



Root bacterial endophytes alter plant phenotype, but not physiology

Henning, Jeremiah A.; Weston, David J.; Pelletier, Dale A.; Timm, Collin M.; Jawdy, Sara S.; Classen, Aimee Taylor

Published in:
PeerJ

DOI:
[10.7717/peerj.2606](https://doi.org/10.7717/peerj.2606)

Publication date:
2016

Document version
Publisher's PDF, also known as Version of record

Document license:
[CC BY](https://creativecommons.org/licenses/by/4.0/)

Citation for published version (APA):
Henning, J. A., Weston, D. J., Pelletier, D. A., Timm, C. M., Jawdy, S. S., & Classen, A. T. (2016). Root bacterial endophytes alter plant phenotype, but not physiology. *PeerJ*, 4, [e2606]. <https://doi.org/10.7717/peerj.2606>

Root bacterial endophytes alter plant phenotype, but not physiology

Jeremiah A. Henning¹, David J. Weston², Dale A. Pelletier²,
Collin M. Timm³, Sara S. Jawdy² and 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 Center for Macroecology, Evolution, and Climate, The Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

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.

Submitted 27 June 2016
Accepted 24 September 2016
Published 1 November 2016

Corresponding author
Jeremiah A. Henning,
jhennin2@vols.utk.edu

Academic editor
David Day

Additional Information and
Declarations can be found on
page 15

DOI 10.7717/peerj.2606

© Copyright
2016 Henning et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

Subjects Ecology, Plant Science

Keywords Bacterial endophytes, *Burkholderia*, Plant functional traits, *Populus trichocarpa*, *Pseudomonas fluorescens*, Trait plasticity, Plant morphology

INTRODUCTION

A recent review exploring microbiome-mediated plant traits found that plant-associated microbes can modify 14 out of 30 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 phenotype (Zamioudis et al., 2013; 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., 2013; 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; Vandenkoornhuysen et al., 2015; Timm et al., 2015; Timm et al., 2016*) and have been the subject of multiple recent reviews (*Friesen et al., 2011; Friesen, 2013; Vandenkoornhuysen 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 response of plant traits and overall plant phenotype to microbial strains remains a research gap.

Here, we inoculated three endophytic bacterial strains (*Pseudomonas fluorescens* GM41, *Pseudomonas fluorescens* GM30, and *Burkholderia* sp. BT03), originally isolated from wild *Populus*, on a single genotype of *Populus trichocarpa* and measured plant phenotypic response to bacterial inoculation. We measured a suite of traits commonly measured in the functional trait ecology literature to explore how phenotype is influenced by bacterial strains within the pre-existing functional trait framework. Plant functional trait ecology has largely ignored microbiome contribution to plant phenotype. 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* strains allows us to explore how plant traits and overall phenotype respond to closely related bacterial strain genomes. 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 *Burkholderia*.

We predicted that aboveground and belowground traits of *Populus trichocarpa* would respond to *Burkholderia* and *Pseudomonas* strains and inoculation of different bacterial strains would result in different plant phenotypes. 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 clusters of orthologous groups (COG) 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 response to bacterial endophytes without measuring the 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” (Labbé *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. We sterilized shoot tips by soaking fresh cut tips 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 them 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, USA) 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% PPMTM (plant protective mixture) (Plant Cell Technology, Washington, DC, USA), 0.5% activated charcoal (Sigma-Aldrich, St. Louis, MO, USA), and 0.15% Gelzan (Plantmedia, bioWORLD, Dublin, OH, USA). Plants were sub-cultured until it was determined, using microscopy and colony formation units with R2A medium, that the plants were axenic.

Plant cultures were rooted in a growth room at 25 °C under a 16 h photoperiod. After root establishment, plants that were similar in size and developmental stage were selected for experimentation. Plants were weighed and scanned to account for initial plant size differences among treatments. To ensure sterility during scanning, plants were placed between two (21.59 × 27.94 cm) sheets of cellulose acetate that were sprayed with 100% ethanol. Scans were performed with a portable scanner (VuPoint Solutions Inc., City of Industry, CA, USA) at 600 × 600 dpi. Scanned images were analyzed in WinRhizo (Regent Instruments, Quebec City, Canada) to determine initial root surface area, root length, stem length, and leaf surface area. After scanning, plants were transferred into experimental microcosms.

Experimental design

We constructed closed microcosms by interlocking two sterile Magenta boxes (Sigma-Aldrich, St. Louis, MO, USA) with a coupler (Sigma-Aldrich, St. Louis, MO, USA). We added 150 ml calcined clay (Pro’s choice Sports Field Products, Chicago, IL, USA) and 70 ml of 1× Hoagland’s nutrient solution (Sigma-Aldrich, St. Louis, MO, USA) to each microcosm. We drilled two 7 mm holes on adjacent sides of the upper magenta box and covered the holes with adhesive microfiltration discs (Tissue Quick Plant Laboratories, Hampshire, United Kingdom) to allow air to flow into and out of the

microcosms and to prevent outside microbial contamination. Prior to microbial addition, we double sterilized each closed microcosm by autoclaving on a 60 m dry cycle on consecutive days. *Pseudomonas fluorescens* strains (GM30 and GM41) and *Burkholderia* sp. (BT03), hereafter termed *Pseudomonas* GM30, *Pseudomonas* GM41, and *Burkholderia* BT03 were isolated from *Populus deltoides* endospheres from east Tennessee and western North Carolina, USA (originally described in [Brown et al., 2012](#)). For full isolate descriptions, see [Brown et al. \(2012\)](#), [Weston et al. \(2012\)](#), [Utturkar et al. \(2014\)](#), [Timm et al. \(2015\)](#) and [Timm et al. \(2016\)](#). We selected these three strains because previous work (*Pseudomonas* GM30–[Weston et al., 2012](#); [Labbé et al., 2014](#); *Pseudomonas* GM41–[Labbé et al., 2014](#); [Timm et al., 2016](#); *Burkholderia* Bt03–[Timm et al., 2016](#)) had given us indication that strains were able to influence traits in *Arabidopsis thaliana* ([Weston et al., 2012](#)), were able to manipulate plant gene expression and hormonal signaling in *P. deltoides* ([Timm et al., 2015](#); [Timm et al., 2016](#)), and were able to influence host interactions with mycorrhizal symbionts ([Labbé et al., 2014](#)). Although strains were isolated from *P. deltoides*, strains from *Pseudomonas* and *Burkholderia* readily colonize natural *P. trichocarpa* tissues ([Moore et al., 2006](#); [Xin et al., 2009](#); [Knott et al., 2014](#); [Khan et al., 2014](#); [Doty et al., 2016](#)). We grew bacterial strains in isolation and at a constant temperature, 25 °C, in 5 ml of R2A medium. After growing overnight they were pelleted and re-suspended in sterile water to an OD600 of 0.01 ($\sim 1.0 \times 10^7$ cells ml⁻¹).

We inoculated each microcosm by adding 10 ml of the bacterial strain (10^7 cells ml⁻¹) to the calcined clay substrate and stirring for 30 s to distribute the bacteria. After inoculation, we planted the *Populus* clones within each microcosm. Each *Populus* was grown in an individual microcosm in combination with one of the bacterial strains. Thus, the experiment had four treatment combinations—*Pseudomonas* GM30 inoculation, *Pseudomonas* GM41 inoculation, *Burkholderia* BT03 inoculation, and a bacteria-free control. In total, there were 32 microcosms with four treatments (n = 8). The experiment was divided into three different establishment dates in 2014 (1 March, three replicated blocks; 25 March, two replicated blocks; and 2 April, three replicated blocks) because microbiome-free plant tissues were difficult to propagate. Plant-bacteria combinations were grown in the microcosms for five weeks with a 16 h photoperiod, at 21 °C and 80% relative humidity.

After 35 days of growth, plants were removed from microcosms, submerged in sterilized deionized H₂O to remove clay from the root system, weighed, and scanned. Scans were analyzed with WinRhizo to determine final 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 cm² d⁻¹). Additionally, each plant was dried for 48 h 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 host physiological response to different bacterial strains, 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, NE, USA) immediately prior to our experimental harvest. The maximum rate of photosynthesis in saturating light under ambient CO_2 (A_{sat}), the maximum rate of photosynthesis in saturating light and saturating CO_2 (A_{max}), 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).

Comparative genomics of microbes

Genomes of *Pseudomonas* GM30 and GM41 and *Burkholderia* BT03 were sequenced at Oak Ridge National Laboratory and genes were identified using Prodigal ([Brown et al., 2012](#); [Utturkar et al., 2014](#)) and are available at NCBI (GM41: [AKJN000000000.2](#); GM30: [NZ_AKJP000000000.2](#); BT03: [NZ_AKKD000000000.2](#)). Genome annotation, genomes statistics, and annotation comparisons were performed using IMG tools (<https://img.jgi.doe.gov/>). Genome statistics and COG functional predictions were extracted from Integrated Microbial Genomes (<https://img.jgi.doe.gov/>) and then they were compared manually for differential inclusion of predicted functions.

Bacterial colonization

To test for endophytic colonization of *Pseudomonas* GM41, *Pseudomonas* GM30, and *Burkholderia* BT03, we planted cuttings of *P. trichocarpa* into a magenta box using similar methodology and treatments described above ($n = 3$). After two weeks of growth, all the plant roots, stems, and 1–2 mature leaves were surface sterilized by dipping them in a ~10% bleach solution, followed by 70% ethanol, and then rinsing in water three times. We recorded wet weight of plant tissues and then separately macerated each plant tissue compartment in a sterile mortar and pestle in 1 ml sterile $1\times$ PBS. We transferred macerated plant tissues to a 24-well plate where we serial diluted each sample by 10% with $1\times$ PBS at $1\times$, $0.1\times$, $0.01\times$ of original sample concentration. Each sample was streaked onto R2A media plates and allowed to grow for 48 h at 20°C . After 48 h, colony formation was counted. We calculated CFU mg^{-1} of plant tissue by multiplying colony number per plate by $10^{(\text{dilution factor} + 1)}$ and then dividing that number by the dry tissue mass (mg^1).

Statistical analyses

We tested all data for normality using the *normalTest* function in the fBasics package (version 3011.87; [R Metrics Core Team, 2014](#)) for R version 3.0.2 ([R Development Core Team, 2013](#)) and RStudio version 0.98.495 ([RStudio Team, 2013](#)). If data were not normally distributed, we performed log transformations or square-root transformations to satisfy the normality assumptions of analysis of variance (ANOVA).

To explore plant trait response (root dry mass, leaf dry mass, shoot dry mass, total dry mass, root: shoot, root growth rates, leaf growth rates, change in leaf number, specific root length, specific leaf area) to bacterial strains, we used linear mixed-effect models using the lme4 package in R ([Bates et al., 2015](#)). Bacterial strain was a fixed effect in the model and experimental block (three establishment dates) was a random factor. For plant

dry mass measures, we incorporated initial measurements of root surface area in the root dry mass model and initial leaf surface area in the aboveground dry mass model as covariates. To test for significance of bacterial strain (fixed effects) and covariate (initial growth measure) we performed a likelihood ratio test to compare models with and without fixed effects and covariates. If including fixed factors (bacterial strain) was significant an improvement to model fit ($p < 0.05$ in likelihood ratio test), we calculated least square means and confidence intervals using the *diffsmeans* function to calculate differences among strains using the *lmerTest* package version 2.0-3 (Kuznetsova, Brockhoff & Christensen, 2014). We measured host response to bacterial inoculation by calculating the percent change in trait values ((mean trait value for *Populus* inoculated with bacterial strain – mean non-inoculated trait value) $\times 100$).

To test physiological responses (carboxylase activity, A_{\max} , A_{sat}) of plant hosts to bacterial inoculation, we used one-way ANOVA using the *Anova* function in the *CAR* package, (version 2.0-22, Fox & Weisberg, 2011) because we collected physiology data on only a single sampling date ($n = 3$). All raw data and R code is available in Tables S2 and S3, respectively.

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, salicylate and ethylene (Table 1). Relative to the *Pseudomonas* genomes, the *Burkholderia* genome encoded for numerous secondary metabolite biosynthesis pathways and more carbohydrate and lipid transporters, suggesting increased metabolic capabilities within *Burkholderia* (Table 1).

Even though *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 strains. The genome of *Pseudomonas* GM41 encoded for phosphorus solubilization and nitrate reduction, which were lacking in the *Pseudomonas* GM30 genome. Additionally, *Pseudomonas* GM41 contained more secondary metabolite biosynthesis elements compared to *Pseudomonas* GM30. We also found that the genome of *Pseudomonas* GM41 contained more genes coding for carbohydrate metabolism, lipid metabolism, and amino acid transport and metabolism, energy production and conversion, suggesting that *Pseudomonas* GM41 may contain more metabolic breadth than *Pseudomonas* GM30

Table 1 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.

	<i>Burkholderia</i> BT03	<i>Pseudomonas</i> GM30	<i>Pseudomonas</i> 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

Note:

NA = not applicable.

(Table 1). Taken together, our results demonstrated that these three bacterial strains differ in genome size and their functional gene content.

Bacterial colonization of *Populus* root tissue

All three of the bacterial strains colonized *Populus* hosts. Colony-forming units were enriched in all three bacterial strains relative to the control in the 0.1× and 0.01× dilutions (0.1× dilution $F = 18.77$, $p < 0.0001$; 0.01× dilution $F = 13.78$, $p < 0.0001$, Table 2), although CFU number was variable across dilutions, tissue types, and bacterial strain. However, we found no difference in CFUs among non-inoculated control and *Pseudomonas* GM30, GM41, and *Burkholderia* BT03 inoculated host plants at the 1× dilution ($F = 1.24$, $p = 0.319$, Table 2). Across nearly all tissue types, we found that *Pseudomonas* GM30, *Pseudomonas* GM41, and *Burkholderia* BT03 inoculated plants had 10–10,000× more CFUs than did non-inoculated control plants (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). Inoculated host plants contained 0–28,809,015 CFU mg⁻¹ in roots, 0–1,166,273 CFU mg⁻¹ in stems, and 0–73,537 CFU mg⁻¹ in leaves compared to 0–400 CFU mg⁻¹ in root tissues, 0 CFU mg⁻¹ in stem tissue, 0–1,000 CFU mg⁻¹ in leaf tissue compared to non-inoculated control plants (Table 2).

Plant structure is modified by bacterial inoculation

Overall, we found that plant trait response to bacterial endophytes was strain specific. Specifically, mean root growth rate increased 184% with *Pseudomonas* GM30 colonization ($t = 3.84$, $p = 0.001$), however root growth rates were unaffected by *Pseudomonas* GM41

Table 2 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	1,080.5	1,871.5	Bact.	1.3E + 12	3	1.24	0.319
GM30	Leaf	1.0E + 01	19,574.7	30,672.7	Tissue	1.4E + 12	2	1.92	0.1699
GM41	Leaf	1.0E + 01	1,141.3	1,809.3	B × T	1.6E + 12	6	0.74	0.6264
BT03	Leaf	1.0E + 01	41,175.9	45,063.1	Resid.	8.1E + 12	24		
Control	Root	1.0E + 01	110.2	131.5					
GM30	Root	1.0E + 01	170,447.1	212,977.7					
GM41	Root	1.0E + 01	2,438.9	1,563.8					
BT03	Root	1.0E + 01	309,628.0	106,958.6					
Control	Stem	1.0E + 01	0.0	0.0					
GM30	Stem	1.0E + 01	1,166,273.0	1,872,593.0					
GM41	Stem	1.0E + 01	1,510.2	2,135.8					
BT03	Stem	1.0E + 01	654,513.2	688,365.7					
Control	Leaf	1.0E – 01	1,044.4	1,809.0	Bact.	1.2E + 13	3	18.77	> 0.001
GM30	Leaf	1.0E – 01	16,643.8	28,827.9	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	60,745.9	54,910.0	Resid.	5.0E + 12	24		
Control	Root	1.0E – 01	402.7	377.6					
GM30	Root	1.0E – 01	120,591.5	111,174.4					
GM41	Root	1.0E – 01	2,851.9	3,319.7					
BT03	Root	1.0E – 01	3,096,279.7	1,069,585.6					
Control	Stem	1.0E – 01	0.0	0.0					
GM30	Stem	1.0E – 01	289,189.7	330,089.7					
GM41	Stem	1.0E – 01	0.0	0.0					
BT03	Stem	1.0E – 01	904,314.7	1,099,508.6					
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	73,537.1	80,004.4	Resid.	4.1E + 14	24		
Control	Root	1.0E – 02	0.0	0.0					
GM30	Root	1.0E – 02	368,195.0	510,398.0					
GM41	Root	1.0E – 02	20,595.2	35,671.9					
BT03	Root	1.0E – 02	28,809,015.5	14,126,689.6					
Control	Stem	1.0E – 02	0.0	0.0					
GM30	Stem	1.0E – 02	227,127.9	252,544.5					
GM41	Stem	1.0E – 02	0.0	0.0					
BT03	Stem	1.0E – 02	1,805,855.2	1,567,125.7					

($t = 1.61$, $p = 0.12$), and *Burkholderia* BT03 ($t = 1.18$, $p = 0.25$) inoculation (Fig. 1; Table S1). Similarly, mean leaf growth rate increased 114 and 138% with *Pseudomonas* GM30 ($t = 2.27$, $p = 0.03$) and *Pseudomonas* GM41 ($t = 2.86$, $p = 0.01$) inoculation,

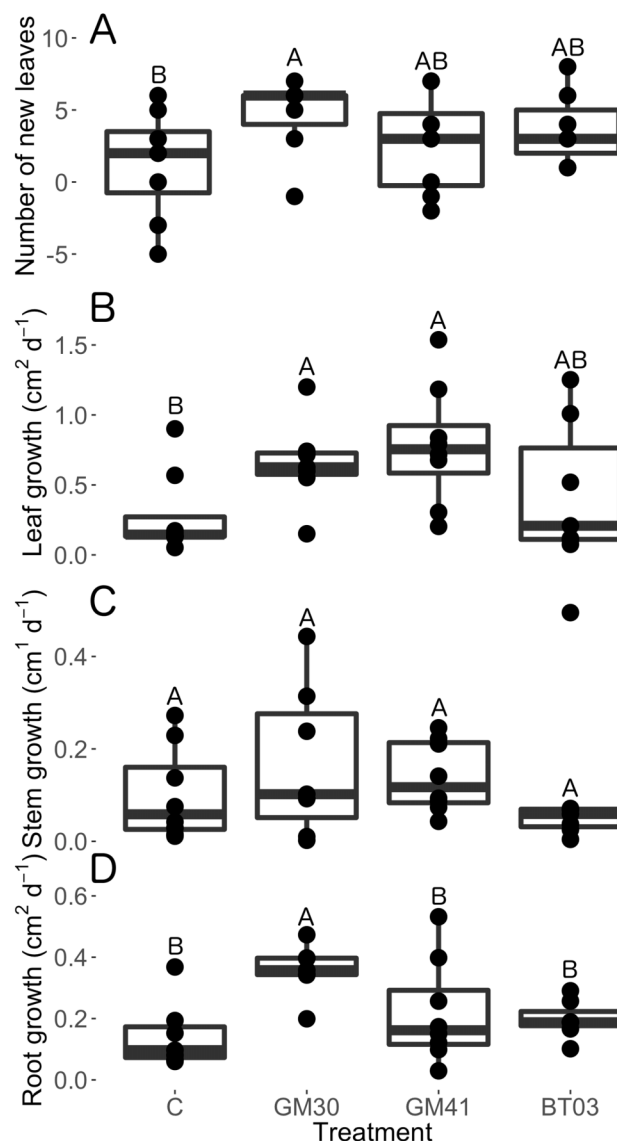


Figure 1 Structural traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) ($n = 8$), were inoculated with *Pseudomonas* GM30 ($n = 7$), *Pseudomonas* GM41 ($n = 8$), or *Burkholderia* BT03 ($n = 7$). (A) Change in leaf number from the first to last day of the experiment.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

but leaf growth rate was unaffected by *Burkholderia* inoculation ($t = 1.02$, $p = 0.32$) (Fig. 1; Table S1). Inoculation by *Pseudomonas* GM30 increased leaf number by 36% ($t = 3.34$, $p = 0.003$) but leaf number was unaffected by *Pseudomonas* GM41 ($t = 0.93$, $p = 0.36$) and *Burkholderia* BT03 ($t = 1.418$, $p = 0.17$) inoculation (Fig. 1). We observed no differences in stem elongation with bacterial inoculation (chisq = 0.06, $p = 0.97$, Table S1).

Interestingly, we observed no differences in total plant dry mass (chisq = 3.27, $p = 0.195$, Fig. 2), root dry mass (chisq = 0.00, $p = 1.00$, Fig. 2), root:shoot ratio

(chisq = 0.00, $p = 1.00$, Table S1) or plant height (chisq = 1.99, $p = 0.158$, Table S1) with bacterial inoculation. However, *Pseudomonas* GM30 inoculation increased leaf dry biomass by 86% ($t = 2.43$, $p = 0.02$) relative to control plants, however leaf biomass was unaffected by *Pseudomonas* GM41 ($t = 0.97$, $p = 0.33$) and *Burkholderia* BT03 ($t = 1.70$, $p = 0.10$) (Fig. 2; Table S1). We observed no differences in specific leaf area with bacterial inoculation (chisq = 2.60, $p = 0.46$, Table S1). Thus, inoculation of *Pseudomonas* GM30 increased leaf surface area ($t = 2.27$, $p = 0.03$) and aboveground dry mass ($t = 2.43$, $p = 0.02$), without changing leaf area:mass ratios. We found no significant differences in root length:dry mass (specific root length, chisq = 1.06, $p = 0.79$) with bacterial inoculation (Table S1). Our results indicate that bacterial strains modify plant resource allocation but not total dry mass production.

Plant physiology is not affected by bacterial inoculation

Bacterial inoculation had no measureable effects on any physiological trait we measured: chlorophyll content (SPAD) (chisq = 2.15, $p = 0.54$), quantum yield of photosynthesis (ϕ) ($F = 1.01$, $p = 0.43$), net photosynthesis at saturating light conditions (A_{sat}) ($F = 0.76$, $p = 0.55$) or maximum net photosynthesis at saturating light and $[\text{CO}_2]$ (A_{max}) ($F = 1.98$, $p = 0.19$) (Fig. 3). In agreement with the total dry mass data, we did not observe significant changes in the measured photosynthetic parameters. Thus, changes in plant structure were not linked with increased photosynthetic capacity, efficiency, or carbon assimilation rates.

DISCUSSION

The plant root microbiome can have a strong influence on plant production and phenotype (Friesen, 2013; Vandenkoornhuysen et al., 2015); yet, less is known about how plant trait expression, production, and physiology are influenced by individual endophytic strains. We explored how plant morphological traits, productivity, and cellular physiology in *Populus trichocarpa* responded to inoculation with three bacterial strains, two closely related *Pseudomonas fluorescens* strains (GM30 & GM41) and a more distantly related *Burkholderia* strain (BT03). We selected bacterial strains that were predicted to differ in metabolic capabilities, plant hormone production and metabolism, and secondary metabolite synthesis in an effort to understand how plant phenotype is influenced by inoculation with different strains of common endophytic bacteria (Table 1, Timm et al., 2015; Timm et al., 2016). Our comparative genomic analysis revealed that while all three strains share many common endophytic functions like plant hormone signal disruption, production of plant hormone auxin, pili, flagella, and chemotaxis, strains potentially differed in their ability to perform these functions. Overall, we found that *Burkholderia* and *Pseudomonas* genomes differed in the carbon substrates they were predicted to degrade, plant hormone production and metabolism, and secondary metabolite synthesis, which led us to predict that plant response to bacterial inoculation would lead to different phenotypes between treatments. All three strains could colonize *Populus* roots, leaves, and stems; however, CFU number was highest within root tissues in all three strains (Table 2).

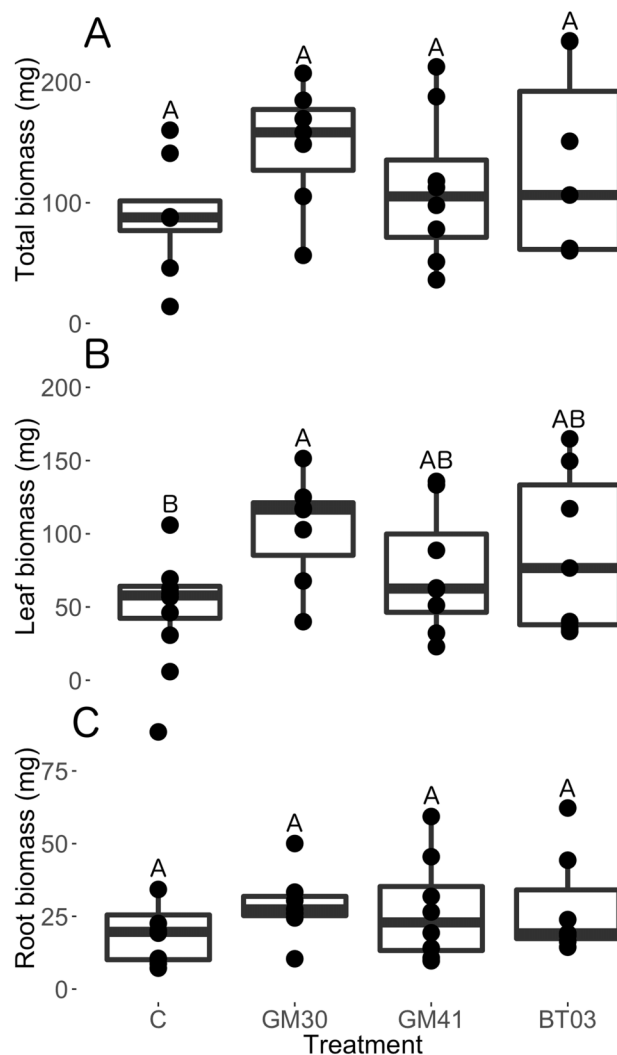


Figure 2 Biomass allocation of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) (n = 8), were inoculated with *Pseudomonas* GM30 (n = 7), *Pseudomonas* GM41 (n = 8), or *Burkholderia* BT03 (n = 7). (A) Total dry mass (mg). (B) Leaf biomass (mg), (C) Root biomass (mg). 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.

Overall, we found root endophyte inoculation altered plant resource allocation patterns without influencing total plant biomass accumulation (Fig. 1). Additionally, we found that plant trait response and overall phenotype differed across bacterial strains in ways that would not have been predicted from our genome analysis. Specifically, *Burkholderia* BT03 was predicted to produce auxin and to metabolize salicylate and ethylene, three plant hormones crucial to plant growth and development (see Yang & Hoffman, 1984; Wasternack & Parthier, 1997; Chen et al., 2009; Dempsey et al., 2011). Additionally, we found the *Burkholderia* genome encoded for multiple transposase elements that degrade poplar-produced aromatics and metabolites (Timm et al., 2015; Timm et al., 2016). Despite the predicted ability of *Burkholderia* to manipulate multiple plant hormonal and

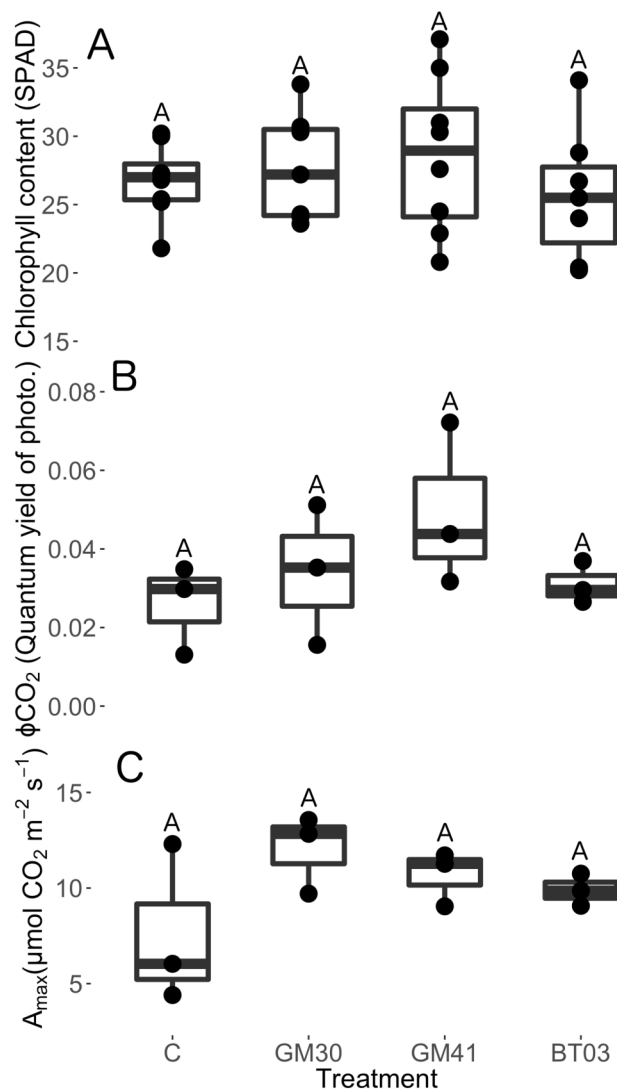


Figure 3 Physiology traits of *Populus trichocarpa* that were not inoculated with bacteria (no microbe control) ($n = 8$), were inoculated with *Pseudomonas* GM30 ($n = 7$), *Pseudomonas* GM41 ($n = 8$), or *Burkholderia* BT03 ($n = 7$). (A) Plant chlorophyll content (SPAD), (B) Φ_{CO_2} (expressed as 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 $1.5 \times$ inner quartile range of our dataset. The dots represent raw data values.

signaling pathways, we observed no measurable changes in any traits when *Populus* was inoculated with *Burkholderia* (Figs. 1–3). This was especially surprising since we consistently measured the highest CFU abundance within *Burkholderia* inoculated individuals (Table 2).

In spite of close genetic relatedness and classification under the same 16S OTU profile, our *Pseudomonas* strains differed in key functional capabilities. Specifically, *Pseudomonas* GM41 encoded for phosphate solubilization and denitrification ability, suggesting these two strains may differentially influence host nutrition, although this remains untested. Our genome analysis revealed that both strains were capable of producing the plant

hormone auxin, however another study found that *Pseudomonas* GM41 produced two times more auxin than *Pseudomonas* GM30 (Timm et al., 2015). Auxin synthesis by endophytic bacteria can increase root branching and lateral root formation and decrease overall plant height, leaf number, chlorophyll content and photosynthetic efficiency (Romano, Cooper & Klee, 1993; Fu & Harberd, 2003; Weston et al., 2012). Thus, we predicted that *Pseudomonas* GM41 would have a strong influence on plant root traits, however we observed no measurable effects of *Pseudomonas* GM41 inoculation on root growth rate or morphology (Fig. 1; Table S1). Belowground, *Pseudomonas* GM30 inoculation increased root surface area growth rate by 184% (Fig. 1) without increasing root biomass (Fig. 2), suggesting *Pseudomonas* GM30 inoculation may change root morphology, leading to longer, thinner, highly-branched roots with similar biomass, as we predicted. Similar patterns have been observed when *Pseudomonas* GM30 is inoculated on both *Arabidopsis* (Weston et al., 2012) and *Populus deltoides* (Timm et al., 2015; Timm et al., 2016). Additionally, inoculation of *Pseudomonas* GM30 increased leaf surface area growth rate by 114% (Fig. 1), leaf number by 36% (Fig. 1), and aboveground biomass by 86% (Fig. 2) but did not influence specific leaf area (Table S1), whereas closely-related *Pseudomonas* GM41 increased leaf surface area growth rate by 138% (Fig. 1) but did not change leaf number (Fig. 1) or aboveground biomass (Fig. 2). Unlike *Burkholderia*, *Pseudomonas* genomes do not contain the genes to directly metabolize salicylate, however inoculation of *Pseudomonas* GM41 can up-regulate salicylic acid synthesis and degradation in *Populus* (Timm et al., 2016). Taken together, our data suggest that predicting plant phenotypic response to bacterial inoculation, even in overly simplified systems using fully sequenced bacterial strains, is extremely difficult.

Contrary to our predictions, leaf physiology (Fig. 3), plant height (Table S1), root:shoot (Table S1), specific leaf area (Table S1), specific root length (Table S1), and total plant dry mass (Fig. 2) were not influenced by bacterial inoculation. It is possible that multiple, overlapping plant signaling and gene expression effects induced by bacterial endophyte inoculation may mask a hosts' phenotype response. For example, endophytes simultaneously up- and down-regulate numerous genes and metabolites in plant host (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 plant responses to endophyte inoculation when measuring down-stream phenotype and functional traits (Bashan, Holguin & de-Bashan, 2004; Timm et al., 2016). Additionally, host physiological response to endophyte inoculation may vary with bacterial strain (Kandasamy et al., 2009; Weston et al., 2012; Timm et al., 2016), plant host (Smith & Goodman, 1999), plant ontogeny (Siddiqui & Shaikat, 2003), or plant stress (Dimkpa, Weinand & Asch, 2009; Yang, Kloepper & Ryu, 2009; Lau & Lennon, 2012). For example, 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; Timm et al., 2016). Thus, biotic and abiotic contexts may drive the phenotypic response of hosts to endophyte inoculation, however this idea requires further testing.

Our study focused on the response of plant functional traits to monoculture associations of common endosphere bacteria, however future studies should focus on plant phenotype response to diverse microbiome communities. With a few well-known exceptions (*Tan & Tan, 1986; Harris, Pacovsky & Paul, 1985; Ma et al., 2003; Lau & Lennon, 2011; Lau & Lennon, 2012*), bacterial community composition in roots has been ignored in studies exploring what drives natural variation in plant traits (*Friesen et al., 2011; Friesen, 2013; Timm et al., 2016*). 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 natural trait variation—especially when conducted across a variety of environmental gradients. Second, once correlations between microbiome composition and plant traits are observed in the field, detailed work constructing communities in the lab and greenhouse would enable a mechanistic understanding of what is underlying the observed patterns. These studies could be especially fruitful when conducted across natural biotic and abiotic environmental gradients in the laboratory, greenhouse, and field settings (*Classen et al., 2015*).

CONCLUSIONS

Our study demonstrates that bacteria living in plant roots can influence plant morphological traits. Increasingly, ecologists are using plant functional traits to explore how changing environments alter plant function (*Wright et al., 2004; Reich, 2014*). Plant traits, such as specific leaf area and specific root length, are often significantly correlated with important plant functions such as carbon fixation and nutrient uptake (*Díaz & Cabido, 2001*). Researchers are using correlations between plant traits and function to extrapolate how plants and ecosystems will respond to global change (*Reich et al., 1999; Wright et al., 2004; Reich, 2014*). While interactions between plant genotype and environment undoubtedly influence plant phenotypic plasticity (*Bradshaw, 1965; Schlichting, 1986; Sultan, 2000; Des Marais, Hernandez & Juenger, 2013*), phenotype is also heavily influenced by biotic factors, like microbiome bacterial endophytes (*Lau & Lennon, 2011; Lau & Lennon, 2012; Wagner et al., 2014; Hacquard & Schadt, 2015*). Given that plant-microbial studies, including ours, have observed strong linkages between microbiome and plant phenotype (reviewed in *Friesen et al., 2011; Friesen, 2013*) interactions among global change drivers, plant genotypes, and plant microbiomes, should be considered in trait-based approaches to ecological questions (*Classen et al., 2015*).

ACKNOWLEDGEMENTS

We thank Lee Gunter, Tse-Yuan Lu, Kelsey Carter, Jesse Labbe, and W. Nathan Cude for their assistance in data collection and plant propagation. The Classen Ecosystem Ecology group and three reviewers provided valuable input on earlier reviews of this manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Funding from Plant-Microbe Interfaces Scientific Focus Area project at Oak Ridge National Laboratory was provided by the U.S. DOE Office of Biological and Environmental Research, Genomic Science Program. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the US Department of Energy under contract no. DEAC05-00OR22725. JH was supported, in part, by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Terrestrial Ecosystem Sciences Program under Award Number DE-SC0010562. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

U.S. DOE Office of Biological and Environmental Research, Genomic Science Program. UT-Battelle, LLC, for the US Department of Energy: DEAC05-00OR22725.
U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Terrestrial Ecosystem Sciences Program: DE-SC0010562.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Jeremiah A. Henning conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- David J. Weston conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Dale A. Pelletier conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Collin M. Timm performed the experiments, reviewed drafts of the paper.
- Sara S. Jawdy performed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Aimée T. Classen conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

Data Deposition

The following information was supplied regarding data availability:

The raw data has been supplied as [Supplemental Dataset Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.2606#supplemental-information>.

REFERENCES

- Bashan Y, Holguin G, de-Bashan LE. 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). *Canadian Journal of Microbiology* 50(8):521–577 DOI 10.1139/w04-035.
- Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67(1):1–48 DOI 10.18637/jss.v067.i01.
- Bisht R, Chaturvedi S, Srivastava R, Sharma AK, Johri BN. 2009. Effect of arbuscular mycorrhizal fungi, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* on the growth and nutrient status of *Dalbergia sissoo* Roxb. *Tropical Ecology* 50(2):231–242.
- Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13:115–155 DOI 10.1016/S0065-2660(08)60048-6.
- Brown SD, Utturkar SM, Klingeman DM, Johnson CM, Martin SL, Land ML, Lu TYS, Schadt CW, Doktycz MJ, Pelletier DA. 2012. Twenty-one genome sequences from *Pseudomonas* species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of *Populus deltoides*. *Journal of Bacteriology* 194(21):5991–5993 DOI 10.1128/JB.01243-12.
- Chen Z, Zheng Z, Huang J, Lai Z, Fan B. 2009. Biosynthesis of salicylic acid in plants. *Plant Signaling & Behavior* 4(6):493–496 DOI 10.4161/psb.4.6.8392.
- Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JAM, Cregger MA, Moorhead LC, Patterson CM. 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: what lies ahead? *Ecosphere* 6(8):1–21 DOI 10.1890/ES15-00217.1.
- Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51(4):335–380 DOI 10.1071/BT02124.
- Dempsey DMA, Vlot AC, Wildermuth MC, Klessig DF. 2011. Salicylic acid biosynthesis and metabolism. *The Arabidopsis Book* 9:e156 DOI 10.1199/tab.0156.
- Des Marais DL, Hernandez KM, Juenger TE. 2013. Genotype-by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. *Annual Review of Ecology, Evolution, and Systematics* 44(1):5–29 DOI 10.1146/annurev-ecolsys-110512-135806.
- Díaz S, Cabido M. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution* 16(11):646–655 DOI 10.1016/S0169-5347(01)02283-2.
- Dimkpa C, Weinand T, Asch F. 2009. Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell & Environment* 32(12):1682–1694 DOI 10.1111/j.1365-3040.2009.02028.x.
- Doty SL, Sher AW, Fleck ND, Khorasani M, Bumgarner RE, Khan Z, Ko AWK, Kim S-H, DeLuca TH. 2016. Variable nitrogen fixation in wild *Populus*. *PLoS ONE* 11(5):e155979 DOI 10.1371/journal.pone.0155979.
- Drogué B, Sanguin H, Chamam A, Mozar M, Llauro C, Panaud O, Prigent-Combaret C, Picault N, Wisniewski-Dyé F. 2014. Plant root transcriptome profiling reveals a strain-dependent response during *Azospirillum*-rice cooperation. *Frontiers in Plant Science* 5(356):607 DOI 10.3389/fpls.2014.00607.
- Fox J, Weisberg S. 2011. *An {R} Companion to Applied Regression*. Second edition. Thousand Oaks: Sage.

- Friesen ML. 2013.** Microbially mediated plant functional traits. In: de Bruijn FJ, ed. *Molecular Microbial Ecology of the Rhizosphere*. Hoboken: John Wiley and Sons, Inc., 87–102.
- Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. 2011.** Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics* **42**(1):23–46 DOI [10.1146/annurev-ecolsys-102710-145039](https://doi.org/10.1146/annurev-ecolsys-102710-145039).
- Fu X, Harberd NP. 2003.** Auxin promotes Arabidopsis root growth by modulating gibberellin response. *Nature* **421**(6924):740–743 DOI [10.1038/nature01387](https://doi.org/10.1038/nature01387).
- Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E, Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW. 2011.** Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. *Applied and Environmental Microbiology* **77**(17):5934–5944 DOI [10.1128/AEM.05255-11](https://doi.org/10.1128/AEM.05255-11).
- Hacquard S, Schadt CW. 2015.** Towards a holistic understanding of the beneficial interactions across the *Populus* microbiome. *New Phytologist* **205**(4):1424–1430 DOI [10.1111/nph.13133](https://doi.org/10.1111/nph.13133).
- Harris D, Pacovsky RS, Paul EA. 1985.** Carbon economy of soybean-Rhizobium-Glomus associations. *New Phytologist* **101**(3):427 DOI [10.1111/j.1469-8137.1985.tb02849.x](https://doi.org/10.1111/j.1469-8137.1985.tb02849.x).
- Jun S-R, Wassenaar TM, Nookaew I, Hauser L, Wanchai V, Land M, Timm CM, Lu TYS, Schadt CW, Doktycz MJ, Pelletier DA, Ussery DW. 2016.** Diversity of *Pseudomonas* genomes, including *Populus*-associated isolates, as revealed by comparative genome analysis. *Applied and Environmental Microbiology* **82**(1):375–383 DOI [10.1128/AEM.02612-15](https://doi.org/10.1128/AEM.02612-15).
- Kandasamy S, Loganathan K, Muthuraj R, Duraisamy S, Seetharaman S, Thiruvengadam R, Ponnusamy B, Ramasamy S. 2009.** Understanding the molecular basis of plant growth promotional effect of *Pseudomonas fluorescens* on rice through protein profiling. *Proteome Science* **7**(1):47 DOI [10.1186/1477-5956-7-47](https://doi.org/10.1186/1477-5956-7-47).
- Kang B, Osburn L, Kopsell D, Tuskan GA, Cheng Z-M. 2009.** Micropropagation of *Populus trichocarpa* ‘Nisqually-1’: the genotype deriving the *Populus* reference genome. *Plant Cell, Tissue and Organ Culture* **99**(3):251–257 DOI [10.1007/s11240-009-9596-9](https://doi.org/10.1007/s11240-009-9596-9).
- Khan Z, Roman D, Kintz T, delas Alas M, Yap R, Doty S. 2014.** Degradation, phytoprotection and phytoremediation of phenanthrene by endophyte *Pseudomonas putida*, PD1. *Environmental Science & Technology* **48**(20):12221–12228 DOI [10.1021/es503880t](https://doi.org/10.1021/es503880t).
- Kloepper JW, Leong J, Teintze M, Schroth MN. 1980.** Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**(5776):885–886 DOI [10.1038/286885a0](https://doi.org/10.1038/286885a0).
- Knoth JL, Kim S-H, Ettl GJ, Doty SL. 2014.** Biological nitrogen fixation and biomass accumulation within poplar clones as a result of inoculations with diazotrophic endophyte consortia. *New Phytologist* **201**(2):599–609 DOI [10.1111/nph.12536](https://doi.org/10.1111/nph.12536).
- Kuznetsova A, Brockhoff P, Christensen R. 2014.** lmerTest: tests for random and fixed effects for linear mixed effect models. R Package Version 2.0-3. Available at <https://CRAN.R-project.org/package=lmerTest>.
- Labbé JL, Weston DJ, Dunkirk N, Pelletier DA, Tuskan GA. 2014.** Newly identified helper bacteria stimulate ectomycorrhizal formation in *Populus*. *Frontiers of Plant Science* **5**:579 DOI [10.3389/fpls.2014.00579](https://doi.org/10.3389/fpls.2014.00579).
- Lau JA, Lennon JT. 2011.** Evolutionary ecology of plant-microbe interactions: soil microbial structure alters selection on plant traits. *New Phytologist* **192**(1):215–224 DOI [10.1111/j.1469-8137.2011.03790.x](https://doi.org/10.1111/j.1469-8137.2011.03790.x).

- Lau JA, Lennon JT. 2012.** Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences of the United States of America* **109**(35):14058–14062 DOI [10.1073/pnas.1202319109](https://doi.org/10.1073/pnas.1202319109).
- Lebeis SL. 2015.** Greater than the sum of their parts: characterizing plant microbiomes at the community-level. *Current Opinion in Plant Biology* **24**:82–86 DOI [10.1016/j.pbi.2015.02.004](https://doi.org/10.1016/j.pbi.2015.02.004).
- Lugtenberg B, Kamilova F. 2009.** Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* **63**(1):541–556 DOI [10.1146/annurev.micro.62.081307.162918](https://doi.org/10.1146/annurev.micro.62.081307.162918).
- Ma W, Sebastianova SB, Sebastian J, Burd GI, Guinel FC, Glick BR. 2003.** Prevalence of 1-aminocyclopropane-1-carboxylate deaminase in *Rhizobium* spp. *Antonie van Leeuwenhoek* **83**(3):285–291 DOI [10.1023/A:1023360919140](https://doi.org/10.1023/A:1023360919140).
- Moore FP, Barac T, Borremans B, Oeyen L, Vangronsveld J, van der Lelie D, Campbell CD, Moore ERB. 2006.** Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. *Systematic and Applied Microbiology* **29**(7):539–556 DOI [10.1016/j.syapm.2005.11.012](https://doi.org/10.1016/j.syapm.2005.11.012).
- Murashige T, Skoog F. 1962.** A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* **15**(3):473–497 DOI [10.1111/j.1399-3054.1962.tb08052.x](https://doi.org/10.1111/j.1399-3054.1962.tb08052.x).
- R Development Core Team. 2013.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at <https://www.R-project.org/>.
- Reich PB. 2014.** The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *Journal of Ecology* **102**(2):275–301 DOI [10.1111/1365-2745.12211](https://doi.org/10.1111/1365-2745.12211).
- Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD. 1999.** Generality of leaf trait relationships: a test across six biomes. *Ecology* **80**(6):1955–1969 DOI [10.1890/0012-9658\(1999\)080\[1955:GOLTRA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[1955:GOLTRA]2.0.CO;2).
- R Metrics Core Team. 2014.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at <https://www.R-project.org/>.
- Romano CP, Cooper ML, Klee HJ. 1993.** Uncoupling auxin and ethylene effects in transgenic tobacco and arabidopsis plants. *Plant Cell* **5**(2):181–189 DOI [10.1105/tpc.5.2.181](https://doi.org/10.1105/tpc.5.2.181).
- RStudio Team. 2013.** *RStudio: Integrated Development for R*. Boston: RStudio, Inc. Available at <http://www.rstudio.com/>.
- Schlichting CD. 1986.** The evolution of phenotypic plasticity in plants. *Annual Review of Ecology, Evolution, and Systematics* **17**(1):667–693 DOI [10.1146/annurev.es.17.110186.003315](https://doi.org/10.1146/annurev.es.17.110186.003315).
- Siddiqui IA, Shaikat SS. 2003.** Plant species, host age and host genotype effects on *Meloidogyne incognita* biocontrol by *Pseudomonas fluorescens* strain CHA0 and its genetically-modified derivatives. *Journal of Phytopathology* **151**(4):231–238 DOI [10.1046/j.1439-0434.2003.00716.x](https://doi.org/10.1046/j.1439-0434.2003.00716.x).
- Smith KP, Goodman RM. 1999.** Host variation for interactions with beneficial plant-associated microbes. *Annual Review of Phytopathology* **37**(1):473–491 DOI [10.1146/annurev.phyto.37.1.473](https://doi.org/10.1146/annurev.phyto.37.1.473).
- Stearns JC, Woody OZ, McConkey BJ, Glick BR. 2012.** Effects of bacterial ACC deaminase on *Brassica napus* gene expression. *Molecular Plant-Microbe Interactions* **25**(5):668–676 DOI [10.1094/MPMI-08-11-0213](https://doi.org/10.1094/MPMI-08-11-0213).
- Sultan SE. 2000.** Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**(12):537–542 DOI [10.1016/S1360-1385\(00\)01797-0](https://doi.org/10.1016/S1360-1385(00)01797-0).
- Tan G-Y, Tan W-K. 1986.** Interaction between alfalfa cultivars and *Rhizobium* strains for nitrogen fixation. *Theoretical and Applied Genetics* **71**(5):724–729 DOI [10.1007/BF00263270](https://doi.org/10.1007/BF00263270).

- Timm CM, Campbell AG, Utturkar SM, Jun S-R, Parales RE, Tan WA, Robeson MS, Lu TYS, Jawdy S, Brown SD, Ussery DW, Schadt CW, Tuskan GA, Doktycz MJ, Weston DJ, Pelletier DA. 2015. Metabolic functions of *Pseudomonas fluorescens* strains from *Populus deltoides* depend on rhizosphere or endosphere isolation compartment. *Frontiers in Microbiology* 6(27):1118 DOI 10.3389/fmicb.2015.01118.
- Timm CM, Pelletier DA, Jawdy SS, Gunter LE, Henning JA, Engle N, Aufrecht J, Gee E, Nookaew I, Yang Z, Lu T-Y, Tschaplinski TJ, Doktycz MJ, Tuskan GA, Weston DJ. 2016. Two poplar-associated bacterial isolates induce additive favorable responses in a constructed plant-microbiome system. *Frontiers in Plant Science* 7(283):497 DOI 10.3389/fpls.2016.00497.
- Utturkar SM, Klingeman DM, Land ML, Schadt CW, Doktycz MJ, Pelletier DA, Brown SD. 2014. Evaluation and validation of de novo and hybrid assembly techniques to derive high-quality genome sequences. *Bioinformatics* 30(19):2709–2716 DOI 10.1093/bioinformatics/btu391.
- Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of the microbiome of the plant holobiont. *New Phytologist* 206(4):1196–1206 DOI 10.1111/nph.13312.
- Verhagen BWM, Glazebrook J, Zhu T, Chang H-S, van Loon LC, Pieterse CMJ. 2004. The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Molecular Plant-Microbe Interactions* 17(8):895–908 DOI 10.1094/MPMI.2004.17.8.895.
- Wagner MR, Lundberg DS, Coleman-Derr D, Tringe SG, Dangl JL, Mitchell-Olds T. 2014. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild *Arabidopsis* relative. *Ecology Letters* 17(6):717–726 DOI 10.1111/ele.12276.
- Walker V, Bertrand C, Bellvert F, Moëgne-Loccoz Y, Bally R, Comte G. 2011. Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*. *New Phytologist* 189(2):494–506 DOI 10.1111/j.1469-8137.2010.03484.x.
- Wang Y, Ohara Y, Nakayashiki H, Tosa Y, Mayama S. 2005. Microarray analysis of the gene expression profile induced by the endophytic plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Molecular Plant-Microbe Interactions* 18(5):385–396 DOI 10.1094/MPMI-18-0385.
- Wasternack C, Parthier B. 1997. Jasmonate-signalled plant gene expression. *Trends in Plant Science* 2(8):302–307 DOI 10.1016/S1360-1385(97)89952-9.
- Weston DJ, Pelletier DA, Morrell-Falvey JL, Tschaplinski TJ, Jawdy SS, Lu T-Y, Allen SM, Melton SJ, Martin MZ, Schadt CW, Karve AA, Chen J-G, Yang X, Doktycz MJ, Tuskan GA. 2012. *Pseudomonas fluorescens* induces strain-dependent and strain-independent host plant responses in defense networks, primary metabolism, photosynthesis, and fitness. *Molecular Plant-Microbe Interactions* 25(6):765–778 DOI 10.1094/MPMI-09-11-0253.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* 428(6985):821–827 DOI 10.1038/nature02403.
- Xin G, Zhang G, Kang JW, Staley JT, Doty SL. 2009. A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. *Biology and Fertility of Soils* 45(6):669–674 DOI 10.1007/s00374-009-0377-8.
- Yang J, Kloepper JW, Ryu C-M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science* 14(1):1–4 DOI 10.1016/j.tplants.2008.10.004.

- Yang SF, Hoffman NE. 1984.** Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology* **35(1)**:155–189 DOI [10.1146/annurev.pp.35.060184.001103](https://doi.org/10.1146/annurev.pp.35.060184.001103).
- Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CMJ. 2013.** Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. *Plant Physiology* **162(1)**:304–318 DOI [10.1104/pp.112.212597](https://doi.org/10.1104/pp.112.212597).
- Zou J, Rodriguez-Zas S, Aldea M, Li M, Zhu J, Gonzalez DO, Vodkin VO, DeLucia E, Clough SJ. 2005.** Expression profiling soybean response to *Pseudomonas syringae* reveals new defense-related genes and rapid HR-specific downregulation of photosynthesis. *Molecular Plant-Microbe Interactions* **18(11)**:1161–1174 DOI [10.1094/MPMI-18-1161](https://doi.org/10.1094/MPMI-18-1161).