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# Effects of fertilization on the growth, photosynthesis, and biomass accumulation in juvenile plants of three coffee (*Coffea arabica* L.) cultivars

Z.X. ZHANG*, Z.Q. CAI*, G.Z. LIU*, H. WANG**, L. HUANG*, and C.T. CAI*,+

*Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan 666303, China**
*Pu'er Oasis Technology Co. Ltd, Pu'er, Yunnan 665000, China***

## Abstract

We carried out a field experiment in order to study effects of fertilization in juvenile plants of three coffee (*Coffea arabica*) cultivars in Yunnan, SW China. Fertilization treatments included a control without fertilizer (CK), combinations of three NPK fertilization rates [high fertilization ($F_H$), medium fertilization ($F_M$), and low fertilization ($F_L$) with 135, 90, and 45 g per plant per year, respectively], and at two $N:P_2O_5:K_2O$ ratios ($R_1$, 1:0.5:0.8; $R_2$, 1:0.8:0.5). The growth in juvenile plants was not altered by fertilization, with two clear growth peaks being observed in both the height and stem growth rates (RGRs) throughout a year. Both $F_M$ and $F_H$ resulted in significantly higher RGRs in both height and stem diameter compared to $F_L$ and CK in all three cultivars. At the same fertilization rate, the leaf area, branch number, longest branch length, internode number, and biomass of $R_2$ were higher than those of $R_1$, and P significantly affected the root biomass and root to shoot ratio. Compared to the $F_L$ treatment, both $F_M$ and $F_H$ treatments resulted in higher net photosynthetic rates and stomatal conductance across seasons, and in higher intrinsic water-use efficiency during the dry season and at the middle of the wet season. Photosynthetic nitrogen-use efficiency at $R_2$ was higher than that at $R_1$, but no significant differences were observed between the different fertilization rates. Among the three coffee cultivars, Caturra exhibited the highest height, stem diameter, longest branch length, and internode number. Our results indicated that the optimal $N:P_2O_5:K_2O$ ratio was 1:0.8:0.5 for the juvenile growth of coffee plants. Both $F_M$ and $F_H$ could help optimize the growth and photosynthetic rate of coffee plants, but $F_M$ is suitable for the ecological friendly agriculture and economic sustainability at coffee plantations.

*Additional key words*: biomass; *Coffea arabica*; fertilization; photosynthesis; relative growth rate; water-use efficiency.

## Introduction

Coffee is currently cultivated in over 80 countries all over the world, and it is a key cash crop for the poverty alleviation and national economic development in many developing countries (Mafusire *et al*. 2010, De Beenhouwer *et al*. 2015). Coffee was introduced to China more than 100 years ago. The majority of the coffee produced in southern China is *Coffea arabica* L. (Long *et al*. 1997), which is the third largest agricultural export of the Yunnan province (Guo *et al*. 2009). *C. arabica* was widely planted in mountainous areas in western Yunnan, where the dry and nutrient-poor soil is one of the main limiting factors for its growth and yield (Long *et al*. 1997, Lang *et al*. 2012). Although nutrient and water uptake are independent processes, the water status of the soil greatly affected nutrient uptake and efficiency, and fertilizer can increase soil water use (Fang *et al*. 2010, Jiao *et al*. 2012).

Both the supplementation and the efficiency of mineral nutrients used are important factors affecting the growth and photosynthesis of plants. Nitrogen (N) demand is the highest for coffee plants among mineral nutrients (Cai *et al*. 2004), since N can increase the photosynthetic rate ($P_N$) and promote the generation of new branch and axillary bud (Nazareno *et al*. 2003, Cai *et al*. 2007). Although phosphorus (P) has a minor impact on the growth, photosynthesis and yield of adult coffee compared to N and K (Cai *et al*. 2007); it plays an important role in the coffee root growth (Yang *et al*. 2010) and the absorption of N, K, Mg (Huang *et al*. 2009) at the juvenile stage.

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+Corresponding author; phone-fax: +86 (0) 871 65161066, e-mail: caict@xtbg.ac.cn
*Abbreviations*: $C_i$ – intercellular $CO_2$ concentration; $g_s$ – stomatal conductance; $P_N$ – net photosynthetic rate; PNUE – photosynthetic nitrogen-use efficiency; RGR – relative growth rate; R/S – root to shoot ratio; $WUE_i$ – intrinsic water-use efficiency.
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The combined application of N and P can increase $P_N$ and water-use efficiency under the drought conditions (Zhang *et al*. 2015). Although seedlings are less sensitive to K deficiency (Li *et al*. 2013), K plays a critical role in adult tree growth and productivity (Li *et al*. 2013). Thus, the proper combination of NPK nutrients may favor the healthy improvement of growth and photosynthetic capacity in juvenile coffee plants. Generally, a positive relationship between nutrient application rate and growth was observed in the field crops (Rotundo and Westgate 2009, Castro-Tanzi *et al*. 2012), but overdosed fertilizers may cause pollution of the environment and produce high greenhouse gases (especially $N_2O$) emission (Capa *et al*. 2015). Meanwhile, the optimal use of fertilizers and ideal fertilizer proportion are strongly linked to the specific stage of plant nutrient demand (Lawlor *et al*. 2001, Lobell 2007). Therefore, the appropriate fertilization rate at a certain growth stage is one of important sustainable agricultural practices for modern nature-friendly agriculture.

The effects of various concentrations of N, P, and K nutrients on the growth, leaf photosynthesis, and yield of adult coffee plants were well documented (Barros *et al*. 1995, Cai *et al*. 2004). However, farmers in China generally overused fertilizers for juvenile coffee plants without a consideration of consequent economic and environmental problems. In this study, we compared the vegetative growth, photosynthetic traits, and biomass allocation in three young *C. arabica* cultivars (*i.e*., Caturra, Catuai, and $\text{Catimor}_{7963}$) under different fertilization treatments with three fertilizer rates and two fertilizer ratios in the field, in order to find the optimal fertilizer NPK rate and ratio.

## Materials and methods

**Study site**: The experiment was carried out during two consecutive years of 2013–2014 in the field in the Coffee Species Garden in Pu'er (22°39′N, 100°56′E, 1,175 m a.s.l.), Yunnan Province, southwest China. The soil (0–20 cm) at this site is characterized as an acidic lateritic red soil with pH 5.22. Organic matter and total N, P, and K contents of the soil were 30.11, 1.71, 0.62, and 18.58 g $kg^{-1}$, respectively, while the available N, P, and K contents were 11.5, 5, and 193.13 mg $kg^{-1}$, respectively. During the experiment, yearly total precipitation was 1,209 mm, and there was a clear alternation of dry (November to June) and wet (late June to October) seasons.

**Experimental material and design**: Three coffee cultivars (*Coffea arabica* cv. Caturra, Catuai, and $\text{Catimor}_{7963}$) were investigated in the present study. Among the three cultivars, Caturra, of a Brazil origin, is a mutant of Red Bourbon. This highly productive and dwarf coffee has been widely cultivated in Colombia and Costa Rica (Frank and Vaast 2009, Zhang *et al*. 2011). Catuai, a hybrid of Yellow Caturra and Mundo Novo with the high yield, dwarf, and strong-resistance traits, is widely cultivated in Central America, and occupies about 50% of coffee planting area in Brazil (Zhang *et al*. 2011). $\text{Catimor}_{7963}$, a hybrid progeny of Hibrido de Timor and Caturra with high rust resistance, is widely cultivated in China, and occupies about 80% of Chinese coffee planting area (Zhang *et al*. 2011). The six-month-old seedlings of each coffee cultivar were transplanted in the field in August 2013 with the planting density of 4,500 plants per ha, and 450 g(organic fertilizer) per plant. All plants were rain fed. In October 2013, fertilization treatments were assigned in each cultivar with a factorial combination of three different NPK fertilization rates and two $N:P_2O_5:K_2O$ ratios ($R_1$, 1:0.5:0.8; $R_2$, 1:0.8:0.5), combined with a control treatment without fertilizer (CK). Thirty coffee individuals were used in each treatment. The three NPK fertilization rates were applied as follows: high fertilization ($F_H$, 135 g per plant per year), medium fertilization ($F_M$, 90 g per plant per year), and low fertilization ($F_L$, 45 g per plant per year). The fertilization treatment was selected according to the recommendation of coffee plantations in the first year (Zou *et al*. 2013). According to Peng *et al*. (2012), fertilizers were applied three times: 20%, 40%, and 40% of the total amount was used in October 2013, June 2014, and August 2014, respectively. The fertilizer used was mineral N from urea (46.4% of total N), P from superphosphate (12% $P_2O_5$), and K from potassium chloride (60% $K_2O$). The organic fertilizer contained 28% of organic matter and the living bacteria count was $3\times10^7$ cfu $g^{-1}$ (*Yunnan Red Sun Chemical Co. Ltd*, Yunnan, China).

**Growth**: In September 2013, 30 coffee plants per treatment were labeled for the growth measurement. The height and stem diameter (2 cm above ground) were determined every month from September 2013 to October 2014. The relative growth rate (RGR) was calculated as: RGR = $(\ln X_{t+1} - \ln X_t)/t$, where t is the time in month (Cai *et al*. 2007). The number of branches, length, and internode numbers of the longest branches were measured and counted in October 2014.

**Biomass and leaf N, P, K content**: In October 2014, five plants per treatment were harvested and the number of fully expanded leaves was counted. Twelve fully expanded leaves were randomly chosen from each plant, the leaf area was scanned using *ImageJ* software (*ImageJ*, *NIH*, USA), and the total leaf area was estimated. Then the harvested trees were divided into leaves, branches, stems, and roots. Plant tissues were dried to a constant mass at 60°C. The total biomass of leaves, branches, stems, and roots were measured and the root to shoot ratio [R/S = root biomass/(leaf biomass + branch biomass + stem biomass)] was calculated. Three leaf samples per treatment were chosen for analysis of leaf N, P, and K contents. Total N content of leaves was analyzed by the semi-micro-

Kjeldahl digestion method, using carbon and nitrogen element analyzer (*Vario Max CN*, *Elementar*, Germany). Leaf P content was measured by molybdenum-antimony spectrophotometric method, and leaf K content was measured by flame photometric determination respectively, using inductively coupled plasma atomic-emission spectrometer (*iCAP6300*, *Thermo Fisher Scientific*, USA)

**Gas exchange**: The photosynthetic traits, including net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), and internal $CO_2$ concentration ratio ($C_i$) were measured with five coffee plants per treatment, using a portable infrared gas analyzer in open-system mode (*Li-6400*, *Li-Cor*, USA) under ambient $CO_2$ concentration and saturating irradiance [900 μmol(photon) $m^{-2}$ $s^{-1}$], provided by a internal *6400-02B* LED radiation source between 09:30 and 11:00 h. Leaf temperature and leaf to air vapor pressure deficit (VPD) were kept at 25–27°C, and <1.0 kPa, respectively. Intrinsic water-use efficiency ($WUE_i$) and photosynthetic nitrogen-use efficiency (PNUE) were calculated by the ratio of $P_N$ to $g_s$ and the ratio of $P_N$ to unit of leaf N, respectively (Da Matta *et al*. 2002, Jiao *et al*. 2012). The photosynthetic traits were determined in the fully expanded and healthy upper leaves (one leaf per tree) in March (dry season), July (the middle of wet season), and October (the end of wet season), respectively.

**Statistical analyses**: All data are presented as mean values ± standard deviation (SD). We used two-way analysis of variance (*ANOVA*) to compare variables between fertilization treatments, different cultivars, and their interactions. We used then LSD contrasts to examine whether each trait differed between fertilization treatments, within and between different cultivars. Relationships between different parameters were determined by *Pearson*'s correlation coefficients (R) using a two-tailed test. All statistical analyses were conducted using *SPSS version 16.0* (*SPSS*, Chicago, USA).

## Results

**Vegetative growth**: The RGRs of plant height and stem diameter in all coffee cultivars showed a similar seasonal fluctuation. During the one-year experimental period, RGRs in both height and stem diameter showed two growth peaks (Fig. 1), with the low and high peak being observed in March 2014 and June 2014, respectively. There were no cultivar and fertilization interactions among the growth traits except for the branch number (Table 1). Thus, cultivar and fertilization were assumed to have independent effects. Among the three cultivars, no differences were found in the RGRs in height and stem diameter across all treatments. But the three coffee cultivars differed greatly in their height, stem diameter, length of the longest branch, and internode number, with the highest values being observed in Caturra (Table 1, Fig. 2). Fertilization significantly affected the growth traits (height, stem diameter, leaf area, branch number, and the length and internode number of the longest branch, Table 1, Fig. 2). The RGRs of height and stem diameter were higher under $F_H$ and $F_M$ compared to $F_L$ and CK. The difference in RGRs in height and stem diameter between $R_1$ and $R_2$ was not significant across all fertilization treatments and cultivars, but the values of the leaf area, length of the longest branch, and internode number were larger in $R_2$ than those in $R_1$ (all $p<0.05$). Among all fertilization treatments, $F_HR_2$ resulted in the greatest growth traits, followed by $F_MR_2$.

The biomass (leaf, branch, stem or root, and individual plant) of the three coffee cultivars significantly increased with the increasing fertilization (Fig. 3). Meanwhile, effects of fertilization on the biomass allocation in the three coffee cultivars were basically identical: leaf biomass accounted for the largest proportion of the coffee plants (about 32.5−40.7%), followed by stems (24.9−29.6%), roots (15.6−24.2%), and branches (14.8−18.5%). Among the cultivars, Caturra coffee plants had the highest values of the individual plant mass, root biomass, and root to shoot ratio (R/S), while leaf, stem, and branch biomass were not different between three cultivars (Table 1, Fig. 3). Although the individual plant biomass, rather than leaf, stem, and branch biomass, in $R_2$ tended to be higher than that in $R_1$, the differences were not significant.

**Leaf photosynthetic traits and leaf nutrient content**: The $P_N$, $g_s$, and $WUE_i$ of three coffee cultivars at the middle and end of the wet season were significantly higher than those during the dry season in all treatments, while $C_i$ was similar in all seasons (Fig. 4). In general, high fertilizer rates resulted in the increases in $P_N$ and $g_s$ of all cultivars and seasons (Table 1, Fig. 4). However, with the increasing rate of fertilization, a significant increase in $WUE_i$ was observed in both the dry season and the middle of the wet season, rather than at the end of the wet season. Both in the dry season and at the end of the wet season, $P_N$ values in $F_H$ and $F_M$ were significantly higher than those in $F_L$ and CK treatments. Across all seasons, the leaf gas-exchange parameters were not significantly different between the $R_1$ and $R_2$ treatments and between the

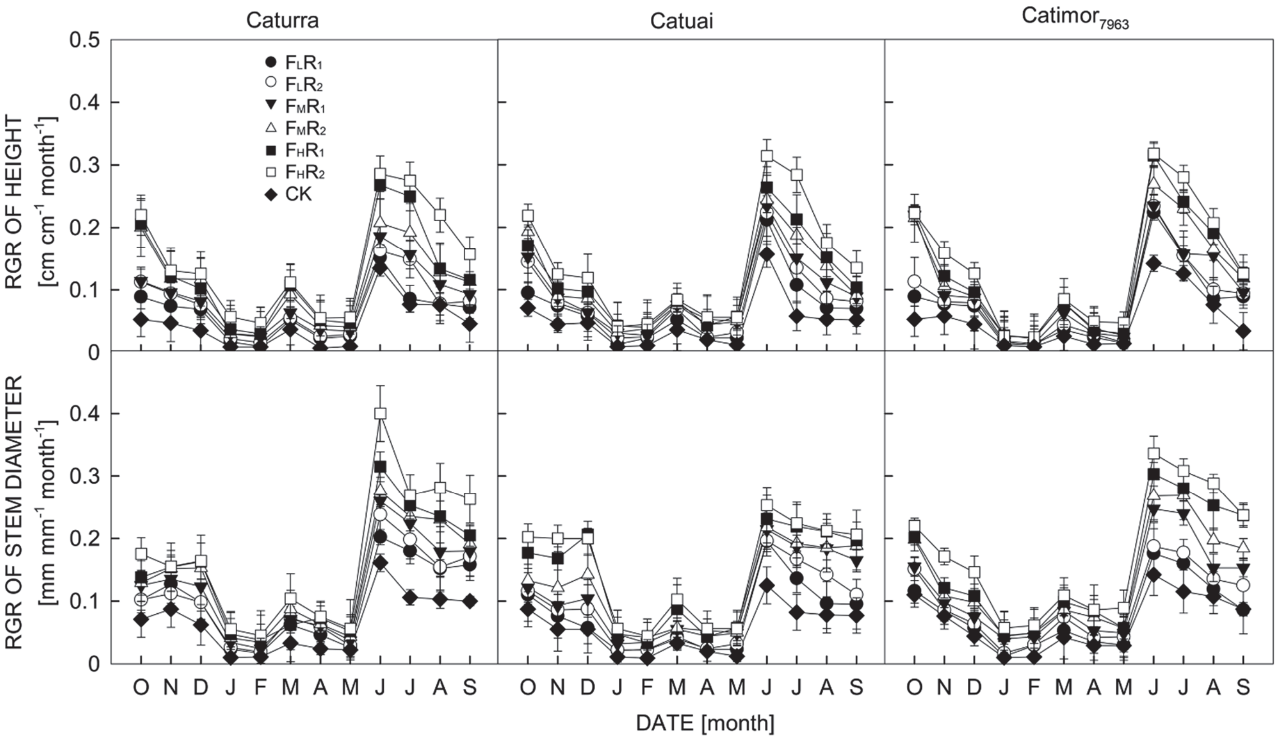


Fig. 1. Effects of fertilization treatments on the relative growth rate (RGR) of height and stem diameter (means ± SD, *n* = 30) in plants of three *Coffea arabica* cultivars from October 2013 until September 2014. $F_L$, $F_M$, and $F_H$ indicated that the NPK fertilization rate applied was 45, 90, 135 g per plant per year, respectively; $R_1$: N:$P_2O_5$:$K_2O$ = 1:0.5:0.8, $R_2$: N:$P_2O_5$:$K_2O$ = 1:0.8:0.5; CK: without fertilizer.

Table 1. Results of two-way *ANOVA* for morphological, growth, and photosynthetic traits of three *Coffea arabica* cultivars. RGR – relative growth rate, R/S – root to shoot ratio, $P_N$ – net photosynthetic rate, $g_s$ – stomatal conductance, $C_i$ – intercellular $CO_2$ concentration, $WUE_i$ – intrinsic water-use efficiency. ns – not significant, * – $p$<0.05, ** – $p$<0.01.

| Traits | | Source of variation<br>Cultivar (C) | Treatment (T) | C × T |
|---|---|---|---|---|
| Morphology | RGR of height | ns | ** | ns |
| | RGR of stem diameter | ns | ** | ns |
| | Height | * | ** | ns |
| | Stem diameter | * | ** | ns |
| | Leaf area | * | ** | ns |
| | Branch number | * | ** | * |
| | Length of the longest branch | * | ** | ns |
| | Internode number | * | ** | ns |
| Biomass | Individual biomass | * | ** | ns |
| | Leaf biomass | ns | ** | * |
| | Stem biomass | ns | ** | ns |
| | Branch biomass | ns | ** | * |
| | Root biomass | * | ** | * |
| | R/S | * | ** | * |
| Photosynthesis | $P_N$ | ns | * | ns |
| | $g_s$ | ns | ** | ns |
| | $C_i$ | ns | ** | ns |
| | $WUE_i$ | ns | ns | ns |

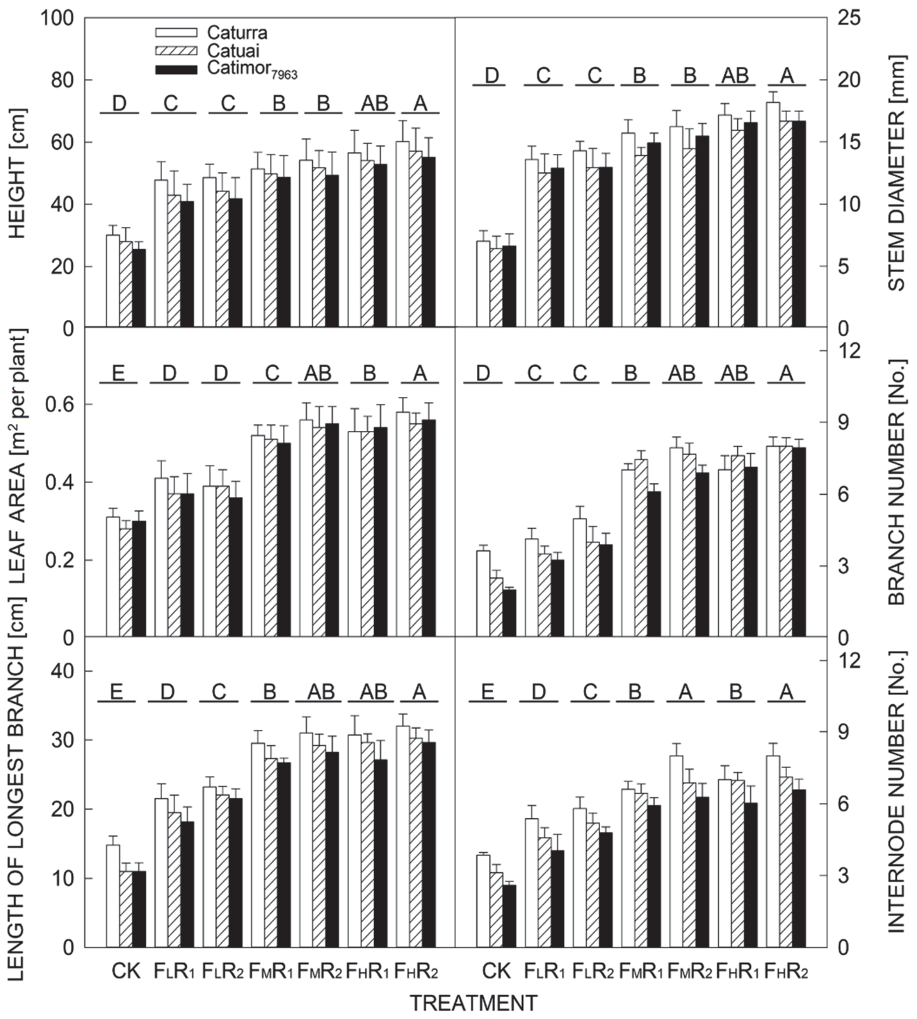


Fig. 2. Effects of fertilization treatments on the growth traits (means ± SD, $n$ = 30, except for the leaf area, which was $n$ = 5) of three *Coffea arabica* cultivars. Different *capital letters* above horizontal lines indicate significant differences between treatments. $F_L$, $F_M$, and $F_H$ indicated that the NPK fertilization rate was applied at 45, 90, 135 g per plant per year, respectively; $R_1$: $N:P_2O_5:K_2O$ = 1:0.5:0.8, $R_2$: $N:P_2O_5:K_2O$ = 1:0.8:0.5; CK: without fertilizer.

three cultivars (Table 1, Fig. 4). $P_N$ was found to correlate positively with $g_s$ across all treatments and seasons ($r$ = 0.86, $p$<0.01).

Leaf nutrient contents of the three coffee cultivars were affected by fertilizer, with the highest values being observed in $F_H$ and $F_M$ treatments (Table 2). Leaf P contents of the three coffee cultivars in $R_2$ tended to be higher than that in $R_1$, but leaf K contents showed the opposite trend. The CK treatment had the largest PNUE in all cultivars, followed by $F_MR_2$ treatment. Under the same fertilization rate, PNUE of Caturra were higher than that of Catuai and Catimor$_{7963}$, but no significant differences were found between $R_2$ and $R_1$.

**Correlation between the growth and photosynthetic traits**: Across different fertilization treatments and cultivars, the branch variables (branch number, length of the longest branch, and internode number) were positively related to the vegetative growth (RGRs in height and stem diameter), individual plant biomass, leaf area, and leaf photosynthetic rate, respectively (Table 3).

## Discussion

Crop productivity requires a sufficient nutrient supplementation. The leaf N, P, K contents in $F_L$ treatment (below 24.67 g kg$^{-1}$, 1.09 g kg$^{-1}$, and 17.89 g kg$^{-1}$, Table 2) were lower than the nutrient-deficit thresholds reported (Long *et al*. 1997, Cai *et al*. 2006), but leaf N, P, K contents in $F_H$ and $F_M$ treatments were within the appropriate range (Cai *et al*. 2006), which indicated that both $F_H$ and $F_M$ could provide well-balanced nutrition for the coffee plants

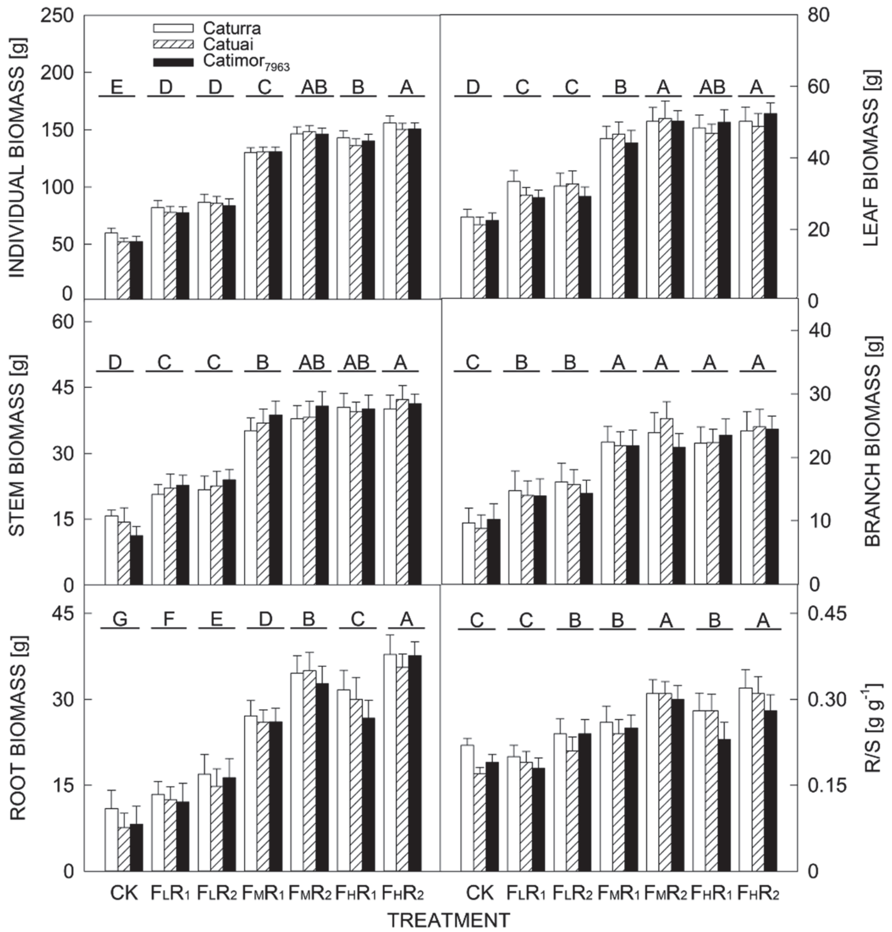


Fig. 3. Effects of fertilization treatments on the biomass and biomass allocation (means ± SD, $n$ = 5) of three *Coffea arabica* cultivars. R/S – root to shoot ratio. *Different capital letters* above horizontal lines indicate significant differences between treatments. $F_L$, $F_M$, and $F_H$ indicated that the NPK fertilization rate applied was 45, 90, 135 g per plant per year, respectively; $R_1$: $N:P_2O_5:K_2O$ = 1:0.5:0.8, $R_2$: $N:P_2O_5:K_2O$ = 1:0.8:0.5; CK: without fertilizer.

in our study. In most coffee plantations worldwide, vegetative growth of coffee plants showed active and quiescent growth periods modulated by the local duration of conductive growing conditions (Da Matta *et al.* 1999). Throughout the growth period in our study, all three cultivars of coffee plants showed a high growth peak in the wet season, and a minor peak in the dry season. Medium and high fertilization enabled the coffee plants to grow faster than the low-fertilized plants, but failed to change the growth rhythm of coffee (Fig. 1). Da Matta *et al.* (1999) also observed that nutrient supply can stimulate the growth during the rainy, warm season, but cannot prevent a growth decline during the dry, cool season.

A major indicator of high yield in coffee at the juvenile stage is a branch trait (Cai *et al.* 2007, Wang *et al.* 2014), and the branch length and internode number are significantly correlated with the later growth and production of coffee (Ou and Jiang 1991, Wang *et al.* 1996, Peng 2012). The height and stem diameter showed significant relationship with the branch variables (branch number, length, and internode number of the longest branch). A proper plant height can prevent branches growing too densely, avoid the branches and leaves shading each other, while stout stems can provide nutrient delivery to ensure healthy growth of coffee plants (Wang *et al.* 2014). In addition, photosynthetic area and capacity are key factors to determine plant growth and yield. However, both the positive and negative correlations between photosynthesis and growth have been reported (Kozlowski 1992, Silva *et al.* 2004). In this work, leaf area and $P_N$ were closely related to branch variables (Table 3), which were constant in our previous research (Cai *et al.* 2007). In $F_HR_2$, the three coffee cultivars exhibited the largest height, stem, leaf area, and branch variables, followed by the $F_HR_1$ and $F_MR_2$ treatments (Fig. 2). Therefore, the high rate of fertilization played an important role in promoting the growth at the juvenile stage.

The shoot to root ratio of trees shifted with age, generally from the 4:1 (one-year-old) assumed for tropical trees to 2:1 (Van Noordwijk *et al.* 2002). In the present study, about 75.8−84.4% of the dry matter of the coffee plants belonged to aboveground parts, similarly as in

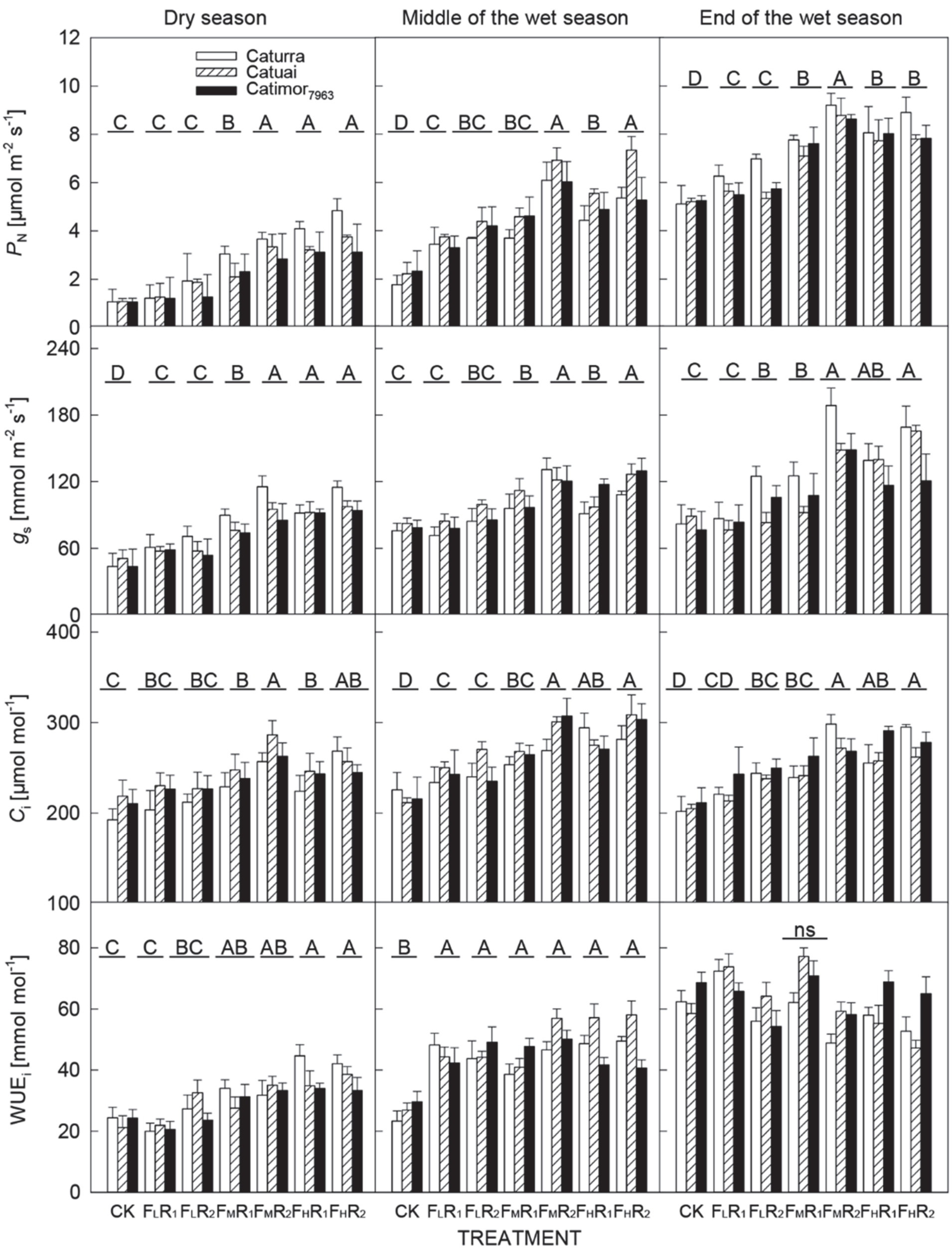


Fig. 4. Effects of fertilization treatments on the photosynthetic traits in leaves (means ± SD, $n$ = 5) of three *Coffea arabica* cultivars. $P_N$ – net photosynthetic rate, $g_s$ – stomatal conductance, $C_i$ – intercellular $CO_2$ concentration, $WUE_i$ – intrinsic water-use efficiency. *Different capital letters* above horizontal lines indicate significant differences between treatments. ns – not significant. $F_L$, $F_M$, and $F_H$ indicated that the NPK fertilization rate was applied at 45, 90, 135 g per plant per year, respectively; $R_1$: $N:P_2O_5:K_2O$ = 1:0.5:0.8, $R_2$: $N:P_2O_5:K_2O$ = 1:0.8:0.5; CK: without fertilizer.

one-year-old Arabica coffee (Chemura *et al*. 2014). In addition, it was found that N is the most important factor influencing the growth and photosynthesis of *C. arabica* plants, followed by K, while P influences adult plants the least (Cai *et al*. 2004). However, we found that P was relatively better than K in promoting the growth and differentiation of roots, shoots, and lateral buds at the juvenile stage in coffee under the same fertilization rate. Our result was different from those of Nazareno *et al*. (2003), but some reports insisted that P can accelerate cell proliferation (Mo *et al*. 2012). They found that P was mainly distributed in meristem of growing points of shoots and roots in coffee plants (Huang *et al*. 2009, Mo *et al*. 2012). Thus, increasing the P application in coffee plants at the juvenile growth stage can indeed promote the growth of roots and new shoots.

Fertilizer supply increased $P_N$ and $g_s$, but slightly affected $C_i$, especially at the end of the wet season (Fig. 4). The change in $P_N$ occurred largely due to stomatal limitations, similarly to that proposed by Silva *et al*. (2004)

in Catuai coffee plants in the field. The proportionally larger increase in $P_N$ than that in $g_s$ might be attributed to the accompanied increases in $WUE_i$, which was associated with the improved action of photosynthetic enzymes in coffee plants affected by $F_M$ and $F_H$.

$P_N$, $g_s$, and $WUE_i$ of coffee plants were lower during the dry season than those in the wet season (Fig. 4), as previously found (Cai *et al*. 2007). The reduction in $g_s$ in dry season reflected avoidance mechanism that minimizes water loss, which have been previously found (Silva *et al*. 2004, Cai *et al*. 2007). After severe drought stress, the photosynthesis of coffee can completely recover after

Table 2. Effects of fertilization treatments on leaf nutrient content and photosynthetic nitrogen-use efficiency (PNUE) of three *Coffea arabica* cultivars (means ± SD, $n = 3$). $F_L$, $F_M$, and $F_H$ indicated that the NPK fertilization rate applied was 45, 90, 135 g per plant per year, respectively; $R_1$: $N:P_2O_5:K_2O$ = 1:0.5:0.8, $R_2$: $N:P_2O_5:K_2O$ = 1:0.8:0.5; CK – without fertilizer. The *different letters* represent significant difference within each cultivar ($p$<0.05). ns – not significant, * – $p$<0.05, ** – $p$<0.01.

| Leaf traits | Treatment | Cultivar<br>Caturra | Catuai | $Catimor_{7963}$ |
|---|---|---|---|---|
| Leaf N content [g kg$^{-1}$] | CK | 18.9 ± 1.74$^{b}$ | 18.5 ± 2.04$^{c}$ | 18.62 ± 1.14$^{c}$ |
| | $F_LR_1$ | 22.5 ± 2.14$^{b}$ | 23.85 ± 2.56$^{c}$ | 24.42 ± 2.8$^{c}$ |
| | $F_LR_2$ | 23.45 ± 2.31$^{b}$ | 24.55 ± 2.63$^{c}$ | 24.67 ± 2.67$^{c}$ |
| | $F_MR_1$ | 27.68 ± 2.44$^{a}$ | 26.78 ± 2.22$^{b}$ | 27.87 ± 2.52$^{ab}$ |
| | $F_MR_2$ | 27.02 ± 2.8$^{a}$ | 26.72 ± 2.26$^{b}$ | 27.69 ± 2.79$^{ab}$ |
| | $F_HR_1$ | 27.74 ± 2.84$^{a}$ | 27.55 ± 2.86$^{ab}$ | 26.42 ± 2.27$^{b}$ |
| | $F_HR_2$ | 27.5 ± 2.72$^{a}$ | 29.42 ± 2.49$^{a}$ | 28.64 ± 2.38$^{a}$ |
| Leaf P content [g kg$^{-1}$] | CK | 1.0 ± 0.14$^{b}$ | 1.01 ± 0.11$^{c}$ | 1.01 ± 0.17$^{b}$ |
| | $F_LR_1$ | 1.01 ± 0.15$^{b}$ | 1.07 ± 0.15$^{c}$ | 1.11 ± 0.15$^{b}$ |
| | $F_LR_2$ | 1.19 ± 0.11$^{b}$ | 1.14 ± 0.17$^{c}$ | 1.21 ± 0.08$^{b}$ |
| | $F_MR_1$ | 1.33 ± 0.12$^{a}$ | 1.36 ± 0.08$^{b}$ | 1.46 ± 0.1$^{a}$ |
| | $F_MR_2$ | 1.32 ± 0.09$^{a}$ | 1.38 ± 0.09$^{b}$ | 1.5 ± 0.11$^{a}$ |
| | $F_HR_1$ | 1.41 ± 0.17$^{a}$ | 1.48 ± 0.17$^{ab}$ | 1.45 ± 0.09$^{a}$ |
| | $F_HR_2$ | 1.48 ± 0.18$^{a}$ | 1.59 ± 0.11$^{a}$ | 1.56 ± 0.18$^{a}$ |
| Leaf K content [g kg$^{-1}$] | CK | 17.49 ± 1.14$^{c}$ | 17.61 ± 1.46$^{bc}$ | 17.77 ± 1.18$^{bc}$ |
| | $F_LR_1$ | 17.89 ± 1.45$^{c}$ | 19.11 ± 1.86$^{bc}$ | 19.7 ± 1.53$^{bc}$ |
| | $F_LR_2$ | 17.19 ± 1.84$^{c}$ | 17.16 ± 1.49$^{c}$ | 17.81 ± 1.81$^{c}$ |
| | $F_MR_1$ | 21.17 ± 1.72$^{ab}$ | 21.13 ± 1.44$^{ab}$ | 22.16 ± 1.16$^{ab}$ |
| | $F_MR_2$ | 20.16 ± 1.63$^{b}$ | 20.69 ± 1.67$^{ab}$ | 20.77 ± 1.6$^{b}$ |
| | $F_HR_1$ | 22.22 ± 1.22$^{a}$ | 22.12 ± 1.27$^{a}$ | 22.82 ± 1.35$^{a}$ |
| | $F_HR_2$ | 21.01 ± 1.26$^{ab}$ | 21.18 ± 1.08$^{b}$ | 21.78 ± 1.38$^{ab}$ |
| PNUE [µmol g$^{-1}$(N) s$^{-1}$] | CK | 3.8 ± 0.14$^{a}$ | 3.69 ± 0.17$^{a}$ | 3.75 ± 0.18$^{a}$ |
| | $F_LR_1$ | 3.42 ± 0.23$^{ab}$ | 2.59 ± 0.24$^{b}$ | 2.86 ± 0.13$^{b}$ |
| | $F_LR_2$ | 3.61 ± 0.09$^{a}$ | 2.95 ± 0.34$^{b}$ | 2.88 ± 0.21$^{b}$ |
| | $F_MR_1$ | 3.21 ± 0.16$^{b}$ | 2.9 ± 0.14$^{b}$ | 3.09 ± 0.16$^{b}$ |
| | $F_MR_2$ | 3.74 ± 0.21$^{a}$ | 3.48 ± 0.16$^{a}$ | 3.41 ± 0.26$^{ab}$ |
| | $F_HR_1$ | 3.18 ± 0.22$^{b}$ | 2.99 ± 0.27$^{b}$ | 2.92 ± 0.35$^{b}$ |
| | $F_HR_2$ | 3.56 ± 0.26$^{a}$ | 3.17 ± 0.08$^{ab}$ | 3.29 ± 0.13$^{b}$ |
| Two-way *ANOVA* (significant level) | | | | |
| | | Cultivar (C) | Treatment (T) | C×T |
| Leaf N content | | ns | ** | Ns |
| Leaf P content | | * | ** | Ns |
| Leaf K content | | ** | ** | Ns |
| PNUE | | ** | ** | Ns |

Table 3. Coefficient index between the branch variables and the growth parameter, biomass and photosynthetic rate of three *Coffea arabica* cultivars. * – $p$<0.05, ** – $p$<0.01. RGR – relative growth rate; $P_N$ – net photosynthetic rate.

| Correlation efficiency ($R$) | RGR of height | RGR of stem diameter | Height | Stem diameter | Leaf area | Individual biomass | $P_N$ |
|---|---|---|---|---|---|---|---|
| Branch number | 0.79* | 0.78* | 0.84* | 0.83* | 0.98** | 0.98** | 0.93** |
| Length of the longest branch | 0.80* | 0.86* | 0.85* | 0.86* | 0.97** | 0.96** | 0.92** |
| Internode number | 0.78* | 0.79* | 0.83* | 0.86* | 0.97** | 0.93** | 0.93** |

rehydration (Cai *et al*. 2005). $P_N$ and $WUE_i$ in the middle of the wet season were lower than those at the end of the wet season, because the prolonged dry season (from November 2013 to early June 2014) caused severe drought stress in coffee plants and resulted in the decrease of photosynthetic capacity, in accordance with the results of Yang *et al*. (2014).

$P_N$ and photosynthetic nitrogen-use efficiency (PNUE) of all treatments was considerably lower than the values obtained in other woody species (Rosati *et al*. 1999, Hikosaka and Hirose 2000). Compared to species with high PNUE, coffee plants exhibited relatively low PNUE and $P_N$, and high N requirement, likely due to higher N expenditures for nonphotosynthetic functions, *i.e.*, caffeine (Da Matta *et al*. 2002). Coffee plants under N deficiency appeared to be able to utilize N more efficiently, through increasing N investment into photosynthetic components; and thus to increase PNUE with decreasing N (Da Matta *et al*. 2002). However, we did not find increasing PNUE with decreasing N supply (Table 2). This result may be due to high and medium nutrient supply reached a good balance in increasing Rubisco content and decreasing Rubisco activation. In addition, PNUE in $R_2$ were higher than that in $R_1$, implying that P was better than K to promote the use efficiency of N fertilizer. Meanwhile, Caturra had the highest PNUE among the three cultivars, which partially indicated that a high N-use efficiency and use of N-fertilizer with limited environmental risks from N loss by volatilization and/or leaching.

In conclusion, enhancement of the P application could maximize the use of N, promote branch and root growth in juvenile coffee plants. Both medium and high fertilization rates could significantly promote gas exchange in leaves of the three coffee cultivars during all seasons, and show similar effects on vegetative growth and biomass. However, considering the high fertilizer may also increase costs and induce high environmental contamination, we recommend application of medium fertilization rate ($F_M$, 90 g per plant per year), together with NPK ratio of 1:0.8:0.5 in the plantation of juvenile coffee plants.

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