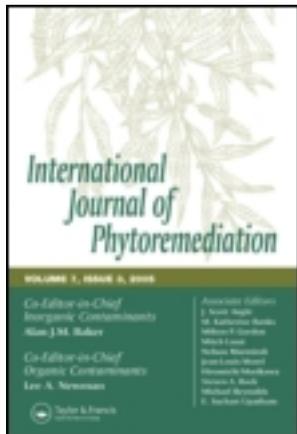


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### Effects of High Nutrient Supply on the Growth of Seven Bamboo Species

Julien Piouceau <sup>a b</sup>, Grégory Bois <sup>a</sup>, Frédéric Panfili <sup>a</sup>, Matthieu Anastase <sup>a</sup>, Laurent Dufossé <sup>b</sup> & Véronique Arfi <sup>a</sup>

<sup>a</sup> PHYTOREM S.A., site d'Areva, chemin de l'autodrome, Miramas, France

<sup>b</sup> Université de La Réunion, Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, site ESIROI-IDAI, Département Agroalimentaire, Sainte-Clotilde, France

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## EFFECTS OF HIGH NUTRIENT SUPPLY ON THE GROWTH OF SEVEN BAMBOO SPECIES

Julien Piouceau,<sup>1,2</sup> Grégory Bois,<sup>1</sup> Frédéric Panfili,<sup>1</sup>  
Matthieu Anastase,<sup>1</sup> Laurent Dufossé,<sup>2</sup> and Véronique Arfi<sup>1</sup>

<sup>1</sup>PHYTOREM S.A., site d'Areva, chemin de l'autodrome, Miramas, France

<sup>2</sup>Université de La Réunion, Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, site ESIROI-IDAI - Département Agroalimentaire, Sainte-Clotilde, France

*Over the last decade, bamboo has emerged as an interesting plant for the treatment of various polluted waters using plant-based wastewater treatment systems. In these systems, nitrogen and phosphorous concentrations in wastewater can exceed plant requirements and potentially limit plant growth. The effects of two nutrient rates on the growth of seven bamboo species were assessed in a one-year experiment: Dendrocalamus strictus, Thyrsostachys siamensis, Bambusa tuldoidea, Gigantochloa wrayi, Bambusa oldhamii, Bambusa multiplex and Bambusa vulgaris. Nutrient rates were applied with a 20:20:20 NPK fertilizer as 2.6 and 13.2 t.ha.yr<sup>-1</sup> NPK to three-year-old bamboo planted in 70 L containers. Morphological characters, photosynthetic responses, and NPK content in bamboo tissues were investigated. Under high-nutrient supply rate, the main trend observed was an increase of culm production but the culms' diameters were reduced. For the seven species, the aboveground biomass yield tended to increase with high-nutrient rate. Increasing in nutrient rates also improved the photosynthetic activity which is consistent with the increase of nitrogen and phosphorus contents measured in plant tissues. All the bamboo species tested appears suitable for wastewater treatment purposes, but the species Bambusa oldhamii and Gigantochloa wrayi showed the higher biomass yield and nutrient removal.*

**KEY WORDS:** Bamboo species, specific leaf area, chlorophyll *a* fluorescence, high nutrient rate, bamboo biomass

### INTRODUCTION

Over the last decades, wastewater treatment systems using phytoremediation principles have been developed (Adams *et al.* 2000; McCutcheon and Schnoor 2003). Most of these systems are constructed wetlands which use aquatic plants (Vymazal 2011). Another type of system uses terrestrial plants like poplar, willow and more recently bamboo species. As a plant for treating wastewater, bamboo is interesting in many respects. For mature bamboo plantations of giant species, the aboveground biomass yield can reach 25 and 47 t DM.ha<sup>-1</sup>.yr<sup>-1</sup> under temperate and tropical climate respectively (Scurlock, Dayton, and

Address correspondence to Julien Piouceau; PHYTOREM S.A., site d'Areva, chemin de l'autodrome, 13140 Miramas, France. E-mail: julienpiouceau@phytorem.com

Hames 2000). Mature bamboo plantations have also interesting evapotranspiration rates that range from 9 to 13 mm.day<sup>-1</sup> under tropical climate (Kleinhenz and Midmore 2002). In addition, the bamboo species' dense root system favors the rhizodegradation (McCutcheon and Schnoor 2003) of organic matter contained in wastewater.

In wastewater treatment system using bamboo species, the wastewater is directly spread on the soil surface of the plantation (Perttu and Kowalik 1997; Rosenqvist and Ness 2004; Thawale, Juwarkar and Singh 2006; Singh *et al.* 2008; Arfi *et al.* 2009). Therefore an over application of wastewater on the plantation can lead to a release of nutrients such nitrate and phosphorus in the groundwater that contribute to the eutrophication of water resources (Smith, Tilman, and Nekola 1999; Smith 2009). So it is important to determine the amount of nutrients, especially the amount of N and P that can be stored in the plant biomass. By selecting the species that are most adapted in terms of nutrient removal, growth rate and biomass yield, it may be possible to optimize the treatment system. For wastewater treatment systems using bamboo, young bamboos are planted on the existing soil (Arfi *et al.* 2009). Obviously, a good initial growth stage of young plantations is required to ensure an optimal treatment. Domestic wastewater application provide high amount of nutrient, especially nitrogen and phosphorus (Pescod 1992) for a young bamboo plantation. For crops, an excess of nitrogen induces a rapid stem elongation and results in sensitive crops to stem breakage; excess of nitrogen can also induces a decrease of biomass and grain yield (Morishita 1988; Bennett 1993; Elia and Conversa 2012). High inputs of phosphorous can induce zinc, iron and copper deficiencies (Forsee and Allison 1944; Bingham 1973) and hence reduce the biomass yield. Few studies deal with the effect of high nutrient application on the growth of bamboos (Li *et al.* 2000; Fernandez *et al.* 2003; Kleinhenz *et al.* 2003). Li *et al.* (2000) showed that fertilization increase the shoot number but had no effect on diameter and height for *P. Pubescens*. Conversely, Azmy *et al.* (2004) showed that fertilization increase significantly the height and diameter and tend to increase the shoot number for *G. scortechinii*. The mentioned studies suggest that fertilization induced various morphological modifications which depend on the bamboo species. However, the nutrient rates applied in these studies remain low as compared to the rates that can be reached in a context of wastewater treatment. In addition, these studies report applications of fertilizer on mature bamboo plantations and not on young bamboo.

Our study aims to provide new insights on the effect of high-nutrient supply rates, as it occurs in wastewater treatment system, on the growth of young bamboos species and its consequences on the biomass yield and on the potential storage of the major nutrients (i.e., NPK) in the biomass.

Bamboo represents over 70 genera and 1200 species in the *Bambusoideae* sub-family and are present all over the world (Kleinhenz and Midmore 2001). Bamboo species use a rhizomatous vegetative growth strategy. New shoots, or culms, arising from rhizomes and grow rapidly achieving their final height within one to two months. Once the culms are fully developed their external diameter did not increase anymore. Thus, the growth of bamboo is defined by the number of new shoots that emerged each year, generally during the pluvial season. Each year, culms increase in number and size forming a clump (set of culms). In order to assess the growth of young bamboos under high-nutrient rates, a one-year experiment was carried out using seven species of clumping bamboo (sympodial bamboo; Stapleton 1998), selected for their high biomass yield at mature age. Three-years-old bamboo seedlings were planted in containers receiving two nutrients rates: a "low" and a "high" nutrient rate. A commercial fertilizer was used to mimic the concentration of nitrogen and phosphorus contained in domestic wastewater. The response of bamboo species to high

nutrient rates was determined using the number of culms produced during the experiment and their diameter. The final aboveground biomass yield was then quantified by allometric equations. Chlorophyll *a* fluorescence measurements were performed to determine the photosynthetic activity of the bamboo species. Chlorophyll *a* fluorescence measurements are now widespread to examine photosynthetic performance and stress in plants and to explain the growth variations that may occur between species (Baker 2008). In the same way, the specific leaf area (SLA) was measured to explain variations in growth rate between species. Indeed SLA is an indicator trait of resource-use strategies (Amanullah *et al.* 2007) and relative growth rate (Reich, Michael and Ellsworth 1997). Many studies have demonstrated that the increase in NPK fertilization increased the nitrogen concentration and are positively correlated with leaf photosynthetic activity (Jin *et al.* 2011). So, the total nitrogen, phosphorous and potassium contents in leaves and culms were also measured to explain growth patterns.

## MATERIALS AND METHODS

### Experimental Conditions

The experiment was conducted over one year, from March 25th, 2008 to March, 27th, 2009 (367 days), on Reunion Island, an overseas French department in the south-west Indian Ocean. The experimental site was located at 21°03' S; 55°19' E, at an elevation of 1043 m. During the experiment, the site had a temperature range of 13.2 to 26.2 C°, a total rainfall of 1240 mm.yr<sup>-1</sup> and an average relative humidity of 81.2%.

Seven species of clumping bamboo were selected for the experiment: *Bambusa vulgaris* Schrad. (**BVV**), *Bambusa oldhamii* Munro (**BO**), *Bambusa multiplex* (Lour.) Raeusch. ex Schult. (**BMA**), *Bambusa tuldoides* Munro (**BV**), *Thyrsostachys siamensis* Gamble (**TS**), *Dendrocalamus strictus* (Roxb.) Nees (**DS**) and *Gigantochloa wrayi* Gamble (**GW**). These species have been chosen for their high biomass yields and because these species are the most studied in literature (Suwannapinunt and Thaitutsa 1988; Tripathi and Singh 1996; Singh and Singh 1999; Kleinhenz and Midmore 2001; Castaneda-Mendoza *et al.* 2005; Kibwage *et al.* 2008).

For each species, a cutting of mature culm from a mother clump was taken and planted in soil to allow the sprouting of roots and rhizomes over a one-year period. Grown cuttings were transplanted into 3 liter containers for one year, and then into 15 liter containers for a further before being planted in 70 liter containers for the present experiment. At the beginning of the experiment bamboo species were three years-old.

The growing media used was a 3:1:1 (v:v:v) mix of soil, scoria and sugar cane fibers. Containers were then arranged in a fully randomized block design (7\*2) based on species (seven levels) and nutrient rates (two levels), with 3 replicates per treatment. Each of the three blocks contained each treatment combinations, giving a total of 42 containers of bamboo.

Two stock solutions were prepared using a commercial fertilizer (20:20:20 NPK Soluplant, Duclos International, France) diluted in tap water. The use of fertilizer solutions allowed keeping steady the nutrient concentration of the solution all along the experiment, that it would have not been the case if we had used domestic wastewater. Three kilograms of the fertilizer were dissolved in 100 liters of tap water for the low-nutrient treatment, and fifteen kilograms of fertilizer in 100 liters of tap water for the high-nutrient treatment. One liter of a trace element solution (Oligo Drip 25 Fe, Duclos International, France) was

added to each of the two stock solutions. Plants were watered once a week with tap water and twice a week with nutrient solutions using a drip irrigation system (Netafim<sup>TM</sup>, Israel). The average height of water (with or without fertilizer) added at each watering session was about 70 mm per container. Both stock solutions were diluted inline using a metering pump (Dosatron, France) to provide the nutrient solution to the drip irrigation system via two separate networks, one for each nutrient treatment. The dilution was 1% in the dry season (May to November) and 1.6% in the wet season (December to April). The concentration of the low-nutrient treatment corresponded approximately to the nitrogen concentration found in domestic wastewater, which ranges between 20 and 85 mg.l<sup>-1</sup> (Pescod 1992); the nitrogen concentration for the high-nutrient treatment was five times higher than in domestic wastewater. The concentration range of phosphorous in domestic wastewater is 6 to 20 mg.l<sup>-1</sup> (Pescod 1992). In detail: during the dry season, the concentration of the low-nutrient treatment was 60 mg.l<sup>-1</sup> of nitrogen (N), 60 mg.l<sup>-1</sup> of phosphorus (P) and 60 mg.l<sup>-1</sup> of potassium (K) (electric conductivity of 0.5 mS.cm<sup>-1</sup>) and the concentration of the high-nutrient treatment was 300 mg.l<sup>-1</sup> of N, 300 mg.l<sup>-1</sup> of P and 300 mg.l<sup>-1</sup> of K (electric conductivity of 1.3 mS.cm<sup>-1</sup>); during the wet season, the concentration of the low-nutrient treatment was 96 mg.l<sup>-1</sup> of N, P, and K (0.7 mS.cm<sup>-1</sup>) and the concentration of the high-nutrient treatment was 480 mg.l<sup>-1</sup> of N, P, and K (1.8 mS.cm<sup>-1</sup>). During the experiment, a total of 45.6 g and 228 g N, P, and K per pot were added for the low and high nutrient rate treatments respectively, that represents 2.6 and 13.2 t ha<sup>-1</sup> yr<sup>-1</sup> N, P and K taking the pot area as the surface unit.

### Bamboo Biomass Calculation

In order to determine the total number of culms produced during the experiment, all culms initially present in each 70-liter container were counted and their diameter measured with a digital caliper. The culms were then labeled with a paint spray. At the end of the experiment, all unlabeled culms produced during the experiment were counted in each container, their height measured with a tape measure, their basal diameters measured with a digital caliper at the middle of the first internode and the number of internodes counted.

The biomass yield was determined using allometric equations (Shanmughavel and Francis 2001). For each bamboo species, the allometric equations were established using the basal diameter. In each container, four culms were randomly sampled to measure basal outer diameter, inner diameter (*i.e.* wall thickness), total fresh biomass, fresh leaf biomass and fresh culm plus branch biomass. Weights were measured with a 0.1g-precision scale (Kern & sohn GmbH, Germany). Sub-samples of leaves and culms (including branches) were taken to determine the dry mass (DM) of each part and for chemical analysis once dried.

Regression equations were established between the fresh mass ( $y$ ), the dry mass ( $y$ ) and the basal diameter ( $x$ ). Raw data was log-transformed to normalize the data distribution and to linearize the regression functions according to the equation (1)

$$\text{Log}(y) = a + b \log(x) \quad (1)$$

Regression equations were computed with Minitab 15 software (Minitab Inc., USA). This equation (1) was transformed to obtain the standard form of the allometric equation (2) (Navar 2010):

$$y = bx^a \quad (2)$$

A correction factor was then applied to the final biomass result ( $y$ ) to correct the bias engendered by the logarithm transformation using the following equation (3) (Sprugel 1983)

$$CF = \exp^{(SEE^2/2)} \quad (3)$$

where CF is the correction factor and SEE the standard error of the estimate of the regression.

The total aboveground biomass produced during the year of experimentation was determined for each species thanks to the allometric equation obtained.

### Plant Material, Water Content, and Chemical Analysis

To determine the dry mass, sub-samples of leaves, branches and culms were oven-dried at 70 °C for 48 hours in a drying oven (Memmert, GmbH & Co, Germany) to preserve the nutrients for chemical analysis.

Nitrogen content was determined using the Dumas method by means of an element analyzer (CN 2000, LECO Corporation, USA). For phosphorus and potassium analysis, plant samples were turned into ash (500°C for two hours). The ashes were cooled at ambient air temperature and then digested in two milliliters of hydrochloric acid solution (HCL) at 50%, before being heated on a hot plate to total evaporation of HCL. Two milliliters of HCL were added *de novo* and the ashes were allowed to stand in the HCL for ten minutes. The solution was filtered and then diluted with distilled water in a 50 ml flask. The phosphorus content was determined by a colorimetric method using the ammonium molybdate method with a colorimeter (Proxima, Alliance Instruments Italy). Potassium content was determined by atomic absorption spectrophotometry (220FS, Varian inc., USA).

Only the species *B. oldhamii* (BO), *G. wrayi* (GW), *B. vulgaris* (BVV), *D. strictus* (DS) were analysed.

### Chlorophyll *a* Fluorescence Measurements

Fluorescence measurements were done using a pulse amplitude modulation portable fluorometer (Mini-PAM, Walz GmbH, Germany). All measurements were made on mature leaves from culms produced during the experiment. The maximum quantum yield of photosystem II (PSII) -noted  $F_v/F_m$  in the following- was obtained by dark-adapting leaves for 20 minutes, as recommended by Rascher *et al.* (2000), before applying a saturation pulse of 8000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 800 milliseconds. The effective quantum yield of PSII ( $\Delta F/F'_m$ ) -noted  $\Phi_{PSII}$  in the following-, was obtained per the same protocol but on light-adapted leaves.

Fluorescence measurements were done at the beginning and at the end of the experiment, one block per day (in March 24, 25, 26, 2008 and in March 24, 25, 26, 2009) and always in the morning to avoid the diurnal photoinhibition of midday (Fernandez-Baco *et al.* 1998; Kumar, Pal and Teotia 2002). The measurements of  $F_v/F_m$  and  $\Phi_{PSII}$  were performed twice in the morning on three randomly selected leaves in each experimental unit. As for the nutrient content analysis of tissues, we focused on the following species: *B. oldhamii* (BO), *G. wrayi* (GW), *B. vulgaris* (BVV), *D. strictus* (DS) for the fluorescence measurements.

### Specific Leaf Area Measurements

Specific Leaf Area (SLA) is the ratio between the total leaf area divided by the total dry mass of the leaves sampled. Leaf samples were taken randomly along the culms on the following species: BO, GW, BVV, DS. Leaves were immediately scanned with a scanner (Mustek Scanexpress, Mustek Systems Inc., Taiwan), weighted and then oven-dried at 105 C° for 48 hours. The leaf area was calculated using scan images processed using Adobe Illustrator CS4 software (Adobe Systems Inc., USA).

### Statistical Analyses

Regression trend lines and the coefficient of determination ( $R^2$ ) were calculated using Minitab 15 software (Minitab inc., USA). A two-way ANOVA was performed with SPSS software (SPSS inc., IBM, USA) to test the effect of the “species” factor and the effects of “nutrient rates” factor on the mean number of culms (produced during the experiment), their diameters, total aboveground biomass, nutrient concentration in leaves and culms, fluorescence parameters and SLA. The LSD test was used to compare nutrient rate effects across bamboo species on the mean number of shoots, their diameters, total aboveground biomass, nutrient concentration in leaves and culms, fluorescence parameters and SLA.

## RESULTS

### Effect of the Nutrient Rate on the Number of Culms and on Culms Morphology

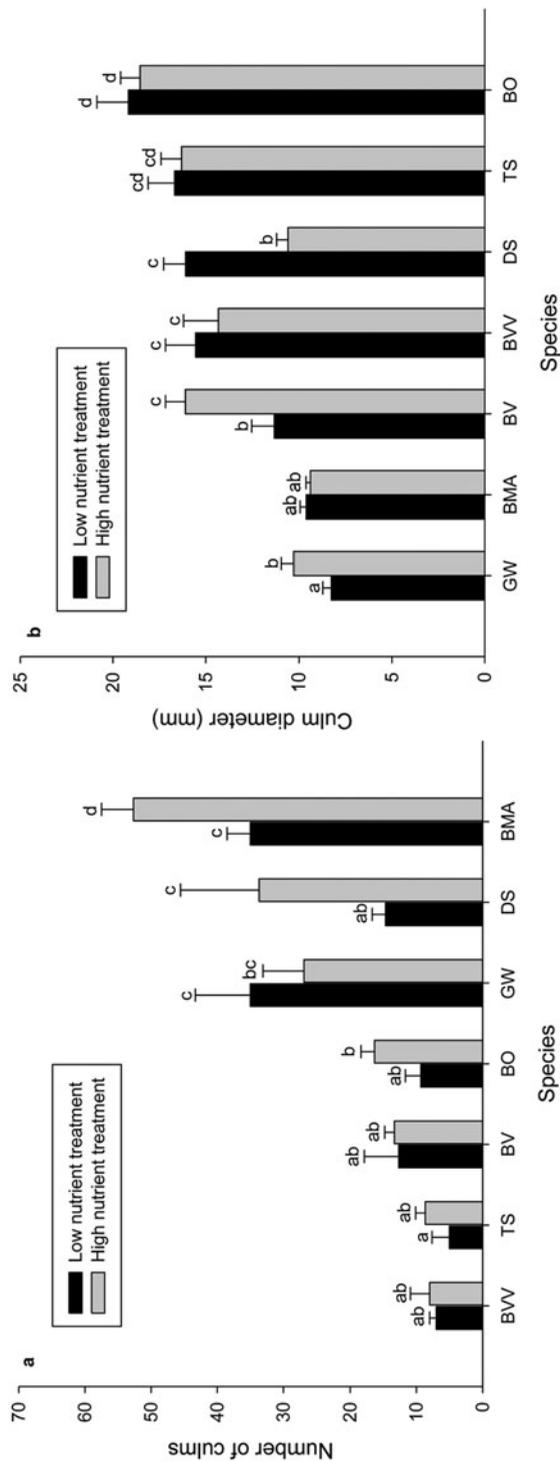
On the whole, the mean number of new culms per species increased significantly with the increase in nutrient rate (Figure 1a;  $p < 0.01$ ). This increase in culm number is significant for BMA ( $p < 0.01$ ), from 35 to 52 new culms and for DS ( $p < 0.001$ ) from 14 to 33 new culms produced, for the low and high-nutrient treatments respectively. On the contrary, high-nutrient treatment tended to reduce the number of culms of GW from 35 culms with the low-nutrient treatment to 27 culms with the high-nutrient treatment.

On the whole, the mean diameter of culms tended to decrease with the increase in nutrient rate (Figure 1b). This decrease is significant for DS ( $p < 0.001$ ) from 16 to 10.5 millimeters. Conversely the mean diameter of culms significantly increased for GW ( $p < 0.05$ ) and BV ( $p < 0.001$ ) from 8.2 to 10.2 and 11.3 to 16.1 millimeters respectively with the high-nutrient treatment. For the high nutrient rate, no significant effect was noticed on the height of culms and their wall thickness (data not shown).

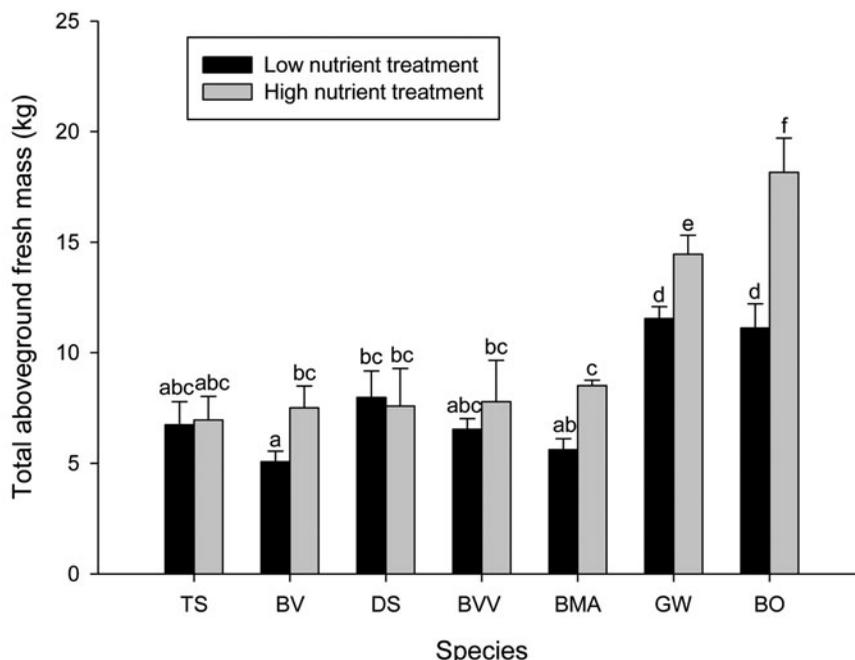
The aboveground biomass yield was determined with allometric equations listed in Table 3. The increase in nutrient rate significantly increased total aboveground fresh biomass (Figure 2;  $p < 0.001$ ). A significant increase of the biomass ( $p < 0.05$ ) by 25, 48, 51, and 63% was observed for GW, BV, BMA, and BO respectively. The species GW produced 5.9 to 7.5 kg, and the species BO produced 9.6 to 11.2 kg more aboveground fresh biomass with the high-nutrient treatment than the other species.

### Photosynthetic Activity and SLA

All bamboo species showed  $F_v/F_m$  ratios above 0.700 (Table 1). The  $F_v/F_m$  ratio tended to be higher for the high-nutrient treatment. This trend was even significant for the BVV species ( $p < 0.001$ ). The values of  $\Phi_{PSII}$  tended to be higher for GW, BVV, and



**Figure 1** Mean number (a) and diameter (b) of new bamboo culms produced during the experiment for the low and the high-nutrient treatment for the species, *B. vulgaris* (BVV), *T. siamensis* (TS), *B. tuldooides* (BV), *B. oldhamii* (BO), *G. wrayi* (GW), *D. strictus* (DS), and *B. multiplex* (BMA). Letters indicate significant differences among the two nutrient treatments (LSD,  $p < 0.05$ ).



**Figure 2** Total aboveground fresh mass (kg) produced over the one-year experiment for low and high-nutrient treatments for *T. siamensis* (TS); *B. tuldoidea* (BV); *D. strictus* (DS); *B. vulgaris* (BVV); *B. multiplex* (BMA); *G. wrayi* (GW); *B. oldhamii* (BO). Letters indicate significant differences between the two nutrient treatments (LSD,  $p < 0.05$ ).

DS under the high-nutrient treatment. In decreasing order, the  $\Phi_{PSII}$  is higher with the high-nutrient treatment for DS, BVV, BO, and GW.

The SLA increased significantly ( $p < 0.05$ ) with the high nutrient treatment. The SLA increased significantly for DS ( $p < 0.05$ ) from 231.4 to 279.3  $\text{g}\cdot\text{cm}^{-1}$  for the low and high-nutrient treatments respectively (Table 1). The SLA tended to increase with the

**Table 1** Chlorophyll *a* fluorescence measurements and specific leaf area (SLA) for the low and high-nutrient treatment

Species	Nutrient rate	Fv/Fm <sup>a</sup>	Yield ( $\Phi_{PSII}$ ) <sup>b</sup>	SLA ( $\text{g}\cdot\text{cm}^{-1}$ ) <sup>c</sup>
<i>D. strictus</i>	Low	0,758 ± 0,014bc	0,744 ± 0,065b	231,40 ± 18,10a
	High	0,757 ± 0,008bc	0,770 ± 0,022b	279,30 ± 32,70b
<i>B. vulgaris</i>	Low	0,700 ± 0,016a	0,650 ± 0,043ab	192,60 ± 23,20a
	High	0,787 ± 0,009c	0,705 ± 0,048ab	215,00 ± 16,70a
<i>G. wrayi</i>	Low	0,714 ± 0,019ab	0,561 ± 0,050a	206,75 ± 5,46a
	High	0,720 ± 0,023ab	0,649 ± 0,087ab	209,98 ± 7,30a
<i>B. Oldhamii</i>	Low	0,723 ± 0,027ab	0,751 ± 0,034b	187,67 ± 6,85a
	High	0,742 ± 0,010abc	0,672 ± 0,080ab	203,15 ± 7,15a

<sup>a</sup> Fv/Fm maximum quantum yield of photosystem II; <sup>b</sup> Yield effective quantum yield of photosystem II; <sup>c</sup> SLA specific leaf area.

Values are means ±SE. The values with different letters indicate significant differences between the species and the nutrient rates (LSD,  $p < 0.05$ ).

high-nutrient treatment for BO, GW, and BVV. In decreasing order, the SLA was higher with the high-nutrient treatment for DS, BVV, GW, and BO.

### Nitrogen and Phosphorus Contents

Applying a higher nutrient rate induced a significant increase of nitrogen in leaves and culms ( $p < 0.001$ ). The nitrogen content in culms increased for all species by a factor of 2 to 4 with the high-nutrient treatment compared to the low-nutrient treatment. For the high-nutrient treatment the nitrogen content ranged from 29.4 to 36 g.kg<sup>-1</sup> for the leaves and from 14.1 to 18.8 g.kg<sup>-1</sup> DM for the culms. DS was the species which store the higher amount of nitrogen in leaves and culms (Table 2) and the species BO showed the best response to the high-nutrient treatment with an increase in the leaves' nitrogen content of 38% and 349% for the culms.

The phosphorus content was significantly increased in leaves and culms with the high-nutrient treatment ( $p < 0.001$ ). The phosphorus contents between leaves and culm was close and ranged from 1.8 to 4.3 g.kg<sup>-1</sup> for the leaves and from 1.9 to 3.5 g.kg<sup>-1</sup> DM for the culms. BO was the species which store the higher amount of phosphorus in leaves and culms (Table 2) and GW showed the best response to a high nutrient treatment with an increase in the leaves and culms' phosphorus content of 81%.

Depending on the species and the plant part, the potassium contents increased or decreased with the high-nutrient treatment. For the DS species, the potassium content in culms increased significantly from 7.7 to 14.6 g.kg<sup>-1</sup> DM ( $p < 0.01$ ) and tended to increase in leaves from 16.5 to 17.3 g.kg<sup>-1</sup> DM. For the GW species, the potassium content tended to increase in leaves from 12.6 to 14 g.kg<sup>-1</sup> DM and in culms from 5.5 to 6.9 g.kg<sup>-1</sup> DM. Conversely, for the BVV and BO species, potassium decreased in leaves and culms.

**Table 2** Average nitrogen, phosphorus and potassium contents in leaves and culms in five bamboo species, for the low and the high-nutrient treatment.

Species	Plant part	Nutrient rate	Nitrogen (g/kg DM)	Phosphorus (g/kg DM)	Potassium (g/kg DM)
<i>D. strictus</i>	Leaf	Low	30, 7 ± 1, 3c	1, 8 ± 0, 1a	16, 5 ± 1, 8cd
		High	36 ± 1, 5d	2, 5 ± 0, 3b	17, 3 ± 1, 6d
	Culm	Low	6, 2 ± 0, 9ab	1, 3 ± 0, 3a	7, 7 ± 2ab
		High	18, 8 ± 2, 4e	1, 9 ± 0, 4abc	14, 6 ± 3, 6c
<i>B. vulgaris</i>	Leaf	Low	28, 4 ± 0, 9c	1, 7 ± 0, 0a	16, 5 ± 1, 1cd
		High	30, 7 ± 1, 3c	1, 8 ± 0, 1a	15, 4 ± 0, 5cd
	Culm	Low	8, 8 ± 0, 9b	1, 5 ± 0, 2a	10, 6 ± 1, 7bc
		High	17, 8 ± 0, 3de	2, 3 ± 0, 2bc	9, 5 ± 1, 8ab
<i>G. wrayi</i>	Leaf	Low	25, 4 ± 1, 1b	1, 8 ± 0, 1a	12, 6 ± 0, 7ab
		High	29, 4 ± 0, 9c	3, 2 ± 0, 1c	14 ± 1bc
	Culm	Low	6, 6 ± 0, 6ab	1, 9 ± 0, 2ab	5, 5 ± 0, 6a
		High	14, 1 ± 1, 1c	3, 3 ± 0, 3d	6, 9 ± 1, 5ab
<i>B. oldhamii</i>	Leaf	Low	22, 2 ± 1, 7a	2, 6 ± 0, 0b	9, 8 ± 1, 4a
		High	30, 6 ± 1, 0c	4, 3 ± 0, 2d	10, 8 ± 1a
	Culm	Low	3, 4 ± 0, 2a	2, 6 ± 0, 3c	8, 9 ± 2, 2ab
		High	14, 9 ± 1, 9cd	3, 5 ± 0, 3d	7, 6 ± 1, 7ab

Values are means ±SE. The values with different letters indicate significant differences between the species and the nutrient rates (LSD,  $p < 0.05$ )

**Table 3** Allometric equations for fresh biomass determination by species

Species	Equation*	R <sup>2</sup> (%)
<i>T. siamensis</i>	$y = 1,015 (1,052 * x^{2,320})$	91,6
<i>B. tuldooides</i>	$y = 1,113 (3,604 * x^{1,897})$	84,4
<i>D. strictus</i>	$y = 1,080 (0,441 * x^{2,469})$	89,5
<i>B. vulgaris</i>	$y = 1,025 (0,900 * x^{2,323})$	98,3
<i>B. multiplex</i>	$y = 1,008 (0,034 * x^{3,332})$	97,3
<i>G. wrayi</i>	$y = 1,062 (1,083 * x^{2,347})$	94,5
<i>B. oldhamii</i>	$y = 1,012 (6,359 * x^{1,688})$	95,3

\*y = Total above ground fresh biomass (g); x = Basal diameter (mm).

### Aboveground Biomass and Nutrient Uptake

From the biomass results obtained using allometric equations (Table 3), and from the number of culms counted after one year of growth, the annual aboveground biomass yield and the density of culms per hectare was estimated, assuming a plantation density of 400 clumps/ha (Table 4). From the tissue's nutrient content (Table 2) and the total aboveground dry biomass, the nitrogen and phosphorus stored in the bamboo biomass was estimated using a density of 400 clumps/ha (Table 4). This table shows that four years old bamboo in containers produced between 1.1 and 2.6 t DM ha<sup>-1</sup>yr<sup>-1</sup> for the low-nutrient treatment, and between 1.4 and 3.4 t DM ha<sup>-1</sup>yr<sup>-1</sup> for the high-nutrient treatment. The species TS, BV, and DS produced the lowest amount of biomass with 1.4 t DM ha<sup>-1</sup>yr<sup>-1</sup>, and GW and BO produced the highest amount of biomass with 3.1 and 3.4 t DM ha<sup>-1</sup>yr<sup>-1</sup> respectively. The density of culms per hectare varied between species. The species TS showed the lowest culm density with the high-nutrient treatment, with a yield of 6268 culms.ha<sup>-1</sup>, and the species BMA showed the highest culm density with a yield of 38,520 culms.ha<sup>-1</sup>. In Table 4, the lowest amount of nitrogen stored in the aboveground biomass with the high-nutrient treatment was for the species DS (30 kg.ha<sup>-1</sup>), followed by BVV (32 kg.ha<sup>-1</sup>), GW (55 kg.ha<sup>-1</sup>), and BO (73 kg.ha<sup>-1</sup>). The species which stored the lowest amount of phosphorus was DS (3 kg.ha<sup>-1</sup>), followed by BVV (4 kg.ha<sup>-1</sup>), GW (10 kg.ha<sup>-1</sup>), BO (15 kg.ha<sup>-1</sup>). The species which stored the lowest amount of potassium was BVV (17 kg.ha<sup>-1</sup>), followed by DS (22 kg.ha<sup>-1</sup>), GW (27 kg.ha<sup>-1</sup>), and BO (33 kg.ha<sup>-1</sup>).

## DISCUSSION

### Growth of Clumping Bamboo Species under High Nutrient Rates

The high-nutrient treatment had an effect on both the number of culms produced and the culm diameter. We did not observe any effect on culm height and culm wall thickness, contrary to Shunshan (1994), who observed that culm wall thickness decreased with the application of mineral fertilizer with *Phyllostachys pubescens* J. Houz. As shown in Figures 1a and 1b, the morphological responses mainly depended on the bamboo species. For the species BVV, TS, BO and DS, we observed an increase in the number of culms produced as previously described in other studies (Kleinhenz and Midmore 2002; Kleinhenz *et al.* 2003; Razak and Ismail 2006). Our results showed that culms diameter was also decreased for these species, a response that has never been observed to our knowledge. Conversely, for the species GW high nutrient rates decreased the number of culms produced, but culm

**Table 4** Estimate of biomass yield, density of culms and nutrient uptake during the year of experimentation for a plantation of 400 clumps/ha

Species	Nutrient rate	Fresh aboveground biomass (t/ha/yr)	Dry aboveground biomass (t/ha/yr)	Initial culm density (culm/ha)	Final culm density (culm/ha)	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)
<i>T. siamensis</i>	Low	2,7 ± 0,5	1,6 ± 0,3	4000	6000	—	—	—
	High	2,8 ± 0,5	1,4 ± 0,3	2800	6268	—	—	—
<i>B. tuldoidea</i>	Low	2,1 ± 0,2	1,1 ± 0,1	8132	13200	—	—	—
	High	3,1 ± 0,4	1,4 ± 0,2	5732	11068	—	—	—
<i>D. strictus</i>	Low	3,2 ± 0,5	1,6 ± 0,3	4932	10800	14 ± 4	2 ± 1	13 ± 4
	High	3,1 ± 0,7	1,4 ± 0,3	10280	23720	30 ± 9	3 ± 1	22 ± 7
<i>B. vulgaris</i>	Low	2,7 ± 0,2	1,4 ± 0,1	6268	9068	17 ± 1	2 ± 1	16 ± 2
	High	3,2 ± 0,8	1,5 ± 0,4	3732	6932	32 ± 8	4 ± 1	17 ± 3
<i>B. multiplex</i>	Low	2,3 ± 0,2	1,2 ± 0,1	16132	30132	—	—	—
	High	3,5 ± 0,1	1,7 ± 0,1	17468	38520	—	—	—
<i>Gigantochloa wrayi</i>	Low	4,7 ± 0,3	2,6 ± 0,2	13868	27880	29 ± 1	5 ± 1	18 ± 2
	High	5,8 ± 0,4	3,1 ± 0,2	12268	23068	55 ± 6	10 ± 1	27 ± 6
<i>B. oldhamii</i>	Low	4,5 ± 0,5	2,2 ± 0,3	8800	12532	18 ± 3	6 ± 2	20 ± 1
	High	7,3 ± 0,7	3,4 ± 0,3	10400	16932	73 ± 9	15 ± 2	33 ± 5

Values are means ± Standard Error.

diameter was significantly larger. Azmy *et al.* (2004) reported a similar increase of *G. scortechinii* culm diameter in response to fertilization.

The growth of bamboo mainly depends on the rhizome. Li *et al.* (2000) suggest that the increase in nutrient rate releases more buds from dormancy from the rhizome, increasing the number of shoots. However, in this study the shoots developing from these buds ultimately had the same size despite fertilization. In our study, the diameter of shoots was also affected suggesting a change in the ontogeny of culms on the contrary to Li *et al.* (2000). The change in the ontogeny is probably a characteristic of the sympodial bamboo (pachymorph rhizome) that cannot colonize their environment by the rhizome elongation as well as the monopodial bamboo (leptomorph rhizome) studied by Li *et al.* (2000).

Our results suggest that an increase in culm number implies a decrease of the diameter of shoots and vice-versa. These results may reveal two separate growth strategies in response to a nutrient-rich environment for bamboo species to colonize their environment. In the first, the bamboo species produce more culms to rapidly colonize their environment and in the second, the bamboo produce culms with larger diameters, and therefore taller culms (Yen, Ji, and Lee 2010) that can reach the light more easily in a dense forest. These speculations are consistent when the different Asian origins with different ecological constraints are factored in.

Despite changes in growth strategies the high-nutrient treatment tended to increase the biomass yield of the species BV, BVV, BMA, GW, and BO, especially for BMA and BO which showed a 50–60% increase in biomass. Conversely, the high-nutrient treatment had little or no effect on the biomass yield of the TS and DS species. The final biomass yield depended not only on the combination of each type of morphological pattern (i.e., number of new culms, diameter), but also on their magnitude. Indeed, the number of culms in DS clumps significantly increased with nutrient rates, while culm diameter was strongly reduced. However, the result of these two morphological responses in terms of biomass yield was balanced, since the aboveground biomass remained similar for both the low and high nutrient rate treatments (Fig. 2). Moreover, despite the high increase in biomass (51%) and high density of culms (38520 culms; Table 4) of BMA, this species does not produce the highest biomass yield among the seven species studied. These results indicate that, whatever the species, the intrinsic morphological characteristics of the species, which actually conditions the mass of culm, are an important factor in the global biomass yield.

All the species showed  $F_v/F_m$  values higher than 0.700 (Table 1), that is close to the maximum value for a plant's quantum yield (i.e., 0.83; (Björkman and Demmig 1987)). These results reveal that all the bamboos were in a growth state during the experiment and were not limited by any stress caused by the high nutrient rate. Indeed, the yield parameter ( $\Phi_{PSII}$ ) of bamboo tended to increase between the beginning and the end of the experiment (data not shown), and between the low and high-nutrient treatment for all bamboo species (Table 1). These results are in compliance with the SLA increase for the high-nutrient treatment. Several studies demonstrated that SLA is positively correlated to the leaves' photosynthetic activity and to the increase of their nitrogen content (Reich, Michael and Ellsworth 1997; Wright *et al.* 2004; Jin *et al.* 2011). Indeed, applying the high-nutrient treatment increased the leaves' nitrogen content significantly (Table 2), as observed by several authors (Li *et al.* 1998; Kleinhenz and Midmore 2001; Kleinhenz *et al.* 2003).

The nitrogen content in leaves range from 29.4 and 36 g.kg<sup>-1</sup> DM with the high nutrient rate, that is close to the value of 3.0% recommended by Kleinhenz *et al.* (2003) for optimal yields. For the culms, the nitrogen content was two to four times higher for

the high-nutrient treatment than for the low-nutrient treatment. The maximum phosphorus content in leaves and culms was 4.3 and 3.5 g.kg<sup>-1</sup> DM respectively for the species BO. These results are four times higher than those found in existing literature. Shanmughavel and Francis (1997) reported, for a mature natural forest (without fertilization), that the nitrogen content in culms and branches ranged from 6.0 to 8.9 g.kg<sup>-1</sup>, and the phosphorus content in leaves from 0.8 to 1, and 0.6 to 0.9 g.kg<sup>-1</sup> in culm and branches for *Bambusa bambos* (L.) Voss. These results suggest that when a high nutrient rate is supplied to the soil, bamboo species are able to increase their ability to store nutrients in their tissues, especially in the culms. Indeed the culms showed an increase of 102 to 349% in the nitrogen stored in their tissue with the high-nutrient treatment. This increase corresponds to the storage ability of old culms to store nutrients before the shoot emergence (Li *et al.* 2000; Kleinhenz and Midmore 2001). Indeed, carbohydrates are stored in old culms' parenchyma cells before being remobilized and rapidly translocated through the rhizome system to provide the growth of new culms.

We observed a significant increase of nitrogen and phosphorus content in leaves and culms with the high-nutrient treatment but, for potassium, the increase was only significant for the species DS. These results comply with Li *et al.* (1998) who report no significant increase in potassium content in leaves with increasing fertilization for *Phyllostachys pubescens* J. Houz. According to Kleinhenz and Midmore (2001), potassium is the most element stored by bamboo tissues and the management of K application is the most important measure to improve bamboo productivity. In our study the potassium content did not show variations between treatments indicating that bamboo growth was not limited by the lack of this element.

### **Biomass Yield and Sizing of Bamboo Plantation for Wastewater Treatment**

The species GW and BO were the most productive bamboo species among the seven species studied, with a biomass yield range of 2.6 to 3.1 and 2.2 to 3.4 t DM ha<sup>-1</sup> yr<sup>-1</sup> respectively. They were also the species that stored the highest amounts of nitrogen and phosphorus in the total aboveground biomass per hectare, with the high-nutrient treatment (Table 4). In its aboveground parts, the species GW may contain 55, 10, and 27 kg.ha<sup>-1</sup> of N, P, and K respectively, and the species BO 73, 15, and 33 kg.ha<sup>-1</sup> of N, P, K, based on a 4 year-old plantation with a plantation density of 400 clumps/ha. For a plantation of 1600 clumps/ha, we could expect an uptake of 290 kg.ha<sup>-1</sup> for nitrogen and 57 kg.ha<sup>-1</sup> for phosphorus in the aboveground part of the species BO. These values are for a young plantation, but a mature plantation will absorb more nitrogen and phosphorus. Kleinhenz *et al.* (2001) reported an accumulation of 131 to 619 kg.ha<sup>-1</sup> of nitrogen, 54 to 97 kg.ha<sup>-1</sup> of phosphorus and 178 to 277 kg.ha<sup>-1</sup> of potassium. According to these values, the surface needed for wastewater treatment could be optimized according to the bamboo species' ability for storing nitrogen and phosphorus in their aboveground parts. Thus, the optimal volume of wastewater spread on the bamboo plantation could be determined to limit the leaching of nitrogen and phosphorus away from the rhizosphere.

### **CONCLUSION**

No adverse effects on the growth of young bamboos were observed by the application of high nutrient rates. All the bamboo species studied produced more or the same amount

of aboveground biomass with the higher nutrient supply. Applying high nutrient rates on several bamboo species induced different morphological responses depending on the species. With the increase of nutrient rate, two growth strategies were observed: either the number of culms increased and their diameter decreased, or the number of culm decreased and their diameter increased. The latter result has never been reported to the best of our knowledge. Both types of morphological pattern tended to increase the aboveground biomass yield. Our results suggest that all the bamboo species studied can be used for wastewater treatment. However, the species *Gigantochloa wrayi* Gamble and *Bambusa oldhamii* Munro were the most productive bamboo species, storing the highest amounts of nitrogen and phosphorus in their aboveground parts. These results need to be confirmed by field trials, in order to quantify the nutrients stored by mature bamboo plantation and those retained in the soil.

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