pod in mucilage layer	Canopy Stem	husk & beans	Ripe

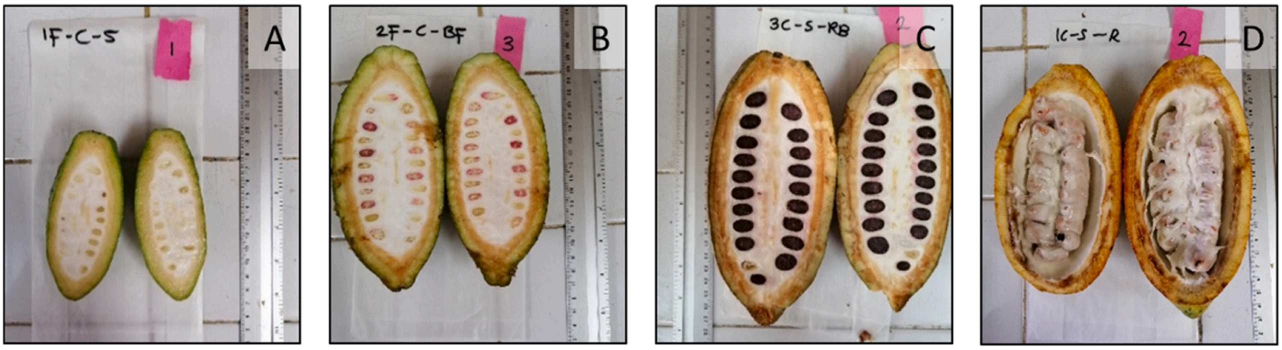

Fig. 1. Overview of the developmental stages for cocoa pods. A. large cherelle, B. pod in bean filling stage, C. pod in filled bean stage, D. ripe pod.

inclination point of the curve in days after pollination which corresponds to the point of most rapid growth, d affects near which asymptote maximum growth occurs and t is time in days after pollination (DAP) (ten Hoopen et al., 2012). The actual inflection point (t_{max}), the time (DAP) at which maximum growth occurred, was calculated by ((Eq. 2)):

$$t_{max} = \frac{b - \ln(d)}{c} \tag{2}$$

For each of these parameters, we tested whether they were dependent on the harvest season, the fertilizer treatment and their interaction. Additionally, a random effect for L_{max} was included to correct for the repeated measurements on pods. Model comparison based on Akaike Information Criterion (AIC) was used to test whether the effects of fertilizer application, harvest season and their interaction were significant for each of the four parameters.

For all other analyses, linear mixed-effects models were fitted using the glmmTMB package (Brooks et al., 2017). An overview of the response variables, the fixed effects, the tested interactions and the random effects can be found in Table 2. The explanatory variables that were included as fixed effects in all models (where applicable) were: fertilizer application (yes/no), location in the tree (stem/canopy) and pod stage (cherelle/filling/filled/ripe). For the models for N, P and K concentration and content in the husk and beans, we added one of four pod size measurements in addition to the explanatory variables mentioned above: pod volume, pod fresh weight, pod dry weight, dry weight of beans or husk depending on the tissue modelled. We did not combine all size measurements in one model as they were strongly correlated.

A random intercept was included for country, representing the experiment in Côte d'Ivoire and Ghana. If measurements were taken at the tree level, an additional random intercept per tree, nested within country, was included. For the yield measurements, which were only taken in Côte d'Ivoire, only a random intercept for each tree was included. Plots did not explain any variation, as variation amongst trees (in the tree random effect) already explained most of the variance in the random effects. Therefore, we did not include a random intercept per plot.

Fixed effects significantly influencing the response variable were identified using model selection, based on the Akaike Information Criterion, with correction for small sample sizes (AICc). We compared models for all possible combinations of fixed effects and their two-way interactions, including an intercept-only model. We regarded models with less than a 2-unit difference in AICc as equally supported (Burnham and Anderson, 2002) and from these best models we selected the most parsimonious model. For the models for nutrient content and concentration (Table 2, models 5–19) of developing pods, we first selected the best full model from a set of models that just varied in the size measurement included, based on AICc, before comparing all possible models. Note that not all possible interactions have been tested for all models; in some cases overfitting led to convergence issues (Table 2, models 2–5, 11).

To calculate the annual pod and dry bean yield and the resulting offtake of nutrients for 2020, we first calculated the average number of pods, per tree, per season and per pod location, using the 3-weekly pod counts. Then, we used the fitted fresh weight model (Table 3, model 26) to predict the fresh weight for the pods harvested on the stem and canopy in the minor and major season. Thereafter, we predicted fresh bean weight (Table 3, model 27) harvested per tree for 2020 based on the fitted fresh bean weight model. Finally, we used the average husk and bean dry matter content of the dried pod samples harvested in Côte d'Ivoire in May 2019 and September 2019 to convert fresh bean weight to dry bean yield (on average 0.38% dry matter) to calculate the annual yield for 2020.

3. Results

3.1. Pod development

3.1.1. Pod growth

To determine the effect of fertilizer application on the rate of pod development, the length, width and (calculated) volume of hand-pollinated stem pods were recorded weekly on fertilized and unfertilized trees during the minor and major harvest. The expansion of the pods followed S-shaped curves over time (days after pollination; DAP). Pod width showed less variation than pod length and was therefore used for further exploration. The final pod width (L_{max} parameter), and the parameter influencing the point of most rapid growth (*c*) differed with the harvest season, the treatment and their interaction. Pods developing in the major season were larger than pods developing in the minor season, especially on fertilized trees (Fig. 2). Additionally, larger pods (with a higher L_{max}) took longer to reach the point of most rapid growth (*c*) and had a higher growth rate (b). The calculated inflection points (t_{max}) for the fertilized pods were 96 DAP during the major and 86 DAP during the minor season, and 90 DAP during the major and 87 DAP during the minor season for the curves of the unfertilized pods.

3.1.2. Nutrient concentration in developing pods

We assessed whether application of fertilizer, the location of the pod in the tree, the bean/husk dry matter and the developmental stage of the pod influenced N, P, and K concentrations in the husk and the beans of large cherelles, filling pods, filled pods and ripe pods. The nutrient concentrations in the beans were all significantly influenced by bean dry matter, fertilizer application, pod stage and the interaction between bean dry matter and fertilizer application (Table 3, models 8–10). The smaller pods (with low and medium total bean dry weight) developing on fertilized trees had a lower N, P and K concentration in their beans throughout all development stages (Fig. 3, A-C). For pods developing on both fertilized and unfertilized trees, the nutrient concentrations decreased with increasing total bean dry weight, however, this reduction was stronger without fertilizer application. This led to a higher P

Table 2
An overview of all the full mixed-effect models tested including all response variables and all fixed effects, interactions and random intercepts. These full models were the starting point for the model comparisons to identify the fixed effects significantly influencing the response variables.

Experiment		Response Variable				Fixed effects	Random effects***
Pod Development	1	Pod Width (cm)				L_{max}, b, c, d, t (in DAP) with all factors except for DAP dependant on harvest season, fertilizer_Y/N and harvest season x fertilizer_Y/N	Pod ID
	2	Bean	N, P, K	concentration	(%)	husk dry matter* (g) - fertilizer_Y/N - location in tree - pod stage - fertilizer_Y/N x husk dry matter* (g) - fertilizer_Y/N x pod stage **	Country
	3	Husk	N, P, K	concentration	(%)	husk dry matter* (g) - fertilizer_Y/N - location in tree - pod stage - fertilizer_Y/N x husk dry matter* (g) - fertilizer_Y/N x pod stage **	Country
	4	Cherelle	N, P, K	content per pod	(g)	bean dry matter* (g) - fertilizer_Y/N - location in tree - pod stage - fertilizer_Y/N x bean dry matter* (g) - fertilizer_Y/N x pod stage **	Country
	5	Husk	N, P, K	content per pod	(g)	husk dry matter* (g) - fertilizer_Y/N - location in tree - pod stage - fertilizer_Y/N x husk dry matter* (g) - fertilizer_Y/N x pod stage **	Country
	6	Bean	N, P, K	content per pod	(g)	bean dry matter* (g) - fertilizer_Y/N - location in tree - pod stage - fertilizer_Y/N x bean dry matter* (g) - fertilizer_Y/N x pod stage **	Country
Pod Load	7	Number of pods developing simultaneous				fertilizer_Y/N - harvest season - location in tree - pod stage - fertilizer_Y/N x harvest season - fertilizer_Y/N x location in the tree - fertilizer_Y/N x pod stage - fertilizer_Y/N x harvest season x location in the tree**	Country/Tree
	8	N, P, K content in pods on the tree			(g)	fertilizer_Y/N - harvest season - fertilizer_Y/N x harvest season	Country/Tree
Yield	9	Annual pod production per tree				fertilizer_Y/N - location in tree - fertilizer_Y/N x location in tree	Tree
	10	Seasonal pod production per tree				fertilizer_Y/N - location in tree - harvest season - fertilizer_Y/N x location in tree - fertilizer_Y/N x harvest season - harvest season x location in tree	Tree
	11	Pod fresh weight			(g)	fertilizer_Y/N - location in tree - harvest season - fertilizer_Y/N x location in tree - fertilizer_Y/N x harvest season - harvest season x location in tree	Tree
	12	Bean fresh weight			(g)	pod fresh weight (g) - fertilizer_Y/N - location in tree - harvest season - fertilizer_Y/N x location in tree - fertilizer_Y/N x harvest season **	Tree
	13	Beans per 100 g pod fresh weight			(g)	fertilizer_Y/N - location in tree - harvest season - fertilizer_Y/N x location in tree - fertilizer_Y/N x harvest season - harvest season x location in tree	Tree

* selected from all tested size variables (pod volume, pod fresh weight, husk/bean dry weight, pod dry weight).
** not all two-way interactions included, interactions causing convergence issues due to overfitting were left out.
*** Pod ID was included as a random effect for the asymptote (Lmax) in Eq. (1). All other random effects were random intercepts.

Table 3
Overview of all optimal models showing the response variable, selected fixed effects and the random effects, and the marginal and conditional R^2. For all these models we selected the most parsimonious model as optimal model with (with ΔAIC <2). All selected fixed effects are significant as removing them would increase the model AIC with more than 2 units.

	Response Variable				Fixed Factors	Random factor	R^2_{mar}	R^2_{con}
	Pod Development							
1	Pod Width			(cm)	L_{max} (-harvest season x fertilizer_Y/N), b (-harvest season), c (-harvest season x fertilizer_Y/N), d (-harvest season), t	Pod (for L_{max})	–	–
5	Husk	N	concentration	(%)	fertilizer_Y/N - location in tree - pod stage - fertilizer_Y/N x pod stage	Country	0.76	0.77
6	Husk	P	concentration	(%)	fertilizer_Y/N - pod stage - fertilizer_Y/N x pod stage	Country	0.70	0.71
7	Husk	K	concentration	(%)	fertilizer_Y/N - pod stage - fertilizer_Y/N x pod stage	Country	0.61	0.65
8	Bean	N	concentration	(%)	bean dry matter* (g) - fertilizer_Y/N - pod stage - fertilizer_Y/N x bean dry matter* (g)	Country	0.37	0.48
9	Bean	P	concentration	(%)	bean dry matter* (g) - fertilizer_Y/N - pod stage - fertilizer_Y/N x bean dry matter* (g)	Country	0.32	0.36
10	Bean	K	concentration	(%)	bean dry matter* (g) - fertilizer_Y/N - pod stage - fertilizer_Y/N x bean dry matter* (g)	Country	0.78	0.78
11	Cherelle	N	content per pod	(g)	total dry matter (g) - cherelle stage - fertilizer_Y/*N* + cherelle stage x fertilizer_Y/N	Country	0.97	0.97
12	Cherelle	P	content per pod	(g)	total dry matter (g) - cherelle stage	Country	0.95	0.95
13	Cherelle	K	content per pod	(g)	total dry matter (g) - cherelle stage - fertilizer_Y/*N* + cherelle stage x fertilizer_Y/N	Country	0.98	0.98
14	Husk	N	content per pod	(g)	husk dry matter* (g) - fertilizer_Y/N - location in tree - pod stage - fertilizer_Y/N x pod stage	Country	0.91	0.91
15	Husk	P	content per pod	(g)	husk dry matter* (g) - fertilizer_Y/N - pod stage - fertilizer_Y/N x pod stage	Country	0.84	0.86
16	Husk	K	content per pod	(g)	husk dry matter* (g) - fertilizer_Y/N - pod stage - fertilizer_Y/N x pod stage	Country	0.95	0.95
17	Bean	N	content per pod	(g)	bean dry matter* (g) - fertilizer_Y/N - pod stage - fertilizer_Y/N x bean dry matter* (g)	Country	0.99	0.99
18	Bean	P	content per pod	(g)	bean dry matter* (g) - fertilizer_Y/N - pod stage	Country	0.98	0.98
19	Bean	K	content per pod	(g)	bean dry matter* (g) - fertilizer_Y/N - pod stage	Country	0.95	0.95
	Pod Load							
20	Number of pods				fertilizer_Y/N - harvest season - location in tree - pod stage - fertilizer_Y/N x harvest season - fertilizer_Y/N x location in the tree - fertilizer_Y/N x pod stage - fertilizer_Y/N x harvest season x location in the tree	Country/ Tree	0.34**	0.67**
21	N in pods on the tree			(g)	fertilizer_Y/N - harvest season - fertilizer_Y/N x harvest season	Country/ Tree	0.17	0.28
22	P in pods on the tree			(g)	fertilizer_Y/N - harvest season - fertilizer_Y/N x harvest season	Country/ Tree	0.18	0.28
23	K in pods on the tree			(g)	fertilizer_Y/N - harvest season - fertilizer_Y/N x harvest season	Country/ Tree	0.17	0.28
	Yield							
24	Annual pod production per tree				location in tree	Tree	0.46	0.46
25	Seasonal pod production per tree				fertilizer_Y/N - location in tree - harvest season - fertilizer_Y/N x harvest season - harvest season x location in tree	Tree	0.32	0.32
26	Pod fresh weight*			(g)	fertilizer_Y/N - location in tree - harvest season - fertilizer_Y/N x location in tree - harvest season x location in tree	Tree	0.20	0.50
27	Bean fresh weight**			(g)	pod fresh weight (g) - fertilizer_Y/N - location in tree - fertilizer_Y/N x location in tree	Tree	0.65	0.72
28	Beans per 100 g pod fresh weight				fertilizer_Y/N - harvest season	Tree	0.05	0.23

* Pod fresh weight model, details in Appendix 2.1, model was used for harvest calculations.
** Bean fresh weight model, details in Appendix 2.2, model was used for harvest calculations.

and K concentration in large pods (with high bean dry matter) for pods from fertilized trees whereas N concentration remained higher for unfertilized trees. The N and P concentration in the beans increased with the subsequent stages of the ripening process, whereas K concentration decreased with maturation of the pod. We did not find a significant difference in bean nutrient concentrations between pods harvested from the stem and canopy, as pod location was not included in the selected model.

The N, P and K concentration in the husk were not dependant on total dry matter, but were significantly influenced by pod developmental stage, fertilizer application and their interaction only (Table 3, models 5–7). The N and P concentration in the husk reduced with increasing maturity, whereas the K concentration decreased marginally from cherelle to filling pod but then increased strongly when pods were filled and when pods ripened, which was the exact opposite to the trends observed in the beans, (Fig. 3, E-F). Pods developing on fertilized trees had a lower N concentration in their husk in the large cherelle, filled and ripe stage. The husk P concentration was lower for fertilized pods in the cherelle and filled pod stage, but not in ripe pods. The K concentration of the husk was lower for pods on fertilized trees in the cherelle stage and filled stage but was equal or marginally higher in the filling and ripe stage. Canopy pods had a marginally (0.05%), but significantly higher husk N concentration than stem pods.

Nutrient concentrations, contents and changes over time were very similar for Côte d'Ivoire and Ghana, and across trees, as differences between the marginal R^2 and conditional R^2 values were marginal (Table 3) indicating that the random effects explained hardly any variation.

3.1.3. Total nutrient content of developing pods

We investigated whether fertilizer application, total dry matter and location of the pods in the tree influenced the total N, P and K content for young pods that were still prone to wilting (small and large cherelles). The nutrient content of cherelles was significantly influenced by total

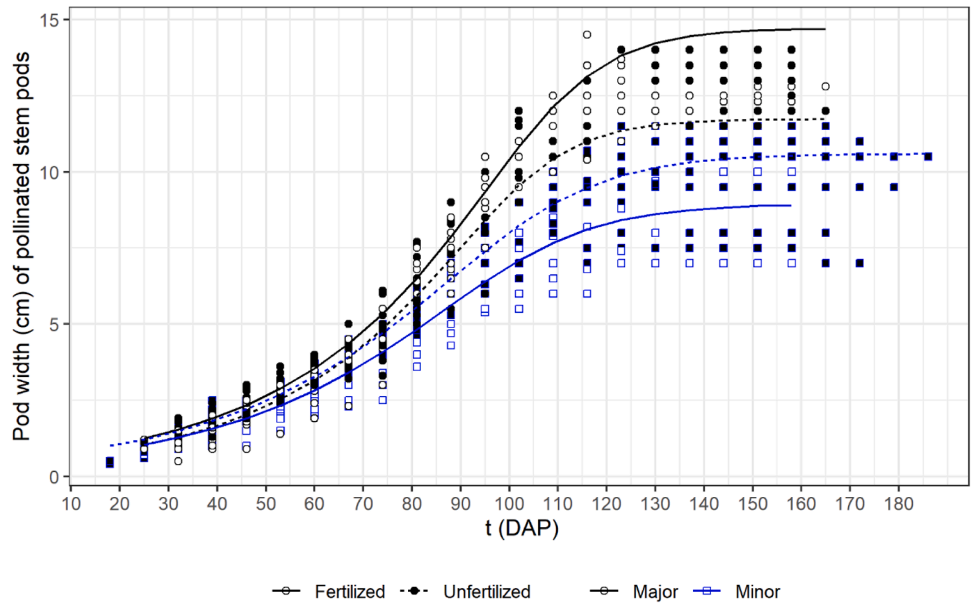

Fig. 2. Pod width measured during the development of stem pods for the minor and major harvest season in Côte d'Ivoire.

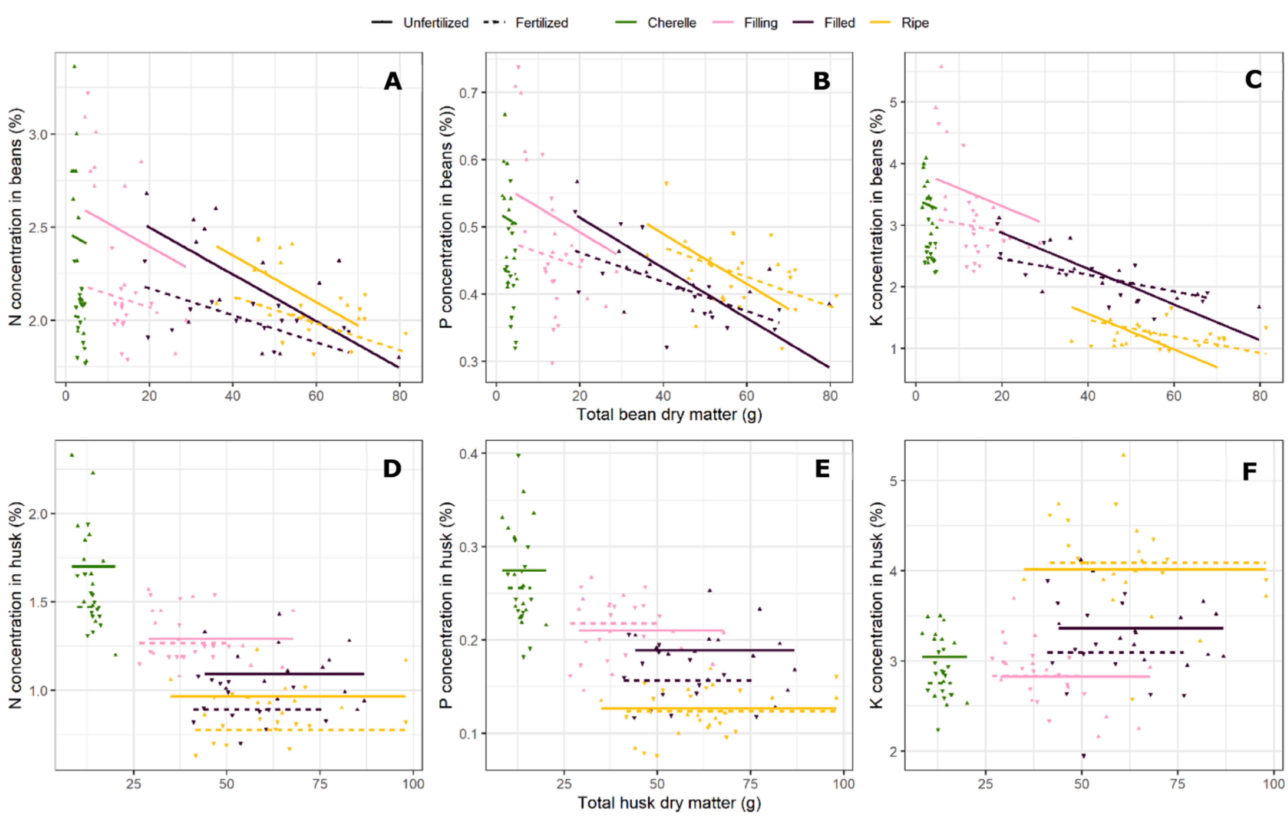

Fig. 3. The N (A), P (B) and K (C) concentrations of the beans of large cherelles, filling pods, filled pods and ripe pods and their relation to total bean dry matter. Below the N (D), P (E) and K (F) concentration of the husks of developing pods for Côte d'Ivoire and Ghana. Lines are based on the predicted values of the linear mixed-effects models for Côte d'Ivoire. For the N concentration in the husk, the predicted lines are for stem pods, canopy pods have a 0.03% lower concentration.

pod dry matter and cherelle stage, and for N and K also by fertilizer and the interaction between fertilizer and cherelle stage (Table 3, models 11–13). As expected, the N, P and K content in the pods increased with total dry matter and cherelle stage (Supplementary Information; Fig. A1). Fertilizer application had no effect on P content of the cherelles, however application of fertilizer resulted in a lower N and K content of large cherelles, which both decreased with approximately 10% of their total content. The N and K content for small cherelles of fertilized and unfertilized trees was similar. The location of the cherelles in the tree did not influence their nutrient content.

For larger pods, we assessed the effects of the same factors on the N, P and K content but separately for the husk and the beans. The nutrient contents in the beans were all significantly influenced by bean dry matter, fertilizer application and pod stage, and for N additionally the interaction between fertilizer application and bean dry matter (Table 3, models 17–19). Fertilizer application resulted in a marginally lower content of N, P and K in the beans. Furthermore, bean N, P and K content increased with the accumulation of bean dry matter and with increasing pod developmental stage except for the total content of K in beans upon ripening, which showed a strong decrease (Fig. 4, A-C). Location of the pod in the tree did not have a significant effect on nutrient content.

In the husk, the N, P and K content increased with dry matter accumulation. The factors significantly influencing the nutrient content of the husk were: husk dry matter, fertilizer application, pod stage, and the fertilizer pod stage interaction (Table 3, models 14–16). K increased with increasing ripening stage, whereas N and P decreased (Fig. 4E-F). The reduction of husk N was stronger for fertilized trees when the beans filled and ripened. The P content of the husk decreased earlier, at filled (beans) stage, for pods on fertilized trees (and more at ripening), whereas the concentration in the unfertilized pods fell strongly at ripening, resulting in a similar total P content in ripe pods, regardless of fertilizer treatment. Fertilizer application only led to a decreased K content in the husk of filled pods, for all other stages it remained equal for pods of fertilized and unfertilized trees. The N content in the husk of canopy pods was marginally yet significantly higher than the content of pods developing on the stem (0.03 g difference), which was similar to what we observed for the N husk concentration.

3.2. *Fertilizer effects on the number of developing pods*

To test whether fertilized trees produced more pods than unfertilized trees, we counted all the pods developing simultaneously on the selected trees in the different developmental stages before/in the minor and major harvest season. The number of pods developing was significantly influenced by all tested factors: fertilizer application, harvest season, location in the tree, stage of pod development, all possible interactions with fertilizer and the three-way interaction between fertilizer, harvest season and location in the tree (Table 3, model 14). Fertilizer effects were visible during the development of the major harvest, when fertilized trees had more pods of all developmental stages, with the largest increase in pods developing in the canopy, both in Côte d'Ivoire and Ghana (Fig. 5). In the minor season, fewer pods were produced in both experiments and the differences between the number of pods on fertilized and unfertilized trees were generally marginal, except for ripe pods in Côte d'Ivoire (Fig. 5A).

3.3. *Allocation of N, P and K to all developing pods*

Knowing the total nutrient content and the number of pods in different developmental stages developing on the tree (simultaneously), allowed us to extrapolate the total allocation of N, P and K to the number of pods per tree per harvest season. The total allocation of nutrients to developing pods was for all nutrients significantly influenced by the fertilizer treatment, the harvest season and the treatment x harvest season interaction. In the minor season (Fig. 6A & C), the amount of nutrients allocated to developing pods was relatively similar for unfertilized and fertilized trees, but in the major season fertilized trees allocated more N, P and K to their developing pods (Fig. 6D & E). We estimated that, in the major season fertilized trees allocated (on average for Côte d'Ivoire and Ghana) 16.8 g of N, 3.1 g of P and 30.7 g of K to developing pods, whereas unfertilized trees allocated on average 12.1 g of N, 2.1 g of P and 21.9 g K.

3.4. *Fertilizer effects on yield*

3.4.1. *Number of harvested pods*

In Côte d'Ivoire, where we conducted the detailed annual harvest in 2020, the annual production of pods per tree was only significantly

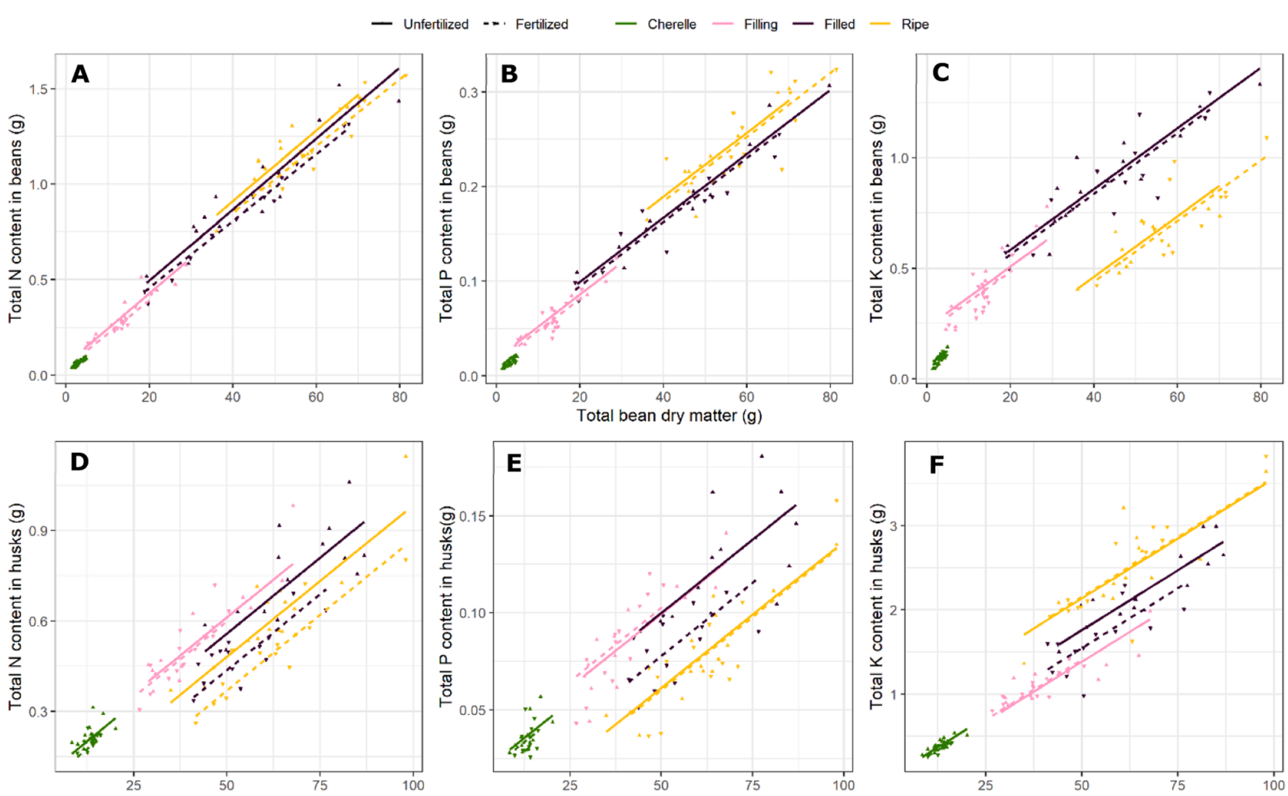

Fig. 4. The N, P and K content in the beans (A, B and C respectively) and husks (D, E and F respectively) of large cherelles, filling pods, filled pods and ripe pods in Côte d'Ivoire and Ghana. The lines are based on the predicted values of the linear mixed-effect models for Côte d'Ivoire and are based on pod volume, treatment and the ripening stage of the pods.

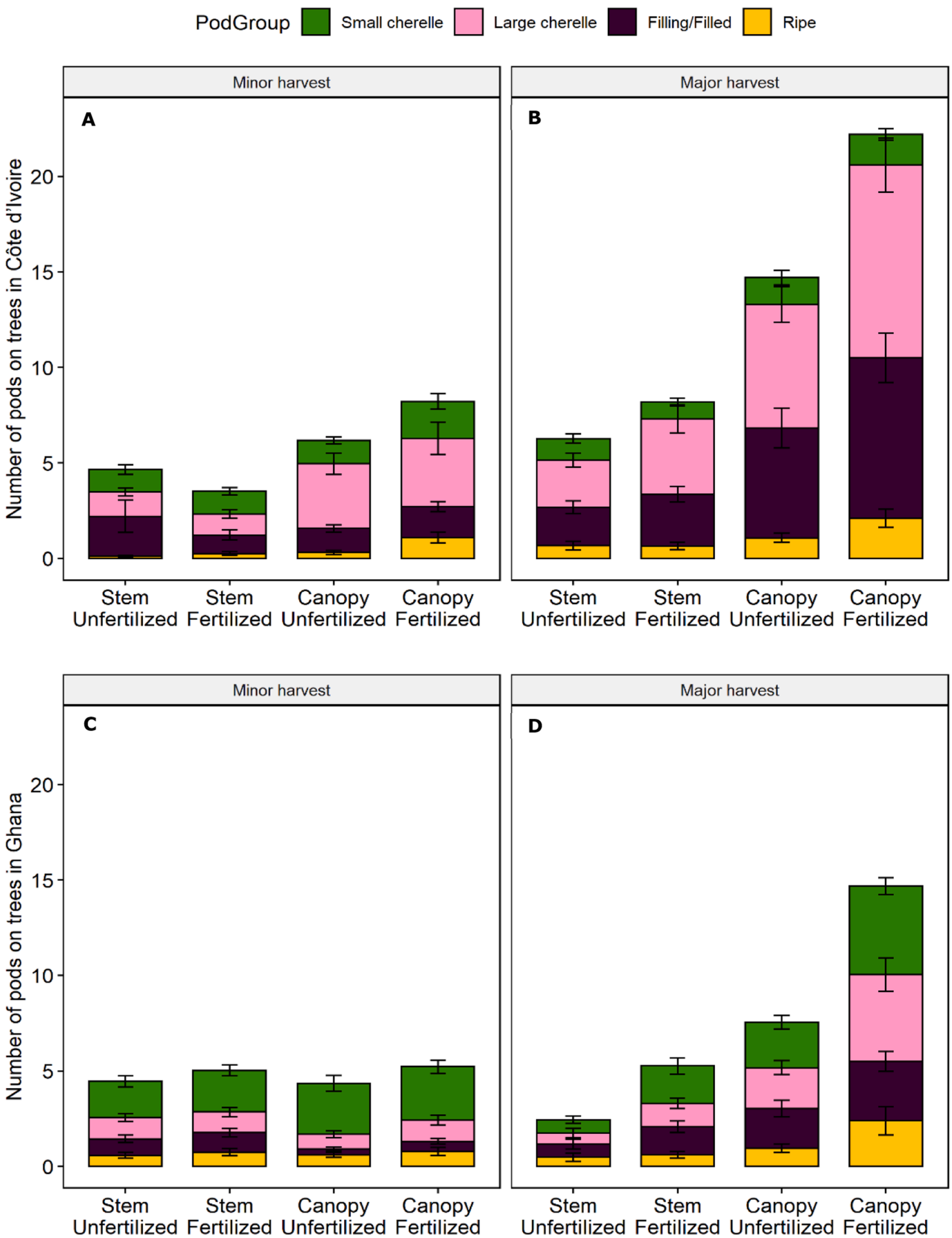

Fig. 5. Number of pods in the measured development stages counted on the stem and in the canopy of fertilized and unfertilized trees in the early rain season just before the minor harvest and in the late rain season just before the onset of the major harvest in Côte d'Ivoire (A & B) and Ghana (C & D).

influenced by the location in the tree (Table 3, model 24). On average 55.3 pods were produced per tree that year, of which 47.2 pods in the canopy and 8.1 pods on the stem (Fig. 7A). Fertilizer application did not significantly increase the number of pods harvested in 2020, neither on the stem, nor in the canopy. However, application of fertilizer did significantly affect the distribution of the canopy pod production over the two harvest seasons. Unfertilized trees produced the majority of their canopy pods in the minor harvest season (an average of 32.6 pods

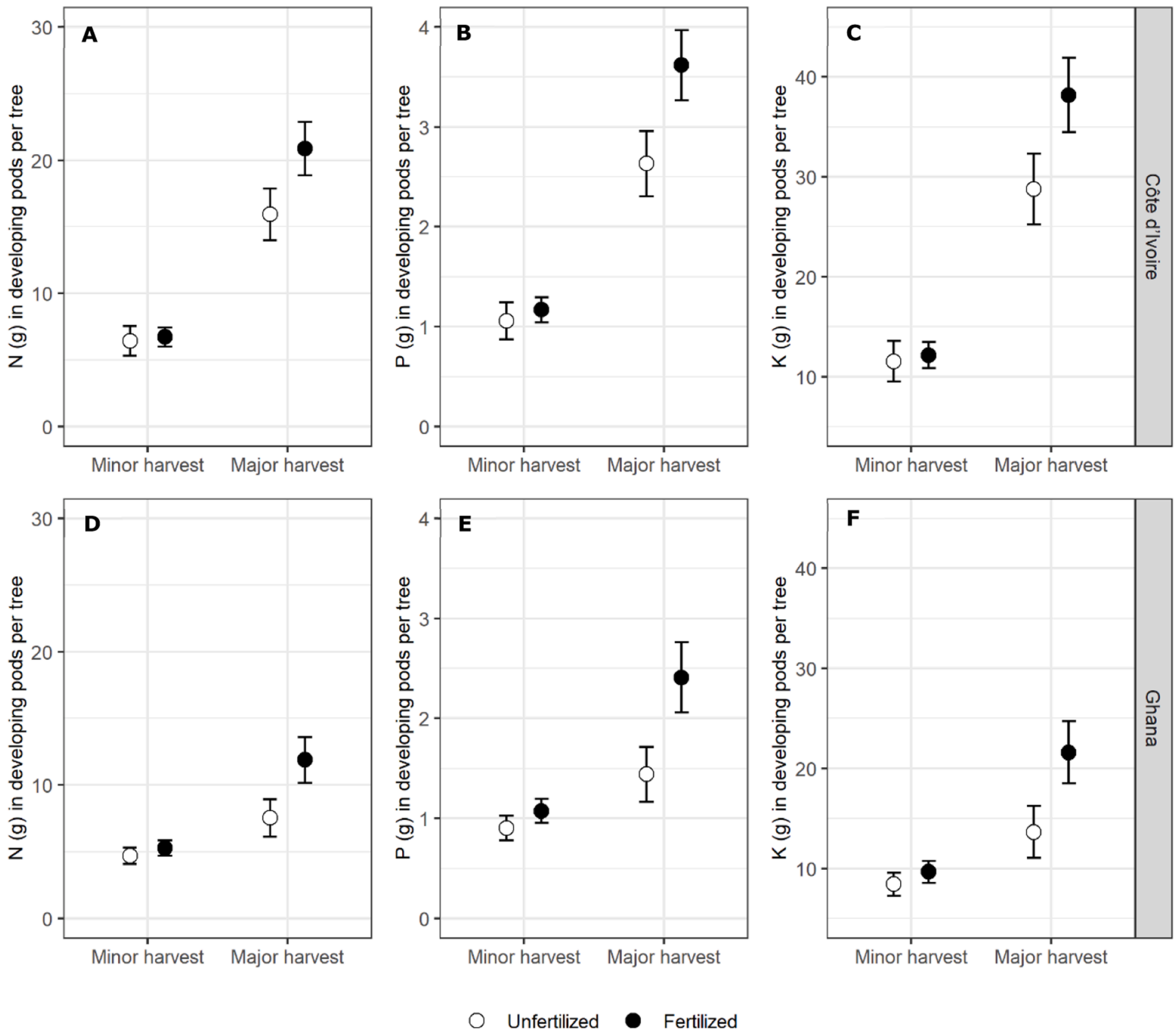

Fig. 6. Nutrient allocation in developing pods in Côte d'Ivoire (top) and in Ghana (bottom). Extrapolated from the nutrient content calculations for 2019, we calculated the N, P and K (in g) stored in developing and ripe pods on the trees developing during the early rain season (before the minor harvest) and in the late rain season (developing for the major harvest) in Côte d'Ivoire (A-C) and Ghana (D-F).

per tree) and significantly less pods (14.2 pods per tree) were produced in the major harvest season. In contrast, fertilized trees significantly increased their canopy pod production from 21.6 pods per tree in the minor season to 26.1 canopy pods per tree in the major season (Fig. 7A). On average, few more stem pods were produced (1.1 in unfertilized and 2.1 for fertilized trees) in the major harvest, with no significant difference between fertilized and unfertilized trees. Seasonal pod production was thus significantly influenced by fertilizer application, location in the tree, harvest season, the interaction between fertilizer application and harvest season and the interaction between harvest season and location in the tree (Table 3, Model 25).

3.4.2. Pod weight

Pod fresh weight was significantly influenced by fertilizer application, the location in the tree, the harvest season, the interaction between fertilizer application and location in the tree and the interaction between harvest season and the location in the tree. Pods produced in the major season, with an average weight of 582 g for stem pods and 406 g for canopy pods, were heavier than pods produced in the minor season (with 370 g and 252 g, respectively). Additionally, fertilized trees produced significantly heavier canopy pods (on average 50 g heavier than pods of unfertilized trees) (Table 3; Supplementary Information 2.1), whereas the fresh weight of stem pods did not significantly differ between the two treatments (Fig. 7B).

3.4.3. Bean content

We produced an allometric model (Table 3;Supplementary Information 2.2) to predict fresh bean weight in the pod based on pod fresh weight, location of the pod in the tree, fertilizer treatment and the interaction between fertilizer treatment and location of the pod in the tree. As expected, bean content increased significantly with increasing pod fresh weight. Fertilizer application resulted in a relatively higher bean content, as a result of a higher bean to husk ratio. In the minor season, the average bean content was similar for fertilized and unfertilized trees, but in the major season the fresh weight of the beans per 100 g pod weight decreased for unfertilized trees, whereas it remained stable for fertilized trees. In the major season, fertilized trees produced on average 7.4 g more fresh beans for stem pods and 2.3 g for canopy pods per 100 g fresh pod weight than unfertilized trees (Fig. 7C). For stem pods in the major season, with an average pod weight of 586 g,

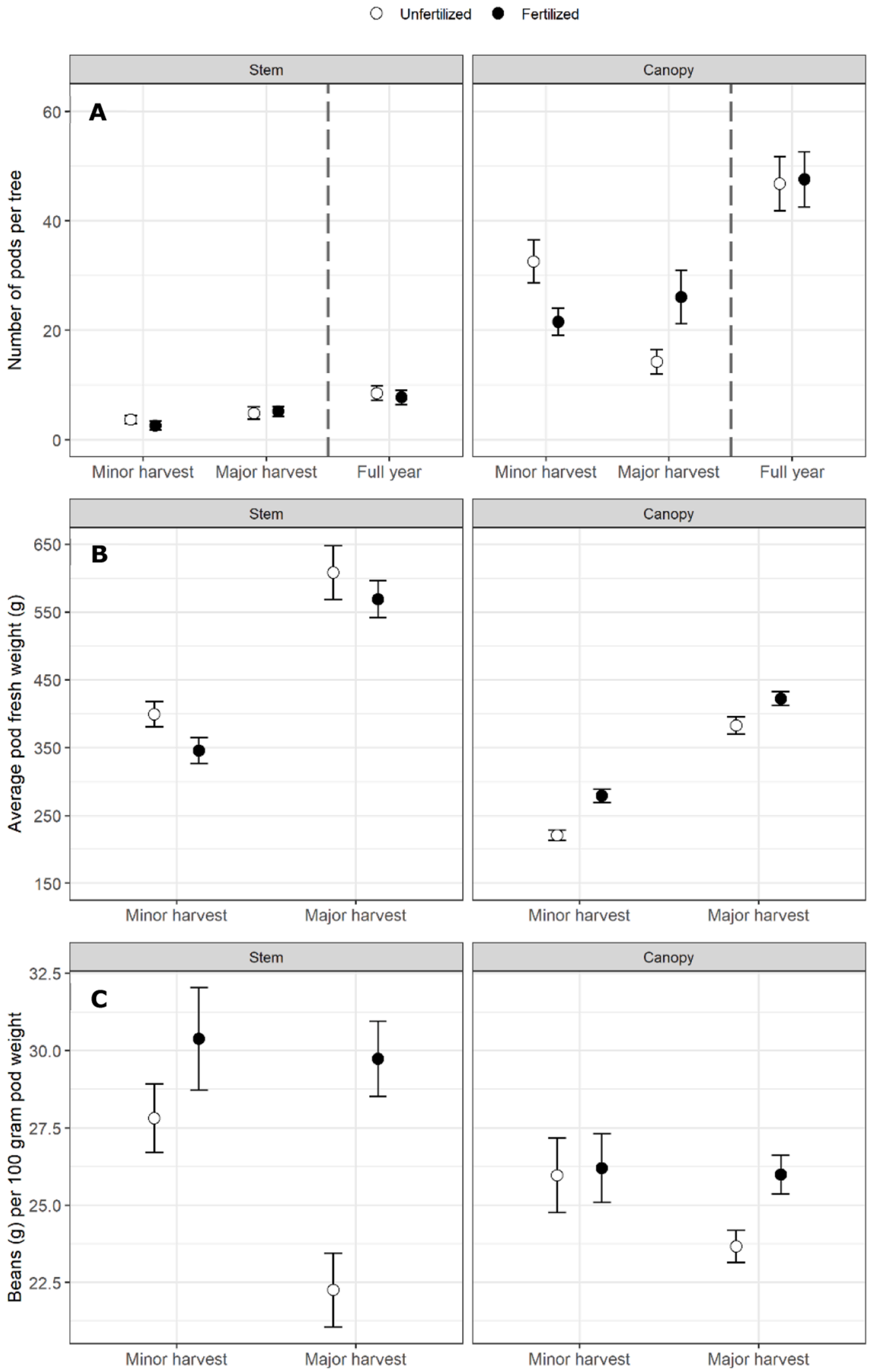

Fig. 7. Pod production per tree (mean ± SE) (A), pod fresh weight (mean ± SE) (B) and bean/husk fresh weight ratio (mean ± SE) of pods produced in Côte d'Ivoire in the minor and major harvest for the stem and canopy (C).

unfertilized trees would contain 131 g of fresh beans whereas fertilized trees would contain 174 g of fresh beans. In canopy pods produced in the major season with an average weight of 413 g, pods of unfertilized trees would contain 98 g, and canopy pods on fertilized trees 107 g of fresh beans.

3.5. *Yield*

Combining the average pod count data with the linear (prediction) model for pod weight and the allometric model for bean content (Supplementary Information 2.1 & 2.2) we estimated the fresh bean yield per tree in 2020 (Table 4). Despite no significant increase in the number of produced pods, we estimated that on average each fertilized tree

Table 4
Annual yield calculations per tree for Ivory Coast.

	Minor harvest			Major harvest			Annual harvest		
	Nr. of pods	FW pod[1]	FW beans[2]	Nr. of pods	FW pod[1]	FW beans[2]	FW pods[1]	FW beans[2]	DW beans[3]
	av. 2020	*(g/pod)*	*(g/pod)*	*av. 2020*	*(g/pod)*	*(g/pod)*	*kg/tree*	*kg/tree*	*kg/tree*
Unfertilized									
Stem	3.7	388.4	98.2	4.8	543.8	131.5	4.0	1.0	0.4
Canopy	32.6	265.8	66.9	14.2	368.3	88.8	13.9	3.4	1.3
Total							17.9	4.4	1.7
Fertilized									
Stem	2.6	378.5	113.3	5.7	533.9	146.6	4.0	1.1	0.4
Canopy	21.6	307.7	82.7	26.6	410.2	104.6	17.6	4.6	1.8
Total							21.6	5.7	2.2

[1] Predictions of the pod fresh weight based on the harvest season, the fertilizer treatment and the location in the tree using the fresh weight prediction model.
[2] Predictions of the fresh bean weight per pod, using the allometric model for bean weight and the estimated fresh weight.
[3] Dry weight of the beans calculated using the bean dry matter constant of 38.3%.

produced 3.7 kg more pod fresh weight, resulting in 1.3 kg more fresh beans. Using the average bean dry matter content of 38.3% as found in this study, led to an average increase of 0.5 kg dry beans per tree after fertilizer application.

Combining these yield calculations with the predicted nutrient concentrations for ripe pods, we extrapolated the total offtake in N, P and K with the yield for 2020 (Table 5). The total nutrient offtake of N, P and K in harvested pods was respectively 7.8 g, 2.6 g and 0.9 g higher for fertilized trees.

4. Discussion

In this study, we investigated the detailed effects of increased available nutrients on pod development and yield in cacao. We assessed the effect of increased available nutrients on pod development rates, the total allocation of nutrients within and towards developing pods, and how these together influenced annual pod and bean yield.

4.1. Final pod size depends on pod growth rate rather than on pod development time

Following the expansion of hand pollinated stem pods in Côte d'Ivoire showed that pods grew larger in the major harvest season and that this seasonal effect was increased by the application of fertilizer. In the major harvests, due to infestations of *Heteroptera*, many of the pods were lost before ripening, but the fertilized pods already grew larger than unfertilized pods in the first 120 days after pollination. In contrast, in the minor season the pods on unfertilized trees grew larger. In both case this was due to a higher pod growth rate rather than a longer pod development time. Pods on unfertilized trees did not develop slower or needed longer to ripen than pods on fertilized trees, as we had initially hypothesized. Apparently, once pods have passed the physiological wilting stage their development rate is not delayed by nutrient deficiencies.

The rate of pod development in our study, and the shape of the curve, were in agreement with the major physiological processes of pod development. Generally, up to 75 days after pollination, pods enlarge in conjunction with the ovules (McKelvie, 1956). Initial expansion is slow up to 40 days after pollination (DAP) whereafter growth starts to increase rapidly, and at about 75 DAP the pod begins to swell (McKelvie, 1956). Around 85 days after pollination, the embryos start growing, causing pod width to increase rapidly whereafter ovule growth slows down, beans start to fill and the jelly-like endosperm is being consumed. This is in accordance with the calculated inflection points (t_{max}) of the curves modelled in this experiment; where growth rates decreased at 87 and 86 DAP for pods in the minor season and 90 and 96 DAP for the larger pods developing in the major season for unfertilized and fertilized trees, respectively (Fig. 2). The point where our pod width measurements started to plateau coincides with the period in which the bean filling process is complete, at approximately 140 DAP (McKelvie, 1956). From 85 DAP onwards, during the filling and filled stages of the beans and the ripenining proces, the accumulation of dry matter, and therewith N, P and K, increases rapidly (Humphries, 1939).

4.2. Fertilizer application influenced nutrient dynamics of developing pods

In small cherelles and large cherelles, which are still susceptible to wilt, fertilizer application had no effect or a negative effect on the nutrient content. Thus, in fertilized trees the same amount or less nutrients were available per developing cherelle, yet more pods manage to reach maturity in the major season. This strongly suggests other factors than competition for nutrients play a larger role in the regulation of cherelle wilt.

To enhance understanding on the nutrient dynamics during pod development, we evaluated both nutrient concentrations and content. Stable or increasing nutrient concentrations and increasing nutrient content with each consecutive developmental stage indicated that nutrients were transported to the tissue at rates equal to, or higher than, those for other dry matter components of cacao, including fats, carbohydrates, fibres and proteins that make up the dry matter of cacao beans (Torres-Moreno et al., 2015) and husks (Campos-Vega et al., 2018). This was the case for N and P in the beans and K in the husk, for which concentration and content continued to increase with increasing pod maturity. For K in the beans up to the filled stage and for N and P in the husks of pods in the large cherelle and bean filling stage, content increased but concentration decreased. This so-called chemical dilution

Table 5
Estimated N, P and K offtake with the yield in Côte d'Ivoire 2020 for fertilized and unfertilized trees.

	Annual Harvest		Beans			Husk			Total offtake		
	DW beans	DW husk	N	P	K	N	P	K	N	P	K
	kg/tree	*kg/tree*	*g/tree*	*g/tree*	*g/tree*	*g/tree*	*g/tree*	*g/tree*	*g/tree*	*g/tree*	*g/tree*
Unfertilized	1.7	2.7	37.5	7.3	21.7	21.9	2.5	90.9	59.4	9.9	112.6
Fertilized	2.2	3.5	44.7	9.5	22.7	22.5	3.1	110.8	67.2	12.5	133.5

(Nachtigall and Dechen, 2006; Paramasivam et al., 2000) occurs when the accumulation of nutrients in the tissue is slower than the accumulation of the other constituents of the dry matter.

The actual reduction in total N and P content in the husk, total K content in the beans and their decreasing concentrations indicated that nutrients not only had been diluted, but had been remobilized and reallocated within the pods or to other developing pods. The large reduction of K content in the beans coincided with the large increase of husk K content at that stage, which strongly suggested that besides import of nutrients via the vascular system, K was reallocated from within the pod. A similar shift was observed for N and P in the husk, when their content in the beans increased. Meeting nutrient demands of filling pods by the reallocation of mobile nutrients (including N, P and K) from other tissues has been demonstrated in other tree crops (Quartieri et al., 2002; Smith, 2009).

Fertilizer had a limited effect on the nutrient dynamics of pod development. Only during the filled stage of pod development, fertilizer application resulted in a slower increase in K concentration and content in the husk, and a stronger decrease in N and P concentration and content. It is not surprising that in this stage differences were largest, as the late filling/filled stage is the period with the highest nutrient demand and nutrient intake of the pod during its whole development (E. C. Humphries, 1939). Our findings suggest that fertilized trees cope with their higher pod load and larger pods by having either a slightly lower influx of nutrients or a higher influx of carbohydrates, fats and metabolites, and resort to earlier reallocation of mobile nutrients when the nutrient demand of developing pods is at its peak.

4.3. Competition for nutrients leads to dilution of nutrients in the bean dry matter in heavy pods

The effects of fertilizer application on the nutrient concentration in the beans was not only related to the pod developmental stage but also to pod size. As expected, total pod dry matter was the main driver of the total N, P and K contents in both the husk and beans, thus heavier pods contained more nutrients regardless of their developmental stage. In the husk, the increase in nutrient content in heavier pods was proportional to the increase in husk biomass, keeping the concentration of the nutrients stable. For the beans, the nutrients in the dry matter were diluted in pods with more beans. This dilution was less strong for pods on fertilized trees, which increased bean N, P and K concentration in larger pods of fertilized trees, but not of unfertilized trees. The lower nutrient concentration in smaller-sized pods on fertilized trees is likely the result of a higher nutrient demand at the tree level, resulting from both the larger number of pods (a larger sink) and the on average larger pods with more beans that fertilized trees produced (Fig. 7A&B).

4.4. Nutrient content of husks and beans in ripe pods are not altered by fertilizer application

Overall, fertilizer application influenced nutrient dynamics during pod development, but did not have a lasting effect on final nutrient concentrations or content of ripe pods. Only the N content in the husks differed between fertilized and unfertilized ripe pods, but it remains unclear if the lower N content in the husk of fertilized pods has any adverse effects, or that in unfertilized pods there is a more luxurious use of N, as the total N demand by the developing pods was lower. Likewise, Lockard and Burridge (1965) found very similar concentrations for ripe pods and only found slightly increased P contents in ripe beans after fertilizer application. Comparing the values of this experiment between the two experimental locations and with the results of previous experiments (Aikpokpodion, 2010; Lockard and Burridge, 1965), we conclude that pod nutrient content is quite conserved. As beans are seeds, it may be evolutionarily favourable if they are well supplied with nutrients, improving the chance of succesful offspring establishment and continuation of the genetic traits.

4.5. Increased numbers of developing pods in the major season after application of fertilizer

There was large variation between trees in pod growth and in the number of pods they could sustain. As the experimental plots in both sites were planted with genetically diverse populations (like most farmers plots), these differences could be partially genetic and partly caused by the heterogeneity that was present in these plots. Missing trees, uneven shading (in Ghana) and border effects (in Côte d'Ivoire) affected the available light and the microclimate, creating unequal competition pressure that resulted in large differences in canopy size and aboveground biomass amongst trees. This uneven competition has been shown to significantly affect pod yields (Trebissou et al., 2021).

We hypothesized that physiological cherelle wilt would be the main driver of the reduced number of pods on unfertilized trees, but our results showed that competition for mineral nutrients was unlikely the main cause of physiological cherelle wilt. Therefore, cherelle wilt is most likely caused by a shortage of assimilates in unfertilized trees, as has been found for other fruit tree species (Bote and Vos, 2016; Engels et al., 2012; Hawkesford et al., 2012). As physiological wilt affects young pods only, we expected that the number of small cherelles would be high, since 75% of the cherelles is lost in cacao (Melnick, 2016), and that the number of cherelles would be similar for fertilized and unfertilized trees. We found similar numbers of small cherelles for fertilized and unfertilized trees, but in far lower quantities than expected (Fig. 5). Flowering, and therewith cherelle formation, seemed repressed when pods reached the filling stage (Table 1) (personal observation), which agrees with the higher flowering intensity that was found after pod removal in cacao trees (Valle et al., 1990). We expected fertilized trees to carry more large cherelles and pods in the stages after the onset of bean filling (filling/filled and ripe), as less wilting occurred. This was confirmed by our observations, as the higher pod load of fertilized trees was caused by a higher number of large cherelles, filling/filled and ripe pods, predominantly located in the canopy (Fig. 5). Yet, this difference only occurred in the major harvest, in the minor season pod production was lower in general, and similar for fertilized and unfertilized trees. This seasonal yield increase was larger in Côte d'Ivoire than in Ghana. This could be due to known interaction between shading and fertilizer response; that fertilizers have a stronger positive effect on cocoa yield in unshaded plots (van Vliet and Giller 2017). Alternatively, it could be an effect of shading alone, as it has been demonstrated that yields of the major season, but not the minor cropping season, are higher in unshaded plots in certain cocoa production regions (Asare et al. 2016). However, as many other factors including precipitation and the mineral nutrient levels in the soil were different between the two plots, the yield difference cannot with certainty be ascribed to a single factor.

Our findings for the total nutrients (N, P and K) allocated to developing pods on the trees followed the same trend (Fig. 6). As cherelles have low biomass and nutrient content, this difference is predominantly caused by a higher number of the more mature (unripe and ripe) pods.

4.6. Seasonal effect of fertilizer application on pod production

Fertilizer application positively affected pod production and yield in the canopy especially, during the major harvest season only, as was found both for the annual yield measurements in Côte d'Ivoire, as well as for pod counts in Ghana and Côte d'Ivoire. Pod counts of developing pods suggested that fertilized trees produced more pods, as the counts in the minor season were similar to those of unfertilized trees and in the major season pod counts for fertilized trees were higher. However, the number of annually harvested ripe pods was equal for both treatments. Unfertilized trees produced more pods in the minor season, while fertilized trees produced more pods in the major season, which suggests that the higher production of unfertilized trees in the minor season may be spread out over a longer period with less pods being produced simultaneously. The absence of a fertilizer effect in the minor season and the strong positive effect in the major season is corroborated by similar findings from a 3-year trial investigating the effect of fertilizer and its interaction with shade on yield in Ghana (Asomaning et al., 1971).

The seasonality of the fertilizer effect may be attributed to seasonal vegetative growth and the competition for assimilates, which for example results in biannual production cycles in coffee and mango too (Bote, 2016; El-Motaium et al., 2019). In cacao, the onset of the rain season is one of the major cues for cacao trees to start flushing heavily (De Almeida and Valle, 2007). This in combination with the common farmers practice of applying fertilizer in March, will stimulate more vigorous vegetative growth in fertilized trees. However, this period of strong vegetative growth coincides with the cherelle-wilt stage of the pods developing for the minor season. Application of fertilizer will thus increase competition for assimilates between young cherelles and developing leaf flushes and therewith increase cherelle wilt (Valle et al., 1990). This could explain the observation of no, or a negative, fertilizer effect in the minor season, especially since young developing leaves seem to be a stronger sink than cherelles (Astuti et al., 2011).

Nevertheless, the increased yield for fertilized trees in the major season most likely resulted from increased vegetative growth. The increased number, size and bean content of pods produced by fertilized trees was most likely the result of a higher assimilate availability. Senescence and leaf abscission during the dry season (De Almeida and Valle, 2007) reduces canopy density and therewith light interception (Tosto et al. unpublished) during the filling of the pods in the minor season (2.5 months after pollination), as the loss of leaves will only be compensated after several flushing cycles. The pods in the minor season therefore develop with a lower leaf to fruit ratio, which reduces fruit size in many fruit tree species (Fischer et al., 2012). This, most likely, resulted in smaller pods in the minor season for both fertilized and unfertilized trees. Similarly, pods of unfertilized trees in the major season may remain smaller, as unfertilized trees are expected to recover their leaf area and canopy density slower, due to less stimulation by nutrient supply and suppression of vegetative growth by the larger number of developing pods in the minor harvest season. Additionally, the suboptimal nutrient supply might lead to low rates of net photosynthesis and/or insufficient cell expansion as a result of N or P deficiencies or a combination of both (Marschner, 2011), all decreasing assimilate production and decreasing its capacity to fill and sustain developing crops.

The actual bean content per pod is dependant on many factors, including the size of the pod, the location of the pod (stem/canopy), pollen quantity and quality and environmental and genetic factors (Lachenaud, 1994; Toxopeus and Wessel, 1970). Moreover, our results showed that fertilizer application positively affects the bean content of pods both on the stem and in the canopy. Fertilizer application caused a shift in pod allometry, increasing the bean to husk ratio. This could be the result of a larger number of the embryos developing which would result in a higher number of beans (Lachenaud, 1995) or the result of larger seeds and larger embryos. For future research it would therefore be interesting to include bean number and (single) bean weight to the measurements.

Overall, we found that fertilizer application had a positive effect on yield by influencing the timing, the available assimilates and nutrients, and the allometry of the pods. All these positive effects together resulted in 1.3 kgs more fresh bean yield, and 0.5 kg more dry bean yield per tree. With a planting density of 1333 trees per hectares, as was the case in the plot in Côte d'Ivoire, this increase would add up to almost 670 kg additional dry beans per hectare, increasing the estimated yields from 2260 without to 2930 kg ha^{-1} with fertilizer application.

5. Conclusions and implications

Fertilizer application did not increase the rate of cacao pod development, nor did it have a strong effect on the nutrient concentration or content of developing pods, except for N where the total concentration and content in both the husks and beans was slightly, but significantly lower, for all stages of pod development. Fertilizer application, however, did increase the number of pods that a tree can sustain in the major harvest season, and increased the total N, P and K sequestered in developing pods. The increased pod production in the major season in response to fertilizer application resulted in a higher yield, despite no absolute difference in pod numbers, as pods produced in the major season were larger. Additionally, fertilizer application changed the allometry of the pods and increased the bean to pod weight ratio.

Cacao fertilizer responses are complex and continued efforts are necessary to produce optimal fertilization strategies. We showed that detailed yield recordings can contribute to unravelling the variation in cacao fertilizer responses. Conducting similar experiments in different locations, with different fertilizer compositions and quantities and with different application times will be essential to provide specific and detailed fertilizer recommendations in the future. Increasing the productivity and the longevity of cacao plantations, with proper fertilizer recommendations, could increase the cost-benefit ratio of fertilizer application. This is crucial in Ghana and Côte d'Ivoire where "free" nutrients from forest rent (Ruf and Zadi, 1998) have been "consumed" over time and new expansion of cacao in forests is no longer possible due to lack of unprotected forests (Odijie, 2016). If fertilizers are available and affordable, and their application is profitable, they could decrease the need for expansion of cacao into forests in areas where forests are still present such as in Cameroon (Kenfack Essougong et al., 2020).

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Authors contribution

Study conception and design; E.G, M.S - Methodology implementation; E.G, A.T - Experiment execution; E.G – Data collection; E.G, A.T - Data analysis/interpretation: E.G, D.R - Manuscript writing/revision: E. G, D.R, M.S, A.T.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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