Quantification and diversity of cultivated bacteria in root endosphere and rhizosphere of bamboo species *Fargesia nitida* in association with the tree succession

Short Title: Endo-/rhizo-sphere bacteria of bamboo

ABSTRACT

Fargesia nitida is a cold-resistant evergreen bamboo and is a pioneer plant in the secondary succession after the native trees were destroyed in the eastern Tibetan Plateau. However, little is known about the effects of this plant on soil conditions and about its microbiomes. Aiming at learning the interactions among the soil characteristics, the plants and the microbes in relation to the plant succession, a study on cultivated microbes associated with the rhizocompartments of *F. nitida* was performed in the present study to reveal the preference of this plant to the root associated microbes, in comparison with that associated with the successive spruce (*Picea asperata* Mast.) trees. The results demonstrated that growth of *F. nitida* could improve the soil nutrient contents, especially increasing total nitrogen, NH_4^+-N, total carbon, and microbial biomass carbon, and maintained more soil bacteria than the successive spruce trees. Based upon the study of *F. nitida* root-associated cultivated microbial community, the nutrient improvement in *F. nitida* growing soils might be from the root endophytic bacteria, which presented greater abundance (3.8, 1.7, and 12.6 folds) than that of bacteria in its rhizosphere, root zone soil, and spruce root zone soil, respectively. *Pseudomonas* members, especially species related to *P. baetica* and *P. vancouverensis,* were strongly selected by *F. nitida* as root endophytes.

Keywords: Bamboo, Cultivated Microbiome, Plant Succession, Soil, *Pseudomonas*

INTRODUCTION

Species in the genus *Fargesia*, with the common name of 'jian zhu' (arrow bamboo), are small to medium clumping bamboos (up to 5 m in tall, 20 mm in diameter, and solid internodes of 15-18 cm long) natively distributed in alpine conifer forests of East Asia. Some of them serve as food for giant panda, as materials for handicraft and textile (fiber) industry, as ornamentals plant, as well as vegetable (tender bamboo shoot) for cooking (Editorial Committee of Chinese Flora 1996). Among the *Fargesia* species, *Fargesia nitida* (Mitford) Keng f. ex Yi is a cold-resistant evergreen bamboo native to the eastern Sichuan Province and western Hubei Province of China, at the edge of pine (*Pinus tabuliformis* Carrière) and spruce (*Picea asperata* Mast.) forest in humid subtropical climate. In natural succession after the local coniferous forest or ecosystem was destroyed, *F. nitida* is usually recovered as one of the pioneer plants, similar with another bamboo *Fargesia spathacea* (Xu *et al*. 2016). Up-to-date, few studies about *F. spathacea* covering its nutrient effects on giant panda (Wang *et al*. 2017) and its population diversity (Li & Manfred 2002) have been reported. Although antibacterial activities of tissues from several bamboo species (Ramful *et al*. 2022) and diversity of endophytic bacteria in moso bamboo (*Phyllostachys edulis*) based on 16S rDNA sequencing (Liu *et al*. 2017) have been reported, no information is available about the microbes associated with *F. nitida*.

Diverse microbes including bacteria and fungi associated with different plants have been detected by either culture dependent or independent approaches (Román-Ponce *et al*. 2016; Zhang *et al*. 2019a). The microbial communities associated with roots (endosphere and rhizosphere) have the potential to improve growth and production of their host plants by ameliorating the nutrient supply and recycle, or by enhancing the resistance of plants to diseases

and adverse environments (Compant *et al*. 2010). Therefore, the root associated microbes are potential bioresource for biopesticides and biofertilizers (Orozco-Mosqueda *et al*. 2018). To explore these bioresources, isolation and characterization of the microbes are the basal procedure to learn their potential effects on growth and production of plants. Previously, studies on rhizosphere and endosphere microbes associated with crops have documented *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* as dominant phyla in root endosphere, while *Gemmatimonadetes*, *Firmicutes*, and *Acidobacteria* as dominant phyla in rhizosphere of crops in Saskatchewan, Canada (Cordero *et al*. 2020). In the same study, *Pseudomonas* and *Stenotrophomonas* as common predominant genera in the rhizosphere and root endosphere were found, while more dominant genera including *Acinetobacter*, *Arthrobacter*, *Rhizobium*, *Streptomyces*, *Variovorax*, and *Xanthomonas* were described as root endophytes (Cordero *et al*. 2020). In addition, the composition of plant associated microbes in rhizosphere, endosphere, and phytoplane is affected by both the environmental factors (soil pH, salinity, humidity, cropping systems, temperature, etc.) (Luo *et al*. 2022; Zhang *et al*. 2011a) and the plant characteristics (genotypes/varieties and growth stages) (Yu & Hochholdinger 2018; Zhang *et al*. 2011b). Since the root-associated microbes are more important for plant nutrient supply, the effects of root compartments on microbial communities have been recently studied, and the effect order of root endosphere >rhizosphere >root zone >blank soil on the microbes was demonstrated, meanwhile the soil characteristics as the main determinants for the microbial communities in rhizosphere and root zone, and plant species as main determinants for the endophytes were described (Xiao *et al*. 2017).

It has been evidenced that the plant roots could regulate the microbial community in rhizosphere

and in root zone; meanwhile, the microbes in soil or in rhizosphere also could regulate the gene expression in plant roots, even in leaves of plants by long distance signal transportation (Xiao *et al.* 2019). Based on these interactions among the soil characteristics, the plants and the microbes, a study on microbes associated with the rhizocompartments of *F. nitida* might reveal the preference of this plant to the root associated microbes, which might be used to estimate their impacts on growth of the *F. nitida* plants. In addition, growth of *F. nitida* as pioneer plant in the disturbed alpine conifer forests might offer a suitable microbial community in soil for the successive spruce (*Picea asperata* Mast.) trees, which were artificially planted as the typical cultivated tree species in the subalpine region of eastern Tibetan Plateau (including the area of eastern Sichuan Province) in 1980's. With this postulation, we performed the present study to evaluate the diversity and distribution of cultivable bacterial community in root endosphere and rhizosphere of *F. nitida*, as well as to compare the microbial communities in the root zone soils of adjacent *F. nitida* and *P. asperata* trees.

MATERIALS AND METHODS

Sampling site and sample preparation

The sampling area is a mountain near the Experimental Station of Ecology in Maoxian County (103°54′E, 31°42′N, with altitude of 1826 m) of Sichuan Province, which is located on the eastern edge of Tibetan Plateau, with soil type of Calcic Luvisol according to the classification of IUSS Working Group WRB (WRB 2006). Nine plots (400 m^2 each) with distance about 500 - 100 m were selected in the alpine forest with mixed spruce trees (*P. asperata*) and arrow bamboos (*F. nitida*). In each plot, five randomly selected *F. nitida* plants were sampled by uprooting the bamboo roots together with soil (0-30 cm in depth) on 11th of October, 2019.

Correspondingly, nine spruce root zone soils were collected from the nearby spruce trees (about 20 m from bamboo in distance). Each sample containing about 0.5 kg of soil and 5 root systems was maintained in plastic bag for transporting to the laboratory within 48 h. In laboratory, the roots were shaken vigorously to separate soil not tightly adhering to the roots as root zone soil. The tightly adhering soil was collected by brushing as rhizosphere soil. The fine roots of bamboo were cut off from the root system and washed several times with tap water to eliminated the attached soils. In total, nine rhizosphere soil samples, nine root samples and nine root zone soils of bamboos and nine spruce root zone soils were obtained and were kept at 4 °C for maximum 1 week before subsequent analyses.

Analyses of soil physicochemical traits

Water content of soil was determined by the oven drying method, while pH and electrical conductivity (EC) of soil were determined in water suspensions of 1:2.5 and 1:5 (w/w, soil/water), respectively. For physicochemical analysis, a part of the root zone soil was air dried and passed through a 0.15 mm sieve. Then, the total carbon (TC) and total nitrogen (TN) contents were measured with a vario MACRO cube CN Elemental analyzer (Elementar Analysensysteme, Germany) (Wang *et al*. 2016). The NH_4^+-N and NO_3^--N were extracted from soil with 1 M KCl and their contents were measured by continuous flow analysis with a SEAL AutoAnalyzer 3 (SealAnalytical, German). Available phosphorus (AP) content was determined by Olsen method with Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP-OES) (Olsen and Cole 1954). Available potassium (AK) content was estimated with the standard method of Mc Lean & Watson (1985). The chloroform fumigation extraction method (Vance *et al*. 1987) was applied for soil microbial biomass carbon (MBC) and microbial biomass nitrogen

(MBN) determination, by comparing the extraction of carbon and nitrogen in the fumigated and unfumigated samples, while 0.45 and 0.54 were used as the efficiency correction factors (Kec and Ken) for C and N, respectively (Fließbach *et al*. 2006).

Enumeration of mesotrophic aerobic bacteria and fungi

Cultural mesotrophic aerobic bacteria and fungi were quantified for all the samples. For endosphere microbes, the root sample (1.0 g) was surface sterilized with 1% sodium hypochlorite solution and was ground in 9 ml of sterile $MgSO_4$ solution (10 mM) as dilution 10^{-1} (Wang *et al*. 2006). For microbes in rhizosphere and root zone soil, 1 g of the rhizosphere soil or root zone soil was suspended in 9 ml of the $MgSO_4$ solution as dilution 10^{-1}. The root extract or soil dilution was further diluted separately up to 10^{-4}. Aliquot of 0.1 ml of the dilutions 10^{-2}-10^{-4} is spread separately on plates of media TSA (g L^{-1}: Pancreatic digest of casein, 17.0; Papaic digest of soybean meal, 3.0, NaCl, 5.0; Dextrose, 2.5; K_2HPO_4, 2.5; Agar, 15.0) and PY (Peptone, 5; yeast extract, 3; $CaCl_2 \cdot 6H_2O$; Agar, 18.0; pH7.2) in duplicate, which were incubated 3-5 days at 28 °C for bacterial counting. For fungal quantification, 0.1 ml of the dilutions 10^{-1} to 10^{-3} was dispersed separately on plates of commercial Potato dextrose agar (PDA) medium (pH 5.0) (Sigma-Aldrich) in duplicates, and the plates were incubated at 28 °C for 5-7 days. Colony forming units (CFUs) were counted and abundance in CFU g^{-1} of fresh roots or dry soil was calculated.

Enumeration of bacteria related to the N-cycle in root zone soils

The microbes for N_2 fixation, ammonification, ammonium oxidation, and denitrification were quantified by most probable number in liquid media. For enumeration of the **nitrogen-fixing**

142 **bacteria**, 1 ml of each dilutions of 10^{-1}-10^{-5} was inoculated in triplicate to the tubes containing
143 10 ml of liquid OAB nitrogen-free medium (Okon *et al*. 1977) (in 1 L: K_2HPO_4, 0.6 g; KH_2PO_4,
144 0.4 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; $CaCl_2$, 0.02 g; DL-malitate, 2.5 g; glucose, 2.5 g;
145 $FeCl_3$, 10.0 mg; $NaMoO_4 \cdot 2H_2O$, 2.0 mg; $MnSO_4$, 2.1 mg; H_3BO_3, 2.8 mg; $CuSO_4 \cdot 5H_2O$, 0.04 mg
146 and $ZnSO_4 \cdot 7H_2O$, 0.24 mg; pH 6.8). The inoculated tubes were incubated under stationary
147 condition at 28 °C for 7 days and the tubes with surface or subsurface growth (turbid) was
148 subcultured for two additional times. The tubes kept growth in the third subculture were
149 considered as positive for N_2-fixation. The **ammonification** microbes were quantified in
150 triplicate by inoculating 1 ml of the soil dilutions 10^{-2}-10^{-5} to the tubes containing 10 ml Stuart
151 medium (KH_2PO_4 9.10 g, Na_2HPO_4 9.50 g, Yeast Extract 0.10 g, Urea (Ultrapure) 20 g, phenol
152 red 0.01 g, distilled water 1 L, pH 6.8). Incubation conditions were same as that for N_2-fixers.
153 The **nitrification** (ammonium oxidizing) microbes were quantified in medium for autotrophic
154 ammonium oxidation (Kundu *et al*. 1987) (g L^{-1}: $CaCO_3$ 5.0; K_2HPO_4 1.0; $MgSO_4 \cdot 7H_2O$ 0.2;
155 NaCl 0.1; $(NH_4)_2SO_4$ 1.0; (mg/l) $MnSO_4$ 4.4; Na_2MoO_4 4.0; KI 0.75; $CuSO_4 \cdot 5H_2O$ 0.25;
156 $ZnSO_4 \cdot 7H_2O$ 1.5 and $CoCl_2 \cdot 6H_2O$ 0.25) and in medium for heterotrophic ammonium oxidation
157 (same medium supplied with 20 mM sodium malate). The tubes containing 10 ml medium were
158 inoculated with 1 ml of the dilutions 10^{-1}-10^{-3} in triplicate and incubated at 28 °C for 3 weeks
159 under stationary condition. Positive results (nitrite production) were visualized by spot tests for
160 total oxidized-N (nitrite and nitrate) using the standard chemical method (Griess-Ilosvay reagent)
161 (Charlot 1965). For **denitrification** bacteria, 1 ml of the soil dilutions 10^{-4}-10^{-6} were inoculated
162 separately into the tubes with Dulan vial and 10 ml of medium for denitrification (g L^{-1}: KNO_3
163 1.00, Asparagine 1.00, sodium citrate 8.50, KH_2PO_4 1.00, $MgSO_4 \cdot 7H_2O$ 1.00, $CaCl_2 \cdot 6H_2O$ 0.20,

$FeCl_3 \cdot 6H_2O$ 0.05; supplied with 5 ml of 1% w/v bromothymol blue in ethanol). Tubes were incubated under stationary condition at 28 °C for 7 days.

The proteolytic bacteria were quantified with the milk agar (Sigma) that contained agar, 15 g; milk solids (equivalent to 10 ml fresh milk), 1 g; peptone, 5 g; yeast extract, 3 g; distilled water, 1 L; pH 7.2. Aliquots of 0.1 ml of soil dilutions 10^{-2}-10^{-4} were distributed on plates of the milk agar that were then incubated at 28 °C for 3 to 7 days. Colonies surrounding by transparent ring were counted as proteolytic bacteria.

Estimation of fungal colonization in root endosphere

In this analysis, fine roots were cut into 5 mm fragments after surface sterilized, and the root fragments were lied on surface of PDA plates for incubation at 28 °C for 7 to 14 days. Formation of mold colonies from the root fragment was recoded as positive for fungal colonization.

Isolation of bamboo root endophytic and rhizosphere bacteria

After quantified the bacteria, representative colonies with different colony types (size, shape, texture, color, translucence) were picked up from the plates inoculated with root extracts and rhizosphere soils, and the colonies were purified by repeated cross streaking on PY plates. All the purified isolates were stored in 30% (w/v) glycerol at -80 °C and were used in further study for identification by 16S rRNA gene sequencing and for phenotypic characterisation.

Phylogenetic identification of bacteria

To identify the bacteria, single colonies of the isolates were picked up from the PY plates for cell lysing in sterilized water (van Berkum *et al*. 1996) and the lysates were used as DNA template to amplify the 16S rRNA gene with the primers fD1 and rD1 (Weisberg *et al*. 1991). The amplicons were sequenced commercially with the same primers. The acquired nucleotide sequences were compared with the sequences in GenBank by blast and 98.6% of the identity was used as threshold of species (Kim *et al*. 2014; Stackebrandt & Ebers 2006). The acquired 16S rRNA genes and the related sequences extracted from the NCBI GenBank database (https://www.ncbi.nlm.nic.gov/) were aligned using the Clustal module in the MEGA7 software (Kumar *et al*. 2016). The phylogenetic tree was reconstructed with maximum-likelihood method (Felsenstein 1981) in the MEGA7 software. Bootstrap analysis with 1000 replicates was applied to evaluate the robustness of the tree topology.

Phenotypic characterization of bacterial isolates

Growth characters and plant growth promoting traits of the isolates were characterized for estimating the adaptation of bacteria to their habitat and the potential impacts of them to the associated plant. The tested growth characters were ranges of pH, salinity, and temperature for growth. The plant growth promoting traits were production of IAA, solubilization of inorganic and organic phosphate, and production of siderophore. All the tests were performed in duplicates.

Ranges of pH, salinity and temperature for growth of the isolates were tested on PY plates by point-inoculation of the fresh cultured strains with sterilized toothpicks, and the inoculated plates were incubated under 28 °C, excepted the temperature tests. For pH range, the pH values

209 of PY medium were adjusted after sterilization by adding 1 N HCl or 1N NaOH to pH values 4
210 through 10 with interval of 0.5 unit. For salinity test, NaCl was added in PY medium at the
211 contents of 0 through 3.0% (w/v) with the interval of 0.5%. The temperature range for growth
212 was tested at 4, 10, 28, 37 and 40 °C. Growth was observed after 3–7 days of incubation.
213
214 **Indole acetic acid (IAA) production** was determined in 5 ml of PY broth supplemented with
215 100 µg ml^{-1} filter sterilized L-tryptophan at 28 °C with continuous shaking for 48 hours. Then,
216 the culture was centrifuged at at 10,000×g and IAA production was measured by mixing 2 ml of
217 culture supernatant with 4 ml of Salkowski reagent (1 ml 0.5 M $FeCl_3$ in 50 ml of 35%
218 perchloric acid) (Glickmann & Dessaux 1995). The absorbance of color developed was
219 measured at 530 nm after 30 min and the relative productivity of the isolates were recorded.
220
221 **Inorganic phosphate solubilization** of the isolates was determined on Pikovskaya nutrient agar
222 plates (Pikovskaya 1948) (g L^{-1}: yeast extract 0.50, dextrose 10.0, calcium phosphate 5.0,
223 ammonium sulphate 0.50, potassium chloride 0.20, magnesium sulphate 0.10, manganese
224 sulphate 0.0001, ferrous sulphate 0.0001, pH 7.0, agar 15). The plates were inoculated by point
225 the bacteria with sterilized tooth-stick and incubated at 28 °C for 3 days. Colony surround by
226 transparent ring was considered as positive for P solubilization.
227
228 **Degradation of organic phosphorus** by the isolates was estimated with modified Pikovskaya
229 medium by adding 20 g of fresh yolk to replace the calcium phosphate. The preparation,
230 inoculation, incubation and degradation ability of the bacteria were same as the phosphate
231 solubilization. For solubilization of both the inorganic and organic P, the diameters (in mm) of

the colony and the clear zone surrounding the colony were measured and the phosphate solubilization activity was presented as phosphate solubilization index (SI) using the formula: SI= Ring diameter/Colony diameter.

Siderophore-producing ability of bacteria was checked qualitatively by Chrome Azurol S **(**CAS) assay (Schwyn & Neilands 1987). CAS reagent was prepared by dissolving 121 mg CAS in 100 ml distilled water and 20 ml of 1 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution (in 10 mM HCl). This solution was further added with stirring to 20 ml hexadecyl trimethyl ammonium bromide (HDTMA) solution that was prepared by dissolving 729 mg HDTMA in 400 ml distilled water. The CAS-HDTMA solution was autoclaved and stored at room temperature for further use. To prepare the medium, 100 ml CAS reagent and 900 ml sterilized PY agar medium were mixed. After spot inoculated with bacteria, plates were incubated at 28 °C for 5 days. The formation of orange zone surrounding the bacterial colonies was recorded as positive for siderophore production (Louden *et al*. 2011).

RESULTS

Soil physicochemical characteristics

In this study, the soils were acid with pH from 4.55-6.81 and humidity from 39.2%-74.8%. In general, the TN, TC, NH_4^+-N and MBC contents were significantly greater in bamboo root zone soils than that in spruce root zone soils (Table 1), while the same tendency was also observed in the values of MBN, NO_3^--N, pH, and EC, although no statistical difference was observed between the soils from root zones of these two plants.

Enumeration of bacteria and fungi

In the enumeration of microorganisms, more abundant and diverse bacteria were counted on the TY plates than that on the TSA plates, so only the results on PY medium were presented in **Table 2**. In general, more bacteria were accounted in bamboo root zone (average 2.2×10^7 CFU g^{-1} dry soil) than in spruce root zone (average 2.9×10^6), while the fungal abundances were similar in root zone of both plants, with averages of 4.2×10^3 ($1.6\text{-}6.9\times10^3$) and 4.0×10^3 CFU g^{-1} dry soil (1.8×10^3 to 1.1×10^4), respectively (Table 2). Clearly, the fungal abundances varied a lot in spruce soils, but relatively stable in bamboo soils. For microbes in N-cycle, all the root zone soils contained nitrogen fixers more than 10^5 MPN g^{-1} of dry soil. The proteolytic, ammonification, and denitrification microbes presented similar abundances (10^6, 10^4, and 10^4 CFU or MPN g^{-1} dry soil, respectively), while the ammonium-oxidizers were 3 folds more in spruce root zone soil than in bamboo soil (1680.6/529.9 MPN g^{-1} in average). However, the abundances of ammonification, denitrification and ammonium-oxidizers presented large variations among the samples of the same plants.

The abundance of endophytic bacteria in bamboo roots ranged from 4.5×10^6 to 9.6×10^7 (average 3.7×10^7) CFU g^{-1} of fresh root tissue, while that of the rhizosphere of bamboo varied from 2.6×10^6 to 1.5×10^7 (average 6.2×10^6) CFU g^{-1} of dry soil. In rhizosphere and root endosphere of bamboo, most of the fungi presented white cotton-like colonies and no inhibition effect was observed between the fungi and the bacteria grown nearby or together. In bamboo roots, the infection ratio of fungi was 23.3% (21 out of 90 root fragments presented growth of fungi). In bamboo rhizosphere, fungi were counted 790-14000 (average 5.6×10^3) CFU g^{-1} of dry soil.

Isolation and identification of bamboo rhizosphere and endosphere bacteria

From the 9 rhizosphere and 9 root samples, a total of 185 isolates were obtained, including 93 from root endosphere and 92 from rhizosphere. Among them, 16S rRNA genes were successfully sequenced for 86 isolates (43 from endosphere and 43 from rhizosphere), with about 1400 pb in size. All the acquired sequences have been deposited in the database of NMDCN (Chinese National Microbiology Data Center) (Table 3). According to the Blast results, all the isolates presented sequence identities greater than 99% with one or more reference strains for defined species, corresponding to 41 species within 18 genera (Table 3). Phylogenetic analysis (Supplementary Fig. 1) revealed that: 1) Most of the endophytic and rhizospheric bacteria associated with *F. nitida* were closely related to the defined species, but the 16S rRNA gene sequences were not sensitive enough to affiliate them into the exact species, since some of them showed identity greater than 99% with more than one species. 2) Contrast with abundance, the diversity and species richness of bacteria were greater in rhizosphere than that in endosphere (34 species in 15 genera of 4 phyla vs 19 species in 7 genera of 3 phyla). 3) In endosphere, only one isolate (Z1R1, 2.3%) was identified as Gram-positive bacterium (*Streptomyces*), and *Pseudomonas* species were absolutely dominant (79.1%); while in rhizosphere 15 isolates (34.9%) were Gram-positive and the dominant genera were *Pseudomonas* (20.9%), *Bacillus* (20.9%), and *Chryseobacterium* (16.3%). 4) *Pseudomonas*, "*Stenotrophomonas rhizophila*", "*Streptomyces venezuelae*", "*Chryseobacterium ureilyticum*" and "*Janthinobacterium lividum*" were common in both endosphere and rhizosphere. 5) In the genus *Pseudomonas*, all the 8 "species" isolated from rhizosphere were detected in endosphere, but another 4 species, "*P. cedrina/azotoformans*" (2 isolates), "*P. helmanticensis*" (1 isolate), *P. baetica* (11 isolates) and *P. vancouverensis* (4 isolates) were only detected in endosphere.

Phenotypic characterization

Characterization of the adaptation and plant growth promoting traits of the bacterial isolates (Fig. 1; Supplementary Table S1) demonstrated that:

1) All strains grew in PY medium supplied with 1% (w/v) of NaCl, and the proportion of salinity tolerant bacteria was greater in root endosphere (100%, 97.6%, 87.8%) than that in rhizosphere (82.9%, 85.4%, 75.6%) at the NaCl concentrations of 2.0, 2.5 and 3% (w/v), respectively.
2) All the isolates could grow at 10-30 °C, while more endophytic bacteria (60.8%) could grow at 4 °C compared with the rhizosphere bacteria (48.1%). And this situation was reverse at high temperature (35 °C and 40 °C), since the growth proportions were 74.3% and 20.3% for endosphere bacteria, and were 87.3% and 34.2% for rhizosphere bacteria, respectively. However, the growth at 4 and 40 °C was weak for most of the bacteria.
3) Majority of the isolates in both endosphere (61.6%-100%, average 89.7%) and rhizosphere (63.4%-97.2%, average 79.5%) could grow at pH 5.5-9.5; small proportion of the isolates grew at strong acid condition: only Z5R1 at pH4.0; 4 strains /1 strain at pH 4.5 and 4 strains /11 strains from endosphere/rhizosphere, respectively, could grow at pH 5.0; and 25.0% from endosphere and 18.3% from rhizosphere could grow at pH 10.0.
4) The yolk-degrading bacteria were abundant in both endosphere (60.5%) and rhizosphere (55.2%), in which, 8 isolates from endosphere and 5 from rhizosphere presented IP >2. Only 3 (4.6%) isolates from endosphere could solubilize $Ca_3(PO_4)_3$, while no isolate in rhizosphere presented this ability. All the tested isolates produced IAA, but more endophytes (24 isolates) presented high production (2+ to 4+) compared with the isolates from rhizosphere (12 isolates

with 2+ and 3+). Also, more endophytes (30.2%) produced siderophore than the rhizosphere bacteria (21.4%).

DISCUSSION

As pioneer plant and subsequent plant, the bamboo *F. nitida* and spruce *Picea asperata* co-exist in the edge of alpine forest in Sichuan. To understand the succession interaction between these two plants, we first compared the physicochemical traits of their root zone soils in the present study. According to the soil features, we could summarize that the studied area has soils with acid pH and low salinity. The TC, TN and AK contents were high, but the AP contents were extremely low, according to the soil fertility evaluation criteria in China (https://max.book118.com/html/2020/1119/8057140003003017.shtm). Comparing with the spruce soils, the significant greater contents of TN, NH_4^+-N, TC and MBC ($P<0.05$) and the tendency of increase of other nutrients (NO_3^--N, MBN, AP and AK) (not significant) in the bamboo soils (Table 1) demonstrated that the bamboo growth could improve soil fertility and increase the soil microbial biomass, which was consistent with its nature of pioneer plants and might offer a condition adequate to grow the subsequent spruce trees.

The enumeration of microbial groups in the bamboo and spruce soils (Table 2) further revealed the microbiological insight of the change in soil traits relating to succession from bamboo to spruce. Firstly, the greater mesotrophic aerobic bacteria (MAB) abundance in bamboo soils (7.6 folds) than that in spruce soil and the similar mold abundances in root zone soils of both plants (Table 2) imply that the greater microbial biomass carbon (MBC) in bamboo soil than that in spruce soil (Table 1) might be mainly caused by the bacteria. It is well known that the soil

bacteria play important role in improving the availability of soil-borne nutrients for the plants (Jacoby *et al*. 2017). So, it could be estimated that the bamboo associated bacteria, together with their host plant, changed the soil traits and made the soil environment suitable for the successively occupied spruce trees. Our data demonstrated that the bacterial community was more sensitive to the plant succession and might be more important contributors in the nutrient accumulation in the studied area, such as less denitrification bacteria and ammonium-oxidizers were detected in bamboo soils than in spruce soils. In this analysis, no difference was detected in abundances of diazotrophs between the bamboo and spruce soils, which was consistent with the results in our simultaneously realized metagenomic analysis (Zhang *et al*. 2023) that similar abundances of *nifB* in bamboo soils and spruce soils were detected, but it was 10 times greater in bamboo root endosphere than that in rhizosphere and soil, which might explain the increased TN in bamboo soil than in spruce soil, and confirmed the effects of plant species on abundance and diversity of diazotrophs in soil (Han *et al*. 2019).

In general, the average soil MBC and MBN in tropic/subtropical forests were 428.4 (368.4-498.0) and 71.4 (60.2-84.0) mg/kg, respectively (Xu *et al*. 2013). And the MBC constitutes about 1-3% of TC and the MBN occupies 3-5% of TN in soils, and they are significantly affected by the agricultural practice and by the cropping system (Moore *et al*. 2000). In our present study, both the MBC/MBN contents and the ratios of MBC/TC (0.6% and 0.5%) and BMN/TN (1.0% and 0.9%) in the tested bamboo and spruce soils were lower than the reported values, implying low microbial biomass or low abundance of microbial cells in the tested soils. Indeed, only $2.2\times10^7/2.9\times10^6$ CFU of bacteria, and $4.2\times10^3/4.0\times10^3$ CFU of fungi were counted in the bamboo/spruce soils, lower than that (10^8 for bacteria and $10^{4\text{-}5}$ for fungi) in forest systems in

Columbia (Silva-Parra *et al*. 2017) and in Brazil (Vieira & Nahas 2005). The lower microbial biomass and abundances in the tested soils might be related to the low temperature in that region (mean annual temperature of 9.3°C, varied from -0.9 °C in January to 18.6 °C in July), which could inhibit the growth of aerobic mesophiles (Vieira & Nahas 2005). A previous study has demonstrated that artificial warming could significantly increase soil microbe biomass (Luo *et al*. 2022). Meanwhile, the difference in abundances of MAB between bamboo and spruce root zone soils might evidence that the litters (leaves and root residues) of spruce inhibited some bacterial groups (Tanase *et al*. 2018).

Up to date, several studies on the potential of some arrow bamboo as food/vegetable for panda and human being (Wang *et al*. 2017), as pioneer plant for reforestation (Xu *et al*. 2016), and on its population diversity (Li & Manfred 2002) have been reported. However, no information is available about their endosphere and rhizosphere microbes, although these microbes are the most important biomes for plant nutrition. Therefore, we further investigated the bacteria in root endosphere and rhizosphere of *F. nitida* to learn the possible mechanism why the growth of this plant could improve soil nutrients. In our study, the average abundances of bacteria associated with *F. nitida* root compartments presented the order of root endosphere ≥ root zone soil > rhizosphere (3.7×10^7, 2.2×10^7, and 6.2×10^6 CFU g^{-1} of fresh root tissue or dry soil, respectively). The greater abundance of endophytes than that of rhizosphere was consistent with previous studies on microbes associated with the root compartments of cotton (Shi *et al*. 2021), but was different from the results of *Salicornia europaea* (Szymańska *et al*. 2016). So, the ratio of bacterial or fungal abundances between root endosphere and rhizosphere might be a plant dependent feature. Furthermore, it could be estimated that the microbial abundance in

393 rhizosphere is mainly affected by root exudates including some antimicrobiotics (Afrin *et al*.
394 2012; Tanaka *et al*. 2013), while the microbial abundance in root zone soils might be mainly
395 determined by contents of organic materials, which was supported by the greater bacterial
396 abundance (2.2×10^7 CFU g^{-1}) and TC (108.58 g kg^{-1}) in bamboo soils than in spruce soils
397 (bacteria: 2.9×10^6 CFU g^{-1}; TC: 65.54 g kg^{-1}).
398
399 In 16S rRNA sequence analysis, 18 genera covering 41 species were detected among 83 tested
400 isolates. However, some isolates shared similarities greater than the species threshold (98.6%
401 similarity) with more than one reference strains belonging to different species, which confirmed
402 the deficiency of 16S rRNA gene sequence analysis in distinguishing closely related bacterial
403 species (Rossi-Tamisier *et al*. 2015). In this case, we presented the isolates sharing great
404 similarities with reference strains of different species within the quotation mark, such as the
405 isolates Z4R7, Z5R7, Z5R10, Z1R7 presented similarities > 98.6% with the reference strains of
406 *Pseudomonas fluorescens, P. jesenii* and *P. tolaasii* and were identified as "*P. fluorescens*". An
407 impressionable point of the present study was that from rhizosphere to endosphere, *F. nitida*
408 strongly selected the bacteria in genus *Pseudomonas*, especially the species *P. baetica*, "*P.*
409 *fluorescens*" and *P. vancouverensis*, but denied Gram-positive bacteria. A possible reason for the
410 low proportion of Gram-positive bacteria might be that the antibiotic compounds inside this
411 bamboo selectively inhibited the Gram-positive bacteria, as reported in a previous report on
412 extracts of other bamboos (Ramful *et al*. 2022). While the great abundance of *Pseudomonas* in *F.*
413 *nitida* root endosphere might imply that they could help the growth or resistance of the host in
414 the sampling area. The fact that no *P. baetica*, "*P. fluorescens*" and *P. vancouverensis* were
415 identified in rhizosphere and the sharing of several rare species (*Pseudomonas simiae*,

Streptomyces venezuelae etc.) in both endosphere and rhizosphere might imply that two mechanisms may functioned for moving the bacteria from rhizosphere to endosphere: 1) by host selection and 2) by chance.

It has been well known that many *Pseudomonas* species could colonize the rhizosphere of plants where they promote plant growth, regulate the nutrient accumulation, fix nitrogen, and increase the stress responses in rhizosphere (Ke *et al*. 2019; Li *et al*. 2017; Samaddar *et al*. 2019). In general, no apparent difference was observed in the growth characters (salinity, pH and temperature ranges) between the *Pseudomonas* populations in endosphere and in rhizosphere. Therefore, the PGPR traits might be the selective factors for the *Pseudomonas* strains to be endophytes. The presence of more *Pseudomonas* isolates with yolk degradation index >3 or with IAA production in endosphere (4 and 3, respectively) than in rhizosphere (1 and 0) in the present study might support this estimation (Fig. 1 and Supplementary Table S1).

Previously, *Pseudomonas* has been frequently isolated from root endosphere of various plants (Xia *et al.* 2021; Zhang *et al.* 2020), including the bamboos (Afriyani *et al.* 2020; Singh *et al*. 2021). In ecosystem, the dominant species are normally the determinant for function. Therefore, the dominance of *P. baetica* and the other species of the same genus in endosphere of *F. nitida* root implied their major contribution to the root function. This species was originally described for a psychrophilic pathogen of the marine fish (*Dicologoglossa cuneata*), which could grow in medium containing 0–6 % (w/v) of NaCl at 4–30 °C (Lopez *et al.* 2012). Subsequently, it was also found as a main root endophyte of a saline-tolerant plant *Messerschmidia sibirica* in beach (Zhang *et al*. 2019b) and as a phosphorus dissolving bacterium in saline soil (Karimzadeh *et al*.

2021). In addition, it has been reported as nitrogen-fixing endophytic bacteria of *Zingiber montanum* plants (Nongkhlaw & Joshi 2014). In the present study, it was the first time to identify this species as bamboo endophyte in the mountain environment and also enlarged its temperature range for growth to 35 °C. Compared with the other endophytes, the ability of yolk degradation, inorganic phosphorus solubilisation and IAA production was not strong or absent in the *P. baetica* isolates. However, the 10 times greater abundance of *nifB* in bamboo root endosphere than that in rhizosphere and soil (Zhang NN, personal communication) might be related to the dominance of *Pseudomonas*, especially *P. baetica*, in root endosphere. So, the real function of this bacterium in the bamboo root needs further study. Another interesting point for the endophytic *Pseudomonas* was that they may also affect the gut bacterial community of the arrow bamboo eaters: the giant panda and insects (Yao *et al*. 2021), since this genus was also found in the gut microbiomes of them.

As another dominant endophyte detected in the present study, *P. vancouverensis* was originally described for soil bacteria isolated from nitroaromatic compounds (Tvrzová *et al*. 2006), and was reported to have multifunction to improve the red T pepper plant growth under salinity stress (Samaddar *et al*. 2019), increase the chilling resistance in tomato (Subramanian *et al*. 2016), and inhibit fire blight (*Erwinia amylovora*) (Mikiciński *et al*. 2020). It has also used as biofertilizer based upon its potassium-solubilizing capacity (Samadi *et al*. 2021) and contains many nitrogen-fixing strains (Surhone *et al*. 2011). The results in our present evidenced that this species was enriched in the endosphere, since it was not identified from the rhizosphere samples; however, the four endophytic isolates of *P. vancouverensis* did not show apparent difference with the other *Pseudomonas* isolates in the tested phenotypical characteristics. In this case, its selection by *F.*

462 *nitida* as dominant endophyte might be related to other traits, such as potassium solubilization
463 and nitrogen-fixation, but it needs further study.
464
465 Compared with the microbiota in other bamboo roots (Han *et al*. 2009; Moshynets *et al*. 2012;
466 Yuan *et al*. 2015), the unique feature of the bacteriome in root endosphere of *F. nitida* is the
467 absence or low abundance of Gram positive bacteria and the super dominance of *Pseudomonas*
468 (Table 2; Supplementary Fig. S1). In root of Moso bamboo (*Phyllostachys edulis*) grown in
469 different regions in China, *Bacillus* were common dominant genus, while *Alcaligenes*,
470 *Arthrobacter*, *Burkholderia*, *Curtobacterium*, *Enterobacter*, *Pseudomonas*, and *Staphylococcus*
471 were site-dependent dominant genera, demonstrating that the bacteriome in bamboo roots might
472 be affected by both the bamboo species and the ecological conditions (Han *et al*. 2009; Yuan *et*
473 *al*. 2015). In addition of the difference in community structure compared from the previous
474 reports, the abundance of root endophytes for *F. nitida* (2.9×10^{7} CFU g^{-1} of root) was also much
475 greater compared with Moso bamboo: $1.75\text{-}4.5\times10^{4}$ CFU g^{-1} of root (Yuan *et al*. 2015) or
476 8.33×10^{5} CFU g^{-1} of root (Han *et al*. 2009). At this moment, it is not clear the abundance
477 differences in endophytes of different bamboo species was due to the species or the ecological
478 conditions.
479
480 Previously, it has been reported that many microbes in root endosphere or rhizosphere could
481 stimulate growth or improve resistance of plants through a range of mechanisms, such as
482 production of phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase,
483 fixation of atmospheric nitrogen, and solubilization of phosphorus or potassium, etc. (Mohanty *et*
484 *al*. 2021). In our present study, it seemed that bacteria with organic phosphorus degradation

485 ability and production of siderosphore were preferred by bamboo root, but inorganic phosphate
486 solubilization and IAA production were rare characters in the bacterial communities associated
487 with *F. nitida* roots in the studied area. These features might be related to the high contents of
488 organic maters (TC) and the low AP contents of the soil, since the organic P degrading bacteria
489 could improve the P supply for the host plant. It was summarized that the strategies of plants
490 used to select the beneficial microbes and the particular microbe partners for a plant to invert the
491 organic N, P, and S into available nutrients were two gaps in the studies on microbe-plant
492 interactions (Jacoby *et al*. 2017), our present study might contribute some information about
493 these gaps.
494
495 **CONCLUSION**
496 Growth of the pioneer plant *F. nitida* could improve the soil nutrient contents, especially
497 increasing total nitrogen, NH_4^+-N, total carbon, and microbial biomass carbon, and harbored
498 more soil bacteria than the successive spruce trees. Based upon the study of *F. nitida* root-
499 associated cultivated microbial community, the nutrient improvement in *F. nitida* growing soils
500 might be from the root endophytic bacteria, which presented greater abundance (3.8, 1.7, and
501 12.6 folds) than that of bacteria in its rhizosphere, root zone soil, and spruce root zone soil,
502 respectively. *Pseudomonas* members, especially species related to *P. baetica* and *P.*
503 *vancouverensis*, were strongly selected by *F. nitida* as root endophytes.
504
505 **Acknowledgements** The authors thanks Mr. Zhongping Tang for the assistants in field sampling.
506 ETW appropriates the Sabbatical Program of IPN and the scholarship of Sistema Nacional de

Investigadores-CONACyT, Mexico. NNZ, CTZ and ETW thanks the support of International Talent Program of Chinese Academy Sciences.

Author contributions All the authors were involved in the design of the project and the manuscript preparation. NNZ, LL and ETW performed the sampling in the fields. JX, LL and ETW performed laboratory experiments. DRA participated data analysis and preparation of the manuscript. NNZ and JX were responsible for the bioinformation analysis. CZZ and FSS supervised the project and offered funding acquisition.

Funding This study was financially supported by the project of National Natural Science Foundation of China (32171644).

Conflicts of interest The authors declare that they have no conflict of interest.

References

Afrin T, Tsuzuki T, Kanwar RK, Wang X (2012) The origin of the antibacterial property of bamboo. J Text Inst 103:844–849

Afriyani, Maulidia V, Alfizar, Sriwati R (2020) Endophytic bacteria (genus: *Pseudomonas* spp.) isolated from Aceh bamboo root as biological agent againts nematode *Meloidogyne* spp. IOP Conf Ser: Earth Environ Sci 425:012074

Charlot G (1965) Colorimetric Determination of Elements. Principles and Methods. Elsevier Publishing Co., New York

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669e678

Cordero J, de Freitas JR, Germida JJ (2020) Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. Can J Microbiol 66:71–85

Editorial Committee of Chinese Flora (1996) Chinese Flora. 9(1):425. Science Publishing, Beijing (in Chinese)

534 Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J
535 Mol Evol 17:368–376
536 Fließbach A, Oberholzer H, Gunst L, Mader P. (2006) Soil organic matter and biological soil
537 quality indicators after 21 years of organic and conventional farming. Agric Ecosyst Environ
538 118:273–284
539 Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski
540 reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ
541 Microbiol 61:793–796
542 Han JG, Xia DL, Li LB, Sun L, Yang K, Zhang LP (2009) Diversity of culturable bacteria
543 isolated from root domains of Moso bamboo (*Phyllostachys edulis*). Microb Ecol 58:363–
544 373
545 Han L-L, Wang Q, Shen J-P, Di HJ, Wang J-T, Wei W-X, Fang Y-T, Zhang L-M, He J-Z (2019)
546 Multiple factors drive the abundance and diversity of the diazotrophic community in
547 typical farmland soils of China. FEMS Microbiol Ecol 95:fiz113
548 Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S (2017) The role of soil
549 microorganisms in plant mineral nutrition—current knowledge and future directions. Front
550 Plant Sci 8:1617
551 Karimzadeh J, Alikhani HA, Etesami H, Pourbabaei AA (2021) Improved phosphorus uptake by
552 wheat plant (*Triticum aestivum* L.) with rhizosphere fluorescent pseudomonads strains
553 under water-deficit stress. J Plant Growth Regul 40:162–178
554 Ke X, Feng S, Wang J, Lu W, Zhang W, Chen M, Lin M (2019) Effect of inoculation with
555 nitrogen-fixing bacterium *Pseudomonas stutzeri* A1501 on maize plant growth and the
556 microbiome indigenous to the rhizosphere. Syst Appl Microbiol 42:248–260
557 Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average
558 nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of
559 prokaryotes. Int J Syst Evol Microbiol 64:346–351
560 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis
561 Version 7.0 for Bigger Datasets. Mol Biol Evol 33:1870–1874
562 Kundu BS, Dadarwal KR, Tauro P (1987) Nitrification and simultaneous denitrification by
563 *Azospirillum brasilense* 12S. J Biosci 12:51–54

564 Li Z-H, Manfred D (2002) Elevational diversity of arrow bamboo (*Fargesia spathacea*)
565 communities on Mount Shennongjia in Central China. J Forest Rese 13:171–176
566 Li HB, Singh RK, Singh P, Song QQ, Xing YX, Yang LT, Li YR (2017) Genetic diversity of
567 nitrogen-fixing and plant growth promoting *Pseudomonas* species isolated from sugarcane
568 rhizosphere. Front Microbiol 8:1268
569 Liu F, Yuan Z, Zhang X, Zhang G, Xie B (2017) Characteristics and diversity of endophytic
570 bacteria in moso bamboo (*Phyllostachys edulis*) based on 16S rDNA sequencing. Arch
571 Microbiol 199:1259–1266
572 López JR, Diéguez AL, Doce A, De la Roca E, De la Herran R, Navas JI, Toranzo AE, Romalde
573 JL (2012) *Pseudomonas baetica* sp. nov., a fish pathogen isolated from wedge sole,
574 *Dicologlossa cuneata* (Moreau). Int J Syst Evol Microbiol 62:874–888
575 Luo L, Guo M, Wang E, Yin C, Wang Y, He H, Zhao C (2022) Effects of mycorrhiza and
576 hyphae on the response of soil microbial community to warming in eastern Tibetan plateau.
577 Sci Total Environ 837:155498
578 Louden BC, Haarmann D, Lynne AM (2011) Use of blue agar CAS assay for siderophore
579 detection. J Microbiol Biol Educ 12:51–53
580 Mc Lean E, Watson M (1985) Soil measurements of plant‐available potassium. In R.D.
581 Munson (ed.) Potassium in Agriculture, pp.277-308. ASA, CSSA, and SSSA Books
582 Mikiciński A, Puławska J, Molzhigitova A, Sobiczewski P (2020) Bacterial species recognized
583 for the first time for its biocontrol activity against fire blight (*Erwinia amylovora*). Eur J
584 Plant Pathol 156:257–272
585 Mohanty P, Singh PK, Chakraborty D, Mishra S, Pattnaik P (2021) Insight into the role of PGPR
586 in sustainable agriculture and environment. Front Sustain Food Syst 5:667150
587 Moshynets O, Brunet J, Potters G (2012) Identification of endophytic bacteria in *Phyllostachys*
588 sp. and *Fargesia* sp. Bamboo Sci Cul 25:19–26
589 Moore JM, Klose S, Tabatabai MA (2000) Soil microbial biomass carbon and nitrogen as
590 affected by cropping systems. Biol Fertil Soils 31:200–210
591 Nongkhlaw FMW, Joshi SR (2014) Epiphytic and endophytic bacteria that promote growth of
592 ethnomedicinal plants in the subtropical forests of Meghalaya, India. Rev Biol Trop
593 62:1295–1308

Okon Y, Albrecht SL, Burris RH (1977) Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. Appl Environ Microbiol 33:85–88

Olsen SR, Cole CV (1954) Estimation of available P in soils by extraction with sodium bicarbonate. USDA Circular 939

Orozco-Mosqueda M, Rocha-Granados M. Glick BR, Santoyo G **(**2018) Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. Microbiol Res 208:25–31

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* 17:362–370

Ramful R, Sunthar TPM, Kamei K, Pezzotti G (2022) Investigating the antibacterial characteristics of Japanese bamboo. Antibiotics 11:569

Román-Ponce B, Ramos-Garza J, Vásquez-Murrieta MS, Rivera-Orduña FN, Chen WF, Yan J, Estrada-de Los Santos P, Wang ET (2016) Cultivable endophytic bacteria from heavy metal(loid)-tolerant plants. Arch Microbiol 198:941–956

Rossi-Tamisier M, Benamar S, Raoult D, Fournier PE (2015) Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. Int J Syst Evol Microbiol 65:1929–1934

Samaddar S, Chatterjee P, Choudhury AR, Ahmed S, Sa T (2019) Interactions between *Pseudomonas* spp. and their role in improving the red T pepper plant growth under salinity stress. Microbiol Res 219:66–73

Samadi A, Hassani A, Gholamhoseini M (2021) Effect of potassium solubilizing biofertilizers application compared to potassium sulfate on growth and some physiological traits of radish (*Raphanus sativus* L.) under drought stress. J Hortic Sci 34:633–643

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56

Shi Y, Yang H, Chu M, Niu X, Wang N, Lin Q, Lou K, Zuo C, Wang J, Zou Q, Zhang Y (2021) Differentiation and variability in the rhizosphere and endosphere microbiomes of healthy and diseased cotton (*Gossypium* sp.). Front Microbiol 12:765269

Silva-Parra A, Mogollón-Ortiz ÁM, Delgado-Huertas H (2017) Soil microbiota: Influence of different land use patterns and soil management factors at Villavicencio Oxisol, East Colombia. Biota Colomb 18:1-10

625 Singh L, Ruprela N, Dafale N, Thul ST (2021) Variation in endophytic bacterial communities
626 associated with the rhizomes of tropical bamboos. J Sustain For 40:111–123
627 Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards.
628 Microbiol Today 33:152–155
629 Subramanian P, Kim K, Krishnamoorthy R, Mageswari A, Selvakumar G, Sa T (2016) Cold
630 stress tolerance in psychrotolerant soil bacteria and their conferred chilling resistance in
631 tomato (*Solanum lycopersicum* Mill.) under low temperatures. PLoS ONE 11:e0161592
632 Surhone LM, Tennoe MT, Henssonow SF (2011) *Pseudomonas vancouverensis.* Betascript
633 Publishing. PP. 68
634 Szymańska S, Płociniczak T, Piotrowska-Seget Z, Hrynkiewicz K (2016) Endophytic and
635 rhizosphere bacteria associated with the roots of the halophyte *Salicornia europaea* L. –
636 community structure and metabolic potential. Microbiol Res192:37–51
637 Tanaka A, Shimizu K, Kondo R (2013) Antibacterial compounds from shoot skins of moso
638 bamboo (*Phyllostachys pubescens*). J Wood Sci 59:155–159
639 Tanase C, Cosarca S, Toma F, Mare A, Cosarca A, Man A, Miklos A, Imre S (2018)
640 Antibacterial activities of spruce bark (*Picea abies* L.) extract and its components against
641 human pathogens. Rev Chim (Bucharest) 69:1462–1467
642 Tvrzová L, Schumann P, Spröer C, Sedláček I, Páčová Z, Šedo O, Zdráhal Z, Steffen M, Lang E
643 (2006) *Pseudomonas moraviensis* sp. nov. and *Pseudomonas vranovensis* sp. nov., soil
644 bacteria isolated on nitroaromatic compounds, and emended description of *Pseudomonas*
645 *asplenii*. Int J Syst Evol Microbiol 56:2657–2663
646 Van Berkum P, Beyene D, Eardl BD (1996) Phylogenetic relationships among *Rhizobium* species
647 nodulating the common bean (*Phaseolus vulgaris* L.). Int J Syst Bacteriol 46:240–244
648 Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial
649 biomass C. Soil Biol Biochem 19: 703–707
650 Vieira FCS, Nahas E (2005) Comparison of microbial numbers in soils by using various culture
651 media and temperatures. Microbiol Res 160:197–202
652 Wang ET, Tan ZY, Guo XW, Rodriguez-Duran R, Boll G, Martinez-Romero E (2006) Diverse
653 endophytic bacteria isolated from a leguminous tree *Conzattia multiflora* grown in Mexico.
654 Arch Microbiol 186:251–259

Wang J, Wu Y, Zhou J, Bing H, Sun H (2016) Carbon demand drives microbial mineralization of organic phosphorus during the early stage of soil development. Biol Fert Soils 52:825–839

Wang H, Zhong H, Hou R, Ayala J, Liu G, Yuan S, Yan Z, Zhang W, Liu Y, Cai K, Cai Z, Huang H, Zhang Z, Wu D (2017) A diet diverse in bamboo parts is important for giant panda (*Ailuropoda melanoleuca*) metabolism and health. Sci Rep 7:3377

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

WRB IWG (2006) World Reference Base for Soil Resources. World Soil Resources Reports, No. 103. FAO, Rome

Xia Q, Rufty T, Shi W (2021) Predominant microbial colonizers in the root endosphere and rhizosphere of turfgrass systems: *Pseudomonas veronii*, *Janthinobacterium lividum*, and *Pseudogymnoascus* spp. Front Microbiol 12:643904

Xiao X, Chen W, Zong L, Yang J, Jiao S, Lin Y, Wang E, Wei G (2017) Two cultivated legume plants reveal the enrichment process of the microbiome in the rhizocompartments. Mol Ecol 26:1641–1651

Xiao X, Li G, Zai X, Bai W, Wang E, Wei G, Chen W (2019) Compositional response of *Phaseolus vulgaris* rhizomicrobiome to a changing soil environment is regulated by long-distance plant signaling. Plant Soil 442: 257–269

Xu X, Peter E, Thornton PE, Post WM (2013) A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Global Ecol Biogeogr 22:737–749

Xu B, Wang J, Shi F, Wu N (2016) Relationships between plant colonization and soil characteristics in the natural recovery of an earthquake-triggered debris flow gully in the Wanglang National Nature Reserve, China. J Mt Sci 13:59–68

Yao R, Dai Q, Wu T, Yang Z, Chen H, Liu G, Zhu Y, Qi D, Yang X, Luo W, Gu X, Yang X, Zhu L (2021) Fly-over phylogeny across invertebrate to vertebrate: The giant panda and insects share a highly similar gut microbiota. Computat Structural Biotechnol J 19:4676–4683

Yu P, Hochholdinger F (2018) The role of host genetic signatures on root–microbe interactions in the rhizosphere and endosphere. Front. Plant Sci. 9:1896

685 Yuan Z-S, Liu F, Zhang G-F (2015) Isolation of culturable endophytic bacteria from moso
686 bamboo (*Phyllostachys edulis*) and 16S rDNA diversity analysis. Arch Biol Sci Belgrade
687 67:1001–1008
688 Zhang YM, Li Y Jr, Chen WF, Wang ET, Tian CF, Li QQ, Zhang YZ, Sui XH, Chen WX
689 (2011a) Biodiversity and biogeography of rhizobia associated with soybean plants grown in
690 the North China Plain. Appl Environ Microbiol 77:6331-6342
691 Zhang YZ, Wang ET, Li M, Li QQ, Zhang YM, Zhao SJ, Jia XL, Zhang LH, Chen WF, Chen
692 WX (2011b) Effects of rhizobial inoculation, cropping systems and growth stages on
693 endophytic bacterial community of soybean roots. Plant Soil 347:147–161
694 Zhang J, Wang ET, Singh RP, Guo C, Shang Y, Chen J, Liu C (2019a) Grape berry surface
695 bacterial microbiome: impact from the varieties and clones in the same vineyard from
696 central China. J Appl Microbiol 126:204–214
697 Zhang CW, Tian XY, Zhang C-S (2019b) Diversity and probiotic activities of endophytic
698 bacteria associated with the coastal halophyte *Messerschmidia sibirica*. Appl Soil Ecol
699 143:30
700 Zhang L, Zhang W, Li Q, Cui R, Wang Z, Wang Y, Zhang Y-Z, Ding W, Shen X (2020)
701 Deciphering the root endosphere microbiome of the desert plant *Alhagi sparsifolia* for
702 drought resistance-promoting bacteria. Appl Environ Microbiol 86:e02863-19
703

LEGEND OF FIGURE

Fig. 1. Summary of phenotypic characterization of the endophytic and rhizospheric bacteria of arrow bamboo (*Fargesia nitida*). Growth tests were performed with PY medium and the other tests were carried out with corresponding media as mentioned in the text. Conc.=Concentration; Degr.=Degradation; Solu.=Solubilization; Prod.=Production.