Root bacterial endophytes alter plant phenotype, but not physiology

Jeremiah A Henning [Corresp., 1] , David J Weston [2] , Dale A Pelletier [2] , Collin M Timm [3] , Sara S Jawdy [2] , Aimée T Classen [1, 4]

1 Department of Ecology & Evolutionary Biology, University of Tennessee - Knoxville, Knoxville, Tennessee, United States

2 Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, United States

3 Joint Institute for Biological Sciences, University of Tennessee, Oak Ridge, TN, United States

4 The Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

Corresponding Author: Jeremiah A Henning
Email address: jhennin2@vols.utk.edu

Plant traits, such as root and leaf area, influence how plants interact with their environment and the diverse, microbiota living within plants can influence plant morphology and physiology. Here, we explored how three bacterial strains isolated from the *Populus* root microbiome, influenced plant phenotype. We chose three bacterial strains that differed in predicted metabolic capabilities, plant hormone production and metabolism, and secondary metabolite synthesis. We inoculated each bacterial strain on a single genotype of *Populus trichocarpa* and measured the response of plant growth related traits (root:shoot, biomass production, root and leaf growth rates) and physiological traits (chlorophyll content, net photosynthesis, net photosynthesis at saturating light - A_{sat}, and saturating CO_2 - A_{max}). Overall, we found that bacterial root endophyte infection increased root growth rate up to 184% and leaf growth rate up to 137% relative to non-inoculated control plants, evidence that plants respond to bacteria by modifying morphology. However, endophyte inoculation had no influence on total plant biomass and photosynthetic traits (net photosynthesis, chlorophyll content). In sum, bacterial inoculation did not significantly increase plant carbon fixation and biomass, but their presence altered where and how carbon was being allocated in the plant host.

1   Title page

2   **Root bacterial endophytes alter plant phenotype, but not physiology**

6   Jeremiah A. Henning[1], David J. Weston[2], Dale A. Pelletier[2], Collin M. Timm[3], Sara S. Jawdy[2],

7   Aimée T. Classen[1, 4]

9   [1] Ecology & Evolutionary Biology, The University of Tennessee, Knoxville, TN USA

10   [2] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN USA

11   [3] Joint Institute for Biological Sciences, University of Tennessee, Oak Ridge, TN USA

12   [4] The Natural History Museum of Denmark, The University of Copenhagen København Ø,

13   Denmark

15   Corresponding author:

16   Jeremiah A. Henning

17   jhennin2@vols.utk.edu

Abstract (193 words)

Plant traits, such as root and leaf area, influence how plants interact with their environment and the diverse, microbiota living within plants can influence plant morphology and physiology. Here, we explored how three bacterial strains isolated from the *Populus* root microbiome, influenced plant phenotype. We chose three bacterial strains that differed in predicted metabolic capabilities, plant hormone production and metabolism, and secondary metabolite synthesis. We inoculated each bacterial strain on a single genotype of *Populus trichocarpa* and measured the response of plant growth related traits (root:shoot, biomass production, root and leaf growth rates) and physiological traits (chlorophyll content, net photosynthesis, net photosynthesis at saturating light - A_{sat}, and saturating CO_2 - A_{max}). Overall, we found that bacterial root endophyte infection increased root growth rate up to 184% and leaf growth rate up to 137% relative to non-inoculated control plants, evidence that plants respond to bacteria by modifying morphology. However, endophyte inoculation had no influence on total plant biomass and photosynthetic traits (net photosynthesis, chlorophyll content). In sum, bacterial inoculation did not significantly increase plant carbon fixation and biomass, but their presence altered where and how carbon was being allocated in the plant host.

37 **Introduction**

38 A recent review exploring microbiome-mediated plant traits found that plant-associated microbes

39 can modify fourteen out of thirty commonly measured functional traits (Cornelissen et al., 2003;

40 Friesen et al., 2011). For example, inoculation with common root-colonizing bacterial strains

41 influenced root and leaf architectural traits, such as specific leaf area and specific root length, as

42 well as plant physiological traits such as carbon fixation and chlorophyll content (Harris,

43 Pacovsky, & Paul, 1985; Ma et al., 2003; Friesen, 2013). Further, inoculation by different

44 members of the plant microbiome may differentially alter plant phenotype (Zamioudis et al.,

45 2013; Timm et al., 2016). The presence of unique bacterial strains in legume genotypes

46 explained more variation in shoot biomass, root biomass, and plant height than plant genotype

47 did (Tan & Tan, 1986). Inoculation of common endophytes can also inhibit primary root

48 elongation and promote lateral root formation and root hair production (Zamioudis et al., 2013;

49 Weston et al., 2012). Recent breakthroughs in the multitude of the –omics fields have allowed

50 for unprecedented mechanistic investigations of microbe-induced changes in host function

51 (Verhagen et al., 2004; Walker et al., 2011; Weston et al., 2012; Vandenkoornhuyse et al., 2015;

52 Timm et al., 2015; 2016) and have been the subject of multiple recent reviews (Friesen et al.,

53 2011; Friesen, 2013; Vandenkoornhuyse et al., 2015; Hacquard & Schadt, 2015; Lebeis, 2015;

54 and many others). This work demonstrated that plant growth promoting bacteria elicit numerous

55 changes in host gene expression through multiple and simultaneous hormonal and immune

56 response pathways (Verhagen et al., 2004; Walker et al., 2011; Weston et al., 2012; Drogue et

57 al., 2014; Timm et al., 2016). However, these studies fall short in explaining how changes in

58 gene expression influence the overall plant phenotype or plant function. Thus, understanding the

59 response of plant traits and overall plant phenotype to microbial strains remains a research gap.

60   Here, we inoculated three endophytic bacterial strains (*Pseudomonas fluorescens* GM41,

61   *Pseudomonas fluorescens* GM30, and *Burkholderia* sp. BT03), originally isolated from wild

62   *Populus*, on a single genotype of *Populus trichocarpa* and measured plant phenotypic response

63   to bacterial inoculation. We measured a suite of traits commonly measured in the functional trait

64   ecology literature to explore how phenotype is influenced by bacterial strains within the pre-

65   existing functional trait framework. Plant functional trait ecology has largely ignored

66   microbiome contribution to plant phenotype. Bacterial strains belonging to the *Pseudomonas*

67   *fluorescens* group are common plant growth promoting bacteria that are abundant in the *Populus*

68   microbiome (see Gottel et al., 2011). *Pseudomonas fluorescens* accounted for approximately

69   34% of the sequences found in the *Populus* endosphere, but only 2-3% of the sequences in the

70   rhizosphere and soil samples originating from the same roots (Gottel et al., 2011). *Pseudomonas*

71   strains can alter plant host function by modifying plant growth (Kloepper et al., 1980;

72   Lugtenberg & Kamilova, 2009; Timm et al., 2015), nutrient allocation (Bisht et al., 2009),

73   hormone signaling (Stearns et al., 2012), up-regulating/down-regulating of gene expression

74   pathways (Timm et al., 2016), and immune function (Verhagen et al., 2004; Weston et al., 2012).

75   Additionally, the *Pseudomonas fluorescens* clade has a large amount of functional diversity (Jun

76   et al., 2016), thus selecting two *Pseudomonas* strains allows us to explore how plant traits and

77   overall phenotype respond to closely related bacterial strain genomes. To contrast with these two

78   strains, we selected a distantly related, but enriched in *Populus* endosphere (Gottel et al., 2011),

79   bacterial strain from the genus *Burkholderi*a.

80   We predicted that aboveground and belowground traits of *Populus trichocarpa* would

81   respond to *Burkholderia* and *Pseudomonas* strains and inoculation of different bacterial strains

82   would result in different plant phenotypes. Further, we predicted that the two *Pseudomonas*

83   strains would produce a plant phenotype that was more similar to one another than to

84   *Burkholderia* because of phylogenetic relatedness, *i.e.* more shared functionality. To test our

85   predictions, we first conducted a genomic comparison using COG (clusters of orthologous

86   groups) database to predict the functional differences among strains. Next, we inoculated each

87   bacterial strain on *Populus trichocarpa* and measured a suite of physiological and architectural

88   plant traits including the root:shoot, biomass production, root and leaf growth rates, chlorophyll

89   content, net photosynthesis, and net photosynthesis at saturating light - A_{sat}, and saturating CO_2 -

90   A_{max}. We chose to measure overall trait response to bacterial endophytes without measuring the

91   pathways involved because we were interested in understanding down-stream consequences of

92   bacterial inoculation on overall plant phenotype.

93

94   **Materials and Methods**

95   *Populus trichocarpa* genotype "93-968" (Labbe et al., 2014) was propagated in tissue culture

96   following standard procedures (see Kang et al., 2009). Briefly, *in vitro* cultures were established

97   from actively growing shoot tips collected from greenhouse-grown *Populus* plants. We sterilized

98   shoot tips by soaking fresh cut tips in a 1% Tween 20 solution for 5 min, 70% Ethanol solution

99   for 1 min, a 0.525% sodium hypochlorite solution for 15 min and then rinsed them three times in

100   sterile H_2O for 5 min. Shoot tips were trimmed to 2 cm in length and transferred to a magenta

101   box (Sigma-Aldrich, St. Louis, MO) containing 80 mL of tissue media ($1\times$ Murashige & Skoog

102   (MS) basal medium (Murashige & Skoog, 1962) supplemented with MS vitamins (Caisson Labs,

103   North Logan, UT, USA), 0.05% 2-(*N*-morpholino) ethanesulfonic acid (MES hydrate) (Sigma-

104   Aldrich, St. Louis, MO, USA), 3% sucrose, 0.1% PPM™ (plant protective mixture) (Plant Cell

105   Technology, Washington, DC, USA), 0.5% activated charcoal (Sigma-Aldrich, St. Louis, MO,

106 USA), and 0.15% Gelzan (Plantmedia, bioWORLD, Dublin, OH, USA). Plants were sub-

107 cultured until it was determined, using microscopy and colony formation units with R2A

108 medium, that the plants were axenic.

109   Plant cultures were rooted in a growth room at 25 °C under a 16 h photoperiod. After root

110 establishment, plants that were similar in size and developmental stage were selected for

111 experimentation. Plants were weighed and scanned to account for initial plant size differences

112 among treatments. To ensure sterility during scanning, plants were placed between two (21.59 x

113 27.94 cm) sheets of cellulose acetate that were sprayed with 100% ethanol. Scans were

114 performed with a portable scanner (VuPoint Solutions Inc., City of Industry, CA, USA) at 600 ×

115 600 dpi. Scanned images were analyzed in WinRhizo (Regent Instruments, Quebec City,

116 Canada) to determine initial root surface area, root length, stem length, and leaf surface area.

117 After scanning, plants were transferred into experimental microcosms.

118

119 **Experimental design**

120 We constructed closed microcosms by interlocking two sterile Magenta boxes (Sigma-Aldrich,

121 St. Louis, MO, USA) with a coupler (Sigma-Aldrich, St. Louis, MO, USA). We added 150 ml

122 calcined clay (Pro's choice Sports Field Products, Chicago, IL, USA) and 70 ml of 1×

123 Hoagland's nutrient solution (Sigma-Aldrich, St. Louis, MO, USA) to each microcosm. We

124 drilled two 7 mm holes on adjacent sides of the upper magenta box and covered the holes with

125 adhesive microfiltration discs (Tissue Quick Plant Laboratories, Hampshire, United Kingdom) to

126 allow air to flow into and out of the microcosms and to prevent outside microbial contamination.

127 Prior to microbial addition, we double sterilized each closed microcosm by autoclaving on a 60

128 m dry cycle on consecutive days. *Pseudomonas fluorescens* strains (GM30 and GM41) and

129   *Burkholderia* sp. (BT03), hereafter termed *Pseudomonas* GM30, *Pseudomonas* GM41, and

130   *Burkholderia* BT03 were isolated from *Populus deltoides* endospheres from east Tennessee and

131   western North Carolina, USA (originally described in Brown et al., 2012). For full isolate

132   descriptions, see Brown et al., 2012; Weston et al., 2012; Utturkar et al., 2014; Timm et al.,

133   2015; 2016. We selected these three strains because previous work (*Pseudomonas* GM30 –

134   Weston et al., 2012, Labbe et al., 2014; *Pseudomonas* GM41 – Labbe et al., 2014, Timm et al.,

135   2016; *Burkholderia* Bt03 – Timm et al., 2016) had given us indication that strains were able to

136   influence traits in *Arabidopsis thaliana* (Weston et al., 2012), were able to manipulate plant gene

137   expression and hormonal signaling in *P. deltoides* (Timm et al., 2015; 2016), and were able to

138   influence host interactions with mycorrhizal symbionts (Labbe et al., 2014). Although strains

139   were isolated from *P. deltoides*, strains from *Pseudomonas* and *Burkholderia* readily colonize

140   natural *P. trichocarpa* tissues (Moore et al., 2006; Xin et al., 2009; Knoth et al., 2014; Kahn et

141   al., 2014; Doty et al., 2016). We grew bacterial strains in isolation and at a constant temperature,

142   25 °C, in 5 ml of R2A medium. After growing overnight they were pelleted and re-suspended in

143   sterile water to an OD600 of 0.01 (~$1.0 \times E7$ cells ml^{-1}).

144   We inoculated each microcosm by adding 10 ml of the bacterial strain (10^7 cells ml^{-1}) to

145   the calcined clay substrate and stirring for 30 s to distribute the bacteria. After inoculation, we

146   planted the *Populus* clones within each microcosm. Each *Populus* was grown in an individual

147   microcosm in combination with one of the bacterial strains. Thus, the experiment had four

148   treatment combinations – *Pseudomonas* GM30 inoculation, *Pseudomonas* GM41 inoculation,

149   *Burkholderia* BT03 inoculation, and a bacteria-free control. In total, there were 32 microcosms

150   with four treatments (n = 8). The experiment was divided into three different establishment dates

151   in 2014 (1 March, 3 replicated blocks; 25 March, 2 replicated blocks; and 2 April, 3 replicated

152   blocks) because microbiome-free plant tissues were difficult to propagate. Plant-bacteria

153   combinations were grown in the microcosms for five weeks with a 16 hr photoperiod, at 21°C

154   and 80% relative humidity.

155   After 35 days of growth, plants were removed from microcosms, submerged in sterilized

156   deionized H_2O to remove clay from the root system, weighed, and scanned. Scans were analyzed

157   with WinRhizo to determine final root surface area, total root length, stem length, and leaf

158   surface area. For each plant, the final measurement of root surface area, total root length, stem

159   length, and leaf surface area was subtracted from the initial measurement and divided by the

160   experiment duration to determine tissue growth rates (cm d^{-1} or cm^2 d^{-1}). Additionally, each plant

161   was dried for 48 hours at 70°C and weighed to measure leaf, shoot (leaf + stem) and root and

162   total dry mass. Specific leaf area and the specific root length of each individual were calculated

163   by dividing leaf area by leaf dry mass or by dividing root length by root dry mass, respectively.

164   To measure host physiological response to different bacterial strains, leaf gas-exchange

165   was measured and used to estimate leaf photosynthesis on our first replicate block (March 1, n =

166   3). For each plant, gas exchange of the largest leaf of the plant was measured (Li-Cor model

167   6400, Li-Cor Biosciences, Lincoln, Nebraska, USA) immediately prior to our experimental

168   harvest. The maximum rate of photosynthesis in saturating light under ambient CO_2 (A_{sat}), the

169   maximum rate of photosynthesis in saturating light and saturating CO_2 (A_{max}), , and the quantum

170   yield of CO_2 fixation (Φ) were all measured. Finally, average leaf chlorophyll content was

171   measured on three fully opened leaves (Konica Minolta Chlorophyll Meter SPAD-S02, Ramsey,

172   NJ, USA).

173

174   **Comparative genomics of microbes**

175   Genomes of *Pseudomonas* GM30 and GM41 and *Burkholderia* BT03 were sequenced at

176   Oak Ridge National Laboratory and genes were identified using Prodigal (Brown et al., 2012,

177   Utturkar et al., 2014) and are available at NCBI (GM41: AKJN00000000.2; GM30:

178   AKJP02000000.2; BT03: NZ_AKKD000000000.2). Genome annotation, genomes statistics, and

179   annotation comparisons were performed using IMG tools (img.jgi.doe.gov). Genome statistics

180   and COG functional predictions were extracted from Integrated Microbial Genomes

181   (img.jgi.doe.gov) and then they were compared manually for differential inclusion of predicted

182   functions.

183

184

185   **Bacterial Colonization**

186   To test for endophytic colonization of *Pseudomonas* GM41, *Pseudomonas* GM30, and

187   *Burkholderia* BT03, we planted cuttings of P. trichocarpa into a magenta box using similar

188   methodology and treatments described above (n=3). After 2 weeks of growth, all the plant roots,

189   stems, and 1-2 mature leaves were surface sterilized by dipping them in a ~10% bleach solution,

190   followed by 70% ethanol, and then rinsing in water three times. We recorded wet weight of plant

191   tissues and then separately macerated each plant tissue compartment in a sterile mortar and pestle

192   in 1 ml sterile $1\times$ PBS. We transferred macerated plant tissues to a 24-well plate where we serial

193   diluted each sample by 10% with $1\times$PBS at $1\times$, $0.1\times$, $0.01\times$ of original sample concentration.

194   Each sample was streaked onto R2A media plates and allowed to grow for 48 hours at 20ºC.

195   After 48 hours, colony formation was counted. We calculated CFU mg^{-1} of plant tissue by

196   multiplying colony number per plate by $10^{(\text{dilution factor} + 1)}$ and then dividing that number by the

197   dry tissue mass (mg^{1}).

198   **Statistical analyses**

199   We tested all data for normality using the *normalTest* function in the fBasics package (version

200   3011.87, R metrics core team 2014) for R version 3.0.2 (R development core team, 2013) and

201   RStudio version 0.98.495 (RStudio, 2013). If data were not normally distributed, we performed

202   log transformations or square-root transformations to satisfy the normality assumptions of

203   ANOVA.

204   To explore plant trait response (root dry mass, leaf dry mass, shoot dry mass, total dry

205   mass, root: shoot, root growth rates, leaf growth rates, change in leaf number, specific root

206   length, specific leaf area) to bacterial strains, we used linear mixed-effect models using the lme4

207   package in R (Bates et al., 2014). Bacterial strain was a fixed effect in the model and

208   experimental block (three establishment dates) was a random factor. For plant dry mass

209   measures, we incorporated initial measurements of root surface area in the root dry mass model

210   and initial leaf surface area in the aboveground dry mass model as covariates. To test for

211   significance of bacterial strain (fixed effects) and covariate (initial growth measure) we

212   performed a likelihood ratio test to compare models with and without fixed effects and

213   covariates. If including fixed factors (bacterial strain) was significant an improvement to model

214   fit (p value < 0.05 in likelihood ratio test), we calculated least square means and confidence

215   intervals using the *difflsmeans* function to calculate differences among strains using the lmerTest

216   package version 2.0-3 (Kuznetsova, Brockhoff, & Christensen, 2014). We measured host

217   response to bacterial inoculation by calculating the percent change in trait values ((mean trait

218   value for *Populus* inoculated with bacterial strain – mean non-inoculated trait value) × 100).

219   To test physiological responses (carboxylase activity, A_{max}, A_{sat}) of plant hosts to

220   bacterial inoculation, we used one-way analysis of variance (ANOVA) using the *Anova* function

221   in the CAR package, (version 2.0-22, Fox & Weisberg 2011) because we collected physiology

222   data on only a single sampling date (n = 3). All raw data and R code is available in Supplemental

223   Table 2 and 3, respectively.

224

225   **Results**

226   **Bacterial strains differ in genomic content**

227   We compared the genomes of *Burkholderia* BT03 and *Pseudomonas* GM30 and GM41 based on

228   predicted enzyme functions using the COG database (Table 1). Overall, our genome comparison

229   demonstrated that the bacterial strains differed in genome size and functional gene content.

230   *Burkholderia* BT03 had a relatively large genome (10.9 Mb) compared to *Pseudomonas* GM30

231   (6.1 Mb) and *Pseudomonas* GM41 (6.6 Mb) (Table 1). We found all three bacterial strains

232   shared functions that were likely critical for establishment and survival in the plant microbiome

233   including the production of the plant hormone auxin, pili, flagella, chemotaxis, increased signal

234   transduction, and secretion systems. However, we found many functional differences among our

235   strains. The genome of *Burkholderia* encoded multiple pathways predicted to be involved in the

236   metabolism of the plant hormones, salicylate and ethylene (Table 1). Relative to the

237   *Pseudomonas* genomes, the *Burkholderia* genome encoded for numerous secondary metabolite

238   biosynthesis pathways and more carbohydrate and lipid transporters, suggesting increased

239   metabolic capabilities within *Burkholderia* (Table 1).

240   Even through *Pseudomonas* GM30 and *Pseudomonas* GM41 were classified as the same

241   16S OTU, their genome size differed as did the predicted functional capabilities of the two

242   strains. The genome of *Pseudomonas* GM41 encoded for phosphorus solubilization and nitrate

243   reduction, which were lacking in the *Pseudomonas* GM30 genome. Additionally, *Pseudomonas*

244   GM41 contained more secondary metabolite biosynthesis elements compared to *Pseudomonas*

245   GM30. We also found that the genome of *Pseudomonas* GM41 contained more genes coding for

246   carbohydrate metabolism, lipid metabolism, and amino acid transport and metabolism, energy

247   production and conversion, suggesting that *Pseudomonas* GM41 may contain more metabolic

248   breadth than *Pseudomonas* GM30 (Table 1). Taken together, our results demonstrated that these

249   three bacterial strains differ in genome size and their functional gene content.

250

251   **Bacterial colonization of *Populus* root tissue**

252   All three of the bacterial strains colonized *Populus* hosts. Colony-forming units were enriched in

253   all three bacterial strains relative to the control in the 0.1× and 0.01× dilutions (0.1× dilution $F =$

254   18.77, p <0.0001; 0.01× dilution $F = 13.78$, p <0.0001, Table 2), although CFU number was

255   variable across dilutions, tissue types, and bacterial strain. However, we found no difference in

256   CFUs among non-inoculated control and *Pseudomonas* GM30, GM41, and *Burkholderia* BT03

257   inoculated host plants at the 1× dilution ($F = 1.24$, $p = 0.319$ Table 2). Across nearly all tissue

258   types, we found that *Pseudomonas* GM30, *Pseudomonas* GM41, and *Burkholderia* BT03

259   inoculated plants had 10-10000× more CFUs than did non-inoculated control plants (Table 2).

260   All three bacterial strains colonized leaf and stem tissues, but the highest CFUs across bacterial

261   treatments were consistently observed in roots (Table 2). Inoculated host plants contained 0-

262   28809015 CFU mg^{-1} in roots, 0-1166273 CFU mg^{-1} in stems, and 0-73537 CFU mg^{-1} in leaves

263   compared to 0-400 CFU mg^{-1} in root tissues, 0 CFU mg^{-1} in stem tissue, 0-1000 CFU mg^{-1} in leaf

264   tissue compared to non-inoculated control plants (Table 2).

265

266   **Plant structure is modified by bacterial inoculation**

267   Overall, we found that plant trait response to bacterial endophytes was strain specific.

268   Specifically, mean root growth rate increased 184% with *Pseudomonas* GM30 colonization ($t = $

269   3.84, $p = 0.001$), however root growth rates were unaffected by *Pseudomonas* GM41 ($t = 1.61$, p

270   $= 0.12$), and *Burkholderia* BT03 ($t = 1.18$, $p = 0.25$) inoculation (Fig 1, Supplemental Table 1).

271   Similarly, mean leaf growth rate increased 114% and 138% with *Pseudomonas* GM30 ($t = 2.27$,

272   $p = 0.03$) and *Pseudomonas* GM41 ($t = 2.86$, $p = 0.01$) inoculation, but leaf growth rate was

273   unaffected by *Burkholderia* inoculation ($t = 1.02$, $p = 0.32$) (Fig 1, Supplemental Table 1).

274   Inoculation by *Pseudomonas* GM30 increased leaf number by 36% ($t = 3.34$, $p = 0.003$) but leaf

275   number was unaffected by *Pseudomonas* GM41 ($t = 0.93$, $p = 0.36$) and *Burkholderia* BT03 ($t = $

276   1.418, $p = 0.17$) inoculation (Fig 1). We observed no differences in stem elongation with

277   bacterial inoculation (chisq= 0.06, $p = 0.97$, Supplemental Table 1).

278   Interestingly, we observed no differences in total plant dry mass (chisq = 3.27, $p = 0.195$,

279   Fig 2), root dry mass (chisq = 0.00, $p = 1.00$, Fig 2), root:shoot ratio (chisq=0.00, $p = 1.00$,

280   Supplemental Table 1) or plant height (chisq = 1.99, $p = 0.158$ Supplemental Table 1) with

281   bacterial inoculation. However, *Pseudomonas* GM30 inoculation increased leaf dry biomass by

282   86% ($t = 2.43$, $p = 0.02$) relative to control plants, however leaf biomass was unaffected by

283   *Pseudomonas* GM41 ($t = 0.97$, $p = 0.33$) and *Burkholderia* BT03 ($t = 1.70$, $p = 0.10$) (Fig 2,

284   Supplemental Table 1). We observed no differences in specific leaf area with bacterial

285   inoculation (chisq= 2.60, $p = 0.46$, Supplemental Table 1). Thus, inoculation of *Pseudomonas*

286   GM30 increased leaf surface area ($t = 2.27$, $p = 0.03$) and aboveground dry mass ($t = 2.43$, $p = $

287   0.02), without changing leaf area:mass ratios. We found no significant differences in root

288   length:dry mass (specific root length, chisq = 1.06, p = 0.79) with bacterial inoculation

289   (Supplemental Table 1). Our results indicate that bacterial strains modify plant resource

290   allocation but not total dry mass production.

291

292   **Plant physiology is not affected by bacterial inoculation**

293   Bacterial inoculation had no measureable effects on any physiological trait we measured:

294   chlorophyll content (SPAD) (chisq = 2.15, p = 0.54), quantum yield of photosynthesis (ϕ) (F =

295   1.01, p = 0.43), net photosynthesis at saturating light conditions (A_{sat}) (F = 0.76, p = 0.55) or

296   maximum net photosynthesis at saturating light and $[CO_2]$ (A_{max}) (F = 1.98, p = 0.19) (Fig 3). In

297   agreement with the total dry mass data, we did not observe significant changes in the measured

298   photosynthetic parameters. Thus, changes in plant structure were not linked with increased

299   photosynthetic capacity, efficiency, or carbon assimilation rates.

300

301   **Discussion**

302   The plant root microbiome can have a strong influence on plant production and phenotype

303   (Friesen, 2013; Vandenkoornhuyse, et al., 2015); yet, less is known about how plant trait

304   expression, production, and physiology are influenced by individual endophytic strains. We

305   explored how plant morphological traits, productivity, and cellular physiology in *Populus*

306   *trichocarpa* responded to inoculation with three bacterial strains, two closely related

307   *Pseudomonas fluorescens* strains (GM30 & GM41) and a more distantly related *Burkholderia*

308   strain (BT03). We selected bacterial strains that were predicted to differ in metabolic

309   capabilities, plant hormone production and metabolism, and secondary metabolite synthesis in an

310   effort to understand how plant phenotype is influenced by inoculation with different strains of

311   common endophytic bacteria (Table 1, Timm et al., 2015; 2016). Our comparative genomic

312   analysis revealed that while all three strains share many common endophytic functions like plant

313   hormone signal disruption, production of plant hormone auxin, pili, flagella, and chemotaxis,

314   strains potentially differed in their ability to perform these functions. Overall, we found that

315   *Burkholderia* and *Pseudomonas* genomes differed in the carbon substrates they were predicted to

316   degrade, plant hormone production and metabolism, and secondary metabolite synthesis, which

317   led us to predict that plant response to bacterial inoculation would lead to different phenotypes

318   between treatments. All three strains could colonize *Populus* roots, leaves, and stems, however

319   CFU number was highest within root tissues in all three strains (Table 2).

320   Overall, we found root endophyte inoculation altered plant resource allocation patterns

321   without influencing total plant biomass accumulation (Fig 1). Additionally, we found that plant

322   trait response and overall phenotype differed across bacterial strains in ways that would not have

323   been predicted from our genome analysis. Specifically, *Burkholderia* BT03 was predicted to

324   produce auxin and to metabolize salicylate and ethylene, three plant hormones crucial to plant

325   growth and development (see Yang & Hoffman, 1984; Wasternack & Parthier, 1997; Chen et al.,

326   2009; Dempsey et al., 2011). Additionally, we found the *Burkholderia* genome encoded for

327   multiple transposase elements that degrade poplar-produced aromatics and metabolites (Timm et

328   al., 2015; 2016). Despite the predicted ability of *Burkholderia* to manipulate multiple plant

329   hormonal and signaling pathways, we observed no measurable changes in any traits when

330   *Populus* was inoculated with *Burkholderia* (Figs 1, 2, 3). This was especially surprising since we

331   consistently measured the highest CFU abundance within *Burkholderia* inoculated individuals

332   (Table 2).

333   In spite of close genetic relatedness and classification under the same 16S OTU profile,

334   our *Pseudomonas* strains differed in key functional capabilities. Specifically, *Pseudomonas*

335   GM41 encoded for phosphate solubilization and denitrification ability, suggesting these two

336   strains may differentially influence host nutrition, although this remains untested. Our genome

337   analysis revealed that both strains were capable of producing the plant hormone auxin, however

338   another study found that *Pseudomonas* GM41 produced two times more auxin than

339   *Pseudomonas* GM30 (Timm et al., 2015). Auxin synthesis by endophytic bacteria can increase

340   root branching and lateral root formation and decrease overall plant height, leaf number,

341   chlorophyll content and photosynthetic efficiency (Romano, Cooper, & Klee, 1993; Fu &

342   Harberd, 2003; Weston et al., 2012). Thus, we predicted that *Pseudomonas* GM41 would have a

343   strong influence on plant root traits, however we observed no measurable effects of

344   *Pseudomonas* GM41 inoculation on root growth rate or morphology (Fig 1, Supplemental Table

345   1). Belowground, *Pseudomonas* GM30 inoculation increased root surface area growth rate by

346   184% (Fig 1) without increasing root biomass (Fig 2), suggesting *Pseudomonas* GM30

347   inoculation may change root morphology, leading to longer, thinner, highly-branched roots with

348   similar biomass, as we predicted. Similar patterns have been observed when *Pseudomonas*

349   GM30 is inoculated on both *Arabidopsis* (Weston et al., 2012) and *Populus deltoides* (Timm et

350   al., 2015; 2016). Additionally, inoculation of *Pseudomona*s GM30 increased leaf surface area

351   growth rate by 114% (Fig 1), leaf number by 36% (Fig 1), and aboveground biomass by 86%

352   (Fig 2) but did not influence specific leaf area (Supplemental Table 1), whereas closely-related

353   *Pseudomonas* GM41 increased leaf surface area growth rate by 138% (Fig 1) but did not change

354   leaf number (Fig 1) or aboveground biomass (Fig 2). Unlike *Burkholderia*, *Pseudomonas*

355   genomes do not contain the genes to directly metabolize salicylate, however inoculation of

356   *Pseudomonas* GM41 can up-regulate salicylic acid synthesis and degradation in *Populus* (Timm

357   et al., 2016). Taken together, our data suggest that predicting plant phenotypic response to

358   bacterial inoculation, even in overly simplified systems using fully sequenced bacterial strains, is

359   extremely complex and difficult.

360   Contrary to our predictions, leaf physiology (Fig 3), plant height (Supplemental Table 1),

361   root:shoot (Supplemental table 1), specific leaf area (Supplemental table 1), specific root length

362   (Supplemental table 1), and total plant dry mass (Fig 2) were not influenced by bacterial

363   inoculation. It is possible that multiple, overlapping plant signaling and gene expression effects

364   induced by bacterial endophyte inoculation may mask a hosts' phenotype response. For example,

365   endophytes simultaneously up- and down-regulate numerous genes and metabolites in plant

366   tissue (see Verhagen et al., 2004; Wang et al., 2005; Walker et al., 2011; Weston et al., 2012;

367   Timm et al., 2016). Thus, counteracting influences among different gene pathways may conceal

368   plant responses to endophyte inoculation when measuring down-stream phenotype and

369   functional traits (Bashan, Holguin, & de-Bashan 2004; Timm et al., 2016). Additionally, host

370   physiological response to endophyte inoculation may vary with bacterial strain (Kandasamy et

371   al., 2009; Weston et al., 2012; Timm et al., 2016), plant host (Smith and Goodman 1999), plant

372   ontogeny (Siddiqui & Shaukat 2003), or plant stress (Dimkpa, Weinand, & Asch 2009; Yang,

373   Kloepper, & Ryu 2009; Lau & Lennon 2012). For example, Root colonization by *Pseudomonas*

374   can reduce chlorophyll content and net photosynthesis (A_{sat}) in a variety of plant hosts (Zou et

375   al., 2005; Weston et al., 2012). However *Pseudomonas* colonization can also increase

376   photosynthetic activity and chlorophyll content (Kandasamy et al., 2009, Timm et al., 2016).

377   Thus, biotic and abiotic contexts may drive the phenotypic response of hosts to endophyte

378   inoculation, however this idea requires further testing.

379   Our study focused on the response of plant functional traits to monoculture associations

380   of common endosphere bacteria, however future studies should focus on exploring how plant

381   phenotype responds to diverse microbiome communities. With a few well-known exceptions

382   (Tan & Tan 1986; Harris, Pacovsky, & Paul 1985; Ma et al., 2003, Lau & Lennon 2011; 2012),

383   bacterial community composition in roots has been ignored in studies exploring what drives

384   natural variation in plant traits (Friesen et al., 2011; Friesen 2013; Timm et al., 2016). We

385   propose a multifaceted approach to investigate linkages among the plant microbiome and natural

386   plant trait variation. First, incorporation of microbiome composition into studies that currently

387   investigate host identity/genotype and environmental parameters may be important for finding

388   patterns in natural trait variation – especially when conduced across a variety of environmental

389   gradients. Second, once correlations between microbiomes and plant traits are observed in the

390   field, detailed work constructing communities in the lab and greenhouse would enable a

391   mechanistic understanding of what is underlying the observed patterns. These studies could be

392   especially fruitful when conducted across natural biotic and abiotic environmental gradients in

393   the laboratory, greenhouse, and field settings (Classen et al., 2015).

394

395   **Conclusions**

396   Our study demonstrates that bacteria living in plant roots can influence plant morphological

397   traits. Increasingly, ecologists are using plant functional traits to explore how changing

398   environments might alter plant function (Wright et al., 2004; Reich 2014). Plant traits, such as

399   specific leaf area and specific root length, are often significantly correlated with important plant

400   functions such as carbon fixation and nutrient uptake (Diaz & Cabido 2001). Researchers are

401   using correlations between plant traits and function to extrapolate how plants and ecosystems

402   will respond to global changes (Reich et al., 1999; Wright et al., 2004; Reich 2014). While

403   interactions between plant genotype and environment undoubtedly influence plant phenotypic

404   plasticity (Bradshaw 1965; Schlichting 1986; Sultan 2000; Des Marais, Hernandez, & Juenger

405   2013), phenotype is also heavily influenced by biotic factors, like the microbiome bacterial

406   endophytes (Lau & Lennon 2011; 2012; Wagner et al., 2014; Hacquard & Schadt 2015). Given

407   that plant-microbial studies, including ours, have observed strong linkages between microbiome

408   and plant phenotype (reviewed in Friesen et al., 2011; Friesen 2013) interactions among global

409   change drivers, plant genotypes, and plant microbiomes, should be considered in trait-based

410   approaches to ecological questions (Classen et al., 2015).

411

412   **Acknowledgements**

413   We thank Lee Gunter, Tse-Yuan Lu, Kelsey Carter, Jesse Labbe, and W. Nathan Cude for their

414   assistance in data collection and plant propagation. The Classen Ecosystem Ecology group and

415   three reviewers provided valuable input on earlier reviews of this manuscript.

416

417

418 **References**

419 Bashan Y, Holguin G, de-Bashan LE. 2004. *Azospirillum*-plant relationships: physiological,

420   molecular, agricultural, and environmental advances (1997-2003). *Canadian Journal of*

421   *Microbiology.* 50:521-577.

422 Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using

423   lme4. *Journal of Statistical Software.* 67:1-48.

424 Bisht R, Chaturvedi S, Srivastava R, Sharma A, Johri B. 2009. Effect of arbuscular mycorrhizal

425   fungi, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* on the growth and nutrient

426   status of *Dalbergia sissoo* Roxb. *Tropical Ecology.* 50:231.

427 Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in*

428   *Genetics.* 13:115-155.

429 Brown SD, Utturkar SM, Klingeman DM, Johnson CM, Martin SL, Land ML, Lu TYS, Schadt

430   CW, Doktycz MJ, Pelletier DA. 2012. Twenty-one genome sequences from *Pseudomonas*

431   species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and

432   endosphere of *Populus deltoides*. *Journal of Bacteriol*ogy. 194:5991-5993.

433 Chen Z, Zheng Z, Huang J, Lai Z, Fan B. 2009. Biosynthesis of salicylic acid in plants. *Plant*

434   *Signaling & Behavior.* 4:493-496.

435 Classen AT, Sundqvist M, Henning JA, Newman GS, Moore JAM, Cregger M, Moorhead LC,

436   Patterson CM. 2015. Direct and indirect effects of climate change on soil microbial and soil

437   microbial-plant interactions: What lies ahead? *Ecosphere.* 6(8):130. doi: 10.1890/ES15-

438   00217.1.

439 Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege

440   H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H. 2003. A handbook of protocols

441   for standardised and easy measurement of plant functional traits worldwide. *Australian*

442   *Journal of Botany.* 51:335-380.

443 Dempsey DMA, Vlot AC, Wildermuth MC, and Klessig DF. 2011. Salicylic Acid Biosynthesis

444   and Metabolism. *The Arabidopsis Book.* 9: e0156 doi: 10.1199/tab.0156.

445 Des Marais DL, Hernandez KM, Juenger TE. 2013. Genotype-by-environment interaction and

446   plasticity: exploring genomic responses of plants to the abiotic environment. *Annual Review*

447   *of Ecology, Evolution, and Systematics.* 44:5-29.

448 Díaz S, Cabido M. 2001. Vive la différence: plant functional diversity matters to ecosystem

449   processes. *Trends in Ecology & Evolution.* 16:646-655.

450 Dimkpa C, Weinand T, Asch F. 2009. Plant–rhizobacteria interactions alleviate abiotic stress

451   conditions. *Plant, cell & Environment.* 32:1682-1694.

452 Doty SL, Sher AW, Fleck ND, Khorasani M, Bumgarner RE, Khan Z, Ko AWK, Kim S-H,

453   DeLuca TH. 2016. Variable Nitrogen Fixation in Wild *Populus*. *PLoS ONE.*

454   doi:11:e0155979.

455 Drogue B, Sanguin H, Chamam A, Mozar M, Llauro C, Panaud O, Prigent-Combaret C, Picault

456   N, Wisniewski-Dyé F. 2014. Plant root transcriptome profiling reveals a strain-dependent

457   response during Azospirillum-rice cooperation. *Frontiers in Plant Science.* 5:607

458   doi:10.3389/fpls:2014.00607.

459 Fox J, Weisberg S. 2011. *An {R} Companion to Applied Regression*, Second Edition. Thousand

460   Oaks CA: Sage.

461 Friesen ML. 2013. *Microbially Mediated plant functional traits molecular microbial ecology of*

462   *the rhizosphere*. John Wiley and Sons, Inc., pp 87-102.

463 Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. 2011.

464   Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and*

465   *Systematics.* 42:23-46.

466 Fu X, Harberd NP. 2003. Auxin promotes Arabidopsis root growth by modulating gibberellin

467   response. *Nature.* 421:740-743.

468 Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E,

469   Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW. 2011. Distinct microbial communities

470   within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil

471   types. *Applied and Environmental Microbiology.* 77:5934-5944.

472 Hacquard S, Schadt CW. 2015. Towards a holistic understanding of the beneficial interactions

473   across the *Populus* microbiome. *New Phytologist.* 205:1424-1430.

474 Harris D, Pacovsky RS, Paul EA. 1985. Carbon economy of soybean-Rhizobium-Glomus

475   associations. *New Phytologist.* 101:427.

476 Jun SR, Wassenaar TM, Nookaew I, Hauser L, Wanchai V, Land M, Timm CM, Lu TYS, Schadt

477   CW, Doktycz MJ, Pelletier DA, Ussery DW. 2016. Diversity of *Pseudomonas* Genomes,

478   Including *Populus*-Associated Isolates, as Revealed by Comparative Genome Analysis.

479   *Applied and Environmental Microbiology.* 82:375-383.

480 Kang B, Osburn L, Kopsell D, Tuskan G, Cheng ZM. 2009. Micropropagation of *Populus*

481   *trichocarpa* 'Nisqually-1': the genotype deriving the *Populus* reference genome. *Plant Cell,*

482   *Tissue and Organ Culture.* 99:251-257.

483 Khan Z, Roman D, Kintz T, delas Alas M, Yap R, Doty S. 2014. Degradation, phytoprotection

484   and phytoremediation of phenanthrene by endophyte *Pseudomonas putida*, PD1.

485   *Environmental science & technology.* 48:12221-12228.

486 Kloepper JW, Leong J, Teintze M, Schroth MN. 1980 Enhanced plant growth by siderophores

487   produced by plant growth-promoting rhizobacteria. *Nature.* 286:885-886.

488 Knoth JL, Kim S-H, Ettl GJ, Doty SL. 2014. Biological nitrogen fixation and biomass

489   accumulation within poplar clones as a result of inoculations with diazotrophic endophyte

490   consortia. *New Phytologist.* 201:599-609.

491 Kandasamy S, Loganathan K, Muthuraj R, Duraisamy S, Seetharaman S, Thiruvengadam R,

492   Ponnusamy B, Ramasamy S. 2009. Understanding the molecular basis of plant growth

493   promotional effect of *Pseudomonas fluorescens* on rice through protein profiling. *Proteome*

494   *Science 7*:1-8.

495 Kuznetsova A, Brockhoff P, Christensen R. 2014. *LmerTest: tests for random and fixed effects*

496   *for linear mixed effect models*. R package, version 2.0-3.

497 Labbé JL, Weston DJ, Dunkirk N, Pelletier DA, Tuskan GA. 2014. Newly identified helper

498   bacteria stimulate ectomycorrhizal formation in *Populus*. *Frontiers of Plant Science.* 5:579

499   doi: 10.3389/fpls.2014.00579.

500 Lau JA, Lennon JT. 2011. Evolutionary ecology of plant–microbe interactions: soil microbial

501   structure alters selection on plant traits. *New Phytologist.* 192:215-224.

502 Lau JA, Lennon JT. 2012. Rapid responses of soil microorganisms improve plant fitness in novel

503   environments. *Proceedings of the National Academy of Sciences.* 109:14058-62.

504 Lebeis SL. 2015. Greater than the sum of their parts: characterizing plant microbiomes at the

505   community-level. *Current Opinion in Plant Biology*. 24:82-86.

506 Lugtenberg B, Kamilova F. 2009. Plant-Growth-Promoting Rhizobacteria. *Annual Review of*

507   *Microbiology.* 63:541-556.

508 Ma W, Sebestianova S, Sebestian J, Burd G, Guinel F, Glick B. 2003. Prevalence of 1-

509   aminocyclopropane-1-carboxylate deaminase in *Rhizobium* spp. *Antonie van Leeuwenhoek.*

510   83:285-291.

511 Moore FP, Barac T, Borremans B, Oeyen L, Vangronsveld J, van der Lelie D, Campbell CD,

512   Moore ERB. 2006. Endophytic bacterial diversity in poplar trees growing on a BTEX-

513   contaminated site: The characterisation of isolates with potential to enhance

514   phytoremediation. *Systematic and Applied Microbiology.* 29:539-556.

515 Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco

516   tissue cultures. *Physiologia Plantarum.* 15:473-497.

517 Reich PB. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto.

518   *Journal of Ecology.* 102:275-301.

519 Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD. 1999.

520   Generality of leaf trait relationships: a test across six biomes. *Ecology.* 80:1955-1969.

521 Romano CP, Cooper ML, Klee HJ. 1993. Uncoupling auxin and ethylene effects in transgenic

522   tobacco and arabidopsis plants. *Plant Cell.* 5:181-189.

523 Schlichting CD. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of*

524   *Ecology, Evolution, and Systematics.* 17:667-693.

525 Siddiqui IA, Shaukat SS. 2003. Plant species, host age and host genotype effects on *Meloidogyne*

526   *incognita* biocontrol by *Pseudomonas fluorescens* strain CHA0 and its genetically-modified

527   derivatives. *Journal of Phytopathology.* 151:231-238.

528 Smith KP, Goodman RM. 1999. Host variation for interactions with beneficial plant-associated

529   microbes. *Annual Review of Phytopathology.* 37:473-491.

530   Stearns JC, Woody OZ, McConkey BJ, Glick BR. 2012. Effects of bacterial ACC deaminase on

531   *Brassica napus* gene expression. *Molecular Plant Microbe Interactions*. 25:668-676 doi:

532   10.1094/MPMI-08-11-0213.

533   Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in*

534   *Plant Science*. 5:537-542.

535   Tan G, Tan W. 1986. Interaction between alfalfa cultivars and *Rhizobium* strains for nitrogen

536   fixation. *Theoretical and Applied Genetics*. 71:724-729.

537   Timm CM, Campbell AG, Utturkar, SM, Jun SR, Parales RE, Tan W, Robeson MS, Lu TYS,

538   Jawdy S, Brown SD, Ussery DW, Schadt CW, Tuskan GA, Doktycz MJ, Weston DJ, Pelletier

539   DA. 2015. Metabolic functions of *Pseudomonas fluorescens* strains from *Populus deltoides*

540   depend on rhizosphere or endosphere isolation compartment. *Frontiers in Microbiology*. 6

541   doi: 10.3389/fmicb.2015.01118.

542   Timm CM, Pelletier DA, Jawdy S, Gunter LE, Henning JA, Engle N, Aufrecht J, Gee E,

543   Nookaew I, Yang Z, Lu TYS, Tschaplinksi TJ, Doktycz MJ, Tuskan GA, Weston DJ. 2016.

544   Two poplar-associated bacterial isolates induce additive favorable responses in a constructed

545   plant-microbiome system. *Frontiers in Plant Science*. 7 doi: 10.3389/fpls.2016.00497.

546   Utturkar SM, Klingeman DM, Land ML, Schadt CW, Doktycz MJ, Pelletier DA, Brown SD.

547   2014. Evaluation and validation of de novo and hybrid assembly techniques to derive high-

548   quality genome sequences. *Bioinformatics*. 30:2709-2716.

549   Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of

550   the microbiome of the plant holobiont. *New Phytologist*. 206:1196-1206.

551 Verhagen BWM, Glazebrook J, Zhu T, Chang HS, van Loon LC, Pieterse CMJ. 2004. The

552 transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Molecular Plant*

553 *Microbe Interactions.* 17:895-908.

554 Wagner MR, Lundberg DS, Coleman-Derr D, Tringe SG, Dangl JL, Mitchell-Olds T. 2014.

555 Natural soil microbes alter flowering phenology and the intensity of selection on flowering

556 time in a wild Arabidopsis relative. *Ecology Letters.* 17:717-726.

557 Walker V, Bertrand C, Bellvert F, Moënne-Loccoz Y, Bally R, Comte G. 2011. Host plant

558 secondary metabolite profiling shows a complex, strain-dependent response of maize to plant

559 growth-promoting rhizobacteria of the genus *Azospirillum*. *New Phytologist.* 189:494-506.

560 Wang Y, Ohara Y, Nakayashiki H, Tosa Y, Mayama S. 2005. Microarray analysis of the gene

561 expression profile induced by the endophytic plant growth-promoting rhizobacteria,

562 *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Molecular Plant Microbe*

563 *Interactions.* 18:385-396.

564 Wasternack C, Parthier B. 1997. Jasmonate-signalled plant gene expression. *Trends in Plant*

565 *Science.* 2: 302-307.

566 Weston DJ, Pelletier DA, Morrell-Falvey JL, Tschaplinski TJ, Jawdy SS, Lu TYS, Allen SM,

567 Melton SJ, Martin MZ, Schadt CW, Karve AA, Chen JG, Yang X, Doktycz MJ, Tuskan GA.

568 2012. *Pseudomonas fluorescens* induces strain-dependent and strain-independent host plant

569 responses in defense networks, primary metabolism, photosynthesis, and fitness. *Molecular*

570 *Plant Microbe Interactions.* 25:765-778.

571 Wright IJ, Reich, PB, Westoby M, Ackerly DD, Baruch Z. 2004. The worldwide leaf economics

572 spectrum. *Nature.* 428:821-827.

573 Xin G, Zhang G, Kang JW, Staley JT, Doty SL. 2009. A diazotrophic, indole-3-acetic acid-

574   producing endophyte from wild cottonwood. *Biology and Fertility of Soils*. 45:669-674.

575 Yang J, Kloepper JW, Ryu C-M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress.

576   *Trends in Plant Science*. 14:1-4.

577 Yang SF, Hoffman NE. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual*

578   *Review of Plant Physiology and Plant Molecular Biology*. 35:155-189.

579 Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CMJ. 2013. Unraveling Root

580   Developmental Programs Initiated by Beneficial *Pseudomonas* spp. Bacteria. *Plant*

581   *Phys*iology. 162:304-318.

582 Zou J, Rodriguez-Zas S, Aldea M, L, M, Zhu J, Gonzalez DO, Vodkin LO, DeLucia E, Clough

583   SJ. 2005. Expression Profiling Soybean Response to *Pseudomonas syringae* Reveals New

584   Defense-Related Genes and Rapid HR-Specific Downregulation of Photosynthesis. *Molecular*

585   *Plant Microbe Interactions*. 18:1161-1168.

Predicted plant-interaction pathways in bacterial strains *Burkholderia* sp. BT03, *Pseudomonas fluorescens* GM30, and *Pseudomonas fluorescens* GM4.

Predicted plant-interaction pathways in bacterial strains *Burkholderia* sp. BT03, *Pseudomonas fluorescens* GM30, and *Pseudomonas fluorescens* GM41. Genome size, relevant pathways, and COG category statistics were identified using IMG tools. Where applicable, gene loci indicating predicted functions in genomes (individual genes or pathways) were included. NA = not applicable.

	Burkholderia **BT03**	*Pseudomonas* **GM30**	*Pseudomonas* **GM41**
Genome size (Mb)	10.9	6.1	6.6
ACC deaminase	PMI06_0002752	PMI25_02765	PMI27_01478
salicylate metabolism	PMI06_001931	*NA*	PMI27_05197
auxin biosynthesis	PMI06_005275	PMI25_03791	PMI27_00952
pili, fimbriae	PMI06_00372-3373	PMI25_00378-0372	*NA*
flagella	PMI06_009483-9498	PMI25_03624 -3649	PMI27_02843-2866
chemotaxis	PMI06_009463-9475	PMI25_05665-5658	PMI27_05395-5382
type 2 secretion system	PMI06_001352-1341	PMI25_00837-00844	*NA*
type 3 secretion system	PMI06_000607-0617	*NA*	*NA*
type 4 secretion system	PMI06_009642-9622	*NA*	*NA*
type 6 secretion system	PMI06_001813-1833	PMI25_012011220	PMI27_02378-2397
carbohydrate metabolism (# of genes)	582	222	291
secondary metabolite metabolism (# of genes)	337	113	148

Colony forming units found in leaf, root, and stem tissue of *Populus trichocarpa* genotypes inoculated with *Pseudomonas* GM30, *Pseudomonas* GM41, or *Burkholderia* BT03 across three dillution factors.

Colony forming units found in leaf, root, and stem tissue of *Populus trichocarpa* genotypes inoculated with *Pseudomonas* GM30, *Pseudomonas* GM41, or *Burkholderia* BT03 across three different dilution factors: 1×, 0.1×, 0.01× concentrations of the original sample. *Pseudomonas* GM41 and *Burkholderia* BT03 data were first published in Timm et al. (2016).

Treatment	Tissue	Dilution	mean CFU	St dev		Sum Sq	Df	F	p
Control	leaf	1.0E+01	1080.5	1871.5	Bact.	1.3E+12	3	1.24	0.319
GM30	leaf	1.0E+01	19574.7	30672.7	Tissue	1.4E+12	2	1.92	0.1699
GM41	leaf	1.0E+01	1141.3	1809.3	B × T	1.6E+12	6	0.74	0.6264
BT03	leaf	1.0E+01	41175.9	45063.1	Resid.	8.1E+12	24		
Control	root	1.0E+01	110.2	131.5					
GM30	root	1.0E+01	170447.1	212977.7					
GM41	root	1.0E+01	2438.9	1563.8					
BT03	root	1.0E+01	309628.0	106958.6					
Control	stem	1.0E+01	0.0	0.0					
GM30	stem	1.0E+01	1166273.0	1872593.0					
GM41	stem	1.0E+01	1510.2	2135.8					
BT03	stem	1.0E+01	654513.2	688365.7					
Control	leaf	1.0E-01	1044.4	1809.0	Bact.	1.2E+13	3	18.77	**>0.001**
GM30	leaf	1.0E-01	16643.8	28827.9	Tissue	3.8E+12	2	9.21	**0.001**
GM41	leaf	1.0E-01	566.2	980.7	B × T	1.1E+13	6	8.91	**>0.001**
BT03	leaf	1.0E-01	60745.9	54910.0	Resid.	5.0E+12	24		
Control	root	1.0E-01	402.7	377.6					
GM30	root	1.0E-01	120591.5	111174.4					
GM41	root	1.0E-01	2851.9	3319.7					
BT03	root	1.0E-01	3096279.7	1069585.6					
Control	stem	1.0E-01	0.0	0.0					
GM30	stem	1.0E-01	289189.7	330089.7					
GM41	stem	1.0E-01	0.0	0.0					

BT03	stem	1.0E-01	904314.7	1099508.6					
Control	leaf	1.0E-02	0.0	0.0	Bact.	6.9E+14	3	13.78	**>0.001**
GM30	leaf	1.0E-02	0.0	0.0	Tissue	4.0E+14	2	11.79	**>0.001**
GM41	leaf	1.0E-02	0.0	0.0	B × T	1.2E+15	6	11.47	**>0.001**
BT03	leaf	1.0E-02	73537.1	80004.4	Resid.	4.1E+14	24		
Control	root	1.0E-02	0.0	0.0					
GM30	root	1.0E-02	368195.0	510398.0					
GM41	root	1.0E-02	20595.2	35671.9					
BT03	root	1.0E-02	28809015.5	14126689.6					
Control	stem	1.0E-02	0.0	0.0					
GM30	stem	1.0E-02	227127.9	252544.5					
GM41	stem	1.0E-02	0.0	0.0					
BT03	stem	1.0E-02	1805855.2	1567125.7					

Figure 1

Structural traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) or were inoculated with *Pseudomonas* GM30, *Pseudomonas* GM4, or *Burkholderia* BT03.

Figure 1: Structural traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) (n = 8), were inoculated with *Pseudomonas* GM30 (n = 7), *Pseudomonas* GM41 (n = 8), or *Burkholderia* BT03 (n = 7). a) Change in leaf number from the first to last day of the experiment. Negative values indicate that leaves senesced during the experiment. b) Leaf surface area growth rates (cm^2 d^{-1}), c) Stem growth rate (cm^1 d^{-1}). d) Root surface area growth rates (cm^2 d^{-1}). Letters represent significant differences of post-hoc least squares means among bacterial treatments. Boxplots display median, first and third quartiles, and vertical lines represent 1.5×inner quartile range of our dataset. The dots represent raw data values.
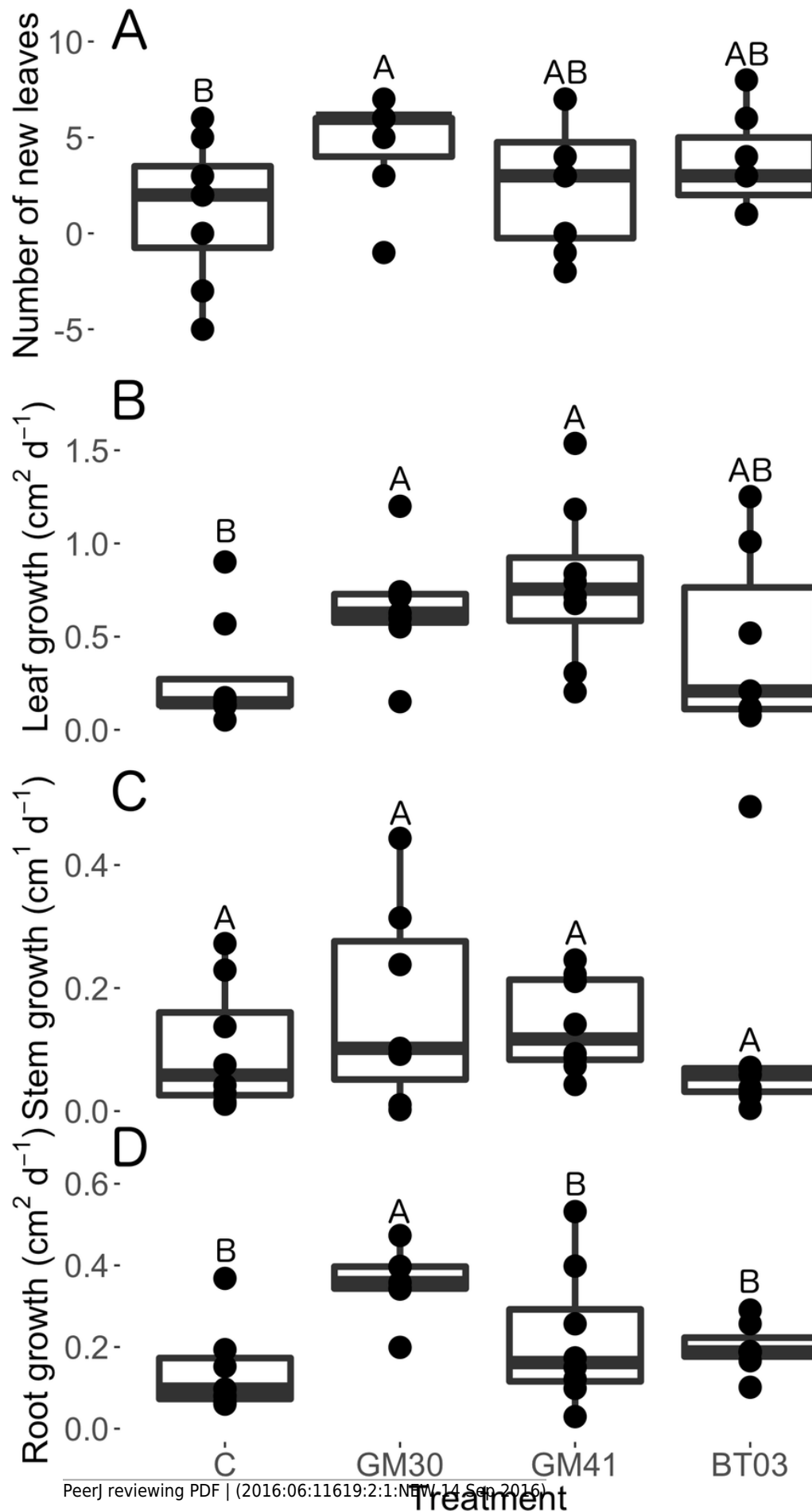
Figure 2

Biomass allocation of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) or were inoculated with *Pseudomonas* GM30, *Pseudomonas* GM41, or *Burkholderia* BT03.

Figure 2: Biomass allocation of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) (n = 8) , were inoculated with *Pseudomonas* GM30 (n = 7), *Pseudomonas* GM41 (n = 8), or *Burkholderia* BT03 (n = 7). a) Total dry mass (mg). b) Leaf biomass (mg), c) Root biomass (mg). Letters represent significant differences of post-hoc least squares means among bacterial treatments. Boxplots display median, first and third quartiles, and vertical lines represent 1.5×inner quartile range of our dataset. The dots represent raw data values.
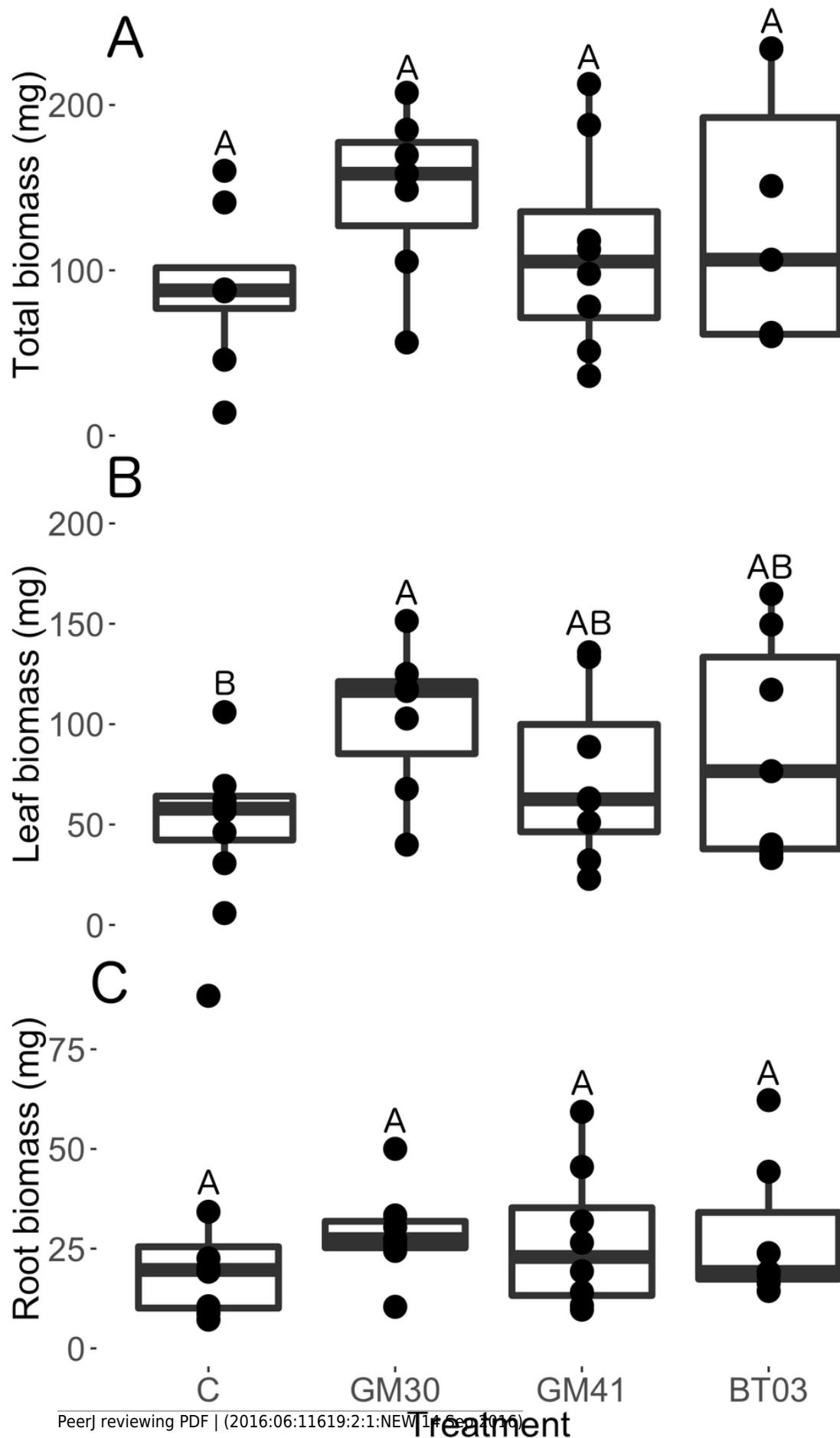
Figure 3

Physiology traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) or were inoculated with *Pseudomonas* GM30, *Pseudomonas* GM41, or *Burkholderia* BT03.

Figure 3: Physiology traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) (n = 8), were inoculated with *Pseudomonas* GM30 (n = 7), *Pseudomonas* GM41 (n = 8), or *Burkholderia* BT03 (n = 7). a) Plant chlorophyll content (SPAD), b) ΦCO_2 (expressed as the slope of carboxylase activity across different light levels), and c) carboxylase activity under maximum light level and CO_2 concentration (A_{max}). Letters represent significant differences of post-hoc least squares means among bacterial treatments. Boxplots display median, first and third quartiles, and vertical lines represent 1.5×inner quartile range of our dataset. The dots represent raw data values.