

1 **Effects of conversion from a natural evergreen broadleaf forest to a Moso bamboo**
2 **plantation on the soil nutrient pools, microbial biomass and enzyme activities in a**
3 **subtropical area**

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25 **Abstract**

26 Converting natural forests to plantations would markedly change soil physiochemical
27 and biological properties, as a consequence of changing plant vegetative coverage and
28 management practices. However, the effects of such land-use change on the soil nutrient
29 pools and related enzymes activities still remain unclear. The aim of this study was to
30 explore the effects of conversion from natural evergreen broadleaf forests to Moso
31 bamboo plantations on the pool sizes and forms of soil N, P and K, microbial biomass, and
32 nutrient cycling related enzyme activities. Soil samples from four adjacent evergreen
33 broadleaf forest-Moso bamboo plantation pairs were collected from a subtropical region in
34 Zhejiang Province, China. The soil organic C (SOC), total N (TN), total P (TP) and total K
35 (TK) concentrations and stocks and different N, P and K forms were measured, and the
36 microbial biomass C (MBC), microbial biomass N (MBN), microbial biomass P (MBP)
37 and four soil enzymes (protease, urease, acid phosphatase and catalase) were determined.
38 The results showed that converting broadleaf forests to Moso bamboo plantations
39 decreased the concentration and stock of SOC but increased those of TK in both soil
40 layers (0–20 and 20–40 cm), and such land-use change increased the concentration and
41 stock of TN and TP only in the 0–20 cm soil layer ($P < 0.05$). This land-use conversion
42 increased the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, resin- P_i , $\text{NaHCO}_3\text{-P}_i$, NaOH-P_i , HCl-P_i ,
43 available K and slowly available K, but decreased the concentrations of water-soluble
44 organic nitrogen (WSON), $\text{NaHCO}_3\text{-P}_o$ and NaOH-P_o ($P < 0.05$). Further, this land-use
45 change decreased the microbial biomass and activities of protease, urease, acid
46 phosphatase and catalase ($P < 0.05$). In addition, the acid phosphatase activity correlated

47 positively with the concentrations of MBP and $\text{NaHCO}_3\text{-P}_o$, and the activities of urease
48 and protease correlated positively with the concentrations of MBN and WSON ($P < 0.01$).
49 To conclude, converting natural broadleaf forests to Moso bamboo plantations had
50 positive effects on soil inorganic N, P and K pools, and negative effects on soil organic N
51 and P pools, and on N- and P-cycling related enzyme activities. Therefore, management
52 practices that increase organic nutrient pools and microbial activity are needed to be
53 developed to mitigate the depletion of organic nutrient pools after the land-use conversion.

54

55 **Keywords:** Evergreen broadleaf forest; Land-use conversion; Microbial biomass; Moso
56 bamboo plantation; Soil nutrient form; Soil enzyme.

58 **1. Introduction**

59

60 Land-use conversion can significantly affect the soil physicochemical and biological
61 properties (Yang et al., 2004; Don et al., 2011; Moghimian et al., 2017). Over the past few
62 decades, in order to gain higher economic benefits and to supply the growing demands of
63 timber, paper and fuel, among other commodities, the conversion from natural forests to
64 plantations is becoming more frequent (Burton et al., 2007; Li et al., 2014; Hu et al., 2018).
65 To increase the growth of plantations after land-use change, intensive management
66 practices, mainly including fertilization, understory vegetation control, and deep
67 ploughing, have been commonly adopted (Li et al., 2013; Zhang et al., 2015a; Dangal et
68 al., 2017; Zhang et al., 2017a). Various studies have revealed that the intensive
69 management practices applied can significantly change the soil pH, nutrient status, and
70 microbial biomass and community composition (Li et al., 2013; Yuan et al., 2015; Xie et
71 al., 2017), and consequently influence soil fertility and plant growth (Pransiska et al., 2016;
72 Tiecher et al., 2017). Therefore, it is great of significance to investigate the effects of land-
73 use change and subsequent management practices on the pool sizes and forms of soil
74 nutrients and associated enzyme activities.

75 The effects of land-use change from natural forest to plantation on soil nutrient status
76 and associated enzyme activities may include the following: (1) the input of exogenous
77 fertilizer can have a direct effect on the pool sizes and forms of soil nutrients (Chang et al.,
78 2007; Sainju et al., 2012; Yang et al. 2017; Li et al. 2018), and (2) the differences in
79 chemical composition and root exudates of different vegetation types may change the

80 microbial growth environment, which affects microbial biomass and soil enzyme activity
81 (Yang et al., 2010; Li et al., 2011; Wang et al., 2013; Yuan et al., 2015). For example, the
82 input of exogenous organic fertilizer and root exudates can increase the availability of
83 water-soluble nitrogen (N) (Scott and Rothstein, 2011; Sainju et al., 2012; Li et al., 2017a).
84 In addition, an increase in N fertilizer application can reduce soil enzyme activity and
85 microbial biomass (Shen et al., 2010; Zhang et al., 2015b). Previous studies showed that
86 understory vegetation plays important roles in cycling nutrients and decreasing soil
87 erosion (Fukuzawa et al., 2006; Zhang et al., 2010).

88 The classification of soil nutrients can help to determine soil nutrient status (Ross et
89 al., 1999; Yang et al., 2010). Different forms of N, such as NH_4^+ -N, NO_3^- -N and water-
90 soluble organic N (WSO_N), can jointly indicate the N supply capacity of soils (Schimel
91 and Bennett, 2004; Chen and Xu, 2008; Yan et al., 2008; Wu et al., 2010). The different
92 forms of phosphorus (P) in soils are formed through the combination of P with different
93 mineral components and can significantly affect N- and P-cycling (Yang et al., 2010; Wei
94 et al., 2017). In addition, the soil potassium (K) supply is closely associated with the
95 transformation rate of different forms of K in soils (Darunsontaya et al., 2012). The
96 different forms of nutrients respond differently to land-use change. For example, Ouyang
97 et al. (2013) reported that after conversion from wetland to paddy field, the total K
98 concentration increased but the available K concentration decreased in soils. Yang et al.
99 (2004) reported that converting secondary forests to rubber plantations increased the
100 concentration of inorganic N but decreased the concentration of total N. In addition, Yang
101 et al. (2010) found that converting natural forests to larch plantations increased the

102 concentrations of total P (TP) and inorganic P (IP) but decreased the concentrations of
103 microbial biomass P (MBP) and organic P (OP). Therefore, exploring the responses of
104 different forms of soil nutrients to land-use change will enable us to elucidate the
105 mechanisms associated with the land-use conversion effects on the soil nutrient status.

106 Soil microbes play an important role in the decomposition and mineralization of soil
107 organic matter (Malchair and Carnol, 2009; Guo et al., 2016; Ge et al., 2017; Li et al.,
108 2017b; Luo et al., 2017). Soil enzymes are closely related to the transformation of soil
109 nutrients, and their activities are closely associated with the level of soil organic matter,
110 soil physicochemical properties and soil microbial biomass (Xu et al., 2010; Liu et al.,
111 2015; Chavarría et al., 2016; Ma et al., 2016). For example, Bhattacharyya et al. (2005)
112 found that there was a pronounced linear correlation between soil urease and microbial
113 biomass. Additionally, Yang et al. (2010) found that acid phosphatase activity was
114 positively correlated with the concentrations of $\text{NaHCO}_3\text{-P}_i$, $\text{NaHCO}_3\text{-P}_o$, and MBP in a
115 subtropical forest soil. Land-use change can markedly affect the soil enzyme activity as
116 well as the soil microbial biomass and nutrient forms (Dawoe et al., 2014; Guo et al.,
117 2016). However, it remains unclear whether the changes in soil enzyme activity caused by
118 land-use change are closely linked with the changes in soil microbial biomass or nutrient
119 forms.

120 Natural evergreen broadleaf forests contribute to maintain biodiversity; these forests
121 are considered to be an important vegetation type in the subtropical regions of China
122 (Wang et al., 2007). However, large areas of natural forests have been transformed into
123 plantations over the past two decades (Yan et al., 2015; Chen et al., 2017), most

124 commonly into bamboo plantations (Guan et al., 2015). The area of Moso bamboo
125 (*Phyllostachys edulis*) plantations has increased to 4.2 million ha due to their substantial
126 economic benefit (Yuen et al., 2017). At present, most of the Moso bamboo plantations
127 are intensively managed, with the application of fertilizers, the removal of understory
128 vegetation, and tillage (Li et al., 2013; Yang et al., 2017). It is expected that conversion
129 from natural evergreen broadleaf forests to Moso bamboo plantations, in combination with
130 subsequent management practices, will markedly change the soil physical, chemical and
131 biological characteristics. However, the effects of the aforementioned land-use change on
132 soil nutrient pools and enzyme activities remain unclear. Therefore, the purposes of the
133 present study were (1) to **analyze** the effects of conversion from evergreen broadleaf
134 forests to Moso bamboo plantations on the pool sizes and different forms of soil nutrients,
135 (2) to investigate the aforementioned land-use conversion effects on the soil microbial
136 biomass and activity of soil enzymes regarding nutrient cycling, and (3) to reveal the
137 relationship between soil enzyme activity and the different forms of soil nutrients or soil
138 microbial biomass.

139

140 **2. Materials and methods**

141

142 *2.1. Experimental site*

143

144 The study was carried out in Congkeng (30°14'N, 119°42'E), Hangzhou, Zhejiang,
145 China. The study area belongs to a subtropical monsoon climate zone with four distinct
146 seasons, with an average annual temperature of 15.8 °C and average annual precipitation

147 of 1420 mm. The annual sunshine duration and frost-free period of this site are 1946 hours
148 and 239 days, respectively. The elevation of the study area is approximately 150 m. The
149 soils at this experimental site are classified as Ferralsols ([World Reference Base for Soil
150 Resources \(WRB\) 2006](#)).

151 We chose two different land-use types, i.e., natural evergreen broadleaf forests and
152 Moso bamboo plantations, to investigate the differences in soil properties. The main tree
153 species in the natural evergreen broadleaf forests were *Cyclobalanopsis glauca*,
154 *Castanopsis eyrie*, and *Castanopsis sclerophylla*, which accounted for approximately 70%
155 of the canopy cover. The understory vegetation in this natural forest was mainly *Litsea*
156 *cubeba*, *Lindera glauca*, and *Camellia cuspidata*, of which the surface cover was
157 approximately 85%. Part of the natural evergreen broadleaf forests had been transformed
158 into Moso bamboo plantations. The Moso bamboo plantation in the present study was
159 established in 2004. The bamboo plantation had been managed intensively for 11 years
160 after the land-use conversion. The stocking density in the bamboo plantation was 3,000
161 stems ha⁻¹, with 10.1 cm mean diameter at breast height. Every year from late June to
162 early July the bamboo plantation was fertilized with urea (200 kg N ha⁻¹), superphosphate
163 (60 kg P ha⁻¹), and potassium chloride (70 kg K ha⁻¹). The fertilizer was usually applied
164 on the soil surface, followed by plowing to a depth of 30–35 cm. The understory
165 vegetation in the bamboo plantation was manually removed each year.

166

167 2.2. Experimental design and soil sampling

168

169 A paired-plot approach was adopted to investigate the effects of land-use conversion
170 on soil properties. One paired-plot included two adjacent plots, i.e., one in the natural
171 evergreen broadleaf forest and the other in the Moso bamboo plantation. Each paired plot
172 had the same geographic and environmental factors, including soil type, slope (15–20°)
173 and aspect (south). We selected four different locations within ~3 km² in the area
174 described above to establish four different paired plots in April 2015; the plot size was 20
175 m × 20 m (400 m²). Within one paired-plot, the distance between the two plots (one in the
176 natural evergreen broadleaf forest and the other in the Moso bamboo plantation) was less
177 than 100 m, and there were 4 replications for each land-use type.

178 In each plot, we collected soil samples from five randomly selected points at the 0–20
179 and 20–40 cm soil layers. For each soil layer, the five samples were thoroughly mixed to
180 form a composite sample. The soil samples were kept on ice before further processing. A
181 2-mm sieve was used to homogenize the samples, and visible roots were removed.
182 Samples were divided into two portions: one portion was stored at 4 °C for further
183 analyses, and the other portion was air-dried. We used a bulk density corer with a 200-cm³
184 volume to collect samples from the two soil layers to determine the bulk density. The
185 average values for the selective physicochemical properties (see methods described below)
186 in the 0–20 cm soil layer for the aforementioned two forest types were listed below: (1)
187 natural evergreen broadleaf forest: pH of 5.67, bulk density of 0.96 g cm⁻³, sand of 301 g
188 kg⁻¹, silt of 413 g kg⁻¹, and clay of 286 g kg⁻¹; (2) Moso bamboo plantation: pH of 5.16,
189 bulk density of 1.06 g cm⁻³, sand of 324 g kg⁻¹, silt of 401 g kg⁻¹, and clay of 275 g kg⁻¹.

190

191 2.3. Determination of soil physicochemical properties

192

193 The soil pH was measured at a soil-to-water ratio of 1:2.5 (w:v) using a pH meter.

194 The soil moisture content was measured by calculating the mass loss after oven drying at

195 105 °C for more than 12 hours. The soil bulk density was determined by collecting a fresh

196 20-g soil subsample from a metal density corer with known volume and oven drying the

197 sample for more than 24 hours at a temperature of 105 °C. The concentrations of soil

198 organic carbon (SOC) and total N (TN) were determined using an elemental analyzer

199 (model CHN-O-RAPID, Heraeus, Germany). The total P (TP) concentration was

200 determined by digesting soil samples with a mixture of concentrated H₂SO₄ and HClO₄,

201 and the molybdate-blue colorimetry method (Murphy and Riley 1962) was used to

202 measure the P concentration in the digest. The soil total K (TK) concentration was

203 determined using the NaOH melting method according to Hanway and Heidel (1952). Soil

204 texture was determined using the pipette method after pre-treating the soil samples with

205 solutions of H₂O₂ and Na₄P₂O₇ (Gee and Bauder 1986). The stocks of SOC, TN, TP and

206 TK were calculated using the following formula:

207

$$208 \quad Y_{\text{stock}} (\text{Mg ha}^{-1}) = X \times BD \times th \times 0.1 \quad (1)$$

209

210 Where X is the concentration (g kg⁻¹) of SOC, TN, TP or TK, BD is the bulk density

211 of the soil layer (Mg m⁻³), and th is the thickness of the soil layer (cm).

212

213 *2.4. Determination of soil N forms*

214

215 The concentrations of NO_3^- -N and NH_4^+ -N in each soil sample were determined
216 according to the method of Li et al. (2014). Briefly, a soil sample was extracted with KCl
217 solution (2 mol L^{-1}), and the concentrations of NO_3^- -N and NH_4^+ -N in the extract were
218 determined using a Dionex ICS 1500 ion chromatograph (Dionex Corp., Atlanta, USA).
219 The WSON concentration was determined according to Jones and Willett et al. (2006). In
220 short, a fresh subsample equivalent to 20 g of oven-dried soil was suspended in 40 mL of
221 distilled water, shaken for 0.5 hours at 150 rpm at 25°C and then centrifuged for 20
222 minutes at $8,000 \times g$. The supernatant was passed through a $0.45\text{-}\mu\text{m}$ membrane filter
223 (Millipore Corp, USA). The concentration of water-soluble N (WSN) in the filtrate was
224 measured using an automated TOC-TN analyzer (TOC-Vcph, Shimadzu, Kyoto, Japan).
225 The concentrations of NH_3^- -N and NH_4^+ -N in the filtrate were determined using an ion
226 chromatograph, and the WSON concentration was calculated using the following formula:

227

$$228 \quad \text{WSON} = \text{WSN} - (\text{NO}_3^- \text{-N}) - (\text{NH}_4^+ \text{-N}) \quad (2)$$

229

230 *2.5. Determination of P forms*

231

232 The concentrations of different forms of P were determined by adopting the Hedley
233 procedure (Hedley et al., 1982; Eriksson et al., 2015). (1) Resin- P_i : a fresh soil sample
234 equivalent to 0.5 g of oven-dried soil was suspended in 30 mL of distilled water. Together

235 with a strip of NaHCO₃-form anion exchange resin membrane (BDH No. 55164)
236 pretreated by the method of Schoenau and Huang (1991), the suspension was shaken for
237 16 hours at 150 rpm at 25 °C, and the P absorbed into the resin membrane (resin-P_i) was
238 recovered by shaking the resin membrane in 50 mL of 0.5 mol L⁻¹ HCl for 1 h. (2)
239 NaHCO₃-P_i and NaHCO₃-P_o: two drops of toluene were added to minimize organic P
240 decomposition to the residue from (1) before shaking in 30 mL of 0.5 mol L⁻¹ NaHCO₃
241 (pH = 8.5) for 16 hours. (3) NaOH-P_i and NaOH-P_o: 30 mL of 0.1 mol L⁻¹ NaOH was
242 added to the residue from (2) and then shaken for 16 hours. (4) HCl-P_i: the residue from (3)
243 was shaken in 30 mL of 1.0 mol L⁻¹ HCl for 16 hours. (5) Residual-P: the residue from (4)
244 was digested in concentrated H₂SO₄ and H₂O₂, followed by shaking for 16 hours. After
245 steps (1) to (5), the suspension was centrifuged at 12,000 × g for 10 minutes. The
246 supernatants were passed through a 0.45-μm membrane filter (Millipore Corp., USA), and
247 the inorganic P concentration in the filtrates were measured using the molybdate-blue
248 method (Murphy and Riley, 1962). In (2) and (3), the TP concentration in the extracts was
249 measured by digesting the extracts in concentrated H₂SO₄ and H₂O₂, and the organic P
250 concentration in the extracts was calculated as the difference between the TP
251 concentration and the inorganic P concentration.

252

253 *2.6. Analysis of K forms*

254

255 The different forms of soil K determined included available K, slowly available K
256 and mineral K. The available K concentration was determined by the method of Zhang et

257 al. (2013). Briefly, 10 g of oven-dried soil sample was suspended in 50 mL of 1.0 mol L⁻¹
258 NH₄OAc, shaken for 0.5 hours at 150 rpm at 25°C, and the suspension was centrifuged at
259 5,000 × g for 10 minutes and then passed through a 0.45-μm membrane filter (Millipore
260 Corp, USA). The concentration of K in the filtrate was measured using a flame
261 photometer (Tiecher et al. 2017). The slowly available K concentration was determined by
262 suspending 10 g of oven-dried soil in 50 mL of 1.0 mol L⁻¹ HNO₃, heated to boiling in an
263 oil bath for 10 minutes, transferred to a 100-mL volumetric flask, and the K concentration
264 was measured using a flame photometer. The soil total K (TK) concentration for each soil
265 sample was determined using the NaOH melting method (Hanway and Heidel, 1952).
266 Slowly available K was calculated as the difference between the concentration of K
267 extracted by the hot HNO₃ solution and the concentration of the K extracted by the
268 NH₄OAc solution. Mineral K was calculated as the difference between the concentration
269 of total K and the concentration of the K extracted by the hot HNO₃ solution.

270

271 *2.7. Analysis of soil microbial biomass and soil enzymes*

272

273 The concentrations of microbial biomass C (MBC) and microbial biomass N (MBN)
274 were determined using the chloroform fumigation-K₂SO₄ extraction method as described
275 in Vance et al. (1987). In brief, fumigated and non-fumigated soil samples were suspended
276 in 40 mL of 0.5 mol L⁻¹ K₂SO₄, shaken at 150 rpm for 30 minutes at 25 °C, centrifuged
277 for 5 minutes at 3500 × g, and the supernatants were passed through a 0.45-μm membrane
278 filter (Millipore Corp, USA). The concentrations of C and N in the extracts were measured

279 in an automated TOC-TN analyzer (TOC-Vcph, Shimadzu, Kyoto, Japan). The soil MBC
280 concentration was calculated as the difference in the concentration of C between the
281 fumigated and non-fumigated samples (Wu et al., 1990; Burton et al., 2010). Similarly, the
282 soil MBN concentration was calculated as the difference in the concentration of N
283 between the fumigated and non-fumigated samples (Li et al., 2014). The concentration of
284 MBP was determined following the method of Brookes et al. (1982). Briefly, the MBP
285 was calculated as the difference between the concentrations of inorganic P extracted with
286 $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ (pH = 8.5) from fumigated and non-fumigated soil.

287 The soil protease (EC 3.4.2.21–24) activity was measured following the method of
288 Ladd and Butler (1972). 1 g of fresh sample was mixed with 2.5 mL of Tris buffer (0.1
289 mol L^{-1} , pH = 8.1) and 2.5 mL of 2% sodium caseinate, and incubated at 50 °C for 2 hours.
290 At the end of the incubation, 1 mL of 17.5% trichloroacetic acid (TCA) was added, then
291 centrifuged for 10 minutes at $5,000 \times g$. 2 mL of the supernatant was mixed with 3.0 mL
292 Na_2CO_3 (1.4 mol L^{-1}) and 1 mL threefold diluted Folin-Ciocalteu reagent. After 10
293 minutes, the absorbance was determined at 700 nm. The protease activity is expressed as
294 the amount of tyrosine released per hour per gram of soil ($\mu\text{mol g}^{-1} \text{ h}^{-1}$).

295 The soil urease (EC 3.5.1.5) activity was determined following the method described
296 in Kandeler and Gerber (1988). Briefly, a fresh soil sample equivalent to 5 g of oven-dried
297 soil was suspended in 2.5 mL of 80 mmol L^{-1} urea and 20 mL of Tris buffer (0.075 mol
298 L^{-1} , pH = 10), and incubated at 37 °C for 2 hours. After incubation, 50 mL of a mixture of
299 1 mol L^{-1} KCl and 10 mmol L^{-1} HCl was added, and the suspension was shaken at 125
300 rpm for 30 minutes. The suspension passed through a filter, and the concentration of

301 ammonia in the filtrate was determined using the colorimetric method described in
302 Marschner et al. (2003). The urease activity is expressed as the amount of $\text{NH}_3\text{-N}$
303 produced per unit mass of soil per hour ($\mu\text{mol g}^{-1} \text{h}^{-1}$).

304 The soil acid phosphatase (EC 3.1.3.2) activity was determined following the method
305 of Tabatabai and Bremner (1969). A fresh soil sample equivalent to 1 g of oven-dried soil
306 was suspended in 0.2 mL of toluene, 4 mL of acetate buffer solution ($\text{pH} = 6.5$) and 1 mL
307 of 50 mmol L^{-1} *p*-nitrophenol phosphate solution. The suspension was shaken at 150 rpm
308 at 37°C for 1 hour, and then 1 mL of CaCl_2 solution (0.5 mol L^{-1}) and 4 mL of NaOH
309 solution (0.5 mol L^{-1}) were added. After shaking for several seconds, the suspension was
310 passed through filter paper, and the absorbance of the filtrate was determined at 400 nm.
311 The acid phosphatase activity is expressed as the amount of *p*-nitrophenyl produced per
312 unit mass of soil per hour ($\mu\text{mol g}^{-1} \text{h}^{-1}$).

313 The soil catalase (EC 1.11.1.6) activity was measured following the method of
314 Johnson and Temple (1964). A fresh soil sample equivalent to 2 g of oven-dried soil was
315 suspended in 40 mL of distilled water and 5 mL of 0.3% H_2O_2 , and shaken at 150 rpm for
316 20 minutes at 25°C . Then, 5 mL of 3 mol L^{-1} sulfuric acid was added, and the mixture was
317 titrated using 0.1 mol L^{-1} KMnO_4 solution. The baseline was determined by titrating a
318 mixture of 5 mL of 0.3% H_2O_2 and 5 mL of 3 mol L^{-1} sulfuric acid with 0.1 mol L^{-1}
319 KMnO_4 . The catalase activity is expressed as the amount of consumption of KMnO_4 per
320 hour per gram of soil ($\mu\text{mol g}^{-1} \text{h}^{-1}$).

321

322 *2.8. Statistical analyses*

323

324 The data presented in this paper are the mean values of four replicates. One-way
325 analysis of variance (ANOVA) and the least significant difference (LSD) test was adopted
326 to determine the land-use conversion effects on the soil physiochemical properties,
327 microbial biomass and enzyme activities. Prior to performing the ANOVA, the normality
328 and homogeneity of variance were evaluated, and data were log-transformed when needed.
329 The relationships between the soil enzyme activity and different soil N and P forms were
330 tested using linear regression analyses. Unless otherwise indicated, differences were taken
331 as statistically significant at $P = 0.05$. Data analyses and visualization were completed
332 using Microsoft Excel 2013 and Origin 9.0, respectively, and the statistical analyses were
333 conducted using SPSS version 18.0 (SPSS, Chicago, IL, USA).

334

335 **3. Results**

336

337 *3.1. Soil total C, N, P and K concentrations and stocks*

338

339 Regardless of soil layer, the SOC concentration in the Moso bamboo plantation was
340 lower than that in the evergreen broadleaf forest (Fig. 1a), while the total K concentration
341 in the bamboo plantation was higher than that in the broadleaf forest (Fig. 1g). The total N
342 and P concentrations in the bamboo plantation were higher than those in the broadleaf
343 forest in the 0–20 cm soil layer, while no differences were observed in the 20–40 cm layer
344 (Fig. 1c, e). The effects of land-use conversion on the total C, N, P and K stocks were
345 similar to the effects on the total C, N, P and K concentrations (Fig. 1).

346

347 3.2. Soil N forms

348

349 Regardless of soil layer, the NO_3^- -N and NH_4^+ -N concentrations in the Moso bamboo
350 plantation were higher than those in the evergreen broadleaf forest (Fig. 2a and b), while
351 the WSON concentration in the bamboo plantation was lower than that in the broadleaf
352 forest (Fig. 2c).

353

354 3.3. Soil P forms

355

356 Regardless of soil layer, the resin- P_i and NaHCO_3 - P_i concentrations in the Moso
357 bamboo plantation were higher than those in the evergreen broadleaf forest, while the
358 NaHCO_3 - P_o concentration in the bamboo plantation was lower than that in the broadleaf
359 forest (Table 1). The NaOH - P_i , HCl - P_i and residual-P concentrations in the bamboo
360 plantation were higher than those in the broadleaf forest in the 0–20 cm soil layer, while
361 no differences were detected in the 20–40 cm layer (Table 1). The NaOH - P_o concentration
362 in the bamboo plantation was lower than that in the broadleaf forest in the 0–20 cm soil
363 layer, while no significant difference was found in the 20–40 cm layer (Table 1).

364

365 3.4. Soil K forms

366

367 Regardless of soil layer, the total K, available K and slowly available K

368 concentrations in the Moso bamboo plantation were higher than those in the evergreen
369 broadleaf forest (Fig. 3a–c). The mineral K concentration in the bamboo plantation was
370 higher than that in the broadleaf forest in the 0–20 cm soil layer, while no difference was
371 found in the 20–40 cm layer (Fig. 3d).

372

373 *3.5. Soil microbial biomass and enzyme activities*

374

375 Regardless of soil layer, the MBC, MBN and MBP concentrations in the Moso
376 bamboo plantation were higher than those in the evergreen broadleaf forest (Fig. 4).
377 Regardless of soil layer, the activities of protease, urease, and acid phosphatase in the
378 bamboo plantation were lower than those in the broadleaf forest (Fig. 5 a–c). The catalase
379 activity in the bamboo plantation was lower than that in the broadleaf forest in the 0–20
380 cm soil layer, while no difference was found in the 20–40 cm layer (Fig. 5d). Acid
381 phosphatase activity correlated positively with MBP and $\text{NaHCO}_3\text{-P}_o$ (Fig. 6), and urease
382 and protease activities correlated positively with the concentrations of MBN and WSON
383 (Fig. 7) ($P < 0.01$).

384

385 **4. Discussion**

386

387 *4.1. Land-use conversion effects on soil nutrient pools*

388

389 Our results revealed that the land-use conversion from evergreen broadleaf forests to

390 Moso bamboo plantations significantly decreased the concentration and stock of SOC (Fig.
391 1). This result coincides with that of Guillaume et al. (2015), who reported that conversion
392 from lowland rainforests to intensively managed rubber plantations significantly decreased
393 the SOC stock. The decrease in concentration and stock of SOC due to the land-use
394 change in our study has two possible explanations. Practices of fertilization and tillage
395 may accelerate the mineralization of SOC and the leaching of soluble soil organic matter
396 (Mancinelli et al., 2010; Sheng et al., 2015; Liu et al., 2018), and the removal of
397 understory vegetation in the plantations may decrease C input into the soil (Wang et al.,
398 2011; Li et al., 2013; Zhang et al., 2014).

399 In addition, this land-use conversion significantly increased the concentrations and
400 stocks of N, P and K (Fig. 1), which is in agreement with Yang et al. (2010) and Zhang et
401 al. (2017b), who reported that conversion from natural forests to larch and pine plantations,
402 respectively, increased the concentrations and stocks of N in the surface soil. Plausibly,
403 the main sources for the increased concentrations and stocks of N, P and K were the
404 synthetic fertilizers applied in the Moso bamboo plantation, as the natural evergreen
405 broadleaf forest did not receive any fertilization.

406

407 4.2. Land-use conversion effects on different soil N forms

408

409 In this study, we found that the conversion from broadleaf forest to bamboo
410 plantation increased the concentrations of NO_3^- -N and NH_4^+ -N (Fig. 2), in agreement with
411 Yang et al. (2004) who reported that the concentration of NO_3^- -N increased after the
412 conversion from secondary forest to a larch plantation. In addition, we also found that the
413 aforementioned land-use change significantly decreased the WSON concentration (Fig. 2),

414 which accords with the result of Li et al. (2014), who reported that the conversion of
415 natural shrub forests to intensively managed Chinese chestnut plantations significantly
416 reduced the WSON concentration in the soil.

417 The changes in soil N forms caused by land-use change can be attributed to a number
418 of possible mechanisms. Intensive **managements** that **include** fertilization, tillage and
419 understory vegetation removal, could lead to a decrease in water and soil conservation
420 ability, and consequently cause the loss of WSON (Yüksek et al., 2009; Sheng et al.,
421 2015). Fertilization can accelerate the mineralization of organic N and enhance the uptake
422 of soluble organic N by plants, consequently reducing the WSON concentration in soils
423 (Schimel and Bennett, 2004; Tao et al., 2018). The increase in NO_3^- -N and NH_4^+ -N
424 concentrations in soils after land-use change are evidently related to the increase in N
425 input from fertilization (Asadiyan et al., 2013).

426

427 *4.3. Land-use conversion effects on different soil P forms*

428

429 **Results of the present study revealed that converting broadleaf forests to Moso**
430 **bamboo plantations increased the resin- P_i and NaHCO_3 - P_i concentrations but decreased**
431 **the NaHCO_3 - P_o concentration (Table 1). Similarly, Yang et al. (2010) found that**
432 **converting** natural secondary forests to larch plantations increased the TP and iron-bound
433 P (Fe-P) concentrations in soils but decreased the MBP and NaHCO_3 - P_o concentrations.

434 The changes in soil P forms caused by land-use change have two possible
435 explanations. Fertilization significantly increased the inorganic P concentration but

436 reduced the organic P concentration in intensively managed rubber and oil palm
437 plantations (Maranguit et al. 2017). In addition, Yang et al. (2012) showed that
438 fertilization caused a significant increase in the inorganic P concentration and a significant
439 decrease in the organic P concentration in soils. Thus, the increase in the concentration of
440 inorganic P fractions is at least partially related to the application of phosphate fertilizer in
441 the Moso bamboo plantation. In addition, the intensive management measures, e.g. deep
442 tillage and fertilization, applied in the Moso bamboo plantation may decrease the organic
443 P concentration since they can promote the mineralization of P-containing organic matter
444 (Yang et al., 2012; Obour et al., 2017).

445

446 4.4. Land-use conversion effects on different soil K forms

447

448 Studying the effects of land-use change on different forms of K can help us to
449 understand the response of K nutrient status to land-use conversion (Wang et al., 2016;
450 Islam et al., 2017). The available K concentration increased after the conversion of natural
451 evergreen broadleaf forests to *Phyllostachys praecox* stands and in the conversion from
452 virgin natural forests to alder and sequoia plantations (Zhang et al. 2013; Moghimian et al.
453 2017). Likewise, our results indicated that conversion from broadleaf forests to Moso
454 bamboo plantations significantly increased the concentrations of total K, available K,
455 slowly available K, and mineral K (Fig. 3).

456 The KCl fertilizer applied in the Moso bamboo plantation was the possible source of
457 the increased K in soils. The fertilizer can quickly increase the available K, and a part of

458 the available K will be transformed to slowly available K and mineral K forms (Rupa et al.,
459 2003; Islam et al., 2017).

460

461 *4.5. Land-use conversion effects on soil microbial biomass and enzyme activity*

462

463 The soil microbial biomass is a sensitive index of the soil nutrient pool, since soil
464 nutrients provide the basis for the survival of soil microbes (Guo et al., 2016; Vitali et al.,
465 2016). In agreement with Fang et al. (2017), who reported that converting natural old-
466 growth broadleaf Korean pine mixed forest to a spruce plantation caused a reduction in the
467 soil MBC concentration, we noticed that the soil MBC concentration was lower in the
468 Moso bamboo plantation than in the broadleaf forest (Fig. 4). In line with our previous
469 study where the MBN concentration decreased significantly 10 years after the conversion
470 from shrub forests to Chinese chestnut plantations (Li et al., 2014), the land-use change in
471 this study decreased the concentrations of MBN and MBP (Fig. 4). The decreased
472 concentrations of MBC, MBN and MBP in the bamboo plantation might have been
473 partially due to the lower pH, which is known to inhibit microbial growth (Luo et al., 2013;
474 Guo et al., 2016; Moghimian et al., 2017). Another possible explanation is the markedly
475 decreased SOC concentration, which might have had a negative impact on the growth of
476 soil microorganisms (Vitali et al., 2016; Moghimian et al., 2017).

477 The soil enzyme activity is one of the most sensitive indicators of soil nutrient status
478 and fertility, and it is greatly affected by land-use conversion and alterations in
479 management practices (Moghimian et al., 2017). Converting tropical forests to rubber

480 plantations decreased the activity of acid phosphatase, catalase activity decreased after the
481 conversion from a broadleaf forest to a *Michelia macclurei* Dandy plantation, and acid
482 phosphatase activity decreased after converting a broadleaf forest to a *Pinus massoniana*
483 Lamb plantation (Yang et al. 2012; Wang et al. 2013). In this study, the activities of
484 protease, urease, acid phosphatase and catalase, involved in N and P cycling, were
485 significantly lower in soils after the land-use change of broadleaf forests to bamboo
486 plantations (Fig. 5). Low soil pH is likely to have a negative effect on the activity of some
487 soil enzymes (Wallenius et al., 2011; Zhang et al., 2015b), which may partially explain the
488 lower activities in bamboo plantation. Also, the lower activities might have resulted from
489 lower microbial biomass. Since soil enzymes originate mainly from soil microorganisms,
490 changes in microbial growth, activity and function resulting from land-use change could
491 affect enzyme activities (Yang et al., 2012; Kader et al., 2017). Zhang et al. (2015b) found
492 a significant relationship between the decrease in acid phosphatase activity and the
493 application of calcium superphosphate fertilizer. Furthermore, urease activity decreased
494 significantly with an increase in nitrogen fertilizer application (Shen et al. 2010). Thus,
495 fertilization in the bamboo plantation might have decreased the soil enzyme activity.

496 Soil enzyme activity is closely associated with the level of soil nutrients (Chen et al.,
497 2003; Islam et al., 2011; Zhang et al., 2015b). Xing et al. (2010) reported that the
498 decomposition of soil organic N into $\text{NH}_4^+\text{-N}$ could be enhanced by increased activities of
499 urease and protease in the subtropics of China. Chen (2003) found that soil acid
500 phosphatase activity in a subtropical fir (*Cunninghamia lanceolata*) plantation in China
501 was closely related with most of the inorganic P fractions except Ca-P. In this study, we

502 found that acid phosphatase activity correlated positively with MBP and $\text{NaHCO}_3\text{-P}_o$ (Fig.
503 6), and urease and protease activities correlated positively with the concentrations of MBN
504 and WSON (Fig. 7). Therefore, the effects of land-use conversion on the soil enzyme
505 activities may be attributed to its effect on the soil nutrient pools.

506

507 **5. Conclusions**

508

509 Converting natural evergreen broadleaf forests to intensively managed Moso bamboo
510 plantation significantly decreased the soil pH and the concentration and stock of SOC, but
511 significantly increased the concentrations and stocks of TN, TP and TK. Further, this land-
512 use conversion increased the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, resin- P_i , $\text{NaHCO}_3\text{-P}_i$,
513 NaOH-P_i , HCl-P_i , residual-P, available K, slowly available K and mineral K but
514 significantly decreased the concentrations of WSON, $\text{NaHCO}_3\text{-P}_o$, and NaOH-P_o , as well
515 as the soil microbial biomass and enzyme activity. These results clearly demonstrate that
516 the aforementioned land-use conversion had positive effects on the soil inorganic N, P and
517 K pools, while the effects on the soil organic N, P and K pools were negative. Therefore,
518 to manage the Moso bamboo plantations sustainably, it is advisable to increase the organic
519 nutrient pools by applying organic fertilizers and re-establishing understory vegetation. As
520 the duration under intensive management will markedly affect the soil nutrient status, both
521 the short- and long-term effects of intensive management on soil N, P and K forms and
522 enzyme activity need to be explored in further studies.

523

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525

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529

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819 **Figure captions**

820

821 **Fig. 1** Effects of conversion from evergreen broadleaf forests to Moso bamboo plantations
822 on the total stocks (a, c, e and g) and concentrations (b, d, **f and h**) of C, N, P and K in
823 soils. Error bars are the standard deviations of the mean ($n = 4$); different lowercase letters
824 within each panel indicate significant differences between different land-use types in each
825 soil layer at $P = 0.05$ level based on the least significant difference (LSD) test.

826

827 **Fig. 2** Effects of conversion from evergreen broadleaf forests to Moso bamboo plantations
828 on the (a) NH_4^+ -N concentration, (b) NO_3^- -N concentration, and (c) water soluble organic
829 N (WSON) concentration in soils. Error bars are standard deviations of the mean ($n = 4$);
830 different lowercase letters within each panel indicate significant differences between
831 different land-use types in each soil layer at $P = 0.05$ level based on the least significant
832 difference (LSD) test.

833

834 **Fig. 3** Effects of conversion from evergreen broadleaf forests to Moso bamboo plantations
835 on the (a) total K concentration, (b) available K concentration, (c) slowly available K
836 concentration, and (d) mineral K concentration in soils. Error bars are the standard
837 deviations of the mean ($n = 4$); different lowercase letters within each panel indicate
838 significant differences between different land-use types in each soil layer at $P = 0.05$ level
839 based on the least significant difference (LSD) test.

840

841 **Fig. 4** Effects of conversion from evergreen broadleaf forests to Moso bamboo plantations

842 on the (a) microbial biomass C, (b) microbial biomass N, and (c) microbial biomass P.
843 Error bars are the standard deviations of the mean ($n = 4$); different lowercase letters
844 within each panel indicate significant differences between different land-use types in each
845 soil layer at $P = 0.05$ level based on the least significant difference (LSD) test.

846

847 **Fig. 5** Effects of conversion from evergreen broadleaf forests to Moso bamboo plantations
848 on the soil (a) protease activity, (b) urease activity, (c) acid phosphatase activity, and (d)
849 catalase activity. Error bars are the standard deviations of the mean ($n = 4$); different
850 lowercase letters within each panel indicate significant differences between different land-
851 use types in each soil layer at $P = 0.05$ level based on the least significant difference (LSD)
852 test.

853

854 **Fig. 6** Relationship between acid phosphatase activity and (a) microbial biomass P, (b)
855 $\text{NaHCO}_3\text{-P}_0$, and (c) $\text{NaHCO}_3\text{-P}_1$ in the evergreen broadleaf forest and Moso bamboo
856 plantation.

857

858 **Fig. 7** Relationships (a-d) between urease activity and the concentrations of $\text{NH}_4^+\text{-N}$,
859 $\text{NO}_3^-\text{-N}$, WSON and MBN, and (e-h) between protease activity and the concentrations of
860 $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, WSON and MBN in the evergreen broadleaf forest and Moso bamboo
861 plantation. WSON: water soluble organic N; MBN: microbial biomass N.

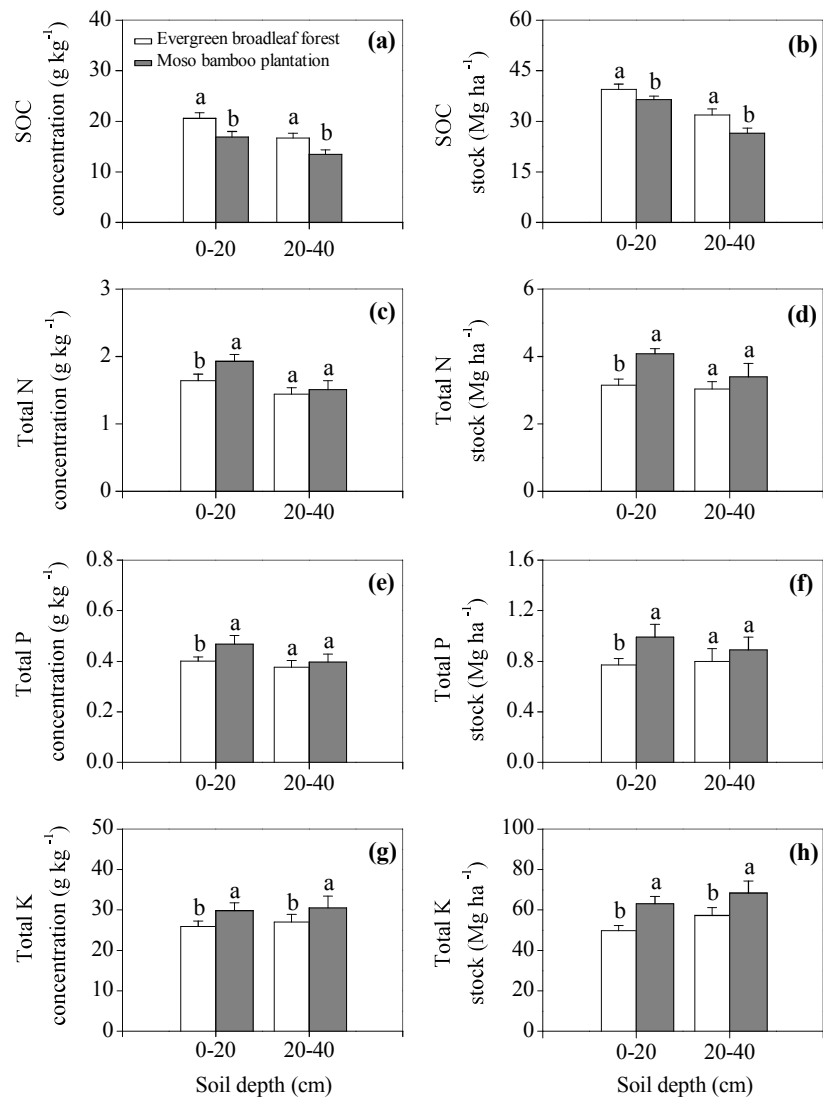
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Table 1 Effect of conversion from evergreen broadleaf forests to Moso bamboo plantations on different P forms in the soil

Forest type	Resin-P _i (mg kg ⁻¹)	NaHCO ₃ -P _i (mg kg ⁻¹)	NaHCO ₃ -P _o (mg kg ⁻¹)	NaOH-P _i (mg kg ⁻¹)	NaOH-P _o (mg kg ⁻¹)	HCl-P _i (mg kg ⁻¹)	Residual-P (mg kg ⁻¹)
	0-20 cm						
Broadleaf forest	2.17 (0.21) b	4.50 (0.26) b	32.1 (2.7) a	47.5 (4.2) b	67.1 (3.9) a	5.89 (0.53) b	241.7 (14.3) b
Bamboo plantation	3.51 (0.18) a	7.23 (0.48) a	24.0 (1.7) b	56.7 (2.6) a	54.7 (2.9) b	9.25 (0.52) a	312.6 (30.4) a
	20-40 cm						
Broadleaf forest	1.12 (0.10) b	4.20 (0.31) b	27.0 (2.1) a	48.3 (3.0) a	63.2 (3.2) a	6.24 (0.52) a	226.0 (24.0) a
Bamboo plantation	1.86 (0.13) a	6.79 (0.37) a	21.3 (2.1) b	52.4 (4.6) a	58.3 (4.3) a	7.12 (0.47) a	249.3 (24.9) a

864 Means with different letters within a column indicate significant differences between the land uses for each parameter within each soil layer at $P = 0.05$ level

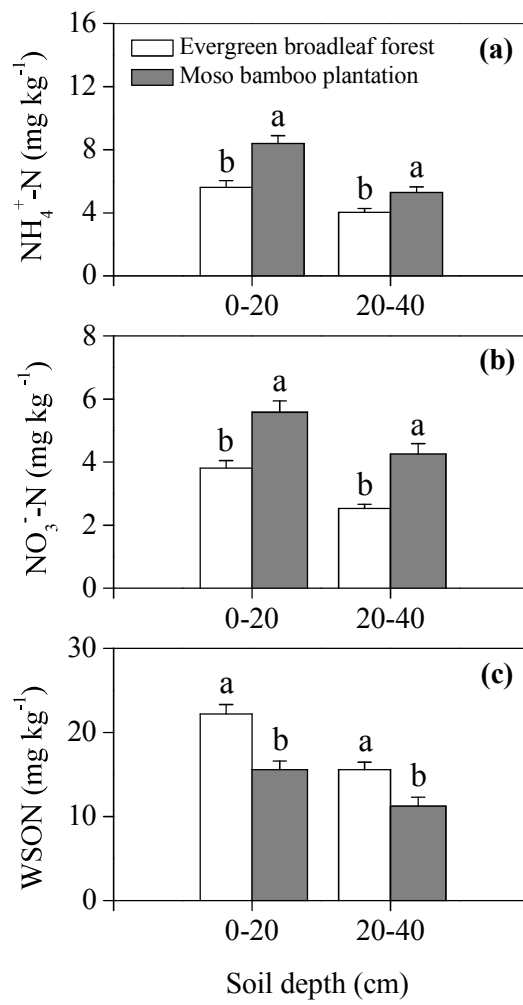
865 according to the least significant difference (D) test.



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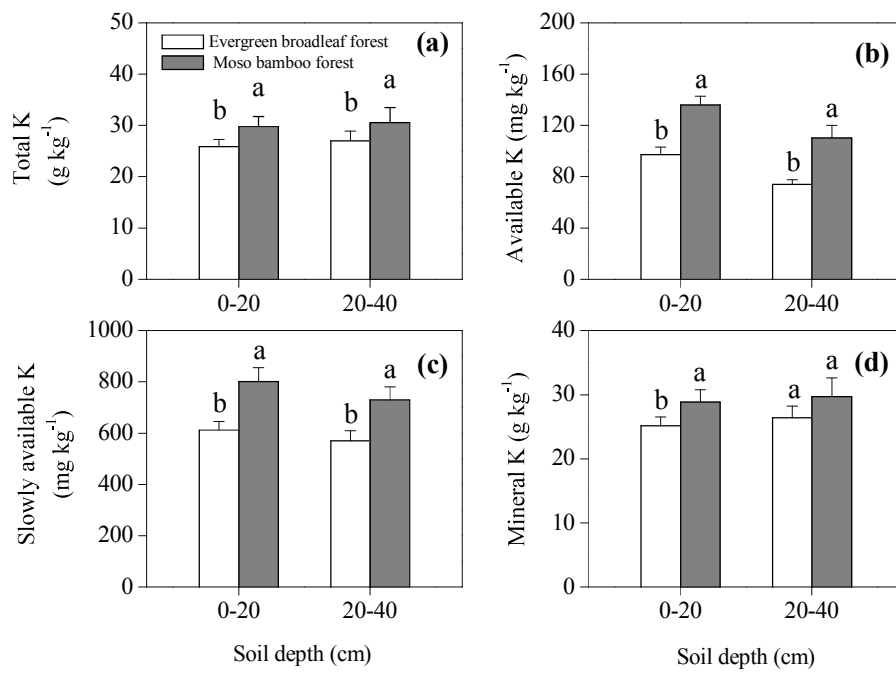
868 **Fig. 1**



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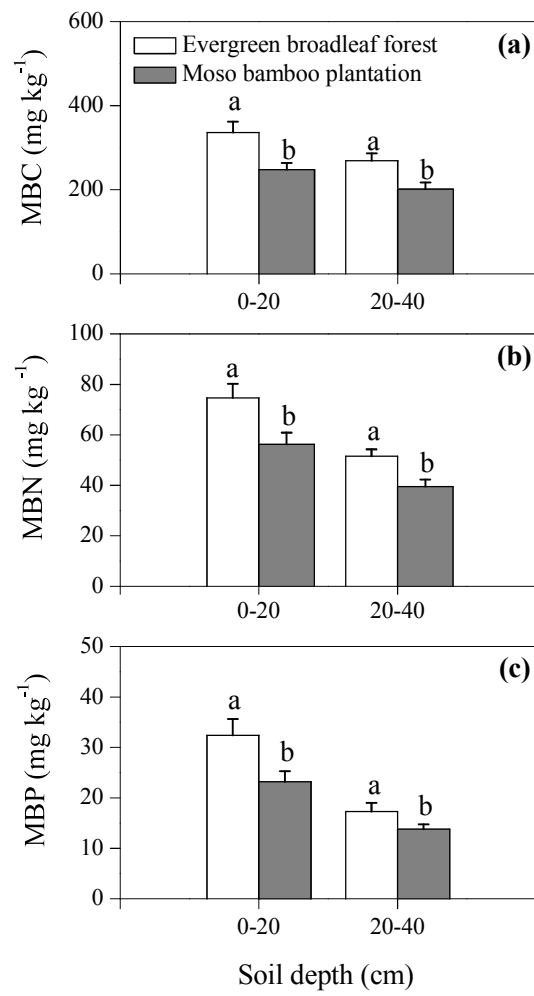
872 **Fig. 2**



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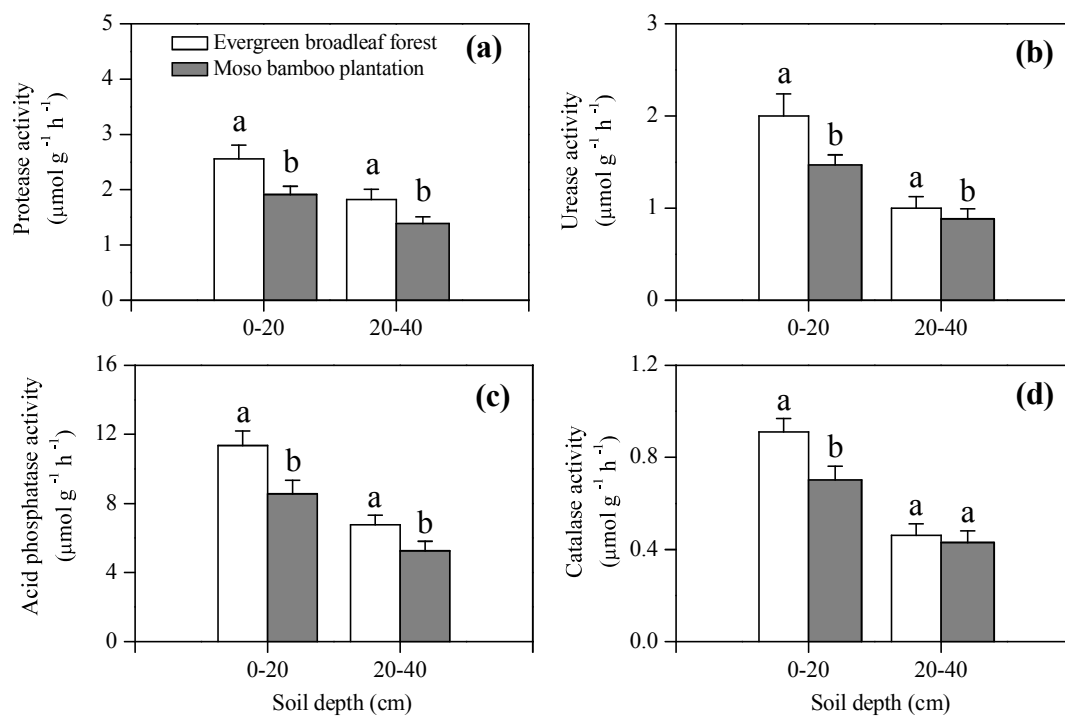
876 **Fig. 3**



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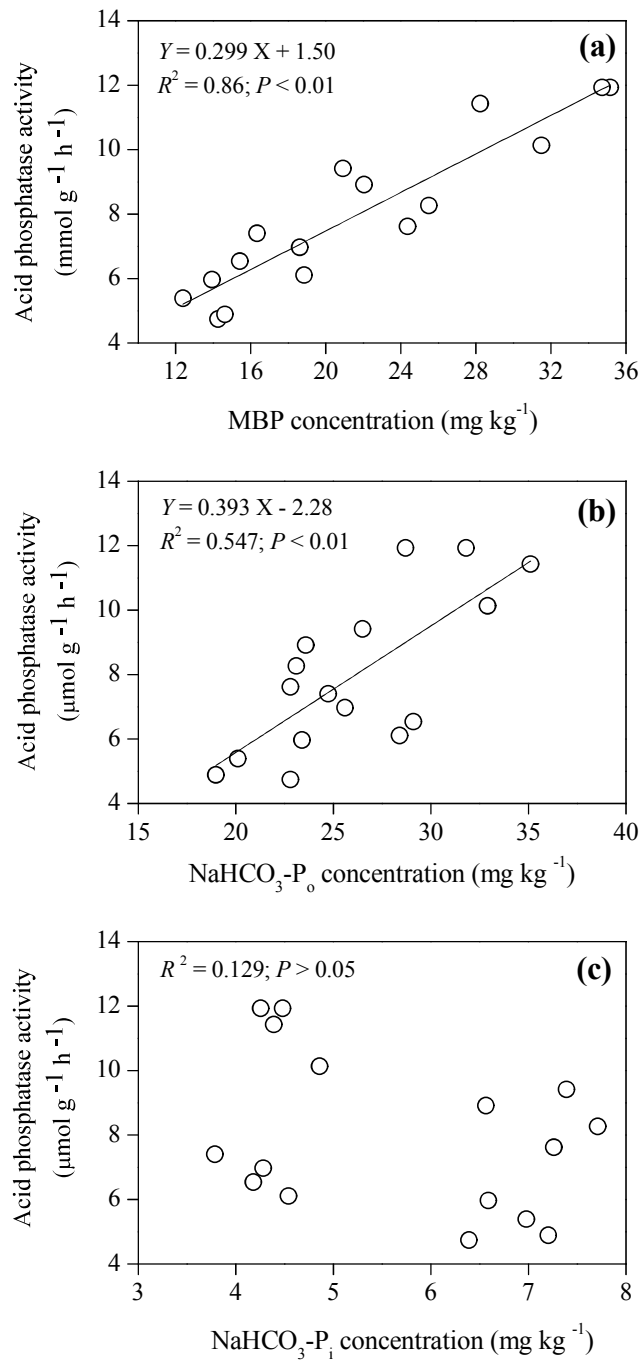
880 **Fig. 4**



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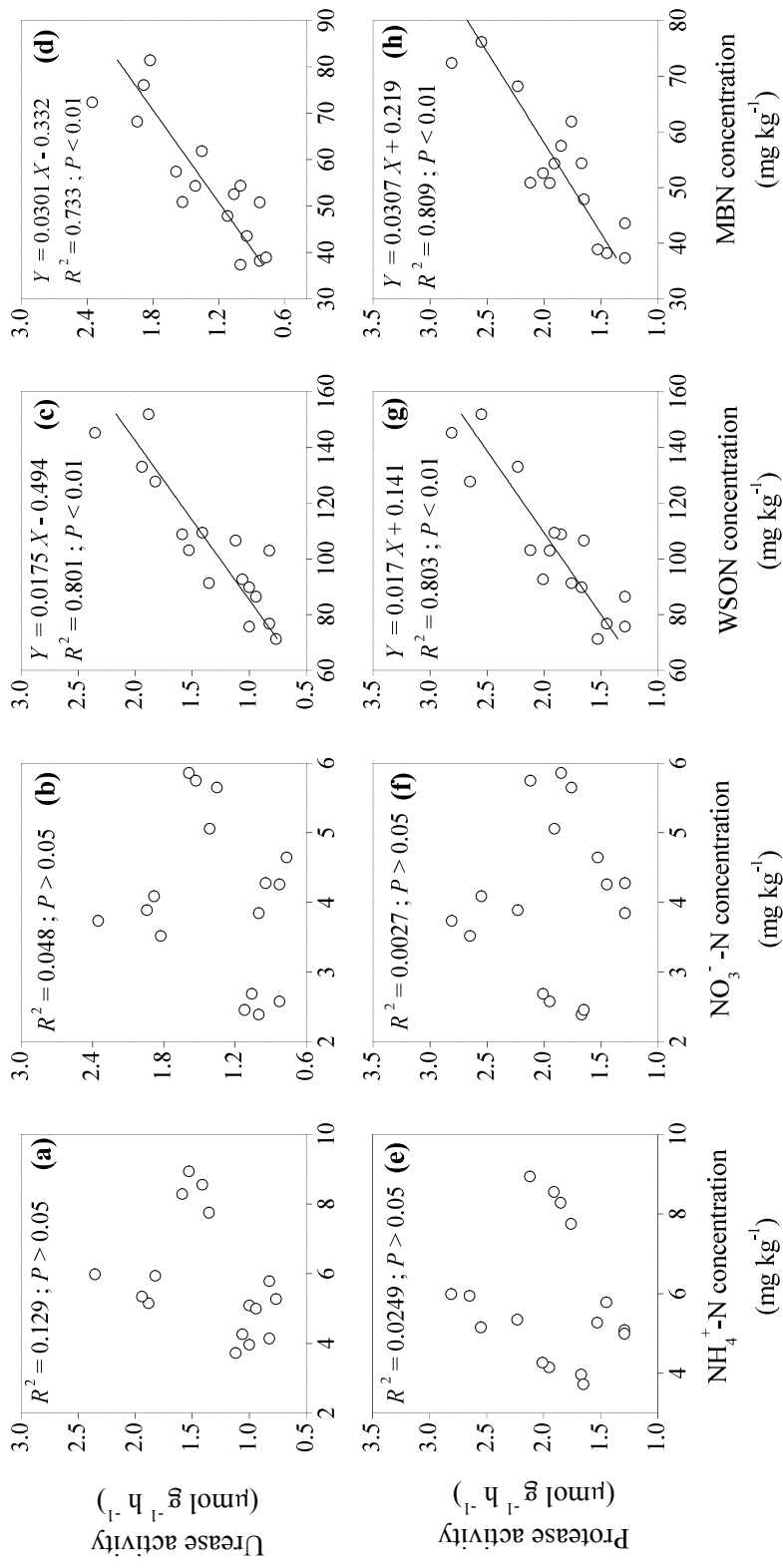
883

884 **Fig. 5**



886

887 **Fig. 6**



888

889 **Fig. 7**