Root bacterial endophytes alter plant phenotypic plasticty, but not physiology

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Plant traits, such as root and leaf area, influence how plants interact with their environment and the diverse, and often unconsidered, microbiota living within plants can influence plant trait plasticity 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 carbon-metabolism breadth, plant hormone production, secondary metabolite synthesis, and predicted strain function. Next, 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 the inoculated bacteria modified plant morphology. However, endophyte inoculation had little 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.

Title page

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Abstract (199 words)

Plant traits, such as root and leaf area, influence how plants interact with their environment and the diverse, and often unconsidered, microbiota living within plants can influence plant trait plasticity 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 carbon-metabolism breadth, plant hormone production, secondary metabolite synthesis, and predicted strain function. Next, 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 the inoculated bacteria modified plant morphology. However, endophyte inoculation had little 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.

Introduction

A recent review exploring microbiome-mediated plant traits found that plant associated microbes can modify the plasticity of fourteen out of thirty commonly measured functional traits (Cornelissen et al., 2003; Friesen et al., 2011). For example, inoculation with common root-colonizing bacterial strains influenced root and leaf architectural traits, such as specific leaf area and specific root length, as well as plant physiological traits such as carbon fixation and chlorophyll content (Harris, Pacovsky, & Paul, 1985; Ma et al., 2003; Friesen, 2013). Further, inoculation by different members of the plant microbiome may differentially alter plant trait plasticity (Zamioudis et al., 2012; Timm et al., 2016). The presence of unique bacterial strains in legume genotypes explained more variation in shoot biomass, root biomass, and plant height than plant genotype did (Tan & Tan, 1986). Inoculation of common endophytes can also inhibit primary root elongation and promote lateral root formation and root hair production (Zamioudis et al., 2012; Weston et al., 2012). Recent breakthroughs in the multitude of the –omics fields have allowed for unprecedented mechanistic investigations of microbe-induced changes in host function (Verhagen et al., 2004; Walker et al., 2011; Weston et al., 2012; Vandenkoornhuyse et al., 2015; Timm et al., 2015; 2016) and have been the subject of multiple recent reviews (Friesen et al., 2011; Friesen, 2013; Vandenkoornhuyse et al., 2015; Hacquard & Schadt, 2015; Lebeis, 2015; and many others). This work demonstrated that plant growth promoting bacteria elicit numerous changes in host gene expression through multiple and simultaneous hormonal and immune response pathways (Verhagen et al., 2004; Walker et al., 2011; Weston et al., 2012; Drogue et al., 2014; Timm et al., 2016). However, these studies fall short in explaining how changes in gene expression influence the overall plant phenotype or plant function. Thus, understanding the links between microbe-induced trait plasticity and overall plant phenotype

remains a research gap. We inoculated three common entophytic bacterial strains onto *Populus trichocarpa* and measured how each bacterial strain altered the plasticity of commonly measured plant functional traits. We selected traits that are commonly measured in the functional trait ecology literature to explore how bacterial strains alter trait plasticity within the pre-existing functional trait framework that has largely ignored microbiome contribution to plant phenotype.

Here, we isolated three endophytic bacterial strains (*Pseudomonas fluorescens* GM41, *Pseudomonas fluorescens* GM30, and *Burkholderia* sp. BT03) from wild *Populus* and inoculated a single genotype of *Populus trichocarpa* with each strain. Bacterial strains belonging to the *Pseudomonas fluorescens* group are common plant growth promoting bacteria that are abundant in the *Populus* microbiome (see Gottel et al., 2011). *Pseudomonas fluorescens* accounted for approximately 34% of the sequences found in the *Populus* endosphere, but only 2-3% of the sequences in the rhizosphere and soil samples originating from the same roots (Gottel et al., 2011). *Pseudomonas* strains can alter plant host function by modifying plant growth (Kloepper et al., 1980; Lugtenberg & Kamilova, 2009; Timm et al., 2015), nutrient allocation (Bisht et al., 2009), hormone signaling (Stearns et al., 2012), up-regulating/down-regulating of gene expression pathways (Timm et al., 2016), and immune function (Verhagen et al., 2004; Weston et al., 2012). Additionally, the *Pseudomonas fluorescens* clade has a large amount of functional diversity (Jun et al., 2016), thus selecting two *Pseudomonas* stains allows us to explore how strain genome differences might influence plant trait plasticity. To contrast with these two strains, we selected a distantly related, but enriched in *Populus* endosphere (Gottel et al., 2011), bacterial strain from the genus *Burkholderi*a.

We predicted that the three bacterial strains used would alter the plasticity of physiological and morphological traits in a single plant genotype. Further, we predicted that the two

Pseudomonas strains would produce a plant phenotype that was more similar to one another than to *Burkholderia* because of phylogenetic relatedness, *i.e.* more shared functionality. To test our predictions, we first conducted a genomic comparison using COG (clusters of orthologous groups) database to predict the functional differences among strains. Next, we inoculated each bacterial strain on *Populus trichocarpa* and measured a suite of physiological and architectural plant traits including the root: shoot, biomass production, root and leaf growth rates, chlorophyll content, net photosynthesis, and net photosynthesis at saturating light - A_{sat}, and saturating CO_2 - A_{max}. We chose to measure overall trait plasticity within several commonly measured functional traits without measuring pathways involved because we were interested in understanding down-stream consequences of bacterial inoculation on overall plant phenotype.

Materials and Methods

Populus trichocarpa genotype "93-968" (Labbe et al., 2014) was propagated in tissue culture following standard procedures (see Kang et al., 2009). Briefly, *in vitro* cultures were established from actively growing shoot tips collected from greenhouse-grown *Populus* plants. Shoot tips were sterilized by soaking in a 1% Tween 20 solution for 5 min, 70% Ethanol solution for 1 min, a 0.525% sodium hypochlorite solution for 15 min and then rinsed three times in sterile H_2O for 5 min. Shoot tips were trimmed to 2 cm in length and transferred to a magenta box (Sigma-Aldrich, St. Louis, MO) containing 80 mL of tissue media (1× Murashige & Skoog (MS) basal medium (Murashige & Skoog, 1962) supplemented with MS vitamins (Caisson Labs, North Logan, UT, USA), 0.05% 2-(*N*-morpholino) ethanesulfonic acid (MES hydrate) (Sigma-Aldrich, St. Louis, MO, USA), 3% sucrose, 0.1% PPM™ (plant protective mixture) (Plant Cell Technology, Washington, DC, USA), 0.5% activated charcoal (Sigma-Aldrich, St. Louis, MO,

105 USA), and 0.15% Gelzan (Plantmedia, bioWORLD, Dublin, OH, USA). Plants were sub-
106 cultured until it was determined, using microscopy and colony formation units with R2A
107 medium, that the plants were axenic.
108 Plant cultures were rooted in a growth room at 25 °C under a 16 h photoperiod. After root
109 establishment plants that were similar in size and development stage were selected for
110 experimentation. Plants were weighed and scanned to account for initial plant size differences
111 among treatments. To ensure sterility during scanning, plants were placed between two (21.59 x
112 27.94 cm) sheets of cellulose acetate that were sprayed with 100% ethanol. Scans were
113 performed with a portable scanner (VuPoint Solutions Inc., City of Industry, CA, USA). Scanned
114 images were analyzed in WinRhizo (Regent Instruments, Quebec City, Canada) to determine
115 initial root surface area, root length, stem length, and leaf surface area for each individual. After
116 scanning, plants were transferred into experimental microcosms.
117 We constructed closed microcosms by interlocking two sterile Magenta boxes (Sigma-
118 Aldrich, St. Louis, MO, USA) with a coupler (Sigma-Aldrich, St. Louis, MO, USA). We added
119 150 ml calcined clay (Pro's choice Sports Field Products, Chicago, IL, USA) and 70 ml of 1×
120 Hoagland's nutrient solution (Sigma-Aldrich, St. Louis, MO, USA) to each microcosm (Fig 1).
121 To allow air to flow into and out of the microcosms, we drilled two 7 mm holes on adjacent sides
122 of the upper magenta box and covered the holes with adhesive microfiltration discs (Tissue
123 Quick Plant Laboratories, Hampshire, United Kingdom) to prevent outside microbial
124 contamination. Prior to microbial addition, we double sterilized each closed microcosm by
125 autoclaving on a 60 m dry cycle on consecutive days. *Pseudomonas fluorescens* strains (GM30
126 and GM41) and *Burkholderia* sp. (BT03), hereafter termed *Pseudomonas* GM30, *Pseudomonas*
127 GM41, and *Burkholderia* BT03 were isolated from *Populus* endospheres (for isolate

128 descriptions, see Brown et al., 2012; Weston et al., 2012; Utturkar et al., 2014; Timm et al.,
129 2015; 2016). The bacterial strains were grown in isolation and at a constant temperature, 25 °C,
130 in 5 ml of R2A medium. After growing overnight they were pelleted and re-suspended in sterile
131 water to an OD600 of 0.01 (~1.0×E7 cells ml^{-1}).
132
133 **Comparative genomics of microbes**
134 Genomes of *Pseudomonas fluorescens* strains GM30 and GM41 and *Burkholderia* sp.
135 BT03 were sequenced at Oak Ridge National Laboratory and genes were identified using
136 Prodigal (Brown et al., 2012, Utturkar et al., 2014) and are available at NCBI (GM41:
137 AKJN00000000.2; GM30: AKJP02000000.2; BT03: NZ_AKKD000000000.2). Genome
138 annotation, genomes statistics, and annotation comparisons were performed using IMG tools
139 (img.jgi.doe.gov). Genome statistics and COG functional predictions were extracted from
140 Integrated Microbial Genomes (img.jgi.doe.gov) and then they were compared manually for
141 differential inclusion of predicted functions.
142
143 **Experimental design**
144 Each *Populus* was grown in an individual microcosm in combination with one of the bacterial
145 strains. Thus, the experiment had four treatment combinations – *Pseudomonas* GM30
146 inoculation, *Pseudomonas* GM41 inoculation, *Burkholderia* BT03 inoculation, and a bacteria-
147 free control. In total, there were 32 microcosms with four treatments (n = 8). We inoculated each
148 microcosm by adding 10 ml of the bacterial strain (10^7 cells ml^{-1}) to the calcined clay substrate
149 and stirring for 30 s to distribute the bacteria. After inoculation, we planted the *Populus* clones
150 within each microcosm. The experiment was divided into three different establishment dates in

2014 (1 March, 3 replicated blocks; 25 March, 2 replicated blocks; and 2 April, 3 replicated blocks) because plant tissues were difficult to propagate. Plant-bacteria combinations were grown in the microcosms for five weeks with a 16 hr photoperiod, at 21°C and 80% relative humidity.

To explore the relationships between bacterial strain and plant trait plasticity each plant was removed from its microcosm after 35 days of growth. Plants were submerged in sterilized deionized H_2O to remove clay from the root system, weighed, and scanned. Scans were analyzed with WinRhizo to determine root surface area, total root length, stem length, and leaf surface area. For each plant, the final measurement of root surface area, total root length, stem length, and leaf surface area was subtracted from the initial measurement and divided by the experiment duration to determine tissue growth rates (cm d^{-1} or cm^2 d^{-1}). Additionally, each plant was dried for 48 hours at 70°C and weighed to measure leaf, shoot (leaf + stem) and root and total dry mass. Specific leaf area and the specific root length of each individual were calculated by dividing leaf area by leaf dry mass or by dividing root length by root dry mass, respectively.

To measure how the presence of an individual bacterial strain influenced host plant physiology, leaf gas-exchange was measured and used to estimate leaf photosynthesis on our first replicate block (March 1, n = 3). For each plant, gas exchange of the largest leaf of the plant was measured (Li-Cor model 6400, Li-Cor Biosciences, Lincoln, Nebraska, USA) immediately prior to our experimental harvest. The maximum rate leaves were able to fix carbon (A_{max}), the maximum rate of photosynthesis in saturating light under ambient CO_2 (A_{sat}), and the quantum yield of CO_2 fixation (Φ) were all measured. Finally, average leaf chlorophyll content was measured on three fully opened leaves (Konica Minolta Chlorophyll Meter SPAD-S02, Ramsey, NJ, USA).

174

175 **Bacterial Colonization**

176 To test for endophytic colonization of *Pseudomonas* GM41, *Pseudomonas* GM30, and
177 *Burkholderia* BT03, we measured colony-forming units (CFUs) from surface sterilized,
178 macerated root, leaf, and stem tissue after 2 weeks of growth. Cuttings of *Populus trichocarpa*
179 were planted into a magenta box containing *Pseudomonas* GM41, *Pseudomonas* GM30,
180 *Burkholderia* BT03, or a bacterial free control, using similar experimental methodology
181 described above (n = 3). After 2 weeks of growth, all the plant roots, stems, and 1-2 mature
182 leaves were surface sterilized by dipping them in a ~10% bleach solution, followed by 70%
183 ethanol, and then rinsing in water three times. We recorded wet weight of plant tissues and then
184 separately macerated each plant tissue compartment in a sterile mortar and pestle in 1 ml sterile
185 1× PBS. We transferred macerated plant tissues to a 24-well plate where we serial diluted each
186 sample by 10% with 1×PBS at 1×, 0.1×, 0.01× of original sample concentration. Each sample
187 was streaked onto R2A media plates and allowed to grow for 48 hours at 20ºC. After 48 hours,
188 colony formation was counted. We calculated CFU mg^{-1} of plant tissue by multiplying colony
189 number per plate by $10^{(\text{dilution factor} + 1)}$ and then dividing that number by the dry tissue mass (mg^{1}).

190 **Statistical analyses**

191 We tested all collected data for normality using the normalTest function in the fBasics package
192 (version 3011.87, R metrics core team 2014) for R version 3.0.2 (R development core team,
193 2013) and RStudio version 0.98.495 (RStudio, 2013). If data were not normally distributed, we
194 performed log transformations or square-root transformations to satisfy the normality
195 assumptions of ANOVA.

196 To explore how microbe strain altered the plant phenotype (root dry mass, leaf dry mass,
197 shoot dry mass, total dry mass, root: shoot, root growth rates, leaf growth rates, change in leaf
198 number, specific root length, specific leaf area) we used linear mixed-effect models. Bacterial
199 strain was a fixed effect in the model and experimental block (three establishment dates) was a
200 random factor using the lme4 package in R (Bates et al., 2014). For plant dry mass measures, we
201 incorporated initial measurements of root surface area in the root dry mass model and initial leaf
202 surface area in the aboveground dry mass model as covariates. To test for significance of
203 bacterial strain (fixed effects) and covariate (initial growth measure) we performed a likelihood
204 ratio test to compare models with and without fixed effects and covariates. If including fixed
205 factors (bacterial strain) was significant an improvement to model fit (p value < 0.05 in
206 likelihood ratio test), we conducted difflsmeans test post-hoc to calculate differences among
207 strains and calculated confidence intervals for our microbial strains using the R package
208 lmerTest version 2.0-3 (Kuznetsova, Brockhoff, & Christensen, 2014). To calculate percent
209 change in trait values we: trait value of non-inoculated host plant was subtracted from trait value
210 in bacterial treatment and divided by trait value of non-inoculated host plant and multiplied by
211 100.
212 To test if bacterial strains altered plant physiology (carboxylase activity, A_{max}, A_{sat}), we
213 used one-way analysis of variance (ANOVA) using the "Anova" function in the CAR package,
214 (version 2.0-22, Fox et al., 2011) because we collected physiology data on only a single
215 sampling date ($n = 3$).
216 All three of the bacterial strains colonized *Populus* hosts. Colony-forming units were
217 enriched in all three bacterial strains relative to the control in the 0.1× and 0.01× dilutions (Table
218 2), although colonization by stains was variable across dilutions, tissue types, and treatments. We

found no difference in CFUs among non-inoculated control and *Pseudomonas* GM30, GM41, and *Burkholderia* BT03 inoculated host plants at the 1× dilution (Table 2). All three bacterial strains colonized leaf and stem tissues, but the highest CFUs across bacterial treatments were consistently observed in roots (Table 2).

Results

Bacterial strains differ in genomic content

We compared the genomes of *Burkholderia* BT03 and *Pseudomonas* GM30 and GM41 based on predicted enzyme functions using the COG database (Table 1). Overall, our genome comparison demonstrated that the bacterial strains differed in genome size and functional gene content. *Burkholderia* BT03 had a relatively large genome (10.9 Mb) compared to *Pseudomonas* GM30 (6.1 Mb) and *Pseudomonas* GM41 (6.6 Mb) (Table 1). We found all three bacterial strains shared functions that were likely critical for establishment and survival in the plant microbiome including the production of the plant hormone auxin, pili, flagella, chemotaxis, increased signal transduction, and secretion systems. However, we found many functional differences among our strains. The genome of *Burkholderia* encoded multiple pathways predicted to be involved in the metabolism of the plant hormones, salicylic acid and ethylene (Table 1). Relative to the *Pseudomonas* genomes, the *Burkholderia* genome encoded for numerous secondary metabolite biosynthesis elements (Table 1). We found that *Burkholderia* contained more carbohydrate and lipid transporters compared to both *Pseudomonas* genomes, suggesting increased metabolic capabilities within *Burkholderia* (Table 1).

Even through *Pseudomonas* GM30 and *Pseudomonas* GM41 were classified as the same 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, nitrate
243 reduction, and secondary metabolite biosynthesis elements compared to *Pseudomonas* GM30.
244 We also found that the genome of *Pseudomonas* GM41 contained more genes coding for
245 carbohydrate metabolism, lipid metabolism, and amino acid transport and metabolism, energy
246 production and conversion, suggesting that *Pseudomonas* GM41 may have more metabolic
247 breadth than *Pseudomonas* GM30 (Table 1). Taken together, our results demonstrated that these
248 three bacterial strains differ in genome size and their functional gene content.
249

250 **Bacterial colonization of *Populus* root tissue**

251 All three of the bacterial strains colonized *Populus* hosts. Colony-forming units were enriched in
252 all three bacterial strains relative to the control in the 0.1× and 0.01× dilutions (0.1× dilution F =
253 18.77, p <0.0001; 0.01× dilution F = 13.78, p <0.0001, Table 2), although colonization by stains
254 was variable across dilutions, tissue types, and bacterial strain. We found no difference in CFUs
255 among non-inoculated control and *Pseudomonas* GM30, GM41, and *Burkholderia* BT03
256 inoculated host plants at the 1× dilution (F = 1.24, p = 0.319 Table 2). We found that
257 Pseudomonas GM30, Pseudomonas GM41, and *Burkholderia* BT03 inoculated plants had 10-
258 10000× more CFUs than did non-inoculated control plants (Table 2). All three bacterial strains
259 colonized leaf and stem tissues, but the highest CFUs across bacterial treatments were
260 consistently observed in roots (Table 2). Inoculated host plants contained 0-28809015 CFU mg^{-1}
261 in roots, 0-1166273 CFU mg^{-1} in stems, and 0-73537 CFU mg^{-1} in leaves compared to 0-400
262 CFU mg^{-1} in root tissues, 0 CFU mg^{-1} in stem tissue, 0-1000 CFU mg^{-1} in leaf tissue compared to
263 non-inoculated control plants (Table 2).
264

Plant structure is modified by bacterial inoculation

Overall, we found that bacterial inoculation increased *Populus* root (chisq = 13.861, $p = 0.003$) and leaf growth rates (chisq = 9.211, $p = 0.01$), independent of strain type (Fig 2). Specifically, mean root growth rate increased 184% ($t = 3.84$, $p = 0.001$), 74% ($t = 1.61$, $p = 0.12$), and 56% ($t = 1.18$, $p = 0.25$) when *Populus* was inoculated with *Pseudomonas* GM30, *Pseudomonas* GM41, and *Burkholderia* BT03 (Fig 2, Supplemental Table 1). Similarly, mean leaf growth rate increased 114% ($t = 2.27$, $p = 0.03$), 138% ($t = 2.86$, $p = 0.01$) and 51% ($t = 1.02$, $p = 0.32$) when *Populus* individuals were inoculated with *Pseudomonas* GM30, *Pseudomonas* GM41, and *Burkholderia* BT03 relative to non-inoculated control plants (Fig 2, Supplemental Table 1). Leaf number differed by bacterial strain inoculation (chisq = 23.396, $p = >0.01$) (Fig 2). Leaf number increased by 36% ($t = 3.34$, $p = 0.003$), 9% ($t = 0.93$, $p = 0.36$) and 15% ($t = 1.418$, $p = 0.17$) when the plants were inoculated with *Pseudomonas* GM30, *Pseudomonas* GM41, and *Burkholderia* BT03 respectively (Fig 2, Supplemental Table 1). We observed no differences in stem elongation (chisq= 0.06, $p = 0.97$, Supplemental Table 1).

Interestingly, we observed no differences in total plant dry mass (chisq = 3.27, $p = 0.195$), root dry mass (chisq = 0.00, $p = 1.00$), root:shoot ratio (chisq=0.00, $p = 1.00$, Supplemental Table 1) or plant height (cm d^{-1}, chisq = 1.99, $p = 0.158$ Supplemental Table 1) among our bacterial treatments (Fig 3). However, we observed an 86% ($t = 2.43$, $p = 0.02$), 33% ($t = 0.97$, $p = 0.33$), and 60% ($t = 1.70$, $p = 0.10$) increase in individual *Populus* dry leaf mass relative to control plants in *Pseudomonas* GM30, *Pseudomonas* GM41, and *Burkholderia* BT03 treatments, respectively (chisq = 6.20, $p = 0.045$) (Fig 3, Supplemental Table 1). We observed no differences in specific leaf area of *Populus* seedlings among our treatments (chisq= 2.60, $p = 0.46$, Supplemental Table 1). Thus, inoculation of *Pseudomonas* GM30 increased leaf surface

288 area and aboveground dry mass, without changing leaf area:mass ratios. We found no significant
289 differences in root length:dry mass (specific root length, chisq = 1.06, p = 0.79) among out
290 treatments (Supplemental Table 1). Our results indicate that bacterial strains modify plant
291 resource allocation but not total dry mass production. However, stains differ in their influence on
292 plant functional traits.
293
294 **Plant physiology is not affected by bacterial inoculation**
295 Bacterial inoculation had no measureable effects on any of the physiological traits measured:
296 chlorophyll content (SPAD) (chisq = 2.15, p = 0.54), quantum yield of photosynthesis (ϕ) (F =
297 1.01, p = 0.43), net photosynthesis at saturating light conditions (A_{sat}) (F = 0.76, p = 0.55) or
298 maximum net photosynthesis at saturating light and [CO_2] (A_{max}) (F = 1.98, p = 0.19) (Fig 4). In
299 agreement with the total dry mass data, we did not observe significant changes in the measured
300 photosynthetic parameters. Thus, changes in plant structure were not leading to increases
301 photosynthetic capacity, efficiency, or carbon assimilation rates.
302
303 **Discussion**
304 The plant root microbiome can have a strong influence on plant production and trait plasticity
305 (Friesen, 2013; Vandenkoornhuyse, et al. 2015); yet, less is known about how individual
306 community members alter plant trait plasticity, production, and physiology. We explored how
307 three bacterial strains, two closely related *Pseudomonas fluorescens* strains (GM30 & GM41)
308 and a more distantly related *Burkholderia* (BT03), altered plant morphological traits,
309 productivity, and cellular physiology. We selected bacterial strains that differed in carbon
310 metabolism breadth, plant hormone production and modification, and secondary metabolite

311 synthesis in an effort to understand how complex molecular plant-microbe interactions influence
312 plant trait plasticity (Table 1, Timm et al., 2015; 2016). Similar to other studies (Weston et al.,
313 2012, Timm et al., 2016), we found that all three strains could colonize *Populus* roots, leaves,
314 and stems (Table 2). Although colonization was highly variable among bacterial strains, plant
315 tissue type, and sample dilution, we found that inoculation of all three bacterial strains
316 consistently lead to higher colony forming units relative to the non-inoculated control plants
317 (Table 2). This suggests that a low level of bacterial colonization occurred in all plants, however
318 inoculated bacteria resulted in 10-10000× CFUs mg^{-1} than non-inoculated plants (Table 2).
319 Overall, root endophyte presence in our study increased the root surface area growth rate up to
320 184% and leaf surface area growth rate up to 138% relative to non-inoculated control plants (Fig
321 2). Bacterial inoculation generally increased leaf production (Fig 2) without changing specific
322 leaf area (Supplemental Table 1), suggesting that aboveground, strains benefit host plant without
323 altering leaf functional traits. However, inoculation of *Pseudomonas* GM30 increased *Populus*
324 root surface area by 184% without increasing root biomass, thus colonization of GM30 may
325 change root morphology leading to longer, thinner, highly-branched roots with similar biomass.
326 Similarly, *Pseudomonas* GM30 inoculation increased root branching in both *Arabidopsis* and
327 *Populus deltoides* (Weston et al., 2012; Timm et al., 2015; 2016). Additionally, bacterial strain
328 presence did not significantly influence total plant dry mass (Fig 3), root:shoot, plant height,
329 specific leaf area, specific root length (Supplemental Table 1) or plant physiology (Fig 4). Taken
330 together, our results suggest that bacterial endophytes may differentially influence the plasticity
331 of aboveground versus belowground plant traits, however this hypothesis needs further testing.
332 The stimulating effect of bacterial strain presence on plant traits varied among the three
333 bacterial strains studied suggesting that strain biochemistry and physiology, not just the presence

of bacteria, can alter plant trait plasticity. Our comparative genomic analysis revealed that even though strains share many common endophytic functions like plant hormone signal disruption, production of plant hormone auxin, pili, flagella, and chemotaxis, strains differed in their ability to perform these functions and each encoded unique functional capabilities. Specifically, the *Burkholderia* genome encoded for an overall higher level of functional diversity and included pathways that alter the salicylic acid and ethylene plant hormonal pathways in *Populus* that are crucial pathways for plant growth and development (see Yang & Hoffman, 1984; Wasternack & Parthier, 1997; Chen et al., 2009; Dempsey et al., 2011). Although *Burkholderia* can produce ethylene, gas-exchange out of the microcosm through our ports may have prevented us from observing the characteristic reduced stem elongation and root growth with increased ethylene concentrations (Burg, 1973; Romano, Cooper, & Klee, 1993). Although *Pseudomonas* genomes do not contain the genes to directly metabolize salicylic acid, inoculation by *Pseudomonas* GM41 can up-regulate salicylic acid synthesis and degradation in *Populus* (Timm et al., 2016). The *Burkholderia* genome also encoded for multiple transposase elements that degraded poplar-produced aromatics and metabolites, functions that are not present in *Pseudomonas* genomes (Timm et al., 2015; 2016). *Burkholderia* and *Pseudomonas* genomes differed in the carbon substrates they might degrade, suggesting strains differ in the niche space occupied within the root, however we found evidence that all three strains could colonize roots, leaves, and stems (Table 2). We found *Burkholderia* had the highest CFU abundance within our colonization study (Table 2). Additionally, *Burkholderia* was predicted to influence three different plant hormonal pathways (auxin, ethylene, salicylic acid). Surprisingly, *Burkholderia* inoculation did not alter plant growth, architectural, or physiological trait plasticity, which suggests there are generalist-specialist tradeoffs present; however this hypothesis needs further testing.

357 In spite of close genetic relatedness, our *Pseudomonas* strains differed in key functional
358 capabilities. Specifically, *Pseudomonas* GM41 encoded for phosphate solubilization and
359 denitficiation ability, suggesting these two strains may differentially influence host nutrition.
360 *Pseudomonas* GM41 produced more sugar transporters and sugar catabolic enzymes, than
361 *Pseudomonas* GM30 potentially allowing *Pseudomonas* GM41 to exploit greater metabolic
362 niche space (Table 1). However, *Pseudomonas* GM41 can produce twice as much auxin than
363 *Pseudomonas* GM30 (Timm et al., 2015). Auxin synthesis by endophytic bacteria can increase
364 root branching and lateral root formation and decrease overall plant height, leaf number,
365 chlorophyll content and photosynthetic efficiency (Romano, Cooper, & Klee, 1993; Fu &
366 Harberd, 2003; Weston et al., 2012). While we did not measure auxin synthesis in the plants we
367 inoculated, it is a possible mechanism for the observed differences in trait plasticity between
368 these closely related strains. Surprisingly, the higher auxin synthesis in *Pseudomonas* GM41 did
369 not result in shifts in root growth rates as observed in *Pseudomonas* GM30. We found
370 *Pseudomonas* GM30 presence in the endosphere increased root growth rates, trended toward
371 increased SRL, increased leaf surface area, and increased leaf production, while *Pseudomonas*
372 GM41 presence had no detectable influence on root traits or leaf production (Fig 2,
373 Supplemental Table 1). This suggests that auxin production alone does not drive host trait
374 plasticity. Complex interactions likely link strain genotype to plant trait plasticity. Contrary to
375 our predictions, bacterial strains that were categorized as the same 16S OTU can have a
376 differential impact on plant trait plasticity. Further, closely related bacterial strains can interact
377 with hosts on a continuum from commensal to pathogenic (Coombs & Franco, 2003).
378 Contrary to our predictions, bacterial endophytes in this study did not significantly
379 influence plant leaf physiology (Fig 4), plant height (Supplemental Table 1), or total plant dry

mass (Fig 3). Root colonization by *Pseudomonas* can reduce chlorophyll content and net photosynthesis (A_{sat}) in a variety of plant hosts (Zou et al., 2005; Weston et al., 2012). However *Pseudomonas* colonization can also increase photosynthetic activity and chlorophyll content (Kandasamy et al. 2009). For example, *Pseudomonas* GM41 colonization can up-regulate cytochrome b6/f and chlororespiration in *Populus deltoides* increasing photosynthetic potential of the plant host (Timm et al. 2016). Given the contrasting results from previous studies, plant carbon dynamics may vary with bacterial strain (Kandasamy et al. 2009; Weston et al. 2012; Timm et al. 2016) or plant ontogeny may interact with bacterial strain to alter plant carbon dynamics (Siddiqui & Shaukat 2003). Similarly, root endophytic bacteria colonization results in the up- and down-regulation of numerous plant genes and leaf metabolites (see Verhagen et al. 2004; Wang et al. 2005; Walker et al. 2011; Weston et al. 2012; Timm et al. 2016). Thus, counteracting influences among different gene pathways may conceal microbe-impacts on plant carbon dynamics when measuring down-stream trait plasticity and plant phenotype (Bashan, Holguin, & de-Bashan 2004; Timm et al. 2016). Finally, host plants may respond differently to even closely related bacterial strains and these interactions may be influenced by plant stress (Lau & Lennon 2011). In fact, during times of biotic and abiotic stress, endophytic bacteria may be critical for maintaining plant function (reviewed in Dimkpa, Weinand, & Asch 2009; Yang, Kloepper, & Ryu 2009). Plants that are growing under optimal conditions, like those in this study, are not undergoing stress and thus the influence of endophytic bacteria on photosynthetic functions may be masked. No matter what the mechanisms were, it appeared that phenotypic responses to microbial inoculation were contingent upon the combinations of bacterial strain type and plant genotype present (Bashan, Holguin, & de-Bashan 2004; Verhagen et al. 2004; Walker et al. 2011).

Clearly, symbiotic, endophytic bacteria can influence plant structure and ability to gain access to nutrients. However, with a few well-known exceptions (Tan & Tan 1986; Harris, Pacovsky, & Paul 1985; Ma et al. 2003, Lau & Lennon 2011; 2012), bacterial community composition in roots has largely been ignored in studies exploring what causes natural variation in plant traits (Friesen et al. 2011; Friesen 2013; Timm et al. 2016). Our study underscores the importance of common root-colonizing bacteria in manipulating root structure of a plant – a trait that has very little genetic or environmental control (Hajek, Hertel, & Leuschner 2013; Fort, Cruz, & Jouany 2014; Slovak et al. 2015; Kramer-Walter et al. 2016). While variation in natural plant root structure may result from soil local microsite variation (Hajek, Hertel, & Leuschner 2013, Kramer-alter et al. 2016), it is possible that the plant microbiome may play a key role in observed root variation (Kothari, Marschner, & George 1990; Rousseau, Sylvia, & Fox 1994; Friesen et al. 2011; Friesen 2013).

Our study focused on monoculture associations of common endosphere bacteria and their influence on plant trait plasticity, however future studies should focus on exploring how the microbiome (as a community) influences plant trait plasticity. We propose a multifaceted approach to investigate linkages among the plant microbiome and natural plant trait variation. First, incorporation of microbiome composition into studies that currently investigate host identity/genotype and environmental parameters may be important for finding patterns in trait variation – especially when conduced across a variety of environmental gradients. Second, once correlations between microbiomes and plant traits are observed in the field, detailed work constructing comminutes in the lab and greenhouse would enable a mechanistic understanding of what is underlying the observed patterns. These studies could be especially fruitful when

425 conducted across natural biotic and abiotic environmental gradients in the laboratory,
426 greenhouse, and field settings (Classen et al. 2015).
427
428 **Conclusions**
429 Our study shows that bacteria living in plant roots can influence plant morphological
430 traits. Increasingly, ecologists are using plant functional traits to explore how changing
431 environments might alter plant function (Wright et al. 2004; Reich 2014). Plant traits, such as
432 specific leaf area and specific root length, are often significantly correlated with important plant
433 functions such as carbon fixation and nutrient uptake (Diaz & Cabido 2001). Researchers are
434 using plant traits to extrapolate how plants will respond to global changes because there are
435 significant correlations between traits and plant function (Reich et al. 1999; Wright et al. 2004;
436 Reich 2014). However, functional trait approaches have been criticized because correlation
437 analyses ignore the mechanisms that drive variation in trait values, mechanisms that might be
438 influenced by global changes. While interactions between plant genotype and environment
439 influence plant functional trait plasticity (Bradshaw 1965; Schlichting 1986; Sultan 2000; Des
440 Marais, Hernandez, & Juenger 2013), biotic influences such as the plant microbiome can also
441 play a role in trait plasticity (Lau & Lennon 2011; 2012; Wagner et al. 2014; Hacquard & Schadt
442 2015). Given that plant-microbial studies, including ours, have observed strong linkages between
443 microbiome and plant trait plasticity (reviewed in Friesen et al. 2011; Friesen 2013) interactions
444 among global change drivers, plant genotypes, and plant microbiomes, should be considered in
445 trait-based approaches to ecological questions (Classen et al. 2015).
446
447 **Author Contributions**

JH, DW, DP, AC developed the project idea, DP provided bacterial isolates, JH and SJ established plant cultures, inoculated plants, and measured plant traits, CT performed the genomic comparisons. JH and AC conducted statistical analyses, JH, AC, CT, DW worked on data interpretation, JH and AC wrote the manuscript, and all authors collectively edited the manuscript.

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469 **References**

470 Bashan Y, Holguin G, de-Bashan LE. 2004. *Azospirillum*-plant relationships: physiological,
471 molecular, agricultural, and environmental advances (1997-2003). *Canadian Journal of*
472 *Microbiology* 50:521-577.
473 Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using
474 lme4. *Journal of Statistical Software* 67:1-48.
475 Bisht R, Chaturvedi S, Srivastava R, Sharma A, Johri B. 2009. Effect of arbuscular mycorrhizal
476 fungi, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* on the growth and nutrient
477 status of *Dalbergia sissoo* Roxb. *Tropical Ecology* 50:231.
478 Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in*
479 *Genetics* 13:115-155.
480 Brown SD, Utturkar SM, Klingeman DM, Johnson CM, Martin SL, Land ML, Lu TYS, Schadt
481 CW, Doktycz MJ, Pelletier DA. 2012. Twenty-one genome sequences from *Pseudomonas*
482 species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and
483 endosphere of *Populus deltoides*. *Journal of Bacteriol*ogy 194:5991-5993.
484 Chen Z, Zheng Z, Huang J, Lai Z, Fan B. 2009. Biosynthesis of salicylic acid in plants. *Plant*
485 *Signalling & Behavior* 4:493-496.
486 Classen AT, Sundqvist M, Henning JA, Newman GS, Moore JAM, Cregger M, Moorhead LC,
487 Patterson CM. 2015. Direct and indirect effects of climate change on soil microbial and soil
488 microbial-plant interactions: What lies ahead? *Ecosphere* 6(8):130. doi: 10.1890/ES15-
489 00217.1.
490 Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege
491 H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H. 2003. A handbook of protocols

492 for standardised and easy measurement of plant functional traits worldwide. *Australian*
493 *Journal of Botany* 51:335-380.
494 Dempsey DMA, Vlot AC, Wildermuth MC, and Klessig DF. 2011. Salicylic Acid Biosynthesis
495 and Metabolism. *The Arabidopsis Book.* **9**: e0156. doi: 10.1199/tab.0156.
496 Des Marais DL, Hernandez KM, Juenger TE. 2013. Genotype-by-environment interaction and
497 plasticity: exploring genomic responses of plants to the abiotic environment. *Annual Review*
498 *of Ecology, Evolution, and Systematics* 44:5-29.
499 Díaz S, Cabido M. 2001. Vive la différence: plant functional diversity matters to ecosystem
500 processes. *Trends in Ecology & Evolution* 16:646-655.
501 Dimkpa C, Weinand T, Asch F. 2009. Plant–rhizobacteria interactions alleviate abiotic stress
502 conditions. *Plant, cell & Environment* 32:1682-1694.
503 Drogue B, Sanguin H, Chamam A, Mozar M, Llauro C, Panaud O, Prigent-Combaret C, Picault
504 N, Wisniewski-Dyé F. 2014. Plant root transcriptome profiling reveals a strain-dependent
505 response during Azospirillum-rice cooperation. *Frontiers in Plant Science* 5:607.
506 doi:10.3389/fpls:2014.00607.
507 Fort F, Cruz P, and Jouany C. 2014. Hierarchy of root functional trait values and plasticity drive
508 early-stage competition for water and phosphorus among grasses. *Functional Ecology.*
509 28:1030-1040.
510 Fox J, Weisberg S. 2011. *An {R} Companion to Applied Regression*, Second Edition. Thousand
511 Oaks CA: Sage.
512 Friesen ML. 2013. *Microbially Mediated plant functional traits molecular microbial ecology of*
513 *the rhizosphere*. John Wiley and Sons, Inc., pp 87-102.

514 Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. 2011.
515 Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and*
516 *Systematics.* 42:23-46.
517 Fu X, Harberd NP. 2003. Auxin promotes Arabidopsis root growth by modulating gibberellin
518 response. *Nature*. 421:740-743.
519 Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E,
520 Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW. 2011. Distinct microbial communities
521 within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil
522 types. *Applied and Environmental Microbiology* 77:5934-5944.
523 Hacquard S, Schadt CW. 2015. Towards a holistic understanding of the beneficial interactions
524 across the *Populus* microbiome. *New Phytologist.* 205:1424-1430.
525 Hajek P, Hertel D, Leuschner C. 2013. Intraspecific variation in root and leaf traits and leaf-root
526 trait linkages in eight aspen demes (*Populus tremula* and *P. tremuloides*). *Frontiers in Plant*
527 *Science.* **4**:1-11. doi: 10.3389/fpls.2013.00415.
528 Harris D, Pacovsky RS, Paul EA. 1985. Carbon economy of soybean-Rhizobium-Glomus
529 associations. *New Phytologist*. 101:427.
530 Jun SR, Wassenaar TM, Nookaew I, Hauser L, Wanchai V, Land M, Timm CM, Lu TYS, Schadt
531 CW, Doktycz MJ, Pelletier DA, Ussery DW. 2016. Diversity of *Pseudomonas* Genomes,
532 Including Populus-Associated Isolates, as Revealed by Comparative Genome Analysis.
533 *Applied and Environmental Microbiology* 82:375-383.
534 Kang B, Osburn L, Kopsell D, Tuskan G, Cheng ZM. 2009. Micropropagation of *Populus*
535 *trichocarpa* 'Nisqually-1': the genotype deriving the *Populus* reference genome. *Plant Cell,*
536 *Tissue and Organ Culture*. 99:251-257.

537 Kloepper JW, Leong J, Teintze M, Schroth MN. 1980 Enhanced plant growth by siderophores
538 produced by plant growth-promoting rhizobacteria. *Nature.* 286:885-886.
539 Kandasamy S, Loganathan K, Muthuraj R, Duraisamy S, Seetharaman S, Thiruvengadam R,
540 Ponnusamy B, Ramasamy S. 2009. Understanding the molecular basis of plant growth
541 promotional effect of *Pseudomonas fluorescens* on rice through protein profiling. *Proteome*
542 *Science 7*:1-8.
543 Kothari S, Marschner H, George E. 1990. Effect of VA mycorrhizal fungi and rhizosphere
544 microorganisms on root and shoot morphology, growth and water relations in maize. *New*
545 *Phytologist* 116:303-311.
546 Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson SJ, Laughlin DC. 2016.
547 Root traits are multidimensional: specific root length is independent from root tissue density
548 and the plant economic spectrum. *Journal of Ecology* doi:10.1111/1365-2745.12562.
549 Kuznetsova A, Brockhoff P, Christensen R. 2014. *LmerTest: tests for random and fixed effects*
550 *for linear mixed effect models*. R package, version 2.0-3.
551 Labbé JL, Weston DJ, Dunkirk N, Pelletier DA, Tuskan GA. 2014. Newly identified helper
552 bacteria stimulate ectomycorrhizal formation in *Populus*. *Frontiers of Plant Science* 5:579.
553 doi: 10.3389/fpls.2014.00579.
554 Lau JA, Lennon JT. 2011. Evolutionary ecology of plant–microbe interactions: soil microbial
555 structure alters selection on plant traits. *New Phytologist* 192:215-224.
556 Lau JA, Lennon JT. 2012. Rapid responses of soil microorganisms improve plant fitness in novel
557 environments. *Proceedings of the National Academy of Sciences* 109:14058-62.
558 Lebeis SL. 2015. Greater than the sum of their parts: characterizing plant microbiomes at the
559 community-level. *Current Opinion in Plant Biology* 24:82-86.

560 Lugtenberg B, Kamilova F. 2009. Plant-Growth-Promoting Rhizobacteria. *Annual Review of*
561 *Microbiology* 63:541-556.
562 Ma W, Sebestianova S, Sebestian J, Burd G, Guinel F, Glick B. 2003. Prevalence of 1-
563 aminocyclopropane-1-carboxylate deaminase in *Rhizobium* spp. *Antonie van Leeuwenhoek*
564 83:285-291.
565 Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco
566 tissue cultures. *Physiologia Plantarum* 15:473-497.
567 Reich PB. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto.
568 *Journal of Ecology* 102:275-301.
569 Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD. 1999.
570 Generality of leaf trait relationships: a test across six biomes. *Ecology.* 80:1955-1969.
571 Romano CP, Cooper ML, Klee HJ. 1993. Uncoupling auxin and ethylene effects in transgenic
572 tobacco and arabidopsis plants. *Plant Cell* 5:181-189.
573 Rousseau J, Sylvia D, Fox A. 1994. Contribution of ectomycorrhiza to the potential nutrient-
574 absorbing surface of pine. *New Phytol*ogist 128:639.
575 Schlichting CD. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of*
576 *Ecology, Evolution, and Systematics* 17:667-693.
577 Siddiqui IA, Shaukat SS. 2003. Plant species, host age and host genotype effects on *Meloidogyne*
578 *incognita* biocontrol by *Pseudomonas fluorescens* strain CHA0 and its genetically-modified
579 derivatives. *Journal of Phytopathology.* 151:231-238.
580 Slovak R, Ogura T, Satbhai SB, Ristova D, Busch W. 2015. Genetic control of root growth: from
581 genes to networks. *Annals of Botany*. doi: 10.1093/aob/mcv160.

582 Stearns JC, Woody OZ, McConkey BJ, Glick BR. 2012. Effects of bacterial ACC deaminase on
583 *Brassica napus* gene expression. *Molecular Plant Microbe Interactions*. 25:668-676. doi:
584 10.1094/MPMI-08-11-0213.
585 Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in*
586 *Plant Science* 5:537-542.
587 Tan G, Tan W. 1986. Interaction between alfalfa cultivars and *Rhizobium* strains for nitrogen
588 fixation. *Theoretical and Applied Genetics* 71:724-729.
589 Timm CM, Campbell AG, Utturkar, SM, Jun SR, Parales RE, Tan W, Robeson MS, Lu TYS,
590 Jawdy S, Brown SD, Ussery DW, Schadt CW, Tuskan GA, Doktycz MJ, Weston DJ, Pelletier
591 DA. 2015. Metabolic functions of *Pseudomonas fluorescens* strains from *Populus deltoides*
592 depend on rhizosphere or endosphere isolation compartment. *Frontiers in Microbiology* 6.
593 doi: 10.3389/fmicb.2015.01118.
594 Timm CM, Pelletier DA, Jawdy S, Gunter LE, Henning JA, Engle N, Aufrecht J, Gee E,
595 Nookaew I, Yang Z, Lu TYS, Tschaplinksi TJ, Doktycz MJ, Tuskan GA, Weston DJ. 2016.
596 Two poplar-associated bacterial isolates induce additive favorable responses in a constructed
597 plant-microbiome system. *Frontiers in Plant Science* 7. doi: 10.3389/fpls.2016.00497.
598 Utturkar SM, Klingeman DM, Land ML, Schadt CW, Doktycz MJ, Pelletier DA, Brown SD.
599 2014. Evaluation and validation of de novo and hybrid assembly techniques to derive high-
600 quality genome sequences. *Bioinformatics.* 30:2709-2716.
601 Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of
602 the microbiome of the plant holobiont. *New Phytologist* 206:1196-1206.

603 Verhagen BWM, Glazebrook J, Zhu T, Chang HS, van Loon LC, Pieterse CMJ. 2004. The
604 transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Molecular Plant*
605 *Microbe Interactions* 17:895-908.
606 Wagner MR, Lundberg DS, Coleman-Derr D, Tringe SG, Dangl JL, Mitchell-Olds T. 2014.
607 Natural soil microbes alter flowering phenology and the intensity of selection on flowering
608 time in a wild Arabidopsis relative. *Ecology Letters* 17:717-726.
609 Walker V, Bertrand C, Bellvert F, Moënne-Loccoz Y, Bally R, Comte G. 2011. Host plant
610 secondary metabolite profiling shows a complex, strain-dependent response of maize to plant
611 growth-promoting rhizobacteria of the genus *Azospirillum*. *New Phytologist* 189: 494-506.
612 Wang Y, Ohara Y, Nakayashiki H, Tosa Y, Mayama S. 2005. Microarray analysis of the gene
613 expression profile induced by the endophytic plant growth-promoting rhizobacteria,
614 *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Molecular Plant Microbe Interactions*
615 18:385-396.
616 Wasternack C, Parthier B. 1997. Jasmonate-signalled plant gene expression. *Trends in Plant*
617 *Science* 2: 302-307.
618 Weston DJ, Pelletier DA, Morrell-Falvey JL, Tschaplinski TJ, Jawdy SS, Lu TYS, Allen SM,
619 Melton SJ, Martin MZ, Schadt CW, Karve AA, Chen JG, Yang X, Doktycz MJ, Tuskan GA.
620 2012. *Pseudomonas fluorescens* induces strain-dependent and strain-independent host plant
621 responses in defense networks, primary metabolism, photosynthesis, and fitness. *Molecular*
622 *Plant Microbe Interactions* 25:765-778.
623 Wright IJ, Reich, PB, Westoby M, Ackerly DD, Baruch Z. 2004. The worldwide leaf economics
624 spectrum. *Nature.* 428:821-827.

625 Yang J, Kloepper JW, Ryu C-M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress.
626 *Trends in Plant Science*. 14:1-4.
627 Yang SF, Hoffman NE. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual*
628 *Review of Plant Physiology and Plant Molecular Biology* 35:155-189.
629 Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CMJ. 2013. Unraveling Root
630 Developmental Programs Initiated by Beneficial *Pseudomonas* spp. Bacteria. *Plant*
631 *Phys*iology 162:304-318.
632 Zou J, Rodriguez-Zas S, Aldea M, L, M, Zhu J, Gonzalez DO, Vodkin LO, DeLucia E, Clough
633 SJ. 2005. Expression Profiling Soybean Response to *Pseudomonas syringae* Reveals New
634 Defense-Related Genes and Rapid HR-Specific Downregulation of Photosynthesis. *Molecular*
635 *Plant Microbe Interactions* 18:1161-1168.

Table 1(on next page)

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.

1

	***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

2

Table 2 (on next page)

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).

1

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					

2

3

Figure 1

Photo of the experimental design

Photo of the experimental design. We sterilized 150cc of calcined clay in two magenta boxes, added microbial inoculum, planted a single *Populus* clone within each, and connected the boxes with a coupler to make an enclosed system. The experimental setup was four treatments by eight replicates in size. A filtered port was added to the magenta boxes to allow for watering *Populus* clones during the experiment.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

Figure 2

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.

Structural traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) (n = 8) (white) or 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 senescenced during the experiment. GM30 inoculation increased new leaf production by 35% relative to control plants. b) GM30 and GM41 inoculation increased leaf growth rates by 114% and 1138%, respectively and relative to control plants, c) There were no changes in stem elongation rate with bacterial inoculation. d) GM30 increased root growth rates by 184%, relative to control plants. Letters represent significant differences of post-hoc least squares means among bacterial treatments.

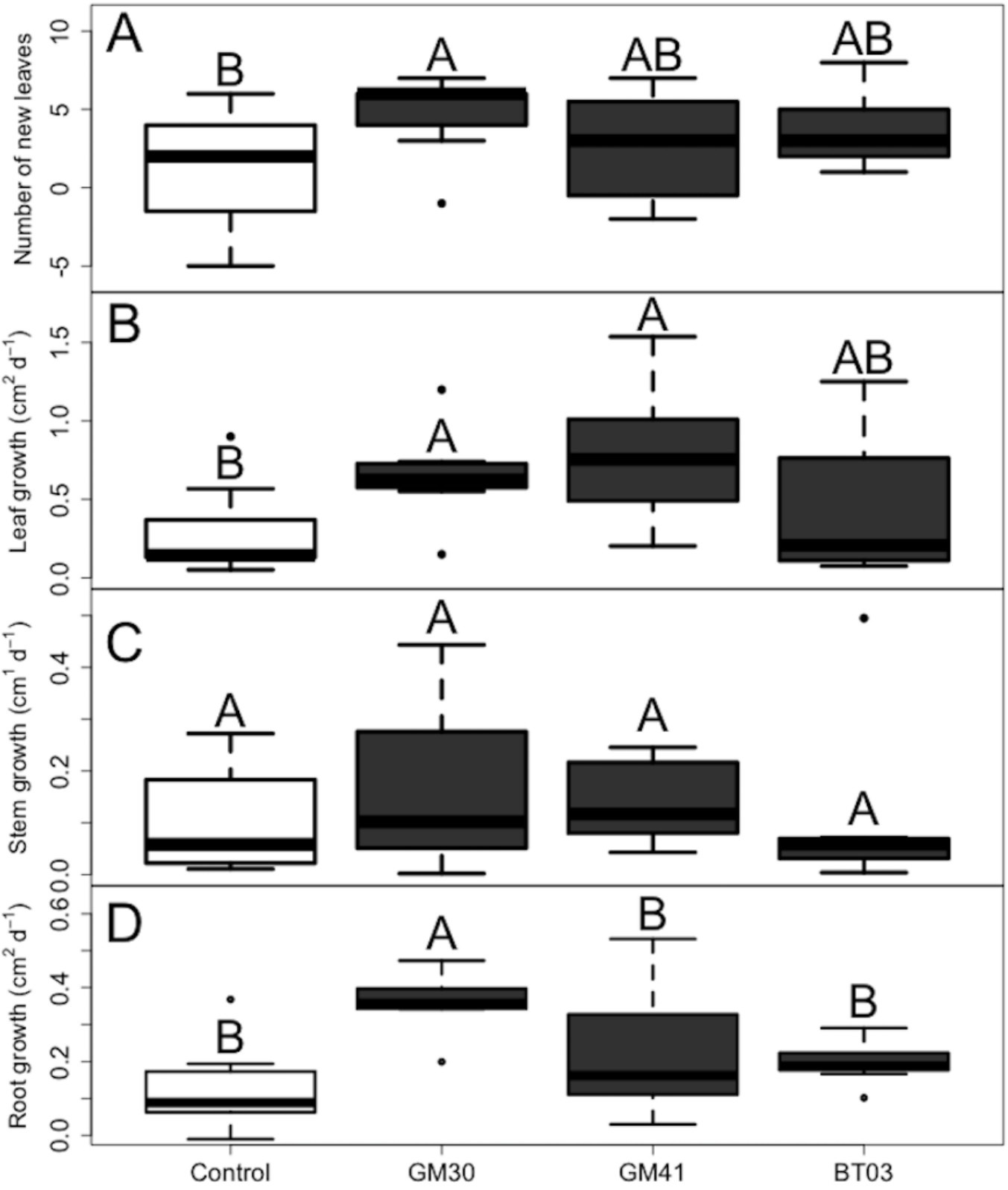

A
B
A
AB
AB
Number of new leaves
-5
0
5
10
B
B
A
A
AB
Leaf growth (cm^2 d^{-1})
0.0
0.5
1.0
1.5
C
A
A
A
A
Stem growth (cm^1 d^{-1})
0.0
0.2
0.4
D
B
A
B
B
Root growth (cm^2 d^{-1})
0.0
0.2
0.4
0.6
Control
GM30
GM41
BT03

Figure 3

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.

Biomass allocation of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) (n = 8) (white) or were inoculated with *Pseudomonas* GM30 (n = 7), *Pseudomonas* GM41 (n = 8), or *Burkholderia* BT03 (n = 7). a) Root entophytic bacteria inoculation does not alter plant total dry mass. b) Inoculation of GM30 increases leaf weight by 86%. c) Bacterial inoculation does not alter dry root weight. Letters represent significant differences of post-hoc least squares means among bacterial treatments.

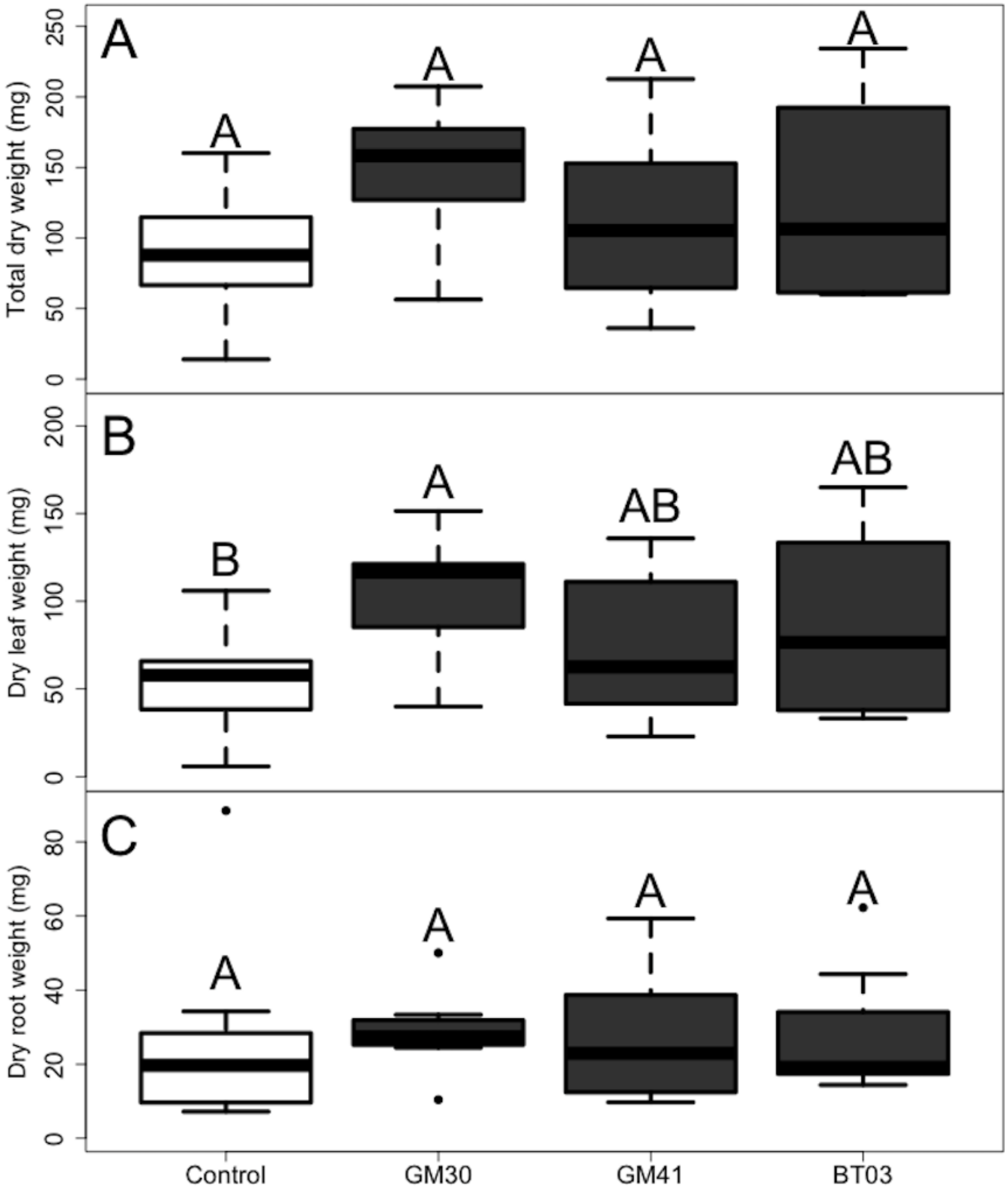
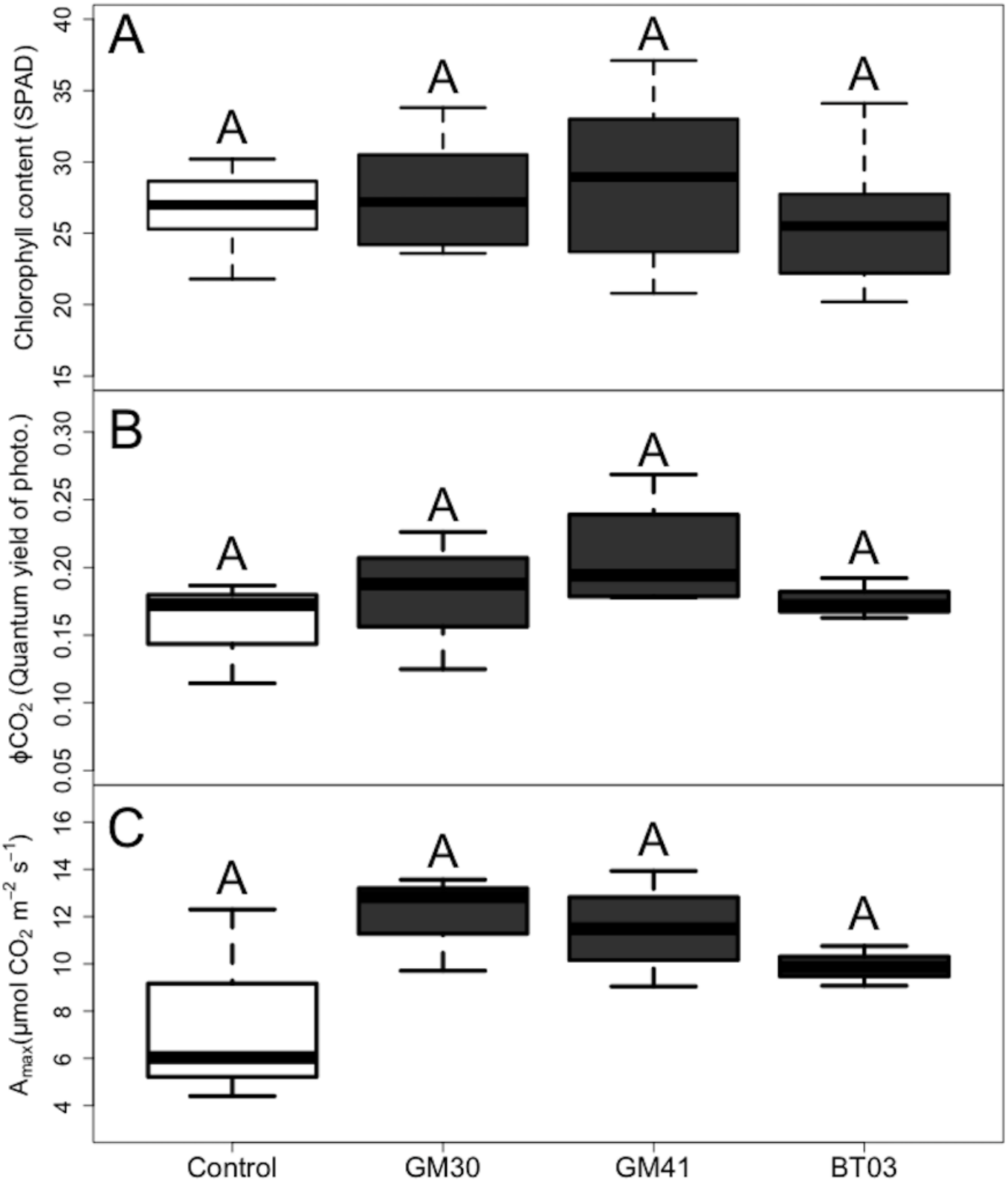

A
Total dry weight (mg)
0
50
100
150
200
250
A
A
A
A
B
Dry leaf weight (mg)
0
50
100
150
200
B
A
AB
AB
C
Dry root weight (mg)
0
20
40
60
80
A
A
A
A
Control
GM30
GM41
BT03

Figure 4

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.

Physiology traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) (n = 8) (white) or were inoculated with *Pseudomonas* GM30 (n = 7), *Pseudomonas* GM41 (n = 8), or *Burkholderia* BT03 (n = 7). Across all treatments there were no significant differences in: 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}).


A
B
C
Chlorophyll content (SPAD)
ϕCO2 (Quantum yield of photo.)
Amax(μmol CO2 m-2 s-1)
Control
GM30
GM41
BT03