



Effect of Fertilization on Anatomical and Physical-mechanical Properties of *Neosinocalamus Affinis* Bamboo

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Abstract: In order to maintain soil fertility of *Neosinocalamus affinis* plantations, fertilizers of N, P, and K were applied. The anatomical and physical-mechanical properties of *N. affinis* bamboo wood from different fertilization treatments were measured. The aim of this study was to elucidate the effect of fertilization practice on the properties of *N. affinis* bamboo wood. The results revealed that the fertilization of P and K resulted in a slight reduction in fiber length. The application of P, K, and low level (0.3 kg/clump) of N fertilizers had no significant effect on the fiber morphology, while high level (0.9 kg/clump) of N fertilizer contributed to short fibers. The specific gravity was significantly decreased by fertilization, while the volume shrinkage was increased. Since the effect of various fertilization treatments had different influence patterns on the properties of *N. affinis*, specific evaluations on the quality of the fertilized bamboo wood should be performed prior to its utilization.

Keywords: fertilization; anatomy; physical-mechanical properties; *Neosinocalamus affinis*

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1 Introduction

Neosinocalamus affinis is a native bamboo species in southwest of China, especially in Sichuan Province. It has been used in the construction, furniture, and artificial craftworks such as basket, container, and farming tools by local residents because of its abundance in the rural regions since ancient times. *N. affinis* bamboo has been considered as the main substitute for wood materials for pulping in Sichuan Province, China, because of its long fiber. With the depletion of wood resources and the development of bamboo processing techniques, small diameter bamboos such as *N. affinis* bamboo have been used as resources for industrial products. Recently, fiber bundle mats have been extracted from *N. affinis* bamboo via a mechanical treatment process, and the extracted fiber bundle mats have been successfully used as reinforcements in the fabrication of bamboo-based composites (Yu et al., 2014). All the advanced techniques that focused on the utilization of small-diameter and thin culm wall *N. affinis* bamboo for high value-added products have obviously promoted the consumption of such bamboo resources.

The application of fertilizer to maintain soil fertility and to satisfy plant nutrients has been practiced in modern agriculture and forestry. Research results have shown that fertilization at midrotation in loblolly pine stands

has been found to have a strong positive influence on volume production (Antony et al., 2009). The results of Makinen and Hynynen's research showed that fertilization increased the radial growth by 37 percent with comparison with the unfertilized Scots pine trees (2014). Jansons et al. also reported that the fertilization could significantly increase the dimensions of Norway spruce in long-term (17% increase in stemwood volume) (2016). In order to promote the growth and harvest large volume production of bamboo, fertilization on bamboo plantations had been also extensively studied, and previous results showed that the addition of fertilizer into the plantations could significantly improve the production of *N. affinis* bamboo (Long et al., 1996). In the view of the high production and the yield gains as demonstrated in the aforementioned research, these fertilization practices on the *N. affinis* bamboo production are encouraging. However, the quality indicated by properties of these fertilized *N. affinis* bamboo wood remains unknown and the basic response of bamboo quality to the fertilization practices should be also clearly clarified.

The main aim of this study was to examine the effect of fertilization and the changes in N rate in the fertilizers on the anatomical characteristics and physical-mechanical properties of *N. affinis* bamboo wood. The obtained results of this research would provide fundamental information on the quality of *N. affinis* bamboo wood harvest-

ed from the fertilized plantations. The results of this study may also provide suggestions on how to make the best use of the fertilized bamboo wood regarding on the quality evaluation.

2 Materials and methods

2.1 Materials

The bamboo wood samples used in this study were harvested from a *N. affinis* bamboo trial established in 2009 on purple soil near Ya'an, Sichuan Province, China. The total nitrogen, phosphorus, potassium, and organic matter content of the soil were 0.094%, 0.054%, 0.297%, 2.653%. Fertilization was carried out in April, July, and September 2013. Urea, calcium superphosphate, and potassium sulfate were used as the N, P, and K sources, respectively. For the control plot, no fertilizers were added. The total amounts of fertilizers added in different treatments are presented in Table 1.

Table 1 Application of fertilizer to the bamboo clumps (units are kg/clump elemental)

Treatment	P	K	N
Control	0	0	0
N0	0.3	0.3	0
N3	0.3	0.3	0.3
N6	0.3	0.3	0.6
N9	0.3	0.3	0.9

Note: N0 represents no addition of nitrogen fertilizer; N3, N6, and N9 represent the addition of 0.3 0.6 and 0.9kg nitrogen fertilizer into each bamboo clump.

There were four bamboo clumps in each treatment plot, and each clump has 20–30culms. The bamboo age ratio in each culm was modified to be 1/1/1 of one-year-old/two-year-old/three-year-old. The bamboo culms were cut down in April 2014. Four bamboo culms were selected for each age from each culm in the five treatment plots. All together of sixty bamboo culms with branches and the top parts removed were harvested from the experimental plots and immediately transported to the laboratory for further usage. Bamboo logs with length of 200 mm were cut from the middle portion of the culms.

2.2 Anatomical characteristics

To soften the samples, bamboo blocks were immersed in distilled water and maintained at a hot plate for 6h. Cross sections with thickness of 30 μ m were sliced from the softened samples using a sliding microtome. Then the cross sections were dehydrated with alcohol and stained with 0.1% safranin-O. Slides with the cross sections were prepared and observed using an Olympus

DP20 digit microphotograph equipped with a tal camera. The images of the cross sections were taken and analyzed by using Image-Pro Plus software (Media Cybernetics, version 6.0). The numbers of vascular bundle on unit area (mm²) were counted for the determination of vascular bundle density.

The chromic acid (10%) and nitric acid were mixed at the ratio of 1 : 1 for the analysis of fiber morphology. Samples with dimension of 20mm \times 2mm \times 2mm were macerated in the pre-prepared solution and maintained at 60°C for 1h, and then carefully rinsed with distilled water until the macerated splints became pure white. Thereafter, a small amount of the splints was dispersed in a drop of glycerol and water mixture (1 : 1). The slides of the splints were prepared and projected using a microscope at 20 \times magnification for the measurement of fiber length. Fibers were randomly selected, and fifty complete and reasonable fibers were measured for each group.

2.3 Physical-mechanical properties

The density, volume shrinkage, compressive strength, and shear strength were determined according to the method in a previous research (Huang et al., 2014). Samples with dimension of 10 mm \times 10 mm \times thickness from the middle portion of the internodes were used for the specific gravity test. For the measurement of the volumetric shrinkage, samples after the determination of the weight were oven dried at (105 \pm 2)°C to a constant weight. The volume dimensions of the green and oven dried samples were measured using the water displacement method.

Samples with size of 35mm \times 20mm \times culm wall thickness and 20mm \times 20mm \times culm wall thickness were prepared for the determination of shear strength (SS) and compressive strength (CS) parallel to grain, respectively. The test was conducted by using a universal testing machine (Reger, RGM-4100, China). For the measurement, a constant loading speed of 0.5 mm/min was given on the specimen and the maximum load or the load at the failure was read. During the test, the force was loaded from top to bottom in the longitudinal direction. Shear and compressive strength were calculated with the following equations:

$$SS = \frac{P_{\max}}{hL} \quad (1)$$

$$CS = \frac{P_{\max}}{bL} \quad (2)$$

where P_{\max} is the maximum load at which the sample fails (N), L represents length of shear surface, b represents the width (mm), h represents the depth (culms wall thickness, mm).

2.4 Statistical analysis

Analysis of variance (ANOVA) was conducted by using

SAS (version 9.1, SAS Institute, Cary, NC) to determine significant differences ($\alpha=0.05$) in bamboo properties among treatments.

3 Results and discussion

3.1 Anatomical characteristics

The fiber length and lumen diameter of the *N. affinis* bamboo showed a decreasing trend as the bamboo age increased, while the fiber wall thickness increased (Table 2). The application of fertilization resulted in a decline in the fiber length for bamboos at all the three ages. No significant difference was detected in fiber morphology between the control samples and those receiving P, K and low level (0.3kg/clump) of N fertilizers. However, fiber length for bamboos receiving 0.9kg of N fertilizer

per clump was significantly decreased compared to the control, decreased by 22.30%, 13.10%, and 15.70% for the one, two, and three-year-old bamboo, respectively.

The fertilization of P and K significantly increased the fiber width for the one-year-old bamboo. The addition of N fertilizer had no significant influence on the fiber width. The combination of P, K, and low level (0.3kg/clump) of N significantly increased the fiber width to 21.18 μ m for the two-year-old bamboo (increased by 9.62%). The application of fertilization had no significant influence on the fiber width for the three-year-old bamboo. Though the treatment with application of P and K and the treatment with the combination of P, K, and high level N (0.9kg/clump) somewhat increased the fiber lumen diameter, the influence was not significant. The effect of fertilization on fiber wall thickness of bamboos at all three ages was insignificant. According to the ANOVA, only the influence of fertilization on fiber length and lumen was significant, and the age by fertilization interaction term had no significant influence on the fiber morphology (Table 3).

Table 2 Means of anatomical properties: fiber length, width, lumen, and wall thickness of *N. affinis* at different ages

Age	Treatment	Fiber length (mm)	Fiber width (μ m)	Fiber lumen (μ m)	Fiber wall thickness (μ m)
1	Control	3.40 \pm 0.53a	17.78 \pm 3.19b	8.53 \pm 2.54a	4.63 \pm 0.70a
	N0	3.17 \pm 0.40ab	20.23 \pm 1.43a	9.66 \pm 2.37a	5.29 \pm 0.89a
	N3	3.09 \pm 0.30ab	16.42 \pm 0.62b	7.45 \pm 2.41a	4.49 \pm 0.98a
	N6	2.83 \pm 0.24ab	18.21 \pm 0.77ab	7.60 \pm 2.46a	5.31 \pm 1.06a
	N9	2.64 \pm 0.61b	16.73 \pm 1.43b	8.75 \pm 2.25a	3.99 \pm 1.21a
2	Control	2.90 \pm 0.19a	19.32 \pm 3.18ab	8.08 \pm 3.53a	5.62 \pm 1.31ab
	N0	2.84 \pm 0.14a	19.40 \pm 1.33ab	9.75 \pm 2.61a	4.83 \pm 1.14b
	N3	2.69 \pm 0.10ab	21.18 \pm 3.73a	6.42 \pm 2.14a	7.38 \pm 2.74a
	N6	2.75 \pm 0.25ab	19.02 \pm 1.34ab	7.24 \pm 2.77a	5.89 \pm 1.57ab
	N9	2.52 \pm 0.20b	17.30 \pm 2.14b	9.35 \pm 2.31a	3.97 \pm 1.92b
3	Control	2.93 \pm 0.29a	18.46 \pm 1.94a	6.26 \pm 2.59ab	6.10 \pm 0.92a
	N0	2.76 \pm 0.23ab	18.89 \pm 2.25a	5.82 \pm 2.73b	6.54 \pm 2.13a
	N3	2.79 \pm 0.28ab	18.79 \pm 2.24a	5.91 \pm 1.92b	6.44 \pm 1.84a
	N6	2.66 \pm 0.32ab	19.58 \pm 5.56a	6.75 \pm 2.13ab	6.42 \pm 2.77a
	N9	2.47 \pm 0.43b	18.18 \pm 1.68a	9.28 \pm 2.46a	4.45 \pm 1.79a

Note: N0 represents no addition of nitrogen fertilizer; N3, N6, and N9 represent the addition of 0.3, 0.6 and 0.9kg nitrogen fertilizer into each bamboo clump. Values followed by the same letter in the same column are not significantly different at 0.05 probability.

3.2 Physical-mechanical properties

Table 4 represents the specific gravity, volume shrinkage, compressive strength, and shear strength of bamboo at the three ages following fertilizations. Both the specific gravity and mechanical strength as determined significantly increased as the bamboo age increased, i.e., the specific gravity, compressive strength, and shear strength increased from 558kg/m³ to 770kg/m³, 42.83MPa to 73.31MPa, and 10.04MPa to 13.28MPa from age 1 to age 3, respectively; while the volume shrinkage decreased. According to the ANOVA results as presented in Table 3, bamboo age had significant influence on physical-mechanical properties. This is due to the hardening of bamboo culm walls with response to mature process (Wahab *et al.*, 2010).

With expectation, the specific gravity and mechanical strength for bamboos at all the three ages demonstrated a drop with the application of fertilizers with P and K, while the volume shrinkage showed an inverse response, i.e., the volume shrinkage was increased with the application of P and K. The physical-mechanical properties for

Table 3 Analysis of variance results for physical-mechanical and anatomical properties of *N. affinis*

Source	Df	Index	Specific gravity	Volume shrinkage	Compressive strength	Shear strength	Fiber length	Fiber width	Fiber lumen	Fiber wall thickness
Age (A)	2	F value	243.040	35.933	73.772	9.239	8.043	1.782	3.836	3.053
		P value	<0.001	<0.001	<0.001	<0.001	0.002	0.186	0.033	0.062
Fertilization (F)	4	F value	78.510	80.171	1.117	0.084	6.843	1.633	3.079	2.661
		P value	<0.001	<0.001	0.367	0.987	<0.001	0.192	0.031	0.052
A×F	8	F value	14.846	5.832	2.278	0.715	0.744	1.040	0.946	0.727
		P value	<0.001	<0.001	0.049	0.676	0.653	0.429	0.495	0.667

Table 4 Means of physical-mechanical properties: specific gravity, volume shrinkage, compressive strength, and shear strength of *N. affinis* at different ages

Age (Year)	Treatment	Specific gravity (kg/m ³)	Volume shrinkage (%)	Compressive strength (MPa)	Shear strength (MPa)
1	Control	558±31.80a	28.17±3.94a	42.83±3.34a	10.04±1.11a
	N0	514±31.30a	28.56±3.36a	42.37±5.60a	9.90±1.11a
	N3	453±5.61b	38.80±5.33b	39.90±4.91a	8.93±1.47a
	N6	408±8.70c	39.27±4.21c	39.86±2.47a	9.19±2.21a
	N9	422±6.20bc	42.29±2.12bc	40.54±4.17a	9.58±1.96a
2	Control	681±30.10a	20.98±6.71a	71.71±14.81ab	11.12±1.81b
	N0	622±24.80b	26.82±4.27b	66.78±6.61b	11.34±0.90ab
	N3	560±7.30c	24.47±3.15c	57.03±3.92c	10.42±1.45b
	N6	670±34.20a	30.63±4.17a	69.48±4.79ab	12.72±1.52a
	N9	523±15.50d	39.78±2.60d	75.11±5.11a	11.68±2.54ab
3	Control	770±33.00a	18.35±3.48a	73.31±8.31a	13.28±1.98a
	N0	593±28.40c	29.51±4.94c	62.44±6.51b	11.27±1.91b
	N3	665±39.20b	30.89±3.50b	65.51±5.62b	12.06±1.04ab
	N6	618±18.80c	36.27±4.45c	71.81±3.53a	12.68±3.18ab
	N9	522±9.00d	38.78±2.52d	61.36±6.80b	12.79±2.30ab

Note: N0 represents no addition of nitrogen fertilizer; N3, N6, and N9 represent the addition of 0.3, 0.6 and 0.9kg nitrogen fertilizer into the each bamboo clump. Values followed by the same letter in the same column are not significantly different at 0.05 probability.

the one-year-old bamboo by given fertilizers with P and K remained insignificant and was lower than those for the control. However significant differences in physical-mechanical properties between the three-year-old bamboos from the treatment with the application of P, K and the control were found. The specific gravity and volume shrinkage of the two-year-old bamboo were significantly affected by the P and K fertilization.

The addition of N fertilizer further decreased the specific gravity, and the changes in the N rate in the fertilizers significantly affected the specific gravity. The response of specific gravity for bamboos at different ages to the N fertilizer and its rate was different. The specific gravity for the one-year-old bamboo reached the minimum at the N level of 0.6kg/clump, and then a slight increment occurred with increasing the N level to 0.9kg/clump. As for the two-year-old bamboo, the specific gravity was significantly decreased by receiving a low level (0.3kg/clump) of N, however with a moderate level of N (0.6kg/clump), the specific gravity for the two-year-old bamboo showed a significant increase and almost reached the same as that of the control. Whereas the application of N with a level of 0.3kg/clump significantly increased the specific gravity for the three-year-

old bamboo with comparison to the control and the fertilization treatment with P and K. But a clear decline in specific gravity was observed as the N level increased, and that for the treatment with 0.9kg/clump was the minimum.

Though a slight decline in mechanical properties for the one-year-old bamboo was observed after receiving the N fertilizer, the changes were insignificant among all the treatments. The application of the low level (0.3kg/clump) of N fertilizer induced a significant decrease in the compressive strength of the two-year-old bamboo. Further increasing the N rate to 0.9kg/clump, the compressive strength was significantly improved and over pass the value for the control. Similar response of shear strength to the N fertilizer and its rate was also observed for the two-year-old bamboo. As for the three-year-old bamboo, the compressive strength was significantly reduced to 61.36MPa by receiving 0.9kg of N fertilizer per clump; however, the application of 0.6kg of N fertilizer per clump showed no significant effect on the compressive strength. The fertilization of N had no significant influence on the shear strength. The application of N led to a large increase in the volume shrinkage. The ANOVA results indicated fertilization had significant influence on the basic density and volume shrinkage. The influence of age by fertilization interaction term on basic density, volume shrinkage, and compressive strength was also significant (Table 3).

4 Discussion

Bamboo age had significant influence on both the anatomical and physical-mechanical properties of *N. affinis* bamboo, i.e., bamboo fiber wall thickness, basic density, and mechanical properties all showed increasing trend with age increment, while the fiber length, lumen, and volume shrinkage decreased. This is mainly due to the fact that as the age increased, mature tissues continue to develop, which changes the properties of bamboo. Though, bamboo age is an unavoidable factor inducing the variance of bamboo properties, the interest of this study is to elucidate the effect of fertilization treatments on the bamboo properties, and the discussion will be restricted to the main effect of fertilization and fertilization by age interaction term.

As for wood, it is more often assumed that the wood quality deteriorates with the treatment of fertilization, and a number of published papers have confirmed this statement (Raymond and Muneri, 2000; Yang et al., 1988; Antony et al., 2001). Several studies have also reported negative responses in bamboo properties following different fertilization treatments (Yang et al., 2013; Zhou et al., 2013). In this study, for *N. affinis* bamboo at all the

three ages, the application of P and K resulted in reduction of mere 2.1%–6.7% in fiber length, and the ANOVA result indicated that the effect was insignificant. The application of N did not show significant influence until a high level (0.9kg/clump) was applied. This implied that fertilization of P, K, and N had a negative influence on the fiber length, and the higher N level induced significant reduction in fiber length. The variation trend that the fiber length decreased with increasing bamboo ages was not altered by fertilization, which was confirmed by the insignificant *P* value (0.653) for the fertilization by age intersection treatment. No specific response in fiber lumen, wall thickness, and width to fertilization treatments was found for bamboos at all the three ages. This may be because of the confounding influence of bamboo growth, ages, climate conditions, and stand soil nutrient. Despite the insignificant effect of N fertilization on the fiber wall thickness, the wall thickness was reduced by 14.03%–29.36% when high level (0.9kg/clump) N fertilizer was used, which may be due to that the high level N application attributed to the increase in foliar growth, resulting in a subsequent reduction in the availability of the photosynthates for second wall thickening (Larson *et al.*, 2001).

The specific gravity was significantly decreased by fertilization as presented in Table 4. Since bamboo specific gravity has close relationship with the fiber morphology such as cell wall thickness and lumen diameter, it can be easily understood that specific gravity could be reduced by fertilization because fertilization induced the decrease in fiber cell wall thickness and the increase in fiber lumen diameter. Another explanation for the decreased bamboo specific gravity is that with the fertilization of N the cell division of bamboo was accelerated, resulting in more parenchyma cells and less thicken wall fibers, which leads to the decrease in the specific gravity (Larson *et al.*, 2001). The influence of fertilization on bamboo specific gravity showed different patterns among bamboo ages, indicating that bamboo age had interaction with fertilization on specific gravity as indicated by the *P* values (Table 3). This result was consistent with the findings of Yang *et al.* (2013), who reported that the long-term N fertilization could significantly decrease the basic density of *Phyllostachys edulis* bamboo. The decrease in lignin content in bamboo caused by N fertilization as demonstrated by Hu *et al.* may also contribute to the reduction in bamboo density as lignin is one of the main cell wall components (2009).

In the negative, the volume shrinkage was increased by fertilization. The bamboo age and fertilization interaction had a significant effect on both specific gravity and volume shrinkage. Since the volume shrinkage is a key reflection of the bamboo stability, the increase in volume

shrinkage induced by fertilization and bamboo age will significantly decrease the bamboo stability. This implied that the influence of fertilization on bamboo physical properties needs to be under consideration for construal utilizations. Although some fertilization treatments declined the mechanical strength, no specific variation trend was established by altering the fertilization treatment. This may be due to that the mechanical properties of bamboo were also related to the anatomical characteristics and the physical properties.

Since fertilization had insignificant influence on the fiber morphologies with the application of low N level (0.3kg/clump) combined with P and K, the effect of such fertilization practice should have no influence on the pulping quality, which may somewhat affect the yield since the practices significantly reduced the specific gravity. As the fertilization employed in the study had an overall reduction on the mechanical properties, the application of the fertilized *N. affinis* bamboo for construal purpose should be carefully evaluated in order to satisfy the construal design requirements.

5 Conclusion

The application of P, K, and low level N (0.3Kg/clump) had no significant influence on the fiber length, however the fiber length for bamboos of all the three ages (age 1, age 2, and age 3) receiving the combination of P, K, and high level (0.9kg/clump) of N was significantly decreased compared to the control. The fertilization had significant influence on the fiber lumen and width, while no significant influence on the fiber wall thickness was observed. The application of P and K had significant influence on the specific gravity and volume shrinkage for the two-year-old bamboo. The effect of N fertilizer on the specific gravity of bamboo was significantly depended on the N level. The volume shrinkage was significantly increased by the application of K, P, and N fertilizers. The fertilization had an overall deduction on the physical-mechanical properties of *N. affinis* bamboo culm. As for the constructional purpose, the effect of fertilization should be carefully taken into consideration.

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