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1 **Biochar addition to forest plantation soil enhances phosphorus availability and**
2 **soil bacterial community diversity**

3

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25

26 **Abstract**

27 Depletion of soil nutrients is a major cause of decline in productivity of forest plantations in
28 successive rotations. Biochar amendment in agricultural systems has been shown to yield various
29 beneficial effects, including increasing soil phosphorus (P) availability. However, the direct and
30 indirect effects of biochar addition on forest soil P dynamics have largely been unexplored. The
31 objective of this study was to examine how biochar produced from harvest residue (leaves and
32 woodchips) affect the P dynamics in second rotation *Cunninghamia lanceolata* (Chinese fir)
33 plantation soil. An incubation experiment which involved mixing of forest soil with 1% or 3% w/w
34 leaf or woodchip biochar, pyrolyzed at 300 °C or 600 °C, was conducted for 80 days at 20 °C. After
35 7, 40 and 80 days of incubation, soil samples were analyzed for total and available P, inorganic and
36 organic P pools, and soil phosphatase activity. At the end of the incubation period, bacterial
37 community composition and diversity were analyzed by 16S rDNA sequencing. The leaf biochar
38 produced at both pyrolysis temperatures was more alkaline and had significantly higher soluble P,
39 nitrogen and calcium contents than the woodchip biochar. Soil total and available P increased
40 significantly in all leaf biochar treatments after 80 days incubation compared to the untreated control
41 soil, but the woodchip biochar treatments had no significant effects. At the end of the experiment, Al-
42 P content was significantly lower and Ca₁₀-P content higher in soil amended with both biochar types
43 compared to the control soil, and Fe-P content was significantly higher in the leaf biochar treatments.
44 Contrary to expectations, acid and alkaline phosphatase enzyme activities were significantly lower in
45 some of the biochar treatments after 80 days incubation compared to the control soil. Nevertheless,
46 the diversity of the bacterial community was higher in leaf biochar-amended forest soil than the
47 woodchip biochar-amended and untreated soil at the end of the experiment. In particular, the
48 abundance increased in the leaf biochar soil treatments of P-solubilizing bacteria, such as
49 *Burkholderia-Paraburkholderia*, *Planctomyces*, *Sphingomonas* and *Singulisphaera*, which can
50 indirectly improve P availability in soil. Thus, conversion of tree harvest residues, particularly leaves,
51 into biochar and recycling back into the soil could be a viable option to boost P availability and help

52 to conserve nutrients or reduce nutrient losses for the next rotation. Before recommending plantation
53 management with biochar, long-term studies are required assessing the life cycle of biochar under
54 field conditions and its promoting effect on growth of *C. lanceolata*.

55

56 **Keywords:** biochar, *Cunninghamia lanceolata*, microbial diversity, phosphate-solubilizing bacteria,
57 phosphorus availability

58

59 1. Introduction

60 A decline in productivity of forest plantations under successive rotations (Tian et al., 2011) has
61 been a major concern among forest managers. Under continuous planting on the same site, depletion
62 of soil nutrients, particularly available phosphorus (P), which is the major growth limiting factor in
63 the tropical and sub-tropical regions, is often associated with productivity decline (Yang et al., 2000).
64 For instance, soil available P has been shown to decrease by more than 50% in second rotation
65 plantation sites compared with that of first generation *Cunninghamia lanceolata* (Lamb.) Hook
66 (Chinese fir) plantations (Wang et al., 2006). The current management practice to conserve site
67 resources during the inter-rotation phase (the time between harvesting of one generation and planting
68 of the next generation) involves burning harvest residues in-situ. However, this practice leads to
69 underutilization of large quantities of plant resources due to combustion loss (Lehmann, 2007) while
70 aggravating soil erosion and air pollution. Although nitrogen (N) and P fertilizers or lime can be added
71 to rectify soil nutrient deficiencies, this practice is not sustainable due to the high economic cost,
72 reduction in downstream water quality due to nutrient runoff and increased emission of soil
73 greenhouse gases (Mitchell et al., 2016).

74 Sustainable P management is, thus, of great importance to maintain plantation productivity while

75 minimizing negative environmental impact. Biochar addition to plantation soils may assist with
76 meeting this aim as there is strong evidence that it increases the growth of woody plants (Thomas and
77 Gale, 2015). Biochar is a predominantly stable, recalcitrant organic carbon-rich material produced by
78 pyrolysis of biomass such as crop straw, sawdust, animal manure, wood, and sludge, at temperatures
79 ranging between 300 and 1000 °C (Verheijen et al., 2010). Biochar can be a direct source of P to soil
80 as biomass xylem tissue releases phosphate during carbonization (de la Rosa et al., 2014). In acidic
81 soil, the availability of P is mainly determined by its interaction with Al, Fe, and Ca. The addition of
82 biochar can increase the availability of P in acidic soil due to the increase in soil pH and Ca content,
83 resulting in decreased soil phosphate sorption capacity by Fe and Al hydrous oxides and Al^{3+}
84 (Chintala et al., 2014; Bornø et al., 2018; Hong et al., 2018). Biochar offers several other benefits,
85 including increased bioavailability of other essential plant nutrients (Haeefe et al., 2011), enhanced
86 soil water and nutrient retention, and improved soil structure and drainage due to its high porosity
87 (Karhuan et al., 2011). Potential detrimental consequences of biochar addition to soil have also been
88 investigated. The environmental risk from metals, metalloids and PAHs contained in biochar has been
89 assessed to be low (Freddo et al., 2012). However, persistent free radicals in biochars have been
90 shown to inhibit seedling germination and growth in soil-free laboratory conditions, although it is
91 possible that these free radicals may be inactivated by natural organic matter and clay in soils (Liao
92 et al., 2014).

93 Additionally, biochar seems to have beneficial effects in increasing the activity of soil bacteria
94 and fungi and altering the soil microbial community, which could increase soil nutrient availability
95 and carbon storage (Anderson et al., 2011; Mitchell et al., 2016). Soil microbes, such as bacteria, have
96 the ability to convert bound P into available P, making it easier for plants to uptake (Anderson et al.,
97 2011). Significant alterations of the microbial community structure in biochar-amended soil have

98 been observed (Lehmann et al., 2011; Muhammad et al., 2016; Huang et al., 2017; Yao et al., 2017a,b;
99 Halmi et al., 2018), which can promote important processes such as P solubilization and P
100 mineralization (Schmalenberger and Fox, 2016). This can result in increased soil available P content
101 through increased activity of soil phosphatase and enhanced microbial dissolution of inorganic fixed
102 P and mineralization of organic P (Gul and Whalen, 2016; Zhu et al., 2018; Xu et al., 2019).
103 Nevertheless, some studies have reported no change in soil microbial community structure after
104 biochar addition (e.g. Yu et al., 2018), whilst in others the observed change has been associated with
105 accelerated soil organic N turnover which might induce N limitation for plants (Tian et al., 2016).

106 Despite increasing understanding of the potential positive effects of biochar amendment in
107 agricultural systems (Atkinson et al., 2010; El-Naggar et al., 2019), the direct and indirect effects of
108 biochar on P dynamics are less well characterized, particularly in forest soils. As biochar properties
109 vary depending on production technology, pyrolysis temperature, and feedstock type (Gul et al., 2015),
110 biochars can have varying effects on soil chemical and biological properties (Bornø et al., 2018).
111 Furthermore, the amount of biochar applied controls the potential beneficial effects of amendment
112 (Noyce et al., 2015). A better understanding of the potential effects of biochar with different properties
113 on P dynamics in forest soil is needed to inform future forest management practices with respect to
114 biochar amendment. Thus, the objective of this study was to examine how biochars produced from
115 the harvest residue of *C. lanceolata* (leaves and woodchips) at different pyrolysis temperatures affect
116 the P dynamics in second generation *C. lanceolata* plantation soil. We hypothesized that biochar
117 addition to forest soil enhances soil P availability with the effect increasing with application rate, and
118 depending on biochar properties, by: (1) increasing soil pH and decreasing the activity or availability
119 of cations (i.e., Al^{3+} , Fe^{3+} and Ca^{2+}) that decrease P sorption or increase P desorption in soil; (2)
120 contributing highly soluble P from the biochar itself that will directly increase soil available P; and

121 (3) increasing activities of phosphatase and the soil bacterial community, which play an important
122 role in transforming inaccessible organic and inorganic P to available P. To test these hypotheses, an
123 incubation experiment, involving two biochar types (leaf biochar and woodchip biochar), produced
124 at two pyrolysis temperatures (300 and 600 °C) and applied at two rates (1% and 3% w/w) was
125 conducted for 80 days to determine soil total P, available P, inorganic and organic P pools, soil
126 phosphatase activity and the composition and diversity of the soil bacterial community.

127

128 **2. Materials and Methods**

129 *2.1. Soil sampling*

130 Soil for the incubation experiment was collected from a second generation *C. lanceolata*
131 plantation at Xinkou Teaching Forest of Fujian Agricultural and Forestry University in Sanming,
132 Fujian province, China (117°27'–118°14'E and 26°07'–27°13'N). The soil was classified as mountain
133 acidic red loam soil based on the Chinese soil classification system, which is equivalent to humic
134 planosols in the FAO system. Soil samples (~50 kg total) were taken from the surface soil (0–20 cm,
135 which contains more than 60% of the fine roots of *C. lanceolata* (Huang et al., 2016) in a 20 m x 20
136 m plot with an S-shape sampling scheme. The soil samples were combined together, homogenized
137 and screened using a 2 mm sieve, and then air-dried (12 h) and stored at 4 °C before starting the
138 incubation experiment 2 weeks later. The nutrient contents and pH of the prepared *C. lanceolata* forest
139 soil were determined as described below.

140

141 *2.2. Biochar production and characterization*

142 Woodchips and leaves after harvesting of *C. lanceolata* at the soil sampling site were used as separate
143 feedstocks for preparation of biochar. In the laboratory, leaves were washed with deionized water to

remove any residual surface soil, dried at 80 °C, crushed using a mechanical pulverizer (Y-800G, Xuman, China) and then screened using a 1 mm sieve. Woodchips were created in the laboratory from branches following the same procedure as for leaves and then pulverized and screened. The prepared material was heated at 300 °C at 600 °C in a muffle furnace with a heat increase of 20 °C min⁻¹ for 4 hours and then left to cool overnight. The Fe, Al and Ca content of biochar and blank digests were determined by ICP-OES (Optima 8000, PerkinElmer, USA) of 0.15 g sub-samples digested with HNO₃ at 120 °C for 24 h (open vessels on a hot plate). The instrument was calibrated with 5 standards and a blank (Millipore water) and a standard was analyzed for quality control every 25 samples. The C and N content were measured by elemental analyzer (vario MAX, Elementar, Germany). Biochar pH was measured with a LL-Ecotrode Plus electrode (Metrohm, Switzerland) in a 1:2.5 (biochar:deionized water) suspension. The ash content was determined by weight loss after heating the biochars at 750 °C for 4 h in a muffle furnace (Yuan et al. 2011). Biochar dissolved organic carbon (DOC) content was determined by shaking the biochar with 2 M KCl (1:25, w/v) for 1 h, filtering through a 0.45 µm PES membrane filter (Jinteng, Tianjin Jinteng Experiment Equipment Co., Ltd., China), and measuring the DOC concentration with a TOC-V_{CPH} (Shimadzu, Japan). Available and total P contents of the biochars was determined using the procedures described in 2.4.

160

161 2.3. Soil incubation experiment

162 To examine the effects of biochar addition to forest soil on P availability, an incubation
163 experiment involving the two biochar types (leaf and woodchip biochars) and two application rates
164 (1% and 3% w/w) was conducted for 80 days. The factorial experimental design involving eight
165 biochar treatments and the unamended soil control is shown in Table 1. The experiment had three
166 timesteps in which samples from each treatment were taken after 7, 40 and 80 days of incubation for
167 analysis of chemical and biological properties. There were four replicates for each treatment and
168 timestep combination, giving a total of 108 incubated soil samples. The air-dry soil (50 g dry weight
169 equivalent) and the relevant biochar type and amount were well-mixed individually for each replicate

170 before adding deionized water to 60% field capacity. Then the biochar-amended samples and control
 171 soil samples were placed in separate glass boxes, sealed, and incubated at 20 °C in the dark. The soil
 172 moisture was maintained at 60% field capacity by weighing and adding deionized water every 2-3
 173 days.

174

175 **Table 1.** The control and different biochar treatments applied to the forest soil. Biochar additions are %
 176 w/w.

177

Treatment abbreviation	Treatment description
CK	unamended soil control
BW3001	1% 300 °C woodchip biochar
BW3003	3% 300 °C woodchip biochar
BW6001	1% 600 °C woodchip biochar
BW6003	3% 600 °C woodchip biochar
BL3001	1% 300 °C leaf biochar
BL3003	3% 300 °C leaf biochar
BL6001	1% 600 °C leaf biochar
BL6003	3% 600 °C leaf biochar

178

179 2.4. Phosphorus characterization in soil/biochar samples

180 Available P was extracted with ammonium fluoride (NH₄F) and hydrochloric acid (HCl) (Liu et
 181 al., 2017). Briefly, 50 mL of a mixture of 0.03 mol L⁻¹ NH₄F and 0.025 mol L⁻¹ HCl were added to
 182 5.0 g sample, and the mixture was agitated on an oscillating shaker (SPH-2102C, Shiping, China) for
 183 5 minutes, before filtration through P-free filter paper (Whatman, China) to separate the solid and

liquid. Total P in soil/biochar samples was determined on digests prepared as follows: 10 mL H₂SO₄ was added to 0.25 g samples, which were left overnight. After adding 1 mL HClO₄, the samples were digested on a hot plate at 300 °C for 2 h (Lu, 1999). Phosphorus concentrations in all extractions were measured using the molybdenum blue method with an ultraviolet-visible spectrophotometer (T6, Puxi, China) at 700 nm (Lu, 1999). The instrument was calibrated with 5 standards and a blank (Millipore water) and a standard was analyzed for quality control every 25 samples. Blank extractions were also conducted for available and total P and the values subtracted from the sample extractions.

Soil samples taken at each incubation timestep were air dried, pulverized and passed through a 0.149 mm sieve. Different forms of phosphorus were determined by sequential extraction of 1 g prepared soil as follows (Lei et al., 2017; Li et al., 2017): (1) Ca₂-P: 50 mL of 0.25 mol L⁻¹ NaHCO₃ (pH 7.5) added and shaken for 1 h; (2) Al-P: 50 mL of 0.5 mol L⁻¹ NH₄F (pH 8.5) added and shaken for 1 h; (3) Fe-P: 50 mL of 0.1 mol L⁻¹ NaOH added and shaken for 4 h; (4) O-Al-P: 50 mL of 1 mol L⁻¹ NaOH added and heated in a water bath at 85 °C for 1 h; (5) O-Fe-P: 50 mL of 0.5 mol L⁻¹ Na₂S₂O₄-Na₃C₆H₅O₇ added and heated in a water bath at 80 °C for 10 minutes; (6) Ca₁₀-P: 50 mL of 0.25 mol L⁻¹ H₂SO₄ added and shaken for 1 h. Mixtures were shaken on an oscillating shaker (SPH-2102C, Shiping, China), then suspensions were centrifuged (5810 R, Eppendorf, Germany) at 3200 g for 5 minutes between each extraction step to separate the supernatant for analysis and the residue. Blank extractions were also conducted for each step and the values subtracted from the sample extractions.

2.5. Determination of soil enzyme activity

The activities of acid and alkaline phosphatase were determined for soil samples at each incubation timestep following the method described by Jin et al. (2016). Specifically, for phosphomonoesterase activities analysis, 1 g moist soil was mixed with 4 mL of universal buffer (pH 6.5 for acid phosphomonoesterase and pH 11 for alkaline phosphomonoesterase) and 1 mL of p-nitrophenyl phosphate, gently shaken and incubated for 1 h at 37 °C. The reaction was terminated by

210 adding 1 mL 0.5 M CaCl₂ and 4 mL 0.5 M NaOH solution. After filtering through Whatman No. 40
211 filter paper, the absorbance of the solution was measured at 410 nm with a spectrophotometer
212 (Puxi/T6, China).

213

214 2.6. Determination of soil bacterial diversity and composition

215 The diversity and composition of the soil bacterial community were determined at the end of the
216 incubation period (80 days) in the control soil and 3% biochar treatments (0.5 g fresh soil) where the
217 greatest changes were expected with the higher biochar application rate. Three of the four replicate
218 samples in each incubation treatment were analyzed.

219 High-throughput sequencing of 16S rDNA PCR products was conducted by Guangzhou
220 Genedenovo Biotechnology Co., Ltd., China. DNA was extracted from 0.25 g field-moist soil using
221 the FastDNA® SPIN Kit for Soil (Bio 101, Vista, CA, USA) according to the manufacturer's
222 instructions, and then stored at -70 °C for subsequent analysis. After genomic DNA was extracted
223 from the samples, the V3 + V4 region of 16S rDNA was amplified with barcode-specific primers.
224 The primer sequences were as follows: 341F: 5'- CCTACGGGNGGCWGCAG -3' and 806R: 5'-
225 GGACTACHVGGGTATCTAAT-3'. The amplifications were conducted in 50 µL reactions
226 consisting of 5 µL KOD buffer (10 x dose concentration), 5 µL 2.5 mmol L⁻¹ dNTPs, 1.5 µL primer
227 (5 µmol L⁻¹), 1 µL KOD polymerase, and 100 ng template DNA. The amplification conditions were:
228 pre-denaturing at 95 °C for 2 min, denaturing at 98 °C for 10 s, annealing at 62 °C for 30 s, and
229 extension at 68 °C for 10 min, repeated for 27 cycles, followed by a final extension at 68 °C for
230 another 10 min.

231 The amplified PCR products were resolved by agarose gel electrophoresis, using 2 % agarose gel
232 stained with ethidium bromide (0.5 µg mL⁻¹), and visualized and documented by fluorimetry (Quanti
233 Fluor™, Promega, USA). The PCR mixture contained 25.0 µL Quanti Fluor™ (1.25 U DNA
234 polymerase, 4 mmol L⁻¹ Mg²⁺, 0.4 mmol L⁻¹ dNTP mixture), 1.0 µL 20 mmol L⁻¹ forward primer, 1.0
235 µL 20 µmol L⁻¹ reverse primer, 1.0 µL template DNA (about 100 ng), and 22.0 µL nuclease-free water

236 to a final volume of 50 μ L. The samples were passed through illustra MicroSpin S-300 HR Columns
237 (GE Healthcare Life Sciences, USA) for PCR purification. For each sample, an 8-digit barcode
238 sequence was added to the 5' end of the forward and reverse primers (Guangzhou Genedenovo
239 Biotechnology Co., Ltd., China).

240 A sequencing library was constructed following the protocols for Illumina platforms (Caporaso
241 et al., 2010), and the amplified products were sequenced using the PE250 mode of Hiseq 2500 (Edgar
242 et al., 2011). The reads with bases having a quality score below 20 were discarded because it was
243 difficult to interpret the reads below this threshold. Then, tags were intercepted and filtered by length
244 (Haas et al., 2011). Finally, the tag sequences were compared with the Gold database r20110519 using
245 the UCHIME algorithm to detect and remove chimeric sequences (Wang et al., 2007; Edgar, 2013)
246 and obtain the final effective tags. The effective tag sequences of all samples were clustered to form
247 operational taxonomic units (OTUs) using Uparseusearch v9.2.64. To construct OTUs, representative
248 sequences were selected based on the 97% similarity threshold, including the tag sequences with the
249 highest abundance of OTUs. The set of representative sequences was annotated by RDP Classifier
250 (version 2.2) with a confidence threshold of approximately 0.8-1. The SILVA taxonomic library
251 (<http://www.arb-silva.de>) was used to assign taxonomy to the sequences (Yilmaz et al., 2014).

252 Venn diagrams of shared OTUs (97% similarity) between the control and biochar-amended soils
253 (Fig. S1) revealed a total of 2432 separate OTUs after 80 days incubation. Sequences were randomly
254 selected based on the relative ratio of known OTUs in obtained sequences, and the rarefaction curve
255 was constructed by plotting the number of OTUs against the number of tags sampled. The gentle
256 slope of the curves (Figs. S2 and S3) indicated that the depth of sequencing had covered all species
257 in the samples.

258

259 2.7. Statistical analysis

260 All statistical analyses were conducted with SPSS v22. Before performing the statistical analyses,

261 data were tested for deviations from normality and homogeneity of variance. One-way ANOVA and
262 comparison of means with Tukey's honestly significant difference post-hoc test ($p < 0.05$) were used
263 to assess any significant differences between the characteristics of the four biochars used in the
264 experiment and between the bacterial community diversity indices of the control and 3% biochar
265 amended soils after 80 days incubation. The effects of biochar addition on available P, total P, different
266 P fractions and phosphatase enzyme activities in forest soil were analyzed by repeated measures
267 ANOVA. When the homogeneity of variance assumption was violated, according to Mauchly's test
268 of sphericity, the degrees of freedom for testing the significance of the within-subject factors were
269 adjusted using the Huynh–Feldt correction factor. Tukey's honestly significant difference test was
270 employed for post-hoc comparisons ($p < 0.05$). For bacterial diversity, QIIME 1.7.0 (Caporaso et al.,
271 2010) was used to calculate the Chao1, ACE, Shannon, and Simpson indices.

272 Principal Component Analysis (PCA) was used to compare soil bacterial community structure
273 between the different treatments. The evolution distances between microbial communities from each
274 sample were calculated using the Jaccard coefficient and represented as an Unweighted Pair Group
275 Method with Arithmetic Mean (UPGMA) clustering tree describing the dissimilarity (1 - similarity)
276 between multiple samples. To compare the membership and structure of communities in different
277 samples, heat maps were generated with the top 20 OTUs using Mothur.

278

279 **3. Results**

280 *3.1. Biochar properties*

281 The biochars produced using leaves and woodchips at different pyrolysis temperatures had
282 significantly different pHs and chemical composition (Table 2). Biochars produced from leaves had
283 higher pH, ash content, available P, and total P, N, and Ca content and lower C:N ratio compared to

the woodchip biochars. For both biochar feedstocks, increasing pyrolysis temperature resulted in higher pH, ash content and DOC, available P and total Ca content at 600 °C than at 300 °C. While the total C and Fe content did not vary between leaf and woodchip biochars, the Al content was significantly higher for the woodchip biochar ($>1900 \text{ mg kg}^{-1}$) than the leaf biochar ($<1100 \text{ mg kg}^{-1}$).

288

3.2. Total and available soil phosphorus contents during the incubation experiment

Soil total P and available P content varied significantly among treatments, incubation time and their interaction (Table 3). The application of biochar from *C. lanceolata* leaves resulted in significantly higher total and available P contents in soil compared to the control and woodchip biochar treatments, with the increase being most pronounced at higher application rates and pyrolysis temperature (Figs. 1 and S4).

The BL3003 and BL6003 treatments increased mean soil total P by 85.4 mg kg^{-1} (28%) and 211 mg kg^{-1} (70%), respectively, and mean available P by 5.86 mg kg^{-1} (45%) and 20.9 mg kg^{-1} (161%), respectively, after 80 days of incubation compared with the control. Soil available P content decreased over time during the experiment within each treatment (Figs. 1 and S4D-F). After 80 days of incubation the mean available P content in the BW3003 and BW6003 treatments was 62% (8.03 mg kg^{-1}) and 60% (7.82 mg kg^{-1}), respectively, that of the control (13.0 mg kg^{-1}), while the total P content of the woodchip biochar treatments varied little with the duration of the study compared with the control (Figs. 1 and S4A-C).

303 **Table 2.** Mean ($\pm$ SE, n=4) chemical compositions of the soil and biochar. Means with different letters across a row indicate significant differences ($p <$
304 0.05) between the biochars.

305

Properties	BW300	BW600	BL300	BL600	Soil
pH (%)	4.05 $\pm$ 0.01a	7.96 $\pm$ 0.01c	7.33 $\pm$ 0.02b	10.4 $\pm$ 0.01d	4.34 $\pm$ 0.06
Ash (%)	1.00 $\pm$ 0.2a	2.70 $\pm$ 0.9a	9.90 $\pm$ 0.5b	22.2 $\pm$ 0.2c	-
DOC* (g kg ⁻¹)	1.26 $\pm$ 0.1c	0.30 $\pm$ 0.03a	2.44 $\pm$ 0.26d	0.96 $\pm$ 0.04b	0.51 $\pm$ 0.02
Total P (g kg ⁻¹)	0.12 $\pm$ 0.02a	0.14 $\pm$ 0.03a	0.82 $\pm$ 0.05b	1.51 $\pm$ 0.11c	0.32 $\pm$ 0.73
Available P (g kg ⁻¹)	0.23 $\pm$ 0.00a	0.62 $\pm$ 0.05b	2.57 $\pm$ 0.16c	3.6 $\pm$ 0.20d	0.014 $\pm$ 0.00
Total C (%)	59.2 $\pm$ 0.07a	67.7 $\pm$ 4.2a	56.3 $\pm$ 3.3a	59.2 $\pm$ 1.0a	1.63 $\pm$ 0.12
Total N (%)	0.39 $\pm$ 0.02a	0.35 $\pm$ 0.04a	1.57 $\pm$ 0.01d	1.28 $\pm$ 0.01c	0.18 $\pm$ 0.02
C:N ratio	151.8 $\pm$ 9.2c	193.4 $\pm$ 11.5d	35.6 $\pm$ 3.9a	46.3 $\pm$ 8.5b	9.1 $\pm$ 1.5
Ca (g kg ⁻¹)	2.31 $\pm$ 0.18a	7.62 $\pm$ 1.34a	33.50 $\pm$ 0.88b	61.58 $\pm$ 0.44c	7.8 $\pm$ 0.09
Fe (g kg ⁻¹)	2.49 $\pm$ 0.54a	2.74 $\pm$ 0.46a	2.21 $\pm$ 0.14a	1.97 $\pm$ 0.18a	672 $\pm$ 41.3
Al (g kg ⁻¹)	1.95 $\pm$ 0.17b	1.99 $\pm$ 0.23b	1.07 $\pm$ 0.15a	0.91 $\pm$ 0.13a	2.24 $\pm$ 0.27

306

* Dissolved organic carbon

Table 3. Summary of repeated measures ANOVA for testing the significance of the between-subject (treatment) and within-subject (time) effects on soil total P, available P, and different P fractions, as well as soil acid phosphatase (ACP) and alkaline phosphatase (ALP) activities.

Variables	Between-subject factor		Within-subject factor		Interaction	
	(d.f. = 8)		(d.f. = 2)		(d.f. = 16)	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Total P	82.9	<0.001	9	<0.001	7.5	<0.001
Available P	169.6	<0.001	84.3	<0.001	5.6	<0.001
Al-P	8.3	<0.001	130.9	<0.001	3.7	<0.001
Fe-P	15.9	<0.001	14.9	<0.001	9.7	<0.001
Ca ₂ -P	22.1	<0.001	134.4	<0.001	8.3	<0.001
Ca ₁₀ -P	33.2	<0.001	133	<0.001	4	<0.001
O-Al-P	6.7	<0.001	391	<0.001	11.2	<0.001
O-Fe-P	3.6	0.006	104.5	<0.001	0.91	0.559
ACP	27.6	<0.001	121.7	<0.001	3.8	<0.001
ALP	11	<0.001	78.9	<0.001	2.5	0.007

312 **Figure 1.** Soil total phosphorus (panel A) and available phosphorus (panel B) contents (mg kg^{-1} , mean
 313 $\pm$ SE, $n=4$) after different incubation times following addition of biochar produced from *C. lanceolata*
 314 leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with
 315 different letter(s) are significantly different among 80 day incubations ($p < 0.05$). Note different y-
 316 axis scales.

3.3. Dynamics of soil P forms in biochar-amended forest soil

Significant differences were detected among treatments, incubation time and their interaction for all soil P forms, except O-Fe-P for which no significant interaction effect was detected (Table 3, Figs. 2, S5, S6 and S7). In all biochar treatments and the control, soil Al-P content increased between 7 and 40 days and then decreased after 80 days incubation (Figs. 2A, S5A, S6A and S7A). At the end of the experiment, soil Al-P content was significantly lower than the control in all the biochar treatments, apart from the 600 °C woodchip biochar treatments. The soil O-Al-P content decreased continuously over time in nearly all treatments (Figs. S5B, S6B and S7B). After 80 days, only the BL3001 and BW3003 treatments had significantly lower soil O-Al-P content than the control (Fig. 2B). The mean soil Fe-P content decreased over time for the control and woodchip biochar treatments and was not significantly different after 80 days of incubation (Figs. 2C, S5C, S6C and S7C). In contrast, the soil Fe-P content in the leaf biochar treatments was significantly higher than in the control after 80 days incubation. Soil O-Fe-P content increased continuously over time during the experiment (Figs. 2D, S5D, S6D and S7D). The only significant difference in soil O-Fe-P content between the treatments after 80 days incubation, was a small but significant increase in the BL3003 treatment soil compared with the control and the BW3001 and BW6003 treatments. The soil Ca₁₀-P content decreased over time for most of the treatments (Figs. 2E, S5E, S6E and S7E). After 80 days incubation, soil Ca₁₀-P content was significantly higher in all the biochar treatments compared with the control, apart from the BW3001 and BL6001 treatments. Mean soil Ca₂-P content generally increased between 7 days and 40 days during the experiment and then stabilized in most treatments (Figs. 2F, S5F, S6F and S7F). After 80 days incubation, compared with the control, the BL6003 treatment was the only treatment with a significantly higher Ca₂-P content, whilst the BW6001 and BW6003 treatments had a significantly lower Ca₂-P content.

343 **Figure 2.** Soil P fractions (panel A is Al-P, panel B is O-Al-P, panel C is Fe-P, panel D is O-Fe-P,
 344 panel E is Ca₁₀-P, and panel F is Ca₂-P) contents (mg kg⁻¹, mean ± SE, n=4) after different incubation
 345 times following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil.
 346 The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly
 347 different among 80 day incubations ($p < 0.05$). Note different y-axis scales.

349 3.4. *Effects of biochar application on soil enzyme activities*

350 Acid phosphatase showed higher activities than alkaline phosphatase in the control and biochar-
351 amended soil samples throughout the experiment. Both soil acid and alkaline phosphatase activities
352 varied significantly among treatments, incubation time and their interaction (Table 3, Figs. 3 and S8).
353 Acid phosphatase activities decreased after 40 and 80 days of incubation compared to 7 days of
354 incubation in almost all treatments (Figs. 3A and S8A-C). After 80 days incubation, among treatments,
355 the activity of acid phosphomonoesterase decreased with biochar pyrolysis temperature and
356 application rate, and significantly for the BW6001, BW6003 BL3003 and BL6003 treatments, but
357 was significantly higher in the BL3001 and BL6001 treatments compared to the control (Fig. 3A).
358 The soil alkaline phosphatase activity also declined over time in most of the treatments (Figs. 3B and
359 S8D-F). After 80 days incubation, the activity of this enzyme was significantly reduced in the
360 BW6003, BL3001 and BL3003 treatments, and increased in the BW3003 and BL6003 treatments,
361 compared to the control (Fig. 3B).

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364 **Figure 3.** Soil acid phosphatase (A) and alkaline phosphatase (B) activities ($\text{mg kg}^{-1} \text{h}^{-1}$, mean $\pm$ SE,
 365 $n=4$) after different incubation times following addition of biochar produced from *C. lanceolata*
 366 leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with
 367 different letter(s) are significantly different among 80 day incubations ($p < 0.05$). Note different y-
 368 axis scales.

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370 3.5. Diversity and composition of soil bacterial community

371 After 80 days incubation, the control, BL3003, BL6003, BW3003, and BW6003 treatments
 372 contained 1451, 1478, 1375, 1328, and 1339 OTUs, respectively, of which BL3003 accounted for the
 373 largest number of OTUs. Compared with the control, the number of unique OTUs was the largest in
 374 the BL3003 treatment, at 403 OTUs, indicating that there were more unique bacterial species
 375 following this treatment. Also, the number of OTUs shared by the BL3003 treatment and the control
 376 was the largest at 1075 (Fig. S1). The alpha diversity indices of soil bacteria across the 3% biochar
 377 addition treatments and the control after 80 days incubation are given in Table 4.

378

379 **Table 4.** Omicsmart MiSeq sequencing bacterial data and bacterial community diversity indices (at
 380 97% sequence similarity) based on the 16S rRNA gene after 80 days incubation. The treatment
 381 abbreviations are shown in Table 1. Different letters within the same column indicate significant
 382 difference between treatments ($p < 0.05$). The treatment abbreviations are shown in Table 1.

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Treatment	Chao1	ACE	coverage	Shannon	Simpson	OTUs
CK	1906±193a	1889±117a	0.996	6.973±0.26a	0.967±0.11a	1451±217a
BL3003	2051±164a	1889±115a	0.996	7.337±0.03b	0.986±0.11a	1478±63a
BL6003	1776±43a	1889±116a	0.996	7.177±0.16ab	0.981±0.181a	1357±14a
BW3003	1838±141a	1832±121a	0.996	6.787±0.06a	0.968 ±0.21a	1328±114a
BW6003	1927±93a	1889±114a	0.996	6.733±0.24a	0.963±0.23a	1339±202a

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386 There was no significant difference in the number of OTUs or any of the indices between the
 387 treatments, with the exception of the Shannon index. The Shannon index, which is a measure of
 388 species richness and evenness, was significantly higher for the BL3003 treatment than the BW3003
 389 and BW6003 treatments and the control. The PCA also showed that the application of the biochar
 390 produced from leaves caused divergence in the community composition (Fig. 4). The contribution of
 391 the first principal component (PC1) was 77.5% and that of the second principal component (PC2)
 392 was 10.8%. The treatments with biochar from *C. lanceolata* leaves were clearly distinguished from
 393 the woodchip biochar treatments and the control along PC1 (the x-axis), whilst the woodchip biochar
 394 and control samples plotted close together. The leaf biochars produced at the two pyrolysis
 395 temperatures were also distinguished along PC2 (the y-axis). These results showed that the
 396 application of biochar made from different *C. lanceolata* harvest residues resulted in different soil
 397 bacterial community structures, with the source of biochar materials having the most significant
 398 influence, followed by the pyrolysis temperature.

400 **Figure 4.** PCA ordination of soil bacterial community structure in biochar-amended soil (3% w/w)
 401 and the control after 80 days incubation. The treatment abbreviations are shown in Table 1.

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The top 25 OTUs accounted for 41-52% of the soil bacteria genus relative abundance in the control and biochar treatments, and are detailed in Table S1. The distinctive bacteria genera in the control and each treatment are highlighted in the heat map in Fig. 5. The control had 171 unique OTUs, of which *Candidatus Solibacter*, *Candidatus Xiphinematobacter*, *Acidobacterium* and *Bacteroides* were more abundant compared to the biochar treatments, while *Jatrophihabitans* and *Bradyrhizobium* were less abundant. The BW3003 treatment contained 123 unique OTUs, of which *Sinomonas* and *Conexibacter* had the highest relative abundance (6.45% and 1.53%, respectively) compared to other treatments, whereas *Pseudomonas*, *Exiguobacterium* and *Acinetobacter* were less abundant. Among the 148 unique OTUs identified in the BW6003 treatment, *Ktedonobacter* had the highest relative abundance (11.9%) compared to other treatments, while *Bryobacter*, *Singulisphaera* and *Bradyrhizobium* were less abundant genera. In the BL3003 treatment, 207 unique OTUs were found of which *Burkholderia-Paraburkholderia*, *Nocardia*, *Paucimonas* and *Amycolatopsis* were the most abundant (6.88%, 1.15%, 1.91%, 1.12%, respectively) compared to other treatments, whereas *Ktedonobacter* and *Candidatus Solibacter* were less abundant. The BL6003 treatment contained 238 unique OTUs, of which the most abundant were *Gemmatimonas*, *Planctomyces*, *Sphingomonasi* and *Niastella* (0.79%, 6.38%, 4.87%, 1.73%, respectively) compared to other treatments, while *Terracidiphilus*, *Sorangium* and *Acidotherrmus* were less abundant.

422 **Figure 5.** z-score hierarchical clustering and heat map of soil bacteria genus abundance in the top 25
 423 OTUs in biochar-amended soil (3% w/w) and the control after 80 days incubation. Each column in
 424 the heat map represents a sample and each row represents a classification level. The color scale
 425 indicates the gene species abundance expressed as standard deviations from the mean (the z-score),
 426 with red for high abundance and blue for low abundance. The treatment abbreviations are shown in
 427 Table 1.
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430 The abundance of several soil P-solubilizing bacteria genera was significantly higher in some of
431 the 3% biochar treatments compared with the control (Fig. 6), particularly for the leaf biochar rather
432 than the woodchip biochar. The abundance of the following P-solubilizing bacteria increased
433 significantly in soil amended with the BL3003 and/or BL6003 treatments compared to the control
434 and the *C. lanceolata* woodchip biochar treatments: *Burkholderia-Paraburkholderia*, *Planctomyces*,
435 *Sphingomonas*, *Singulisphaera*, *Gemmatimonas*, *Nocardia*, *Amycolatopsis*, *Massilia*. The relative
436 abundance of *Burkholderia-Paraburkholderia* increased significantly by 85%, 102%, 959%, and 123%
437 in the BW3003, BW6003, BL3003, and BL6003 treatments, respectively, compared with the control.
438 The relative abundance of *Planctomyces* increased by 91% and 436% in the BL3003 and BL6003
439 treatments, respectively, compared to the control.

440

441 **4. Discussion**

442 The results demonstrate that addition of biochar to second rotation *C. lanceolata* plantation soil
443 maintains higher total and available P contents, depending on the feedstock, pyrolysis temperature
444 and the application rate. The main explanations for the changes in soil available and total P content
445 after biochar addition are: 1) the direct addition of P in the biochar, and 2) the indirect effect of biochar
446 addition in altering soil factors which affect total P content and availability, such as soil pH, the
447 content and activity of soil Al^{3+} , Fe^{3+} , and Ca^{2+} and dissolved organic carbon (DOC) which affect soil
448 P fixation, and the soil microbial community structure and activity. Evidence for the operation of
449 these mechanisms in the present study is explored below.

450 **Figure 6.** The relative abundance (mean $\pm$ SE, n=3) of soil phosphorus-solubilizing bacteria in biochar-amended soil (3% w/w) and the control after 80
 451 days incubation. The treatment abbreviations are shown in Table 1. Bacteria genus labels are: *Burkholderia-Paraburkholderia* (*Burk*), *Sinomonas*
 452 (*Sinom*), *Planctomyces* (*Planc*), *Bradyrhizobium* (*Brady*), *Sphingomonas* (*Sphin*), *Acinetobacter* (*Acine*), *Pseudomonas* (*Pseud*), *Singulisphaera* (*Singu*),
 453 *Conexibacter* (*Conex*), *Gemmatimonas* (*Gemma*), *Nocardia* (*Nocar*), *Amycolatopsis* (*Amyco*), *Sorangium* (*Soran*), *Isosphaera* (*Isosp*), *Massilia* (*Massi*),
 454 *Bacillus* (*Bacil*). Bars with different lower case and upper case letter(s) are significantly different ($p < 0.05$) among treatments.

455

456 Corresponding with the higher available and total P contents of the leaf biochar,
457 the available and total P contents in soil treated with leaf biochar were significantly
458 higher than that treated with woodchip biochar. These differences increased with
459 temperature and application rate at the start of the experiment, although the significance
460 of differences diminished over time. Application of biochars made from rice straw and
461 branches to rice paddy soil showed similar results, where the soil available P increased
462 more with the application of rice straw biochar, which had a higher P content, than the
463 branch biochar (Chao et al., 2015). The P content in leaf biochar was higher than that
464 of woodchip biochar since leaves contain more mineral nutrients and less carbon
465 compared with woody materials in *C. lanceolata* (Ma et al., 2007), resulting in more
466 available P in soil amended with leaf biochar. Furthermore, partially stable P in biochar
467 feedstock may be activated and become soluble after pyrolysis, with pyrolysis
468 temperature determining the element content and surface physical structure of the
469 biochar (Cheng et al., 2006; Gundale and DeLuca, 2006). This influence of pyrolysis
470 temperature on biochar properties was evident in the present study, as total and
471 available P concentrations were higher in each biochar type produced at 600 °C
472 compared to that from the same feedstock pyrolyzed at 300 °C (Table 2).

473 As well as the direct effects of P addition in biochar-amended soils, biochar
474 addition may alter many soil properties which indirectly affect soil P dynamics, content
475 and availability (Bornø et al., 2018). Soil pH is an important control on P availability
476 as it is related to fixation of P by Al and Fe at pH < 5.5 and by Ca at pH > 7.5. Previous

studies have reported that biochar addition may increase soil pH and change the activity or availability of Al^{3+} , Fe^{3+} , and Ca^{2+} , resulting in changed P sorption/desorption in soil (Xu et al., 2014). Most biochars are alkaline because as the pyrolysis temperature increases, surface acidic groups (e.g. carboxyl, hydroxyl and phenolic groups) decrease and surface basic groups (e.g. lactones) increase (Chen et al., 2014). Also, mineral elements such as Na, K, Mg and Ca, are present in the form of oxides or carbonates in the ash (Wu et al., 2019). The biochars examined in this study had $\text{pH} > 7.3$, apart from the woodchip biochar produced at 300°C ($\text{pH} 4.05$), and were added to second rotation forest plantation soil of $\text{pH} 4.3$. In the first half of the incubation experiment, soil pHs in the leaf biochar treatments ($\text{pH} 4.7\text{-}6.1$) were significantly higher than the control soil, but after 80 days soil pH was only significantly enhanced ($\text{pH} 5.5$) in the BL6003 treatment (Table S2). Thus soil pH is unlikely to explain the higher available soil P contents in the leaf biochar treatments.

A further factor in the present study could be increased immobilization of available P in the woodchip biochar treatments due to the higher Al content of the woodchip biochar compared to the leaf biochar, or in the leaf biochar treatments due to the higher Ca content of the leaf biochars (Table 2). Counteracting this effect is that biochar can adsorb ions with which P can precipitate readily in soil, such as Al^{3+} , Fe^{3+} , and Ca^{2+} (Gundale and DeLuca, 2007), or through the formation of chelates between Al^{3+} and Fe^{3+} and organic molecules adsorbed on the surface of biochar (Xu et al., 2014), thus improving soil P availability. The soil Al-P content results after 80 days incubation indicate that increased immobilization of available P by Al^{3+} did not occur in the present

study, since none of the biochar treatments have significantly higher Al-P concentrations than the control, and most are significantly lower. Instead, Al³⁺ inactivation due to biochar addition is most probable. Inactivation of Fe³⁺ and Ca²⁺ by biochar was not evident in this study, since soil Fe-P, Ca₁₀-P and Ca₂-P contents in all biochar treatments were the same as the control or significantly higher (Fig. 2C, E- F). Moreover, the Fe addition in the biochar treatments (~2 g kg⁻¹) was negligible compared to the soil background concentration (~670 g kg⁻¹) (Table 2). There is also no clear evidence of increased formation of chelates with Al³⁺ and Fe³⁺ after biochar addition causing enhanced soil available P, because the soil O-Al-P and O-Fe-P concentrations in most biochar treatments are not significantly different from the control (Fig. 2B, D).

The higher DOC content in the biochar made from *C. lanceolata* leaves than the woodchip biochar, produced at the same pyrolysis temperature (Table 2), could also explain the higher soil available P content in the leaf biochar treatments. Various mechanisms have been suggested by which biochar-derived dissolved organic matter could inhibit P sorption on different soil components, such as: 1) soil colloids due to competition for sorption sites and electrostatic repulsive forces (Schneider and Haderlein, 2016); 2) goethite, particularly in acidic, highly weathered soils (Schneider and Haderlein, 2016); and 3) Fe and Al oxides, due to the increase of anion exchange capacity or cation activity resulting from organic matter addition, as reported following the addition of manure-derived biochar to soil (Yual et al., 2014). Whilst the mineralogy of the study soil was not determined, goethite has been detected in the same soil type in the neighboring county (Chen et al., 2018) and the acidic and highly-weathered

nature of the study soil indicates that the higher DOC inputs from the leaf biochars could help explain the higher soil available P content in these treatments. Although significantly higher soil available P concentrations were maintained in most of the leaf biochar treatments to the end of the 80-day incubation experiment, concentrations decreased over time, probably due to fixation with Ca or chelation with Fe and organic material (see increase in soil O-Fe-P and Ca₂-P contents over time during the experiment, Fig. 2D and F), adsorption to biochar or mineral surfaces, or net immobilization by the microbial biomass (Nguyen and Marschner, 2005; Xu et al., 2019).

Soil enzymes serve several important functions. They are intimately involved in the cycling of nutrients, affect fertilizer use efficiency, and, since they reflect soil microbiological activity, they can act as indicators of soil change. The focus of much soil enzyme research has been to develop methodologies for their measurement and to provide an understanding of their origin and the factors that affect their activity in soil. Comparing enzyme activities between studies can be difficult due to differences in the methodologies used (Peoples and Koide, 2012). The contribution of phosphatase enzymes in increasing soil P availability was minor in the present study. Activities of acid and alkaline phosphomonoesterase decreased significantly in some biochar treatments compared to the control after 80 days incubation with biochar, whilst in others there was an increase in activities or no significant difference between the treatments and the control. These findings of the variable effects of biochar addition on soil phosphatase activities are supported by other studies. Biochar addition to soils has

been reported to increase (Bera et al., 2016; Marzooqi and Yousef, 2017), have no effect (Zhang et al., 2017) or reduce (Foster et al., 2016) phosphatase activity. The lower activities of phosphomonoesterase following biochar amendment of soil has been attributed to several mechanisms (Foster et al. 2016), including: sorption or blockage of the enzyme by biochar, lack of soil liming effect due to biochar addition, and increased soil available P resulting in decreased phosphatase activity. The first two of these explanations are more probable in the present study, since significant differences in soil available P compared with the control did not occur in all of the biochar treatments with reduced enzyme activity.

This study showed that the addition of biochar derived from *C. lanceolata* leaves increased the soil bacterial community diversity. Changes in soil properties after biochar application have been shown to alter the structure of soil bacterial communities (Kolton et al., 2011; Chen et al., 2015; Yao et al., 2017a). Previous studies suggest that, because of its physical properties, such as high nanoporosity and large specific surface area, biochar addition can improve soil bacteria and fungi growth by increasing the overall soil aeration and water retention, and by the biochar itself providing habitat for bacteria and fungi to escape from predators and to live and grow (Quilliam et al., 2013; Yao et al., 2017b; Dai et al., 2018; McCormack., 2019; Zheng et al., 2019). It is hypothesized that, of the biochars used in this study, the leaf-based biochar has characteristics more favorable for enhancing the soil bacterial community (such as larger specific surface area, although not measured) compared to the woodchip biochar. Changes in soil chemical properties, notably soil pH and nutrient content and

availability, caused by biochar application can also alter the bacterial community structure (Rousk et al., 2010; Yao et al., 2017a; Simarani et al., 2018). The increased soil pH (at least for the first 40 days) and P content and availability after the addition of biochar prepared from *C. lanceolata* leaves might stimulate the growth and reproduction of soil bacteria, thereby changing the soil bacterial community structure. The results are consistent with previous studies, which demonstrated a larger number of 16S rRNA gene copies (Chen et al., 2015), and increased microorganism total phospholipid fatty acids (Muhammad et al., 2016) and bacterial diversity (Yao et al., 2017a) in biochar-amended compared to unamended control soil samples.

P-solubilizing bacteria have been shown to enhance the solubilization of P compounds with limited solubility through the release of organic acids and phosphatase enzymes (Alori et al., 2017; Yao et al., 2017a). In the current study, the abundance of some P-solubilizing bacteria increased significantly in soil amended with biochar derived from *C. lanceolata* leaves. Increased abundance of inorganic phosphate-solubilizing bacterial communities has also been reported following soil amendment with straw biochar (Zheng et al., 2019), and the application of citrus wood biochar to soil was shown to increase the root-associated bacterial populations affiliated with the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, which benefit plant growth (Kolton et al., 2011). However, the abundance of genes associated with soil phosphatase synthesis was found to be unaltered 3 months following amendment of agricultural field plots with wood biochar, even though the soil P availability had increased (Gao and DeLuca, 2018). It was therefore concluded that, in these conditions,

P bioavailability was controlled predominantly by abiotic mechanisms related to biochar addition.

5. Conclusions

This study showed that the addition of biochar to second rotation *C. lanceolata* plantation soil enhanced soil P availability, with the effect varying with feedstock type and pyrolysis temperature. Biochar produced from *C. lanceolata* leaves improved soil P availability more than *C. lanceolata* woodchip biochar. Likely explanations for this effect are: 1) direct contribution of soluble P by the leaf biochar itself and of DOC which could have reduced P immobilization; 2) an initial increase in soil pH, thereby reducing the content of sparingly-soluble Al-P; and 3) increased diversity of soil bacterial communities and abundance of P-solubilizing bacteria, resulting from available P and DOC addition in biochar, which may have indirectly improved the soil P availability. However, biochar addition to forest soil had a limited effect on soil phosphatase enzyme activities. Overall, the results demonstrate that conversion of *C. lanceolata* plantation harvest residues into biochar which is recycled back to the soil between rotations could be a viable method to boost soil nutrient availability, particularly P, during subsequent planting. Leaf biochar appears to be more favorable than woodchip biochar for enhancing soil available P in *C. lanceolata* plantation systems. To optimize the use of harvest residues as feedstock for preparation of biochar, different mixtures of leaf and woodchip biochar need to be investigated. As this study was a short-term experiment without plants, long-term field studies of its effect on growth of *C. lanceolata* and life

cycle analysis of this biochar use method should be conducted before recommending
plantation management with biochar.

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Supplementary Material for:

**Biochar addition to forest plantation soil enhances phosphorus
availability and soil bacterial community diversity**

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Fig. S1. Venn diagrams of shared OTUs between the biochar-amended soil (3% w/w) and the control after 80 days incubation. The treatment abbreviations are shown in Table 1.

Fig. S2. Rarefaction curve of OTUs for the biochar-amended soil (3% *w/w*) and the control after 80 days incubation. The treatment abbreviations are shown in Table 1. Each of the three replicates for the control and biochar treatments are shown as -1, -2 and -3.

Fig. S3. The Shannon dilution curve of the sample at 0.03 distance. The treatment abbreviations are shown in Table 1. Each of the three replicates for the control and biochar treatments are shown as -1, -2 and -3.

Fig. S4. Soil total phosphorus (A-C) and available phosphorus (D-F) contents (mg kg⁻¹, mean $\pm$ SE, n=4) after 7, 40 and 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among 80 day incubations ($p < 0.05$).

Fig. S5. Soil P fractions (Al-P, Fe-P, O-Al-P, O-Fe-P Ca₂-P, and Ca₁₀-P) contents (mg kg⁻¹, mean ± SE, n=4) after 7 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$). Note different y-axis scales.

Fig. S6. Soil P fractions (Al-P, O-Al-P, Fe-P, O-Fe-P, Ca₂-P, and Ca₁₀-P) contents (mg kg⁻¹, mean $\pm$ SE, n=4) after 40 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$). Note different y-axis scales.

Fig. S7. Soil P fractions (Al-P, O-Al-P, Fe-P, O-Fe-P, Ca₂-P, and Ca₁₀-P) contents (mg kg⁻¹, mean ± SE, n=4) after 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$). Note different y-axis scales.

Fig. S8. Soil acid phosphatase (panel A-C) and alkaline phosphatase (panel D-F) activities (mg kg⁻¹ h⁻¹, mean ± SE, n=4) after 7, 40 and 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. The treatment abbreviations are shown in Table 1. Bars with different letter(s) are significantly different among treatments ($p < 0.05$) within each timestep. Note different y-axis scales.

Table S1. Soil bacteria community genus relative abundances (% , mean $\pm$ SE, n=3) in the top 25 OTUs for the biochar-amended soil (3% w/w) and the control after 80 days incubation following addition of biochar produced from *C. lanceolata* leaves and woodchips to forest soil. Different letter(s) within the same row indicate significant difference between treatments ($p < 0.05$). The treatment abbreviations are shown in Table 1.

Taxonomy	CK	BW3003	BW6003	BL3003	BL6003
<i>Acidothermus</i>	24.96 $\pm$ 2.98b	22.9 $\pm$ 0.69b	24.71 $\pm$ 2.94b	11.6 $\pm$ 0.7a	5.68 $\pm$ 0.42a
<i>Ktedonobacter</i>	10.05 $\pm$ 1.05ab	8.07 $\pm$ 0.66ab	11.94 $\pm$ 2.24b	5.71 $\pm$ 0.28a	6.76 $\pm$ 0.33a
<i>Sinomonas</i>	1.17 $\pm$ 0.28a	6.45 $\pm$ 0.42b	1.76 $\pm$ 0.51a	1.31 $\pm$ 0.11a	0.89 $\pm$ 0.12a
<i>Bradyrhizobium</i>	1.46 $\pm$ 0.09a	2.47 $\pm$ 0.22b	1.58 $\pm$ 0.12a	2.85 $\pm$ 0.08b	2.64 $\pm$ 0.14b
<i>Exiguobacterium</i>	2.58 $\pm$ 0.15a	1.88 $\pm$ 0.09a	3.26 $\pm$ 0.37a	2.8 $\pm$ 0.17a	3.05 $\pm$ 0.62a
<i>Burkholderia-Paraburkholderia</i>	0.65 $\pm$ 0.03a	1.2 $\pm$ 0.11a	1.27 $\pm$ 0.4a	6.88 $\pm$ 0.58b	1.43 $\pm$ 0.21a
<i>Conexibacter</i>	0.28 $\pm$ 0.04ab	1.53 $\pm$ 0.14c	0.34 $\pm$ 0.06ab	0.5 $\pm$ 0.08b	0.04 $\pm$ 0.009a
<i>Acinetobacter</i>	1.03 $\pm$ 0.11a	0.78 $\pm$ 0.04a	1.33 $\pm$ 0.06a	0.98 $\pm$ 0.11a	1.36 $\pm$ 0.29a
<i>Planctomyces</i>	1.31 $\pm$ 0.33a	1.01 $\pm$ 0.1a	1.13 $\pm$ 0.43a	2.3 $\pm$ 0.45a	6.38 $\pm$ 1.28b
<i>Pseudomonas</i>	1.13 $\pm$ 0.04a	0.75 $\pm$ 0.02a	1.21 $\pm$ 0.21a	1.07 $\pm$ 0.03a	1.25 $\pm$ 0.25a
<i>Sphingomonas</i>	0.98 $\pm$ 0.11a	0.9 $\pm$ 0.23a	0.51 $\pm$ 0.11a	1.26 $\pm$ 0.04a	4.87 $\pm$ 0.94b
<i>Acidobacterium</i>	0.67 $\pm$ 0.07b	0.38 $\pm$ 0.08a	0.28 $\pm$ 0.03a	0.41 $\pm$ 0.02a	0.18 $\pm$ 0.04a
<i>Singulisphaera</i>	0.55 $\pm$ 0.11a	0.55 $\pm$ 0.05a	0.31 $\pm$ 0.08a	0.8 $\pm$ 0.05a	1.16 $\pm$ 0.2b
<i>Candidatus_Solibacter</i>	0.76 $\pm$ 0.01b	0.58 $\pm$ 0.11ab	0.57 $\pm$ 0.05ab	0.41 $\pm$ 0.02a	0.44 $\pm$ 0.03a
<i>Terracidiphilus</i>	0.34 $\pm$ 0.05ab	0.3 $\pm$ 0.03ab	0.41 $\pm$ 0.08b	0.34 $\pm$ 0.01ab	0.15 $\pm$ 0.01a
<i>Jatrophihabitans</i>	0.15 $\pm$ 0.01a	0.39 $\pm$ 0.03b	0.73 $\pm$ 0.09b	0.49 $\pm$ 0.03b	0.61 $\pm$ 0.13b
<i>Sorangium</i>	0.24 $\pm$ 0.01b	0.32 $\pm$ 0.07b	0.38 $\pm$ 0.06b	0.31 $\pm$ 0.05b	0.05 $\pm$ 0.01a
<i>Candidatus_Xiphinematobacter</i>	1.19 $\pm$ 0.21b	0.5 $\pm$ 0.12a	0.38 $\pm$ 0.13a	0.41 $\pm$ 0.07a	0.4 $\pm$ 0.06a
<i>Bryobacter</i>	0.33 $\pm$ 0.02a	0.28 $\pm$ 0.04a	0.19 $\pm$ 0.01a	0.22 $\pm$ 0.03a	0.35 $\pm$ 0.04a
<i>Gemmatimonas</i>	0.18 $\pm$ 0.05a	0.15 $\pm$ 0.05a	0.33 $\pm$ 0.11a	0.26 $\pm$ 0.05a	0.79 $\pm$ 0.16b
<i>Nocardia</i>	0.05 $\pm$ 0.01a	0.08 $\pm$ 0.01a	0.08 $\pm$ 0.02a	1.15 $\pm$ 0.03b	0.05 $\pm$ 0.01a
<i>Amycolatopsis</i>	0.08 $\pm$ 0.02a	0.06 $\pm$ 0.01a	0.05 $\pm$ 0.01a	1.12 $\pm$ 0.49b	0.06 $\pm$ 0.01a
<i>Paucimonas</i>	0.02 $\pm$ 0.001a	0.02 $\pm$ 0.002a	0.02 $\pm$ 0.005a	1.91 $\pm$ 0.14b	0.03 $\pm$ 0.004a
<i>Bacteroides</i>	1.29 $\pm$ 1.25a	0.007 $\pm$ 0.002a	0.01 $\pm$ 0.008a	0.03 $\pm$ 0.02a	0.65 $\pm$ 0.6a
<i>Niastella</i>	0.01 $\pm$ 0.004a	0.004 $\pm$ 0.001a	0.004 $\pm$ 0.001a	0.005 $\pm$ 0.001a	1.73 $\pm$ 0.46b
Total abundance	51.47	51.56	52.78	45.13	41.00

Table S2. Soil pH (mean $\pm$ SE, n=4) for the control and biochar-amended soil at different times during the soil incubation experiment. Different letter(s) within the same row indicate significant difference between treatments ($p < 0.05$). The treatment abbreviations are shown in Table 1.

Days from start of experiment	CK	BW3001	BW3003	BW6001	BW6003	BL3001	BL3003	BL6001	BL6003
7	4.41 $\pm$ 0.03de	4.38 $\pm$ 0.03e	4.33 $\pm$ 0.03e	4.54 $\pm$ 0.02d	4.73 $\pm$ 0.04c	4.73 $\pm$ 0.07c	4.97 $\pm$ 0.08b	5.07 $\pm$ 0.02b	6.14 $\pm$ 0.10a
40	4.36 $\pm$ 0.01d	4.39 $\pm$ 0.05d	4.32 $\pm$ 0.04d	4.44 $\pm$ 0.03d	4.67 $\pm$ 0.07cd	4.96 $\pm$ 0.21bc	4.85 $\pm$ 0.26bc	5.14 $\pm$ 0.17b	5.95 $\pm$ 0.07a
80	4.23 $\pm$ 0.02cd	3.97 $\pm$ 0.01d	4.05 $\pm$ 0.04d	4.03 $\pm$ 0.05d	4.81 $\pm$ 0.55b	4.17 $\pm$ 0.03cd	4.63 $\pm$ 0.06bc	4.42 $\pm$ 0.01bcd	5.54 $\pm$ 0.04a