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Switchgrass Field Performance on Two Soils as Affected by Bacterization of Seedlings with *Burkholderia phytofirmans* Strain PsJN

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Abstract Switchgrass (*Panicum virgatum* L.) is a perennial warm season grass capable of growth on marginal lands without major inputs of water and fertilizers. However, full utilization of its agronomic potential as a bioenergy crop requires improvement of its stand establishment, disease resistance, and prevention of the biomass yield variation from year to year. Our program focuses on the utilization of beneficial bacterial endophytes to enhance switchgrass performance under a low-input production system on marginal lands. We demonstrated earlier that inoculation of switchgrass cv. Alamo with a growth promoting endophyte, *Burkholderia phytofirmans* strain PsJN (PsJN), can significantly enhance seedling vigor and plant growth under both in vitro and greenhouse conditions. In this study, we tested the effects of PsJN bacterization of switchgrass seedlings on stand establishment, plant growth, and biomass yield in three field experiments conducted over 2 years on highly fertile prime land, and on a former tobacco farm with low fertility soil. PsJN bacterization improved growth and development of switchgrass seedlings, significantly stimulated plant root and shoot growth, and tiller number on the low fertility soil ($p<0.001$), and enhanced biomass accumulation on both poor ($p<0.001$) and rich ($p<0.05$) soils, with more effective stimulation of plant growth in low fertility soil than in high fertility soil. The study indicates the potential for the use of PsJN and/or other beneficial bacterial endophytes in the development of low-input switchgrass feedstock production systems.

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Introduction

Fossil fuels have driven world economies since the beginning of the industrial revolution. However, their supply is limited, and peak petroleum production is estimated to have passed [1] while world energy demand is increasing [2]. The growing use of fossil fuels, through increased greenhouse gas emissions, may also further affect climate change [3]. To address this situation, the development and use of renewable forms of energy including solar, wind, and bioenergy are now a major focus of innovation. In the USA, switchgrass (*Panicum virgatum* L.) has been identified as a model renewable bioenergy crop [4, 5], due to its high water use efficiency, carbon sequestration capacity, and ability to grow on marginal lands under low agrochemical inputs [6]. On-farm evaluation of switchgrass performance in the Mid-West highlights its production potential on marginal lands, with 504 % more energy produced than consumed [7]. Switchgrass is taxonomically divided into two ecotypes: cold tolerant upland cultivars which are short stature and yield lower biomass, and lowland cultivars found in milder wet areas, which are higher biomass producers [8]. Lowland cv. Alamo is a prime candidate for bioenergy production in the southeastern US because of its high biomass production [9].

To be economically viable, the biofuel industries will likely be regional due to feedstock biomass handling and transportation logistics [10]. Central and Southern Virginia have a rich farming tradition, primarily built upon the production of tobacco. However, U.S. demand for tobacco has fallen dramatically in the last few decades, leaving many fields fallow and often depleted of nutrients. With little investment and the use of readily available conventional farm forage equipment, such fields can be utilized for switchgrass biomass production for the conversion to biofuels [6].

Beneficial bacterial endophytes have been shown to increase yields of other graminaceous bioenergy crops, such as corn and sugarcane [11, 12]. Endophytes are naturally occurring soil microorganisms that can penetrate plant roots and translocate to the aboveground organs and tissues and, upon colonization, affect plant growth, health, and productivity [13–15]. Multiple mechanisms of plant growth promotion by beneficial bacterial endophytes have been reported over the past 30 years, including production and regulation of plant hormones, synthesis of antimicrobial compounds to inhibit the growth of plant pathogens, helping the host plant to acquire nutrients, and other [16]. A particular endophyte may also convey multiple mechanisms of growth enhancement. *Burkholderia phytofirmans* strain PsJN, for example, has been shown to secrete siderophores for iron acquisition, induce plant host's stress resistance via production of trehalose, stimulate plant growth by production of the plant growth hormone auxin, and by lowering levels of the plant growth inhibitive hormone ethylene [17–20]. In addition to exhibiting multiple mechanisms of action, PsJN effectively colonizes tissues of a broad range of plants including tomato [21–23], potato [24], sweet pepper [21], and grapevine [16, 25]. Under drought conditions, PsJN inoculation increases photosynthesis, chlorophyll content, and efficiency of photosystem II compared to the control treatment [26].

In switchgrass, naturally occurring bacterial endophytes isolated from the plant in the field have been also shown to promote growth [27, 28], improve its stand establishment, and seedling year biomass production [29]. Larger populations of endophytes were found in older stands of switchgrass compared to the more recently established, indicating a change over time [27]. These findings suggest that perennial plants accumulate endophytic populations of rhizospheric bacteria over time [30]. Our studies conducted with PsJN bacterization of switchgrass cv. Alamo under in vitro, growth chamber, and greenhouse conditions demonstrated 57, 46, and 37 % increase of the plant fresh weight, respectively [31]. The objective of this study was to explore the effects of *B. phytofirmans* strain PsJN on switchgrass cv. Alamo seedling establishment, tillering, and plant biomass accumulation during the first 2 years of field growth on two different soils, a prime soil with high organic matter and nutrient content, and a poor former tobacco farm soil in Southern Virginia.

Materials and Methods

Plant Material and Bacterization

Switchgrass (*P. virgatum* L.) seeds of cv. Alamo were purchased from Warner Brothers Seed Co. (Lawton, OK). Seeds were surface sterilized as described in Kim et al. (2012) and germinated for 5–7 days on sterile 7.5 cm wet filter paper (VWR®) in 100 mm×15 mm petri dishes (Fisherbrand®) at 25 °C under white fluorescent light (67 μmol m^{-2} s^{-1}) with 16-h photoperiod [31]. *B. phytofirmans* strain PsJN was obtained from Dr. Angela Sessitsch (Austrian Institute of Technology, Seibersdorf, Austria). PsJN cultures were streaked on King's B (KB) solid medium as previously described [22]. Inoculum was produced by transferring one loop of bacteria from 2-day-old cultures to 5 ml KB broth in a 15-ml culture tube, followed by incubation at 28 °C on a shaker (220 rpm) overnight. Five milliliters of the overnight culture were added to 45 ml KB broth in a 250-ml Erlenmeyer flask and grown to 0.7 at OD_{600}. Bacterial cells were then collected by centrifugation at 3,500 rpm for 7 min at 4 °C, and resuspended in PBS buffer (10 mM NaH_2PO_4 containing 0.8 % NaCl, pH 6.5) after which the OD_{600} was adjusted with PBS buffer to 0.5 at OD_{600} (approx. 10^8 cfu ml^{-1}). Seedlings were soaked in PsJN suspension for 1 min. Control seedlings were treated with PBS buffer alone. The treated seedlings were blot-dried on sterile paper towels and transferred to GA-7 Magenta containers with Murashige and Skoog basal salts plus vitimans (MS+V) (M519, Phytotech Labs, Shawnee Mission, KS) containing 3 % maltose (RPI Inc.) and 0.3 % phytagel (Phytotech labs) at pH 5.8, with five seedlings per container. The plantlets were grown in GA-7 Magenta containers at 25 °C (16-h photoperiod, fluorescent light at 67 μM m^{-2} s^{-1}). After 3 weeks, the seedlings were transferred to a 72-cavity flats filled with Miracle-Gro® Potting Mix (Scotts Miracle-Gro® Company, Marysville, Ohio) and grown in a growth chamber under 28/22 °C day/night temperatures, 16-h photoperiod with fluorescent light at 67 μM m^{-2} s^{-1} for 2 weeks before being transferred to the field or to 4 gal pots containing field soil.

Pot Experiment with Field Soil

To test the effect of PsJN bacterization on the growth and development of switchgrass cv. Alamo, bacterized and non-bacterized transplants were planted into 4 gal pots containing field soil with five plants per pot on September 17, 2011. The experiment was conducted at the Lynchburg Grows greenhouse complex at ambient temperature, with 11 pots per treatment. The pots were watered with an above ground spray system every 3 days delivering 50 ml of tap water per pot. During the test, growth stage was determined by the number of leaves formed and the maturity of the new leaf according to Sanderson (1992) [32].

Field Sites and Soil Fertility

Characteristics of the two field experimental sites were outlined in Table 1. Experiment 1 was conducted in Lynchburg, Virginia, at the Lynchburg Grows Urban Farm and Environmental Education Center (37° 23′ 26″ N, 79° 9′ 57″ W) on Cecil-appling association soil: deep, well drained, with 2–15 % slopes, and firm clay subsoil. Experiment 2 (plots 1 and 2) was conducted in Danville, Virginia, at Walden Farm (36° 36′ 42″ N, 79° 19′ 32″ W) on Cecil-sandy loam soil: deep, well drained, with a 2 to 7 % slope (NRCS, http://websoilsurvey.nrcs.usda.gov). Both soils are classified as Prime Farmland. The field in Lynchburg was historically managed as grassland, and the field in Danville was historically planted with tobacco. No crops were planted on either field over the past 5 years; both were managed by yearly mowing. A broad spectrum herbicide (Roundup®, Scotts Miracle-Gro® Company, Marysville, Ohio) was applied once, 30 days before transplanting according to manufacturers' recommendation, and the sites were cultivated mechanically and hand-weeded. Five soil samples were taken from each plot, approximately 15-cm deep, and combined to form a composite sample for soil fertility analyses. Nitrogen and carbon analyses were done at the Environmental and Agricultural Testing Service (EATS; http://www.soil.ncsu.edu/services/asl/) of the Department of Soil Science at North Carolina State University in Raleigh, NC. Additional analyses were conducted at the Department of Crop and Soil Environmental Sciences—Virginia Tech Soil Testing Lab (http://www.soiltest.vt.edu/).

Experimental Design

A paired experimental design was carried out on both field trials to avoid soil and environmental variation. Lynchburg site plot was 25′×50′, divided into 10 rows spaced 2.5′ apart, with 20 transplants per row spaced 2.5′ apart. Danville plot 1 was 22.5′×60′, divided into 9 rows spaced 2.5′ with 16 plants per row, 2.5′ apart. Plot 2 was 20′×20′, divided into 8 rows, 8 plants per row spaced as above. The Lynchburg field experiment was planted on May 17, 2012, and two subsequent harvests of aboveground biomass were performed—first during vegetative growth at the beginning of the summer (July 6, 2012, n=25), and the second at the end of the growing season at plant dormancy (January 10, 2013, n=50) by cutting at a 5-cm stubble height. Fresh weight and number of tillers were recorded at the first harvest. Dry weights were

Table 1 Trial descriptions and soil characteristics. Ratings are in parenthesis (*VH* very high, *H* high, *M* medium, *L* low). All trace minerals were rated as sufficient

Field soil parameters	Field trial site 1 (Lynchburg field trial)	Field trial site 2 (Walden farm field trial)	Pot trial (pots with field soil)
Location	Lynchburg, VA (37° 23′ 26″ N, 79° 9″ 57″ W)	Danville, VA (36° 36′ 42″ N, 79° 19′ 32″ W)	Lynchburg, VA (37° 23′ 26″ N, 79° 9′ 57″ W)
Description	Cecil-appling association	Cecil-sandy loam soil	NA
Classification	Prime farmland	Prime farmland	NA
Crop History	Managed grassland	Historically tobacco	Rose nursery production
Last Planted	Fallow for more than 20 years	Fallow for more than 5 years	Fallow for more than 5 years
Previous Crops	Managed Grassland	Tobacco	Cut roses
Slope	2–15 %	2–7 %	NA
pH	6.0	5.7	6.6
% nitrogen	0.54	0.07	0.20
% carbon	7.30	0.85	2.84
P (lb/A)	2044 (VH)	4 (L)	1063 (VH)
K (lb/A)	393 (VH)	76 (M-)	924 (VH)
Ca (lb/A)	9979 (VH)	510 (L+)	5880 (VH)
Mg (lb/A)	995 (VH)	175 (H)	1043 (VH)
Zn (ppm)	54.3	0.5	49.3
Mn (ppm)	39.8	2.4	107.9
Cu (ppm)	0.7	0.3	2.7
Fe (ppm)	19	9.9	23.6
B (ppm)	1.4	0.1	1.2
Buffer Index	6.17	6.20	6.45
Acidity (%)	4.9	36.2	3.3

determined after plants were dried for 2 weeks at 21 °C and 35 % humidity. Second year harvests were done on June 5, 2013 (n=23) and November 20, 2013 (n=75). Dry weights were determined as above.

Both Danville plots were planted on August 20, 2012. Plants in plot 1 were bacterized on July 3, 2012 and plants in plot 2 were bacterized on June 21, 2012. Tiller number and height were recorded at the end of the growing season (November 26, 2012). During the second year, root and shoot growth was determined on June 17, 2013 (n=10) after digging up the entire plants and washing roots with tap water. Roots were then blot dried with paper towels and fresh weights of roots and shoots determined. The plants were then dried for 2 weeks at 21 °C and 35 % humidity and dry weights recorded. The final harvest was done on December 04, 2013 after the plants were dormant. Fourteen and 12 pairs of plants were randomly harvested from plots 1 and 2, respectively. The root and shoot fresh and dry weights were determined as described above.

Root Morphology

To determine the effect of PsJN bacterization on root growth and morphology, bacterized and non-bacterized transplants were planted in 4 gal pots containing Miracle Gro® soil mix on March 28, 2013 in a temperature-controlled greenhouse at the Institute for Advanced Learning and Research in Danville, Virginia and harvested on May 14, 2013. The entire plants were harvested, roots washed, and fresh and dry weights of roots and shoots determined as above. The numbers of lateral roots per cm on seminal roots were estimated by counting lateral roots in a 3 cm portion of a randomly selected seminal root and dividing by 3. The number of seminal roots was counted on PsJN and control plants as well.

Statistical Analysis

Statistical analysis was performed using student's paired t test. Values were assigned to each group and reported at 95, 99, or 99.9 % confidence levels.

Results

Description of Field Sites

The objective of this study was to investigate the effects of PsJN bacterization of switchgrass cv. Alamo seedlings on growth and development during the establishment year and subsequent production year on both high fertility and low fertility soils. Previous data indicated that PsJN bacterization of Alamo grown in pots with field soil [31] increased growth greater when compared to plants bacterized and grown in pots with high fertility Miracle Gro® potting mix. To address the objective of this study, two field sites were selected with contrasting soil organic matter and nutrient levels. The site with high fertility soil (site 1) was located in Lynchburg, Virginia, at the Lynchburg Grows Environmental Education Center, and the low fertility soil (site 2) was located at Walden Farm, a historic tobacco farm near Danville, Virginia. Levels of P, K, Ca, and Mg were all rated very high at site 1 compared to low, medium −, low +, and high at site 2, respectively. These levels are not surprising as site 1 had been managed grassland for nearly 50 years whereas site 2 had been planted with tobacco historically. Percentage of nitrogen was 7.7-fold higher at site 1 compared to site 2, and percent carbon and trace nutrients were similarly higher at site 1 (Table 1). Additionally, no fertilizers were applied before or during the trial, and only one initial watering was performed at the time of transplanting.

Comparative Analysis of Biomass Production in Two Fertility Soils

Two harvests were performed on the prime soil (site 1) to determine bacterization effects on biomass yield at midseason and at the end of the growing season. PsJN bacterization significantly enhanced biomass production compared to controls ($p<0.01$ and $p<0.05$, respectively) (Fig. 1a, b). Bacterization effect was more pronounced earlier in the season (35.7 % increase over control in biomass at midseason in the establishment year), than at the end of the season (12 % increase). Similar results were recorded during the second year with a 24 % increase in biomass yield at the end of the season ($p<0.05$) (Fig. 1d). In comparison, fresh and dry weight of plants harvested mid-season during the second year at site 2 (poor soil) differ similarly between the bacterized and control treatments on plots 1 and 2, $p<0.01$ and $p<0.05$, respectively (Fig. 2a, c). However, at the end of the second season on poor soil, the differences were highly significant ($p<0.001$) regarding both above and below ground biomass accumulation. Total dry weight of the above ground biomass in the bacterized treatment was 102.5 % higher on plot 1, and 67.3 % on plot 2.

Effects of PsJN on Plant Development and Morphology

Tiller Number

The benefits of bacterization were also reflected in tiller production, confirming the results of previously reported in vitro and greenhouse study [31]. To explore the effects

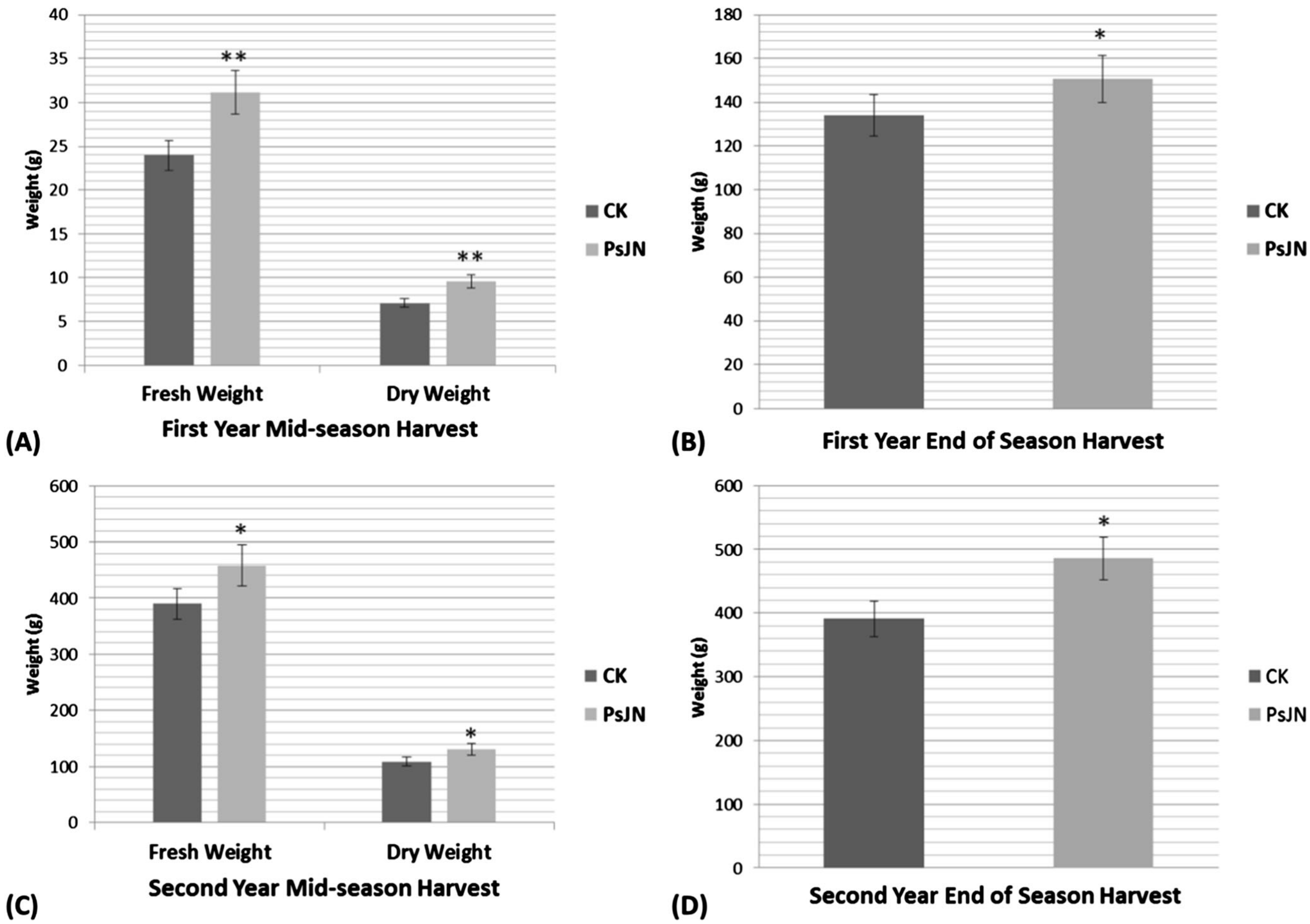

Fig. 1 **a** Results of the first switchgrass harvest in Lynchburg. Above ground plant biomass was harvested on July 06, 2012, at 52 days of growth (n=25, USDA prime farmland, **$p<0.01$). **b** First year end of season harvest. Plants were dried for 2 weeks at 70 °F before weight was measured (*$p<0.05$). **c** First harvest in the second season (June 05, 2013) in Lynchburg. Twenty three pairs of plants were randomly selected, harvested, and weighed for fresh weight. Plants were allowed to dry in a humidity-controlled room for 2 weeks, and dry weight was recorded (*$p<0.05$). **d** Final harvest in second season in Lynchburg (November 20, 2013). The remaining plants (75 pairs) were harvested, allowed to dry in a humidity-controlled room for 2 weeks, and dry weight was recorded (*$p<0.05$). Bars represent standard error

of PsJN bacterization on switchgrass establishment, tiller production was recorded after 3 months of growth in different soil types. Bacterization significantly ($p<0.01$, $p<0.001$) increased tiller number during early vegetative growth on all tested soils (Fig. 3a). Bacterized plants grown in a high nutrient soil in Lynchburg produced almost five times more tillers compared to the plants in pots with field soil and the plants in the field trial on poor soil at Walden farm. Independent of the soil type, PsJN bacterized plants produced significantly ($p<0.01$, $p<0.001$) more tillers than controls.

Plant Development

PsJN effect on switchgrass development was examined in two experiments, at 2.5-month growth (Fig. 3b). Compared to nonbacterized control significant ($p<0.001$) advance of growth stage was recorded in PsJN bacterized plants, with almost an entire new leaf forming in the PsJN treatment.

Root Growth and Morphology

PsJN effect on root growth was evaluated at site 2 (poor soil). In both plots 1 and 2, bacterized plants produced significantly more root biomass ($p<0.001$), averaging 80 % higher over control at the end of the second season. To characterize the effects of bacterization on root morphology, a separate greenhouse pot study was conducted. Significant increases ($p<0.001$) were observed in both fresh and dry root weight as well as root length (Fig. 4). PsJN bacterized plants produced significantly more seminal roots ($p<0.001$) with an average of 3.48 seminal roots per plant while control plants produced 1.7 (Fig. 5). PsJN bacterized plant roots had significantly

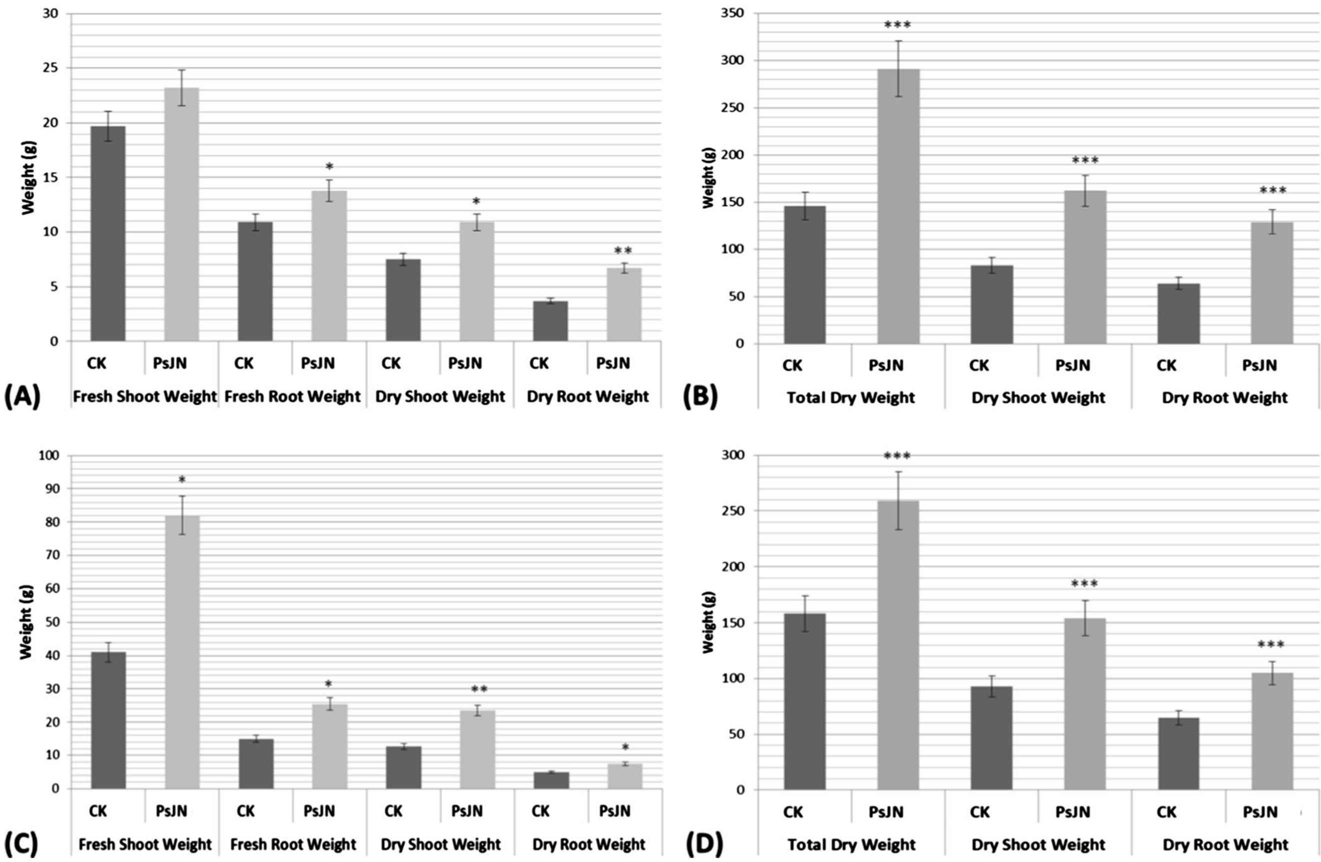

Fig. 2 **a** Plot 1 second year mid-season harvest of cv. Alamo from Walden Farm. Measurements were recorded on June 17, 2013. Ten pairs of plants were dug out completely; the roots were washed and allowed to dry, and then the above ground portion was separated and labeled. Fresh weights were taken within 8 h, and the plants were dried at 28 °C for 2 weeks (** $p<0.01$, * $p<0.05$). **b** Walden Farm plot 1 second year end of season harvest. Fourteen pairs of switchgrass plants were harvested on December 04, 2013. Plants were dug out completely; the roots were washed and allowed to dry, and then the above ground portion was separated and labeled. Dry weights were recorded after plants were dried for 2 weeks (*** $p<0.001$). **c** Walden Farm plot 2 second year mid-season harvest. Ten pairs of switchgrass plants were harvested on June 28, 2013. Ten pairs of plants were dug out completely; the roots were washed and allowed to dry, and then the above ground portion was separated and labeled. Fresh weights were taken within 8 h and the plants were dried at 28 °C for 2 weeks (** $p<0.01$, * $p<0.05$). **d** Walden Farm plot 2 second year end of season harvest. Twelve pairs of switchgrass plants were harvested on December 04, 2013. Plants were dug out completely; the roots were washed and allowed to dry, and then the above ground portion was separated and labeled. Dry weights were recorded after plants were dried for 2 weeks (*** $p<0.001$). Statistical analysis was performed using student's *t* test, and error bars represent standard error

($p<0.001$) more lateral roots per centimeter, compared to control plants, 3.55 vs. 1.95, respectively (Fig. 5).

Discussion

Changing global patterns of temperature and diminishing supply of water and non-renewable fossil fuels have prompted interest in the production of switchgrass as a potential bioenergy feedstock capable of growth on marginal lands under low inputs. However, a broad utilization of switchgrass for biomass feedstock is hampered by its poor stand establishment due to seed dormancy and low seedling vigor, and a consequent overgrowth by weeds [33, 34]. To address this issue, we propose utilization of plant growth promoting bacterial endophytes to enhance seedling vigor and improve switchgrass growth and tolerance to stresses, resulting in better establishment and biomass yields. Earlier studies demonstrated that graminaceous energy crops, including switchgrass, can benefit from inoculation with beneficial bacterial endophytes [11, 12, 15, 27, 28, 31]. One such beneficial endophyte is *B. phytofirmans* strain PsJN [31]. Compared to non-inoculated controls, PsJN bacterization increased switchgrass root development (length and weight), tillering, plant growth, and accumulation of biomass under in vitro and greenhouse growth conditions [31]. To test if these effects can translate into increased seedling establishment and plant performance in the field, we have conducted two field experiments on different fertility soils using cv. Alamo, the responding germplasm to PsJN inoculation in our studies.

At the high nutrient containing soil site in Lynchburg, biomass of PsJN bacterized plants was significantly higher during the stand establishment year, with the most pronounced

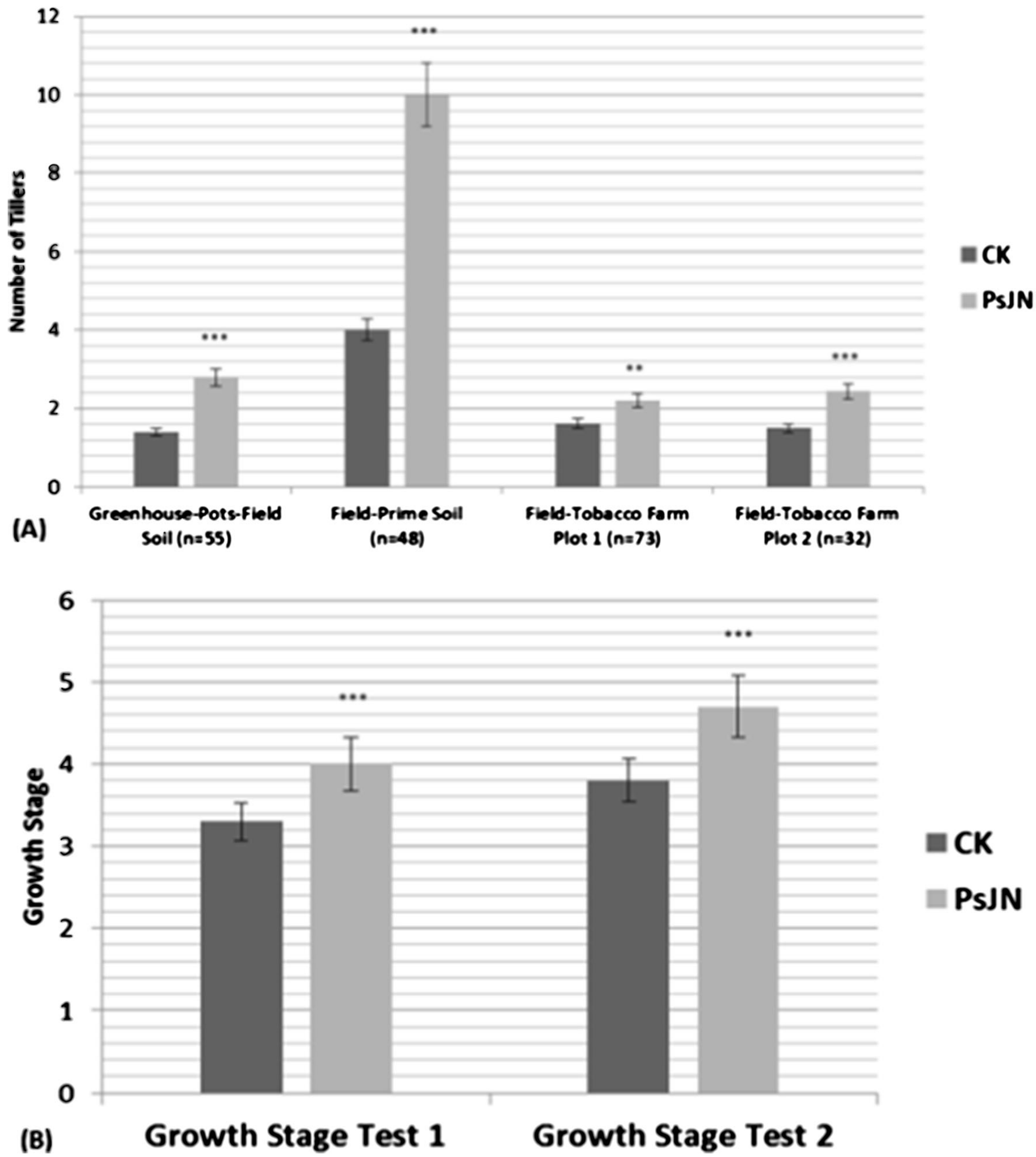

Fig. 3 **a** Switchgrass tiller number during first year establishment. Tiller number of control (CK) and *Burkholderia phytofirmans* strain PsJN bacterized switchgrass was recorded during first year establishment in different types of field soil (***$p<0.001$, **$p<0.01$). **b** PsJN bacterized plants exhibit advanced growth stage. Measurements were recorded at 2.5 months growth (***$p<0.001$, $n=50$). Test 1 was performed in 2010 and test 2 was performed in 2011

difference at 52 days of growth ($p=0.002$, Fig. 1a). The second year regrowth reflected the first year's stand establishment and root growth. Plant biomass in bacterized treatments was significantly ($p<0.05$) higher through the second season; 20 % at the early (06/05/2013) and 24.3 % at the final (11/20/2013) harvest than those of control plants, respectively (Fig. 1c, d). The data indicate that the improvement of seedling establishment and plant performance in the first year persisted through the second year. This effect was even more pronounced in the experiment conducted on the low nutrient containing soil, a former tobacco field. For example, PsJN bacterized plants highly significantly ($p<0.001$) outperformed controls (Fig. 2); 102.5 and 67.3 % increase of biomass yield, respectively, in plots 1 and 2 at the end of the second year. The data support our hypothesis that the benefits of PsJN bacterization of switchgrass could be more pronounced on poor soils and thus integrated into the development of low input biomass feedstock production systems for marginal lands.

Tillering has been considered as an early indicator of stand establishment and future biomass production potential for

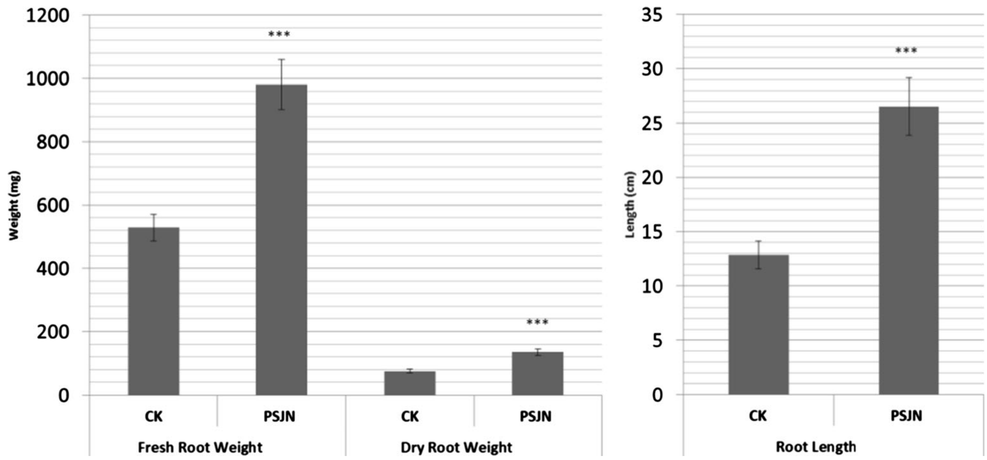

Fig. 4 Root biomass and length at 2.5-month growth in pots. *Burkholderia phytofirmans* strain PsJN bacterized switchgrass increased root biomass and length (***$p<0.001$) at 2.5-month growth in pots with Miracle Gro® soil mix in a temperature-controlled greenhouse

perennial grasses [35, 36]. In this study, we evaluated tillering and root and shoot development in the bacterized and non-bacterized treatments during the establishment year. On high nutrient soils, tiller numbers were increased by 150 % compared to non-bacterized controls. However, on low nutrient soils, the tillering increase was less, 37.5 % and 60 % in plots 1 and 2, respectively ($p<0.001$, Fig. 3a).

In previous experiments, PsJN bacterization resulted in larger root size and weight under greenhouse conditions [31]. Similarly, during the second year of regrowth, PsJN bacterized plants produced higher root biomass than those of control plants on the former tobacco field (Fig. 2b, and d). The PsJN caused root development increase likely allowed the plant to access more nutrients and water, critical to early plant vigor. The PsJN effect on root growth and development was

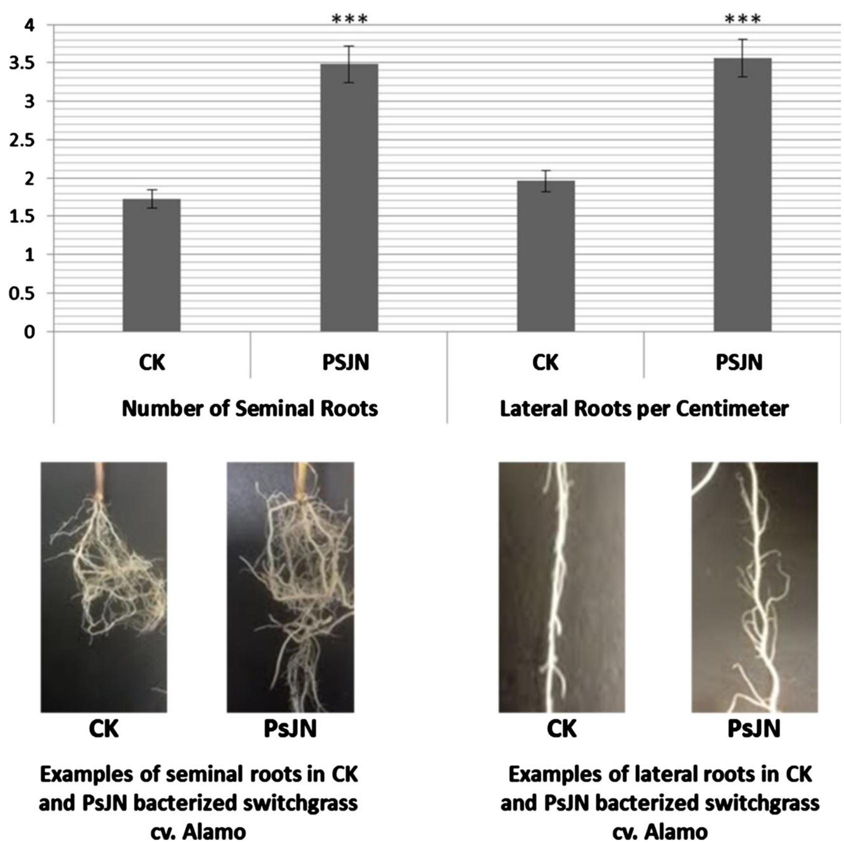

Fig. 5 Root morphology comparison of control (CK) and PsJN bacterized switchgrass. Measurements were recorded at 2.5 months (***$p<0.001$, $n=25$)

also confirmed in a 2.5-month-long study conducted in pots with Miracle-Gro® potting mix in the greenhouse (Fig. 4). The bacterized plants had significantly higher root weight and length ($p<0.001$), similar to those recorded in the field. Root morphology was also changed as PsJN bacterized plants had more seminal (104 %) and lateral (82 %) roots compared to controls (Fig. 5), supporting earlier findings of increased lateral branching of roots in *Arabidopsis* (Poupin et al., 2013)[37]. Our data indicate that PsJN not only increased root size, but also changed its morphology, allowing the plant to better penetrate soil and gain access to water and nutrients. These morphological changes could also aid plants to better tolerate drought stress as reported in maize [26, 38] and enhance its water management properties [17]. Recent evidence also indicates that increased switchgrass root growth caused by the inoculation with endophytic bacteria can contribute to the development of a greater shoot number in the field [29].

Data from the pot experiment with field soil demonstrated that switchgrass bacterization with PsJN accelerated plant development (Fig. 3b) similar to *Arabidopsis* [37]. Bacterized plants formed leaves earlier, and had 21 and 22 % more leaves in two separate experiments compared to controls, likely contributing to higher plant gross photosynthesis and higher biomass yield.

Overall, *B. phytofirmans* strain PsJN bacterization of switchgrass cv. Alamo resulted in plant growth promotion in the field experiment, on two different fertility soils, during the stand establishment year. The benefits of bacterization were more pronounced on the low fertility soil. These beneficial effects were sustained through the second year. Increased root growth, tillering, and early season plant growth vigor contributed to the enhanced productivity of the bacterized stands. The mechanisms underlying PsJN induced switchgrass growth increases may include reduction of ethylene levels through ACC deaminase production [39] and an interplay between ethylene and auxin signaling [40–42]. In the field, PsJN induced plant resistance to biotic stress through ISR, trehalose production to enhance tolerance to abiotic stresses, and production of siderophores to boost nutrient acquisition may also play important roles in seedling establishment and early growth promotion in switchgrass cv. Alamo [17–20, 39–41].

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