

1 **Investigating the role of exudates in recruiting *Streptomyces* bacteria to the *Arabidopsis***
2 ***thaliana* root microbiome**

3

4 Sarah F. Worsley¹, Michael C. Macey², Jake T. Newitt¹, Elaine Patrick¹, Douglas W. Yu^{1,3,4},
5 Barrie Wilkinson⁵, J. Colin Murrell² and Matthew I Hutchings^{1*}

6

7

8 ¹School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich,
9 Norfolk, United Kingdom. NR4 7TJ.

10 ²School of Environmental Sciences, University of East Anglia, Norwich Research Park,
11 Norwich, Norfolk, United Kingdom. NR4 7TJ.

12 ³ State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology,
13 Chinese Academy of Sciences, Kunming Yunnan, 650223 China

14 ⁴ Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences,
15 Kunming Yunnan, 650223 China

16 ⁵Department of Molecular Microbiology, John Innes Centre, Norwich Research Park,
17 Norwich, Norfolk, United Kingdom. NR4 7UH.

18

19 Email: m.hutchings@uea.ac.uk

20

21

22

23

24 **Abstract.** *Arabidopsis thaliana* has a diverse but consistent root microbiome, recruited in part
25 by the release of fixed carbon in root exudates. Here we focussed on the recruitment of
26 *Streptomyces* bacteria, which are well established plant-growth-promoting rhizobacteria and
27 which have been proposed to be recruited to *A. thaliana* roots by the release of salicylic acid.
28 We generated high quality genome sequences for eight *Streptomyces* endophyte strains and
29 showed that although some strains do enhance plant growth, they are not attracted to, and do
30 not feed on, salicylic acid. We used ¹³CO₂ DNA-stable isotope probing to determine which
31 bacteria are fed by the plants in the rhizo- and endosphere and found that streptomycetes did
32 not feed on root exudates *in vivo*, despite the fact that they can use exudate as sole carbon and
33 nitrogen sources *in vitro*. We confirmed increased root colonisation by streptomycetes in plants
34 that constitutively produce salicylic acid, but these plants exhibited a pleiotropic phenotype of
35 early senescence and weak growth. We propose that streptomycetes are attracted to the
36 rhizosphere by root exudates but can be outcompeted for this food source by more abundant
37 proteobacteria and most likely feed off unlabelled complex organic matter.

38

39

40

41

42

43 **Introduction.** Plants form stable and beneficial interactions with bacteria at all stages of their
44 life cycle, but we have a limited understanding of how these interactions are directed or
45 maintained. Young plants can release 30-40% of photosynthetically fixed carbon into the soil
46 as root exudates and these help shape the plant microbiome (Haichar et al 2016, Whipps 1990).
47 Exudates contain a diverse range of compounds, many of which may be metabolized by
48 beneficial bacterial communities (Bais et al 2006, Zhalnina et al 2018). Understanding the
49 signals that recruit different bacterial taxa is economically important because plant-growth-
50 promoting rhizobacteria (PGPR) can increase crop growth and protect against disease (Berg
51 2009). There is growing interest and investment in biological solutions to crop loss, including
52 the application of beneficial microbes as seed coatings and supplements to agricultural soils
53 (Berg 2009, Wei and Jousset 2017). Increasing the presence of antibiotic-producing bacteria
54 such as *Streptomyces* species in agricultural soils has been shown to suppress fungal pathogens
55 in numerous recent studies, (e.g. Cha et al 2016, Inderbitzin et al 2018, Ren et al 2018, Zhou
56 et al 2018) and some *Streptomyces* endophyte species are marketed as fungal growth
57 suppressors (Chaurasia et al 2018, Rey and Dumas 2017, Viaene et al 2016).

58 *Streptomyces* are saprophytic bacteria that play an important ecological role as
59 composters in soil and as prolific producers of antibiotics (van der Meij et al 2017). They
60 evolved filamentous growth ca. 440 mya when plants were first colonising land and it has been
61 suggested that this enabled them to successfully colonise plant roots (Chater 2016). Several
62 studies have reported that streptomycetes are abundant inside the roots of the model plant
63 *Arabidopsis thaliana* (Bai et al 2015, Bodenhausen et al 2013, Bulgarelli et al 2012, Carvalhais
64 et al 2015, Lebeis et al 2015, Lundberg et al 2012, Schlaeppi et al 2014), and others have shown
65 that streptomycetes can protect crop plants such as strawberry, lettuce, rice, and wheat against
66 biotic and abiotic stressors, including drought, salt stress, and fungal pathogens (see Schrey
67 and Tarkka 2008, Viaene et al 2016 for recent reviews). Recent studies have reported that the
68 proportion of *Streptomyces* bacteria in the *A. thaliana* rhizosphere and roots is positively
69 correlated with the levels of phenolic compounds released in *A. thaliana* root exudates,
70 including the defence hormone salicylic acid (SA) (Badri et al 2013, Carvalhais et al 2015,
71 Lebeis et al 2015). It was also reported recently that some phytohormones induce the
72 production of antimicrobial compounds by endophytic streptomycetes (van der Meij et al
73 2018). Not all plant species enrich streptomycetes in their roots, a notable example being
74 barley, and this suggests there are plant-specific signals that shape the root microbiota
75 (Bulgarelli et al 2012, Bulgarelli et al 2015, Haichar et al 2008).

76 In this study we set out to identify which root and rhizosphere bacteria feed off *A.*
77 *thaliana* root exudates and to test the previously published hypothesis that *Streptomyces*
78 bacteria use plant-produced SA as a sole carbon source (Lebeis et al 2015). We report that
79 *Streptomyces* endophytes and other *Streptomyces* strains can indeed grow *in vitro* using *A.*
80 *thaliana* root exudates as a sole carbon and nitrogen source, but *in vivo*, DNA stable isotope
81 probing (DNA-SIP) revealed that *Streptomyces* were not feeding off root exudates in the
82 rhizosphere or the endophytic compartment. We also show that, although root colonisation by
83 *Streptomyces* endophyte isolates increases in *A. thaliana cpr5* plants (which constitutively
84 produce SA) as has been reported previously (Lebeis et al 2015), these isolates were not
85 attracted to SA and could not grow using SA as a sole carbon source. We conclude that
86 colonisation was likely influenced by the pleiotropic effects of the *cpr5* mutation.

87

88 **Materials and Methods.**

89

90 **Isolation of *Streptomyces* endophytes.** Wild-type *A. thaliana* Col-0 seeds were sterilised by
91 washing in 70% (v/v) ethanol for 2 minutes, 20% (v/v) sodium hypochlorite for 2 minutes, then
92 five times in sterile water. Individual seeds were sown into pots of sieved Levington F2
93 compost, placed at 4°C for 24 hours, then grown for 4 weeks under conditions of 12 hours light
94 / 12 hours dark at 22°C. Plants were taken aseptically from pots and roots tapped firmly to
95 remove as much adhering soil as possible. Root material was then placed into sterile PBS-S
96 buffer (PBS-S: 6.33 g NaH₂PO₄.H₂O, 16.5 g Na₂HPO₄.H₂O dissolved in 1L dH₂O, to which
97 200 µl Silwet L-77 was added after autoclaving) for 30 minutes on a shaking platform. Roots
98 were placed into fresh PBS-S and washed for 30 minutes before removing any remaining soil
99 particles with sterile tweezers. Cleaned roots were transferred to 25ml fresh PBS-S and
100 sonicated for 20 minutes in a sonicating water bath to remove any remaining material still
101 attached to the root (as described in Bulgarelli et al 2012). The roots were crushed in sterile
102 10% (v/v) glycerol and serial dilutions were spread onto either soya flour mannitol (SFM) agar,
103 starch casein agar, or minimal medium agar containing sodium citrate (Lebeis et al 2015).
104 Plates were incubated at 30°C for up to 14 days. Colonies resembling streptomycetes were re-
105 streaked onto SFM agar and identified by 16S rRNA gene PCR amplification and sequencing
106 with universal primers PRK341F and MPRK806R (see Table S1 for primers, plasmids and
107 strains used in this work). *Streptomyces* strains were maintained on SFM agar (N1, N2, M2,
108 M3 and *S. coelicolor* M145), Maltose/Yeast extract/Malt extract (MYM) agar with trace

109 elements (L2) or ISP2 agar (*S. lydicus* strains); media recipes are detailed in Table S2. Spore
110 stocks were made as described previously (Kieser et al 2000).

111

112 **Genome sequencing and analysis.** High quality genome sequences were obtained for newly
113 isolated strains N1, N2, M2, M3, and L2, as well as three known strains of *Streptomyces*
114 *lydicus*; one isolated from the commercial plant growth-promoting product Actinovate and two
115 additional *S. lydicus* strains (ATCC25470 and ATCC31975) obtained from the American Type
116 Culture Collection. Strains were sequenced using PacBio RSII sequencing technology at the
117 Earlham Institute, Norwich, UK, (as described in Holmes et al 2018). Biosynthetic gene
118 clusters were predicted using antiSMASH 4.0, and genomes were annotated using RAST (Aziz
119 et al 2008, Weber et al 2015). Amino acid sequences were uploaded to the KEGG Automatic
120 Annotation Server (KAAS) for functional annotation of genes and metabolic pathway
121 mapping. To identify whether isolates carried homologues to known salicylic acid (SA)
122 degradation genes, all experimentally verified pathways involving SA (or salicylate)
123 degradation were identified using the MetaCyc database (<http://www.metacyc.org/>). Amino
124 acid sequences of characterised genes involved in each of the five pathways were then retrieved
125 from UniProt and used to perform Blast searches against predicted ORFs for each
126 streptomycete strain. The results of the best hit (% identity and % query coverage) are given in
127 Table S3.

128

129 **Antibiotic bioassays.** *Streptomyces* spores (4 µl of 10⁶ spores ml⁻¹) were pipetted onto the
130 centre of agar plates and incubated at 30 °C for 7 days before adding indicator strains. *Candida*
131 *albicans*, *Bacillus subtilis*, a clinical isolate of Methicillin Resistant *Staphylococcus aureus*
132 isolated from a patient at the Norfolk and Norwich University Hospital UK (Qin et al 2017),
133 *Escherichia coli* and *Pseudomonas syringae* DC3000 were grown overnight in 10 ml Lysogeny
134 Broth (LB) (1% tryptone, 0.5% NaCl, supplemented with 0.1% glucose for *P. syringae*). These
135 were sub-cultured 1 in 20 (v/v) for a further 4 hours at 30°C for *P. syringae* and 37°C for *E.*
136 *coli*. These cultures were then used to inoculate 100 ml of molten LB (0.5% agar, plus 0.1%
137 glucose for *P. syringae*), of which 3 ml was used to overlay agar plates containing *Streptomyces*
138 colonies. Plates were incubated for 48 hours at 30°C, and bioactivity was indicated by a clear
139 halo around the *Streptomyces* colony. For bioassays using the fungal strains *Lomentospora*
140 *prolificans* or *Gaeumannomyces graminis*, *Streptomyces* species were grown for 7 days, and
141 then a plug of the fungus (grown on potato glucose agar, Sigma Aldrich, for 14 days) was

142 placed at the edge of the agar plate. Plates were incubated at 25°C for up to 14 days to assess
143 inhibition of fungal growth. Bioassays were carried out on a range of different media (Tables
144 S2, S4).

145

146 ***In vitro* Plant growth promotion assays.** *A. thaliana* col-0 seeds were sterilised and plated
147 onto Murashige and Skoog (MS) medium (Duchefa Biochemie, Harlem, Netherlands) (4.43 g
148 L⁻¹ in dH₂O, supplemented with 1% (w/v) sucrose and 0.8% (w/v) agar) which were then left
149 at 4 °C in the dark for 24 hours before being placed, vertically, under long day growth
150 conditions (12 h light / 12 h dark) at 22°C for 10 days. Seedlings were then transferred to square
151 agar plates containing MS agar (1.5% agar, 0% sucrose) and allowed to equilibrate, vertically,
152 overnight at 22 °C. *Streptomyces* spores were diluted to 10⁶ spores ml⁻¹, and 1 µl of this solution
153 was added to the top of the root system of each seedling and allowed to dry. 16 replicate
154 seedlings were inoculated per *Streptomyces* strain. 10% (v/v) glycerol was added to control
155 seedlings. Plates were grown vertically for 16 days, 12 h light/12 h dark at 22°C before
156 measuring plant biomass (dry weight). The biomass of plants with different inocula were
157 compared via ANOVA and Tukey's Honestly Significant Difference (HSD) tests; biomass was
158 log-transformed to ensure normality.

159

160 **IAA production assays** *Streptomyces* isolates were grown on cellophane membranes covering
161 YMD media (Table S2) supplemented with 5 mM tryptophan. After 7 days, cellophane
162 membranes with bacterial biomass were removed and plates were flooded with Salkowskis
163 reagent (as in Bric et al 1991). A red colour indicates that IAA has leached into the medium.

164

165 **Collection of root exudates.** Seeds were sterilised as above and placed at 4°C for 24 hours
166 before being grown, vertically, in conditions of 12 h light/12 h dark for 7 days at 22°C on MS
167 agar. Seedlings were transferred to 12 well plates, containing 3 ml of liquid MS (0% sucrose)
168 in each well and then grown for 10 days before being washed and transferred to new wells
169 containing 3 ml sterile water for 5 days. After removing plant material, liquid from each well
170 was filter-sterilised and added to sterile agarose (0.8% w/v) to make solid growth medium
171 plates. Spores of each *Streptomyces* isolate were streaked onto these plates and incubated for
172 7 days at 30°C. Agarose/water (0.8% w/v) plates were used as a control to assess growth on
173 root exudates.

174

175 **Stable Isotope Probing.** Sterilised *A. thaliana* seeds were sown singly in pots containing 100
176 ml sieved Levington F2 compost, soaked with dH₂O. These were placed in the dark at 4°C for
177 48 hours, then transferred to short-day growth conditions (8 h light/16 h dark) at 22°C for 32
178 days before exposure to CO₂. Each plant was placed in air-tight, transparent 1.9 L cylindrical
179 tubes. Three plants were exposed to 1000 ppmv of ¹²CO₂ and three plants were exposed to
180 1000 ppmv of ¹³CO₂ (99%, Cambridge isotopes, Massachusetts, USA). Three unplanted
181 controls containing only Levington F2 compost were additionally exposed to 1000 ppmv of
182 ¹³C labelled CO₂ to control for autotrophic consumption of CO₂ by soil microbes. CO₂
183 treatments took place over a period of 21 days (ending 53 days after germination). CO₂ was
184 manually injected into tubes every 20 minutes over the course of the 8 hour light period, to
185 maintain the CO₂ concentration at ~1000 ppmv. The volume of CO₂ to be added at each
186 injection was determined by measuring the rate of uptake of CO₂ over 20 minutes every 4 days.
187 CO₂ was measured using an Agilent 7890A gas chromatography instrument with a flame
188 ionisation detector and a Poropak Q (6ft x 1/8”) HP plot/Q (30 m x 0.530 mm, 40 µm film)
189 column with a nickel catalyst and a nitrogen carrier gas. The instrument was run with the
190 following settings: injector temperature 250°C, detector temperature 300°C, column
191 temperature 115°C and oven temperature 50°C. The injection volume was 100 µl and the run
192 time was 5 mins, with CO₂ having a retention time of 3.4 mins. Peak areas were compared to
193 a standard curve (standards of known CO₂ concentration were prepared in 120 ml serum vials
194 that had been flushed with 80%/20% nitrogen-oxygen mixture). At the end of the light period
195 each day, tube lids were removed to prevent the build-up of respiratory CO₂ during the dark
196 period. Just before the next light period, tubes were flushed with an 80%/20% nitrogen-oxygen
197 mix to remove any residual CO₂ before replacing the lids and beginning the first injection of
198 1.9 ml CO₂.

199

200 **Sampling and DNA extraction from CO₂ incubated soil, rhizosphere, and roots.** Two
201 samples of root-free “bulk soil” were collected from each planted and unplanted pot; samples
202 were snap-frozen in liquid nitrogen and stored at -80°C. For the planted pots, roots were tapped
203 until only soil firmly adhering to the root surface remained; the remaining soil was defined as
204 the rhizosphere fraction. To collect this, roots were placed in 25 ml sterile, PBS-S buffer (see
205 above) and washed on a shaking platform at top speed for 30 min before transfer to fresh PBS-
206 S. Used PBS-S from the first washing stage was centrifuged at 3200 x g for 15 minutes, and
207 the supernatant was removed. The resulting pellet (the rhizosphere sample) was snap-frozen

208 and stored at -80°C . The roots were then shaken in fresh PBS-S for a further 30 minutes before
209 removing any remaining soil particles with sterile tweezers. The cleaned roots were finally
210 transferred to fresh PBS-S and sonicated for 20 minutes in a sonicating water bath (as described
211 in Bulgarelli et al 2012). The root (“endophytic”) sample for each plant was then separated into
212 two samples, and snap frozen and stored at -80°C . The endophytic sample consisted of bacteria
213 within the roots and those very firmly attached to the root surface (the “rhizoplane”). A
214 modified version of the manufacturer’s protocol for the FastDNA™ SPIN Kit for Soil (MP
215 Biomedicals) was used to extract DNA from soil, rhizosphere, and root samples. Modifications
216 included pre-homogenisation of the root material by grinding in liquid nitrogen before addition
217 of lysis buffer, an extended incubation time (10 minutes) in DNA matrix buffer, and elution in
218 150 μl of sterile water. DNA yields were quantified using a Qubit fluorimeter.

219

220 **Density gradient ultracentrifugation and fractionation.** DNA samples from the
221 rhizosphere, roots, and unplanted soil were subjected to caesium chloride (CsCl) density
222 gradient separation using an established protocol (Neufeld et al 2007). For each of the replicate
223 rhizosphere samples from both the $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ incubated plants, 1.5 μg of DNA was
224 loaded into the CsCl solution with gradient buffer. For the three unplanted soil sample
225 replicates, 1 μg of DNA was used. For the three root sample replicates under each of the planted
226 treatments ($^{12}\text{CO}_2$ and $^{13}\text{CO}_2$), it was necessary to combine the three replicates as the low DNA
227 yields from each sample did not meet the minimum DNA yield requirement (0.5 μg) for
228 ultracentrifugation on their own. Thus, 0.2 μg of DNA was pooled from each of the three
229 replicates per $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ treatment, and the final 0.6 μg was loaded into the CsCl
230 solution. After ultracentrifugation, the density of each fraction was measured using a
231 refractometer in order to check for successful gradient formation. DNA was precipitated from
232 fractions as detailed in Neufeld et al 2007 and stored at -20°C before use as a template in qPCR
233 and PCR reactions.

234

235 **Fraction selection via qPCR and 16S rRNA gene amplicon sequencing.** To identify
236 fractions containing heavy (^{13}C) and light (^{12}C) DNA for each sample, 16S rRNA gene copy
237 number was quantified across fractions using qPCR. Reactions were carried out in 25 μl
238 volumes. 1 μl of template DNA (either sample DNA or standard DNA), or dH_2O as a control,
239 was added to 24 μl of reaction mix containing 12.5 μl of the 2x Sybr Green Jumpstart Taq
240 Ready-mix (Sigma Aldrich), 0.125 μl each of the primers 341F 806R, 4 μl of 25 mM MgCl_2 ,

241 0.25 μ l of 20 μ g/ μ l Bovine Serum Albumin, and 7 μ l dH₂O. Sample DNA, standards (a dilution
242 series of the target 16S rRNA gene at known quantities), and negative controls were quantified
243 in duplicate. Reactions were run under the following conditions: 96°C for 10 mins; 40 cycles
244 of 96°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min; 96°C for 15 sec; 100 cycles at 75°C-
245 95°C for 10 secs, ramping 0.2°C per cycle. Reactions were performed in 96-well plates (Bio-
246 Rad). The threshold cycle (C_T) for each sample was then converted to target molecule number
247 by comparing to C_T values of a dilution series of target DNA standards. Following
248 quantification of 16S rRNA gene number in each fraction, fractions spanning the peaks in 16S
249 rRNA gene copy number were identified. Equal quantities of each fraction were combined to
250 create a “heavy” buoyant density (labelled) and “light” buoyant density (unlabelled) fraction
251 for each sample, respectively. The 16S rRNA genes were amplified for each of these fractions
252 using the Universal primers PRK341F and MPRK806R (Table S1), and the resulting PCR
253 product was purified and submitted for 16S rRNA gene amplicon sequencing using an Illumina
254 MiSeq at Mr DNA (Molecular Research LP), Shallowater, Texas, USA. Sequence data were
255 then processed at Mr DNA using their custom pipeline (Dowd et al 2008a, Dowd et al 2008b).
256 As part of this pipeline, pair-end sequences were merged, barcodes were trimmed, and
257 sequences of less than 150 bp and/or with ambiguous base calls were removed. The resulting
258 sequences were denoised, and OTUs were assigned by clustering at 97% similarity. Chimeras
259 were removed, and OTUs were assigned taxonomies using *BlastN* against a curated database
260 from GreenGenes, RDPII, and NCBI (DeSantis et al 2006). Plastid-like sequences were
261 removed from the analysis. All data received from Mr DNA were then statistically analysed
262 using *R* 3.2.3 (2015). All the 16S rRNA gene amplicon sequences have been submitted to the
263 European Nucleotide Archive (ENA) database under the study accession number
264 PRJEB30923.

265

266 **Generating eGFP-labelled *Streptomyces* strains.** Plasmid pIJ8660 (Table S1, John Innes
267 Centre, Norwich, UK) containing an optimised eGFP gene under the control of the constitutive
268 *ermE** promoter and the *aac* apramycin resistance marker (Sun et al 1999) was conjugated into
269 *Streptomyces* strains (as described in Kieser et al 2000). Exconjugants were selected and
270 maintained on SFM agar plates containing 50 μ g ml⁻¹ apramycin. For confocal microscopy,
271 *Arabidopsis thaliana* Col-0 seeds were germinated as described for plant growth promotion
272 assays and grown vertically at 22 °C for 9 days under a 12-hour photoperiod. Seedlings were
273 then transferred to MS (1.5% agar, 0% sucrose) and were allowed to equilibrate for 24 hours

274 before being inoculated with 1µl of spore suspension (10^6 cfu ml⁻¹) of either M3 or
275 *Streptomyces coelicolor* M145 (both eGFP-tagged). Inoculated seedlings were then left to grow
276 for 3 days before being washed in a 20% (v/v) solution of glycerol containing 1µg ml⁻¹
277 SynaptoRed™ for 10 minutes. A 20 mm section of root (taken from the base of the petiole)
278 was then mounted onto a slide with 100 µl of the SynaptoRed™/glycerol solution. Samples
279 were imaged using a Zeiss LSM510 META laser-scanning confocal microscope with a
280 PlanApochromat 63x (1.4 NA) objective. Green fluorescent protein was excited at 488 nm and
281 emission collected through a 527.5 ± 22.5 nm bp filter, and FM4-64 excited at 543 nm and
282 emission collected through a 587.5 ± 27.5 nm bp filter.

283

284 **Plant colonisation assays in soil.** Wild-type *A. thaliana* Col-0, *cpr5*, *pad4*, and *sid2-2* were
285 obtained from the Nottingham *Arabidopsis* Stock Centre (NASC). Seeds were sterilised and
286 placed in 500 µl of solutions containing 10^6 pregerminated *Streptomyces* spores ml⁻¹ (either
287 *Streptomyces coelicolor* M145 or isolate M3, both eGFP-tagged) in 2xYT (Table S2, Kieser et
288 al 2000). Spores were pre-germinated at 50°C for 10 minutes. 2xYT was used as a control.
289 Seeds were incubated in the solutions for 90 minutes on a rotating shaker before being
290 transferred to pots of sieved Levington F2 compost, soaked with dH₂O. Another 1 ml of pre-
291 germinated spores (or 2xYT control) was pipetted into the soil to a depth of approximately 2
292 cm below the seed. Pots were then incubated at 4°C for 24 hours, then grown for 4 weeks under
293 a photoperiod of 12h light/ 12h dark at 22°C. Six replicate plants were grown for each plant
294 and streptomycete genotype.

295

296 **Re-isolation of eGFP-tagged bacteria from roots.** Root samples were cleaned as described
297 above and crushed in 1 ml of sterile 10% (v/v) glycerol. 100 µl of the homogenate was plated
298 onto three replicate SFM agar plates containing 50 µg ml⁻¹ apramycin as well as 5 µg ml⁻¹
299 nystatin to inhibit fungal growth. Agar plates were incubated at 30°C for 5 days, then colony
300 forming units (CFU) were counted. CFU counts were converted to CFU per gram of root tissue
301 and log-transformed to normalise residuals. Data were analysed via ANOVA and TSD tests.

302

303 **Testing for sole use of carbon and nitrogen sources.** *Streptomyces* strains were streaked onto
304 minimal medium agar plates (Kieser et al 2000) supplemented with either their preferred
305 carbon source as a positive control (5 g ml⁻¹ of either mannitol, maltose or sucrose), 3.875 mM
306 sodium citrate, or 0.5 mM SA as a carbon source. Plates with no carbon source were used as a

307 control. All plates were incubated for seven days at 30°C. To test for the use of 1-
308 aminocyclopropane-1-carboxylic acid (ACC) as a sole nitrogen source, strains were streaked
309 onto Dworkin and Foster medium (Dworkin and Foster 1958) in which 0.2% (w/v) NH₄SO₄ or
310 0.051% (w/v) ACC was added as a sole nitrogen source, or no nitrogen source as a control.
311 Plates were left for 10 days at 30°C before imaging.

312

313 **Salicylic Acid (SA) as a chemoattractant.** 4 µl of *Streptomyces* spores (10⁶ spores ml⁻¹) were
314 placed in the centre of SFM agar plates and 40 µl of either 1 mM or 0.5 mM filter sterilised SA
315 was inoculated onto 6mm filter paper discs (Whatman) and allowed to dry. Discs were then
316 added to one side of the agar plate, 2 cm from the streptomycete spores. SA solutions were
317 prepared by diluting a 100 mM stock solution to a 1 mM or 0.5 mM SA solution in PBS. The
318 100 mM stock solution was made by dissolving 0.138 g of SA in 2 ml of 100% DMSO before
319 making the solution up to 10 ml with PBS. Thus, the resulting 1 mM and 0.5 mM solutions had
320 a final concentration of 0.2% and 0.1% DMSO, respectively. To check that any observations
321 were not due to the effects of DMSO, control plates were also run alongside the SA experiment,
322 in which discs were soaked in 40 µl of a 0.2% DMSO (in PBS), equivalent to the final
323 concentration of DMSO in the 1 mM SA solution. Plates were incubated for seven days at 30
324 °C.

325

326 **Enumeration of bacteria from soil microcosms after exogenous application of salicylic**
327 **acid.** Levington F2 seed and modular compost (4 ml) was placed into each compartment of a
328 12-well plate and soaked with 0.5 ml of sterile dH₂O or 0.5 mM SA. Each well was then
329 inoculated with 10⁷ spores ml⁻¹ solution of either eGFP-tagged M145 or M3, suspended in
330 dH₂O or 0.5 mM SA. Spores of eGFP-tagged M3 or M145 were used to align with the *in vivo*
331 plant colonisation experiments. There were nine replicate wells for each treatment. Well-plates
332 were placed under a photoperiod of 12 h light/12 h dark for 10 days. 100 mg of soil from each
333 well was then diluted in 900 µl of water and vortexed. Serial dilutions were then plated onto
334 SFM containing 50 µg ml⁻¹ apramycin (for selection), 10 µg ml⁻¹ nystatin, and 100 µg ml⁻¹
335 cyclohexamide (to repress fungal growth). CFU of the *Streptomyces* inoculum were then
336 enumerated on the 10⁻² dilution plates after 4 days to assess whether SA affected growth in
337 soil.

338

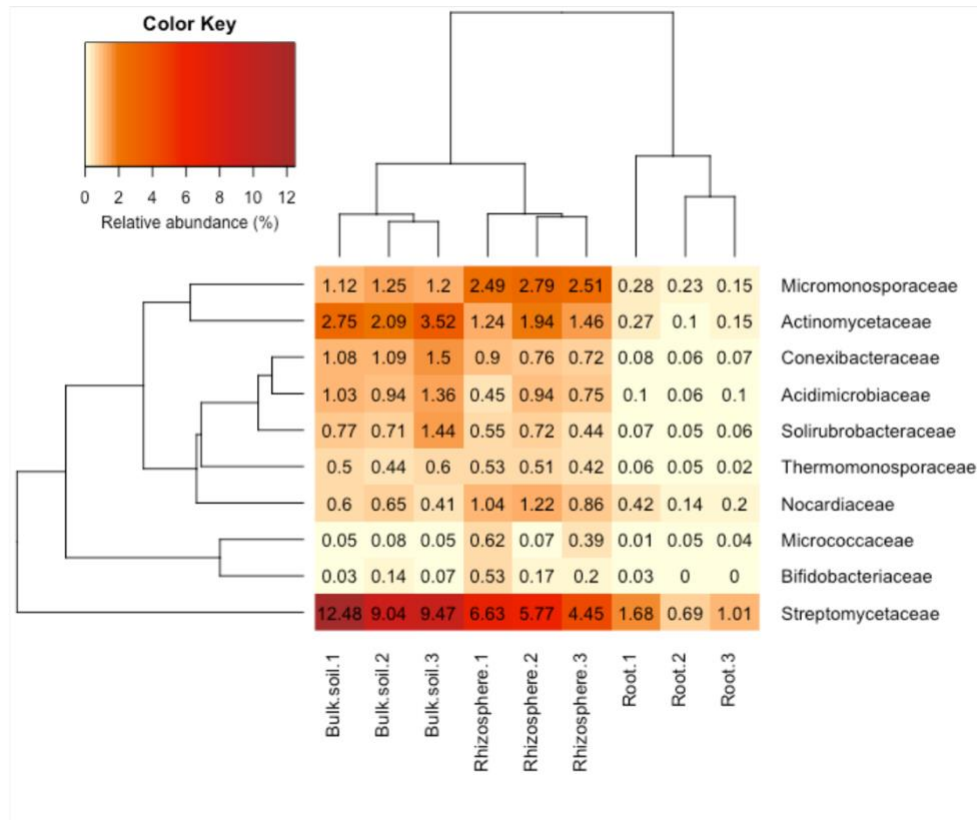
339 **Results**

340

341 **Bacterial 16S rRNA gene profiling of the *A. thaliana* rhizosphere and endosphere.** Several
342 independent studies have reported that *A. thaliana* plants have relatively stable and consistent
343 root bacterial communities, with the enrichment of Actinobacteria within plant roots being
344 predominantly driven by the presence of the family Streptomyetaceae (Bai et al 2015,
345 Bodenhausen et al 2013, Bulgarelli et al 2012, Carvalhais et al 2015, Lebeis et al 2015,
346 Lundberg et al 2012, Schlaeppli et al 2014). To test this on plants grown in our laboratory, we
347 repeated the bacterial 16S rRNA gene profiling using the universal primers PRK341 and
348 MPRK806 (Yu et al 2005) and found the results were in agreement with published studies, in
349 that Streptomyetaceae were the most abundant family of Actinobacteria found in the both the
350 rhizosphere and endophytic compartments (Fig. 1). Although they were not significantly
351 enriched in either compartment compared to the surrounding soil, Streptomyetaceae made up
352 5.62% (\pm 1.10% standard deviation) of the rhizosphere community and 1.12% (\pm 0.50%) of
353 the endophytic community; Actinobacteria as a whole made up 15.15% (\pm 1.47%) and 2.87%
354 (\pm 0.88%) of these compartments, respectively (Fig. 1 and S1).

355

356 **Figure 1.** The relative abundance of Actinobacterial families in soil, rhizosphere and root
357 compartments. N = 3 replicate plants in individual pots. Streptomyetaceae was the most
358 abundant family of Actinobacteria in all three compartments. Clustering represents Bray Curtis
359 dissimilarities.



360

361 **DNA Stable Isotope Probing of the *A. thaliana* rhizosphere and endosphere.** To identify
362 which bacteria feed on root exudates in the rhizo- and endosphere of *A. thaliana*, plants were
363 labelled by growing them (n=3) for 21 days under a short-day photoperiod (8 hours light) in
364 the presence of $^{12}\text{CO}_2$ or $^{13}\text{CO}_2$ in sealed growth chambers (Fig. S2). Unplanted pots were also
365 incubated with $^{13}\text{CO}_2$ to control for autotrophic metabolism. Given the relatively short time
366 frame of CO_2 exposure, the ^{13}C was expected to be incorporated mainly into plant metabolites
367 rather than into plant cell wall material. Bacteria that incorporate ^{13}C into their DNA are
368 therefore likely to be feeding on exuded plant metabolites or directly fixing $^{13}\text{CO}_2$
369 autotrophically. After 21 days, total DNA was extracted from the unplanted soil, rhizosphere,
370 and endosphere compartments of the $^{12}\text{CO}_2$ or $^{13}\text{CO}_2$ incubated plants, and the heavy (^{13}C) and
371 light (^{12}C) DNA were separated by density gradient ultracentrifugation for the rhizosphere and
372 endosphere compartments (Neufeld et al 2007). Heavy and light fractions of the gradient were
373 used for 16S rRNA gene amplicon sequencing using the universal bacterial 16S rRNA primers
374 PRK341 and MPRK806. For the rhizosphere and endophytic samples, bacterial genera were
375 defined as metabolisers of root exudates if they showed at least a two-fold increase in relative
376 abundance in the heavy fraction of $^{13}\text{CO}_2$ plants (^{13}CH) compared to both the $^{13}\text{CO}_2$ light
377 fraction (^{13}CL) and $^{12}\text{CO}_2$ heavy fraction (^{12}CH), respectively. Importantly, the abundance of

378 these bacteria was also less than two-fold greater in the heavy versus the light fractions of
379 $^{13}\text{CO}_2$ unplanted controls which means they are not autotrophically fixing CO_2 .

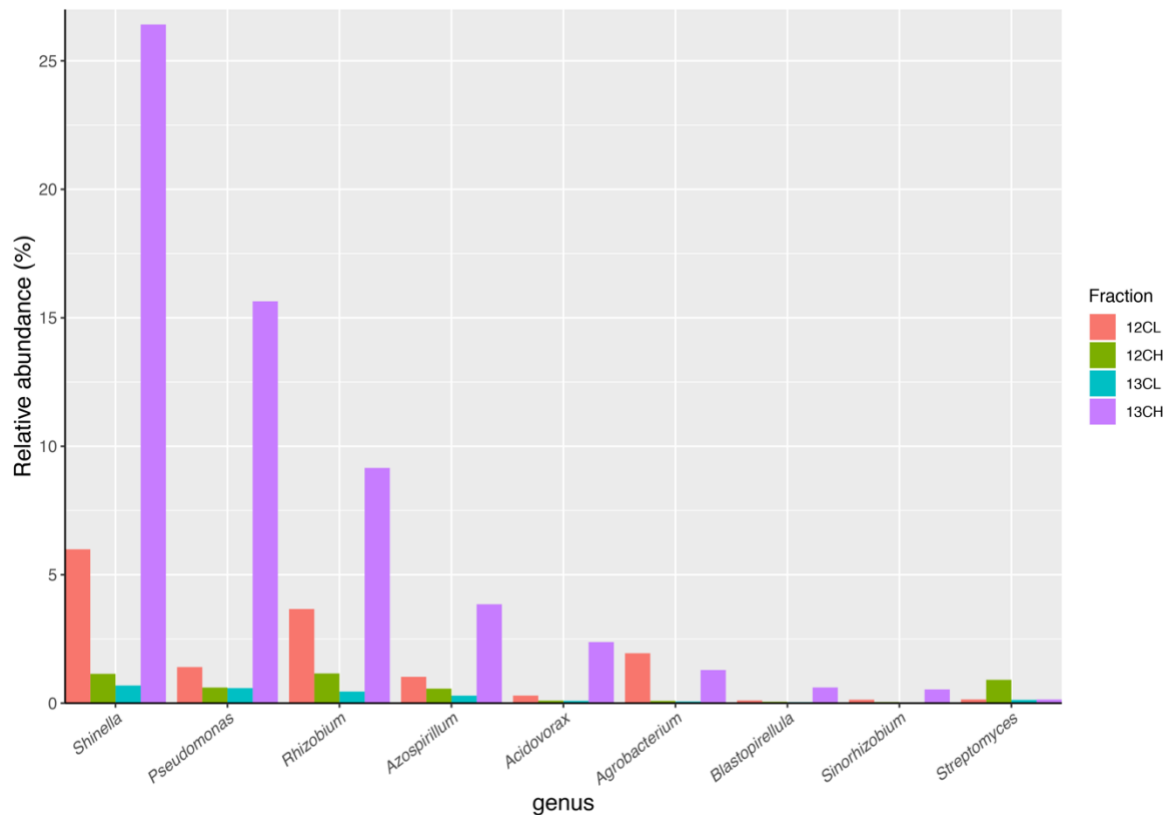
380 Following sorting of the data and the removal of autotrophs (Table S5), 28 genera
381 showed an average of two-fold (or more) enrichment in the ^{13}CH fraction of rhizosphere
382 samples (Fig S3, Table S5). The majority of these taxa were in the phylum Proteobacteria (24
383 out of 28 genera), with only one representative each from the phyla Chloroflexi (*Levilinea*),
384 Firmicutes (*Pelotomaculum*), Cyanobacteria (*Chroococcidiopsis*), and the Planctomycetes
385 (*Pirellula*). The most enriched genus was *Pseudomonas*, which demonstrated a 64-fold
386 enrichment in relative abundance between the ^{13}CH (8.63% average relative abundance) and
387 ^{13}CL (0.13% average relative abundance) fractions and a 23-fold enrichment between the ^{13}CH
388 fraction compared to the ^{12}CH control (0.37% relative abundance). A total of 27 genera
389 demonstrated enrichment in the ^{13}CH fraction of the endophytic compartment (Fig 2, Table
390 S5). Here, the majority of root exudate metabolisers were also Proteobacteria (21 genera in
391 total), with three genera belonging to the phylum Planctomycetes (*Blastopirellula*, *Pirellula*
392 and *Gemmata*), one to the Firmicutes (*Clostridium*), one to the Chloroflexi (*Chloroflexus*), and
393 one to the Actinobacteria (*Jatrophihabitans*). The most abundant genus in the ^{13}CH fraction of
394 the endophytic samples was *Shinella*, which demonstrated a 38-fold enrichment between the
395 ^{13}CH (26.42% relative abundance) and ^{13}CL (0.68% relative abundance) fractions and a 23-
396 fold enrichment between the ^{13}CH and the ^{12}CH control fractions (1.14%). In terms of fold
397 change, however, *Pseudomonas* was the most enriched genus with a 26-fold increase in
398 abundance between ^{13}CH (15.64% relative abundance) and ^{13}CL (0.59% relative abundance)
399 fractions of $^{13}\text{CO}_2$ incubated plants (Fig 2). 15 out of the 28 genera were found to be
400 metabolising exudates in the endosphere but not the rhizosphere, whereas 16 out of 29 genera
401 were metabolising exudates in the rhizosphere but not the endosphere. The remaining 13 genera
402 were enriched in the heavy fractions of both the endophytic and rhizospheric compartments,
403 suggesting that they are able to survive and make use of plant metabolites in both of these
404 niches (Table S5).

405

406 **Figure 2.** Eight genera of bacteria isolated from the endophytic compartment of *Arabidopsis*
407 *thaliana* plants whose relative abundance demonstrated the greatest overall enrichment
408 between the ^{13}CH fraction and the ^{12}CH fraction due to the metabolism of ^{13}C labelled root
409 exudates. In comparison, the genus *Streptomyces* did not demonstrate enrichment between
410 these fractions suggesting they did not metabolise labelled root exudates. $^{12}\text{CL}/^{13}\text{CL}$ and
411 $^{12}\text{CH}/^{13}\text{CH}$ are the buoyant density fractions representing the light/unlabelled (L) and

412 heavy/labelled (H) fractions of DNA from $^{12}\text{CO}_2$ (12C) or $^{13}\text{CO}_2$ (^{13}C) incubated plants
413 respectively.

414



415

416 Surprisingly, there was no enrichment of *Streptomyces* bacteria in the ^{13}CH fractions of either
417 the rhizosphere or endosphere, despite being the most dominant member of the phylum
418 Actinobacteria in both compartments (Figs 1, 2 and S3). Taken together, these data suggest
419 that, in soil, *Streptomyces* bacteria were outcompeted for the root exudates by unicellular
420 bacteria, particularly Proteobacteria, under the conditions used in this experiment. In the
421 current study, Proteobacteria were very abundant in the unfractionated soil (35% average
422 relative abundance), rhizosphere (39%) and endophytic compartment (91%), compared to
423 Actinobacteria (present at 20%, 15% and 3% in the soil, rhizosphere and root compartments,
424 respectively) (Fig S1) and were therefore likely to have the upper hand at the outset of
425 competition (Scheuring and Yu 2012). Accordingly, genera that were found to be metabolising
426 the greatest amount of exudates in the roots were also found to be enriched in the endophytic
427 compartment compared to the surrounding soil (Fig S4). Given their diverse metabolic
428 capabilities, it is likely that streptomycetes are able to persist at low abundance by feeding on
429 more complex organic polymers that contained a much lower level of labelled ^{13}C , under
430 conditions of high competition or in the absence of a preferred type of exudate. *Arabidopsis*

431 *thaliana* has also been shown to have a relatively small “rhizosphere effect” compared to other
432 plant species (Bulgarelli et al 2015, Schlaeppli et al 2014), with weak differentiation in terms
433 of microenvironment and community composition between the rhizosphere and bulk soil.
434 Therefore, complex polymers may have been more readily available in the rhizosphere than
435 exudates, particularly given the fact that compost (used as the plant growth medium in this
436 experiment) contains a high level of organic matter. Alternatively, as they also have spore-
437 forming capabilities, it is possible that *Streptomyces* species were mostly dormant in the
438 rhizosphere and endosphere when the plants were being pulse-labelled with $^{13}\text{CO}_2$.

439

440 **Isolation and genome sequencing of *Streptomyces* endophyte strains.** To analyse plant root
441 interactions with *Streptomyces* species, we selectively isolated these bacteria from *A. thaliana*
442 roots and generated high-quality genome sequences for five phylogenetically distinct
443 *Streptomyces* strains, as judged by initial 16S rRNA gene amplification and sequencing. We
444 also generated high-quality genome sequences for three known endophyte strains (Table 1).
445 These are *Streptomyces lydicus* isolated from the commercial plant growth-promoting product
446 Actinovate and two additional *S. lydicus* strains (ATCC25470 and ATCC31975) obtained from
447 the American Type Culture Collection (Tokala et al 2002). We submitted the genome
448 sequences of all nine strains to the antiSMASH 4.0 server which predicts secondary metabolite
449 biosynthetic gene clusters (Weber et al 2015). The results predicted that all nine strains encode
450 siderophores, antifungal and antibacterial natural products, all of which are common in this
451 genus. To test their bioactivities, agar plate bioassays were performed and showed that all the
452 *Streptomyces* strains inhibited the growth of at least one pathogenic microorganism while
453 some, such as strain N2, show broad spectrum antifungal and antibacterial activity, including
454 against the Gram-negative bacterium, *E. coli* (Table S4).

455

456

457

458 **Table 1.** Features of the *Streptomyces* endophyte genomes sequenced for this study.
459 Biosynthetic gene clusters (BGCs) were predicted using antiSMASH 4.0.

| Strain | Accession no. | Genome size (bp) | ORFs | tRNAs | rRNAs | BGCs |
|--------|---------------|------------------|------|-------|-------|------|
| L2 | QBDT00000000 | 8,073,926 | 7079 | 68 | 18 | 32 |
| M2 | CP028834 | 8,718,751 | 8026 | 72 | 18 | 24 |

| | | | | | | |
|------------|---------------|-----------|------|----|----|----|
| M3 | QANR000000000 | 8,304,843 | 7561 | 74 | 18 | 28 |
| N1 | QBDS000000000 | 7,207,104 | 6239 | 65 | 21 | 22 |
| N2 | CP028719 | 8,428,700 | 7401 | 69 | 21 | 35 |
| Actinovate | RDTC000000000 | 9,139,876 | 7989 | 67 | 21 | 36 |
| ATCC25470 | RDTE000000000 | 7,935,716 | 7084 | 65 | 21 | 26 |
| ATCC31975 | RDTE000000000 | 9,244,118 | 8128 | 66 | 21 | 34 |

460

461 Some plant growth-promoting bacteria are capable of producing plant phytohormones and
462 KEGG pathway analysis revealed that the genomes of all our sequenced *Streptomyces* strains
463 possess genes encoding proteins involved in the biosynthesis of indole-3-acetic acid (IAA),
464 which can contribute to shoot and root growth (Remans et al 2006) (Table S6). For example,
465 all strains have genes encoding key proteins involved in the indole-3-acetamide (IAM)
466 pathway, whereby tryptophan is converted to IAM via a tryptophan 2-monooxygenase enzyme
467 (KEGG reaction R00679). IAM is then further converted to IAA through the action of an
468 amidase enzyme (KEGG reaction R03096). Several strains also possessed genes encoding
469 enzymes involved in the tryptamine (TAM) pathway, which converts tryptamine to IAA via an
470 amine oxidase enzyme (R02173) and an acetaldehyde oxidase enzyme (R02681). *In vitro*
471 colorimetric assays using Salkowski's reagent (Bric et al 1991) qualitatively confirmed the
472 ability of all strains to make IAA (Fig. S5).

473 In addition to IAA, the genomes of all streptomycete isolates possess up to two copies
474 of genes encoding the enzyme aminocyclopropane-1-carboxylate (ACC) deaminase. This
475 cleaves ACC, which is the direct precursor for the plant phytohormone ethylene, into ammonia
476 and 2-oxobutanoate, Kegg reaction: R00997 (Table S6). Bacteria can use the products of this
477 reaction as a nitrogen source, and it was demonstrated *in vitro* that all of the isolates were
478 capable of utilising ACC as a sole nitrogen source when added to minimal medium (Fig. S6).
479 There is evidence that the activity of this enzyme can reduce damage and early-onset
480 senescence caused by excessive ethylene production under prolonged periods of plant stress,
481 by removing the substrate for ethylene biosynthesis (Glick 2014, Palaniyandi et al 2014, Yang
482 et al 2009).

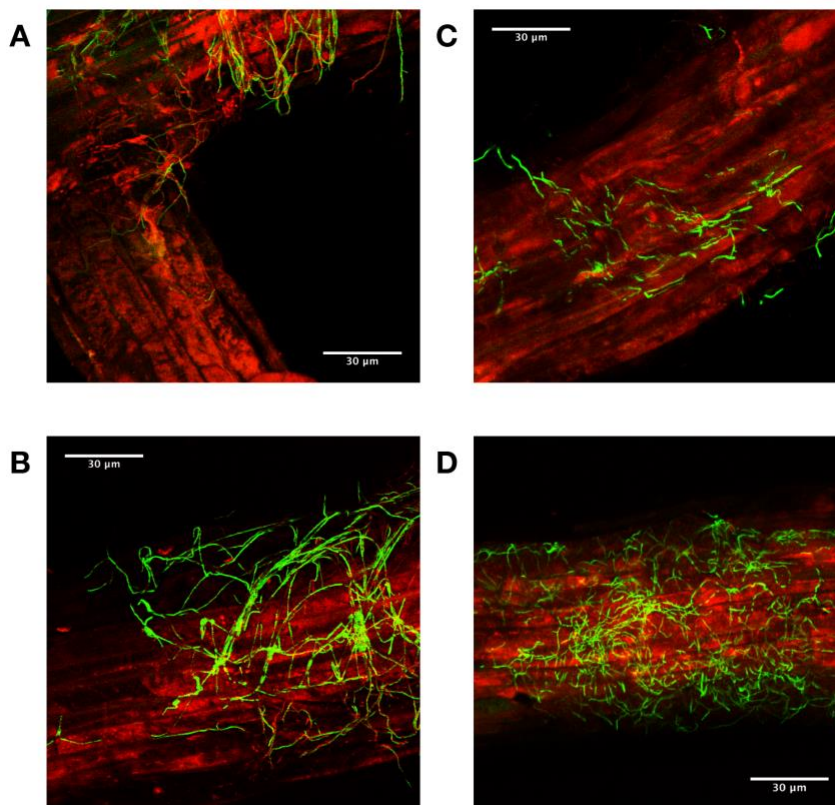
483

484 **Not all *Streptomyces* strains have growth promoting effects in *A. thaliana*.** To investigate
485 whether the sequenced *Streptomyces* strains promote plant growth and fitness, we established
486 root infection assays in which seeds were coated with a suspension of pre-germinated

487 *Streptomyces* spores - these were also added to the soil growth substrate. Tagging the strains
488 with eGFP and the apramycin resistance (*aac*) gene allowed visual confirmation of root
489 infection using confocal microscopy (Fig. 3) and selective re-isolation of the strains on agar
490 plates containing apramycin.

491

492 **Figure 3.** Confocal Laser Scanning Microscopy images of *Arabidopsis thaliana* rhizoplane
493 colonisation by eGFP-tagged *Streptomyces* strains three days after inoculation. A and B show
494 *A. thaliana* roots (red) colonised by eGFP-tagged *Streptomyces coelicolor* M145 (green) and
495 images C and D show *A. thaliana* roots (red) colonised by eGFP-tagged *Streptomyces*
496 endophyte strain M3 (green).



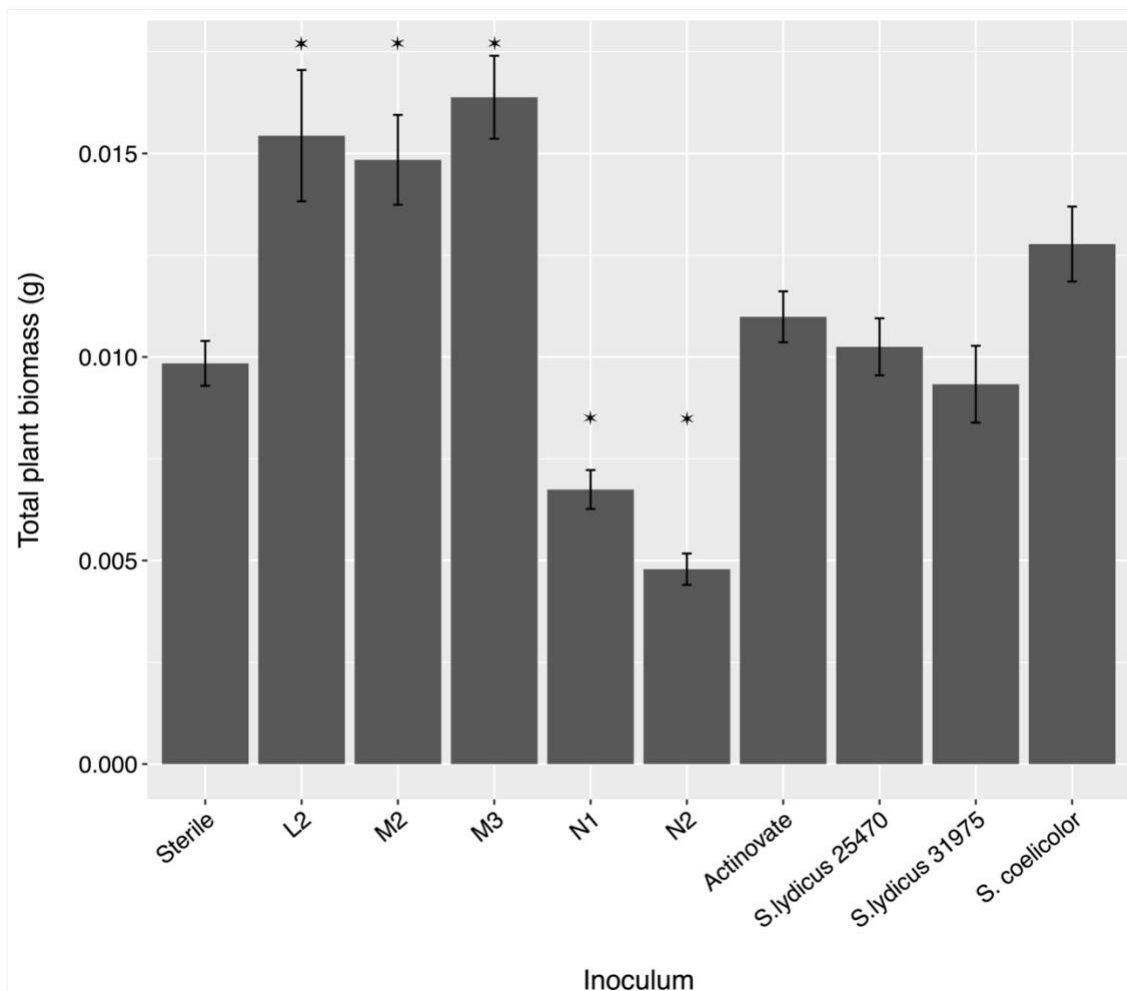
497

498 We tested all the genome-sequenced stains from this study alongside the model organism *S.*
499 *coelicolor* M145. All strains were able to colonise plant roots with varying efficiencies, and
500 several of them significantly increased plant biomass *in vitro* (Fig. 4). However, not all of them
501 promoted plant growth; application of *Streptomyces* strains N1 and N2 significantly reduced
502 the growth of *A. thaliana* ($P < 0.05$ in Tukey's HSD, Fig. 4) and in some cases led to a
503 senescence phenotype with leaf browning; it is possible that some of the biosynthetic gene

504 clusters which have a low percentage homology to other known secondary metabolite clusters,
505 may encode novel herbicidal compounds in these strains.

506

507 **Figure 4.** The impact of *Streptomyces* isolates on *A. thaliana* biomass (grams) 16 days after
508 inoculation. * represents a significant difference in biomass relative to sterile control plants (P
509 <0.05).



510

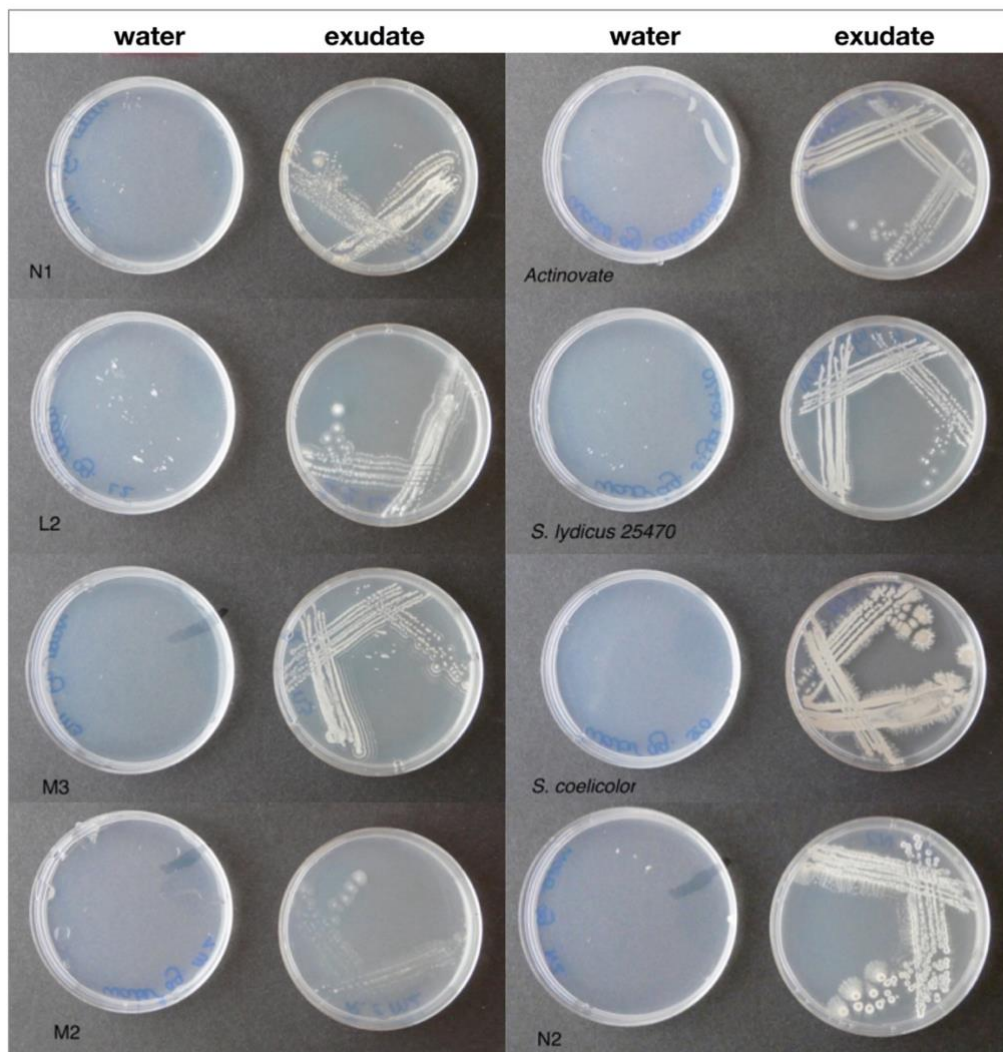
511 **Root exudates promote the growth of *Streptomyces* endophytes *in vitro* but not *in vivo*.**

512 Plants are known to use their root exudates to recruit bacteria from the soil, and these exudates
513 include a complex mixture of sugars, amino acids and phytohormones, including jasmonic acid
514 (JA) and salicylic acid (SA). However, our DNA-SIP experiments suggested that *Streptomyces*
515 species were not feeding on these root exudates in soil-grown plants (Figs 2 and S3). To test
516 whether streptomycetes could use root exudates when grown in monoculture, we collected
517 exudates from hydroponically-grown wild-type *A. thaliana* plants and grew *Streptomyces*
518 isolates on agarose plates containing water only, or water plus sterile root exudates. None of

519 the strains grew on agarose alone, but all grew on agarose plates containing root exudates (Fig.
520 5).

521

522 **Figure 5.** *Streptomyces* strains grown on agarose dissolved in sterile water or agarose dissolved
523 in filter sterilised *A. thaliana* root exudates. Strains M2, M3, N1, N2, and L2 are the
524 *Streptomyces* endophytes isolated and genome sequenced in this study. The other strains are *S.*
525 *lydicus* isolated from Actinovate, *S. lydicus* ATCC25470 and the model laboratory strain *S.*
526 *coelicolor* M145.



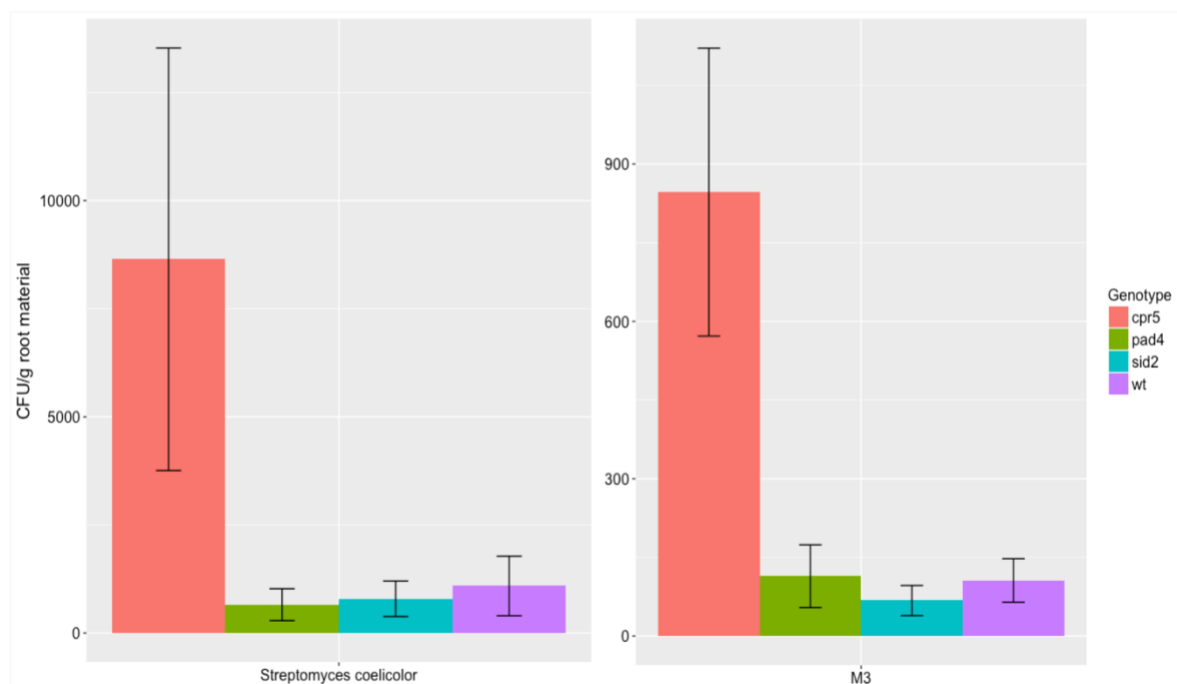
527

528 **Streptomyces do not feed on salicylic acid.** Previous studies have suggested that the
529 defence phytohormone SA plays a key role in modulating root microbiome assembly in *A.*
530 *thaliana*, particularly in recruiting streptomycetes, and that a *Streptomyces* endophyte strain
531 isolated from *A. thaliana* roots could grow using SA as a sole carbon source (Lebeis et al 2015).
532 To test this, we first compared root colonisation efficiency by *S. coelicolor* M145 and
533 *Streptomyces* M3 in wild-type *A. thaliana* col-0, *cpr5* mutant plants that constitutively produce

534 SA, and in *pad4* and *sid2-2* plants that are deficient in SA production (Bowling et al 1994,
535 Jirage et al 1999, Wildermuth et al 2001, Zhou et al 1998). The results confirmed that root
536 colonisation (measured by counting CFU returned per gram of plant root) was significantly
537 affected by plant genotype, irrespective of the *Streptomyces* strain used as an inoculum (plant
538 genotype on log-transformed CFU g⁻¹ was $P < 0.01$, strain genotype interaction was $P = 0.67$
539 in an anova test). Specifically, the colonisation of both M145 and M3 was significantly
540 increased in *cpr5* mutant plants (Fig. 6), which constitutively make SA, compared to the other
541 three genotypes ($P < 0.05$ in all Tukey's HSD tests between *cpr5* and other plant genotypes).

542

543 **Figure 6.** Increased root colonisation of *Arabidopsis thaliana cpr5* plants (constitutively
544 expressing salicylic acid) by *Streptomyces* strains compared to *pad4* and *sid2-2* plants
545 (deficient in salicylic acid production) and wild-type Col-0 plants. Root colonisation was
546 measured as the average colony forming units (\pm SE) of *Streptomyces*, per gram of root, that
547 could be re-isolated from plants 4 weeks after germination. $N = 6$ plants per treatment.



548

549 However, we observed no significant difference between the wild-type, *pad4*, or *sid2-2* plants
550 (Fig. 6). We note that the *cpr5* gene (deleted in *cpr5* plants) has a complex role in regulating
551 plant growth, immunity, and senescence (Jing et al 2007). The *cpr5* plants demonstrated weak
552 growth compared to the other genotypes (Fig. S7), giving rise to the possibility that the
553 observed increase in streptomycete colonisation was due to the complex phenotype of the *cpr5*

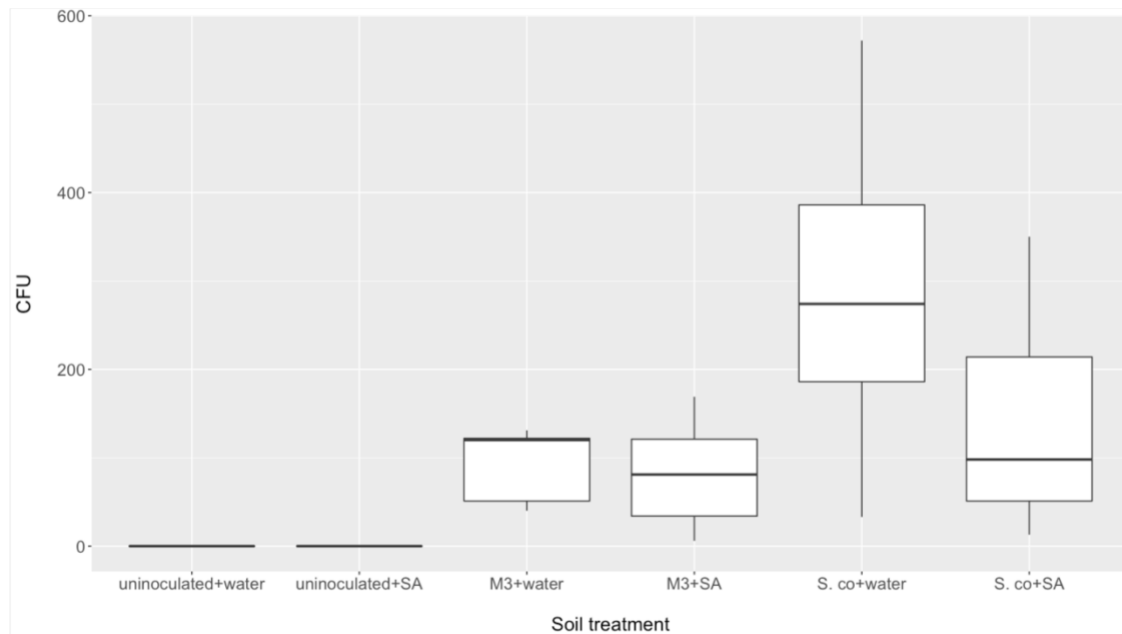
554 mutant plants and not necessarily the higher levels of SA produced by these plants (Jing et al
555 2008).

556 In order to test whether SA could be directly used as a chemoattractant by *Streptomyces*
557 species, we grew all of the sequenced streptomycete strains next to paper disks soaked in either
558 0.5 mM SA, 1 mM SA, or 0.1% (v/v) DMSO (a control for the solvent) but observed no obvious
559 growth towards SA after 10 days. (Fig. S8). We then reasoned that altered exudate SA levels
560 might indirectly benefit streptomycetes by negatively modulating the levels of the other
561 rhizobacteria in the soil. We tested this by establishing soil microcosms in deep 12 well plates,
562 which were then wetted with either sterile water or 0.5 mM SA. Microcosms were inoculated
563 with spores of M145, M3, or left as uninoculated controls. Nine replicates of each wetting
564 treatment were run in parallel for each strain and the control. Strains were recovered on
565 apramycin-containing selective agar medium after 10 days. A GLM with a negative binomial
566 distribution was used to model the effect of strain (M145 or M3), soil treatment (SA or water),
567 and their interaction on bacterial CFU returned from soil wells. Although there was overall a
568 significantly greater number of CFU of M145 recovered from soil wells than for the M3 strain
569 ($P < 0.01$), there was no significant effect of soil treatment ($P = 0.67$) on CFU number. The
570 interaction term was also insignificant ($P = 0.20$), indicating that this did not differ between
571 the two inoculated strains (Fig. 7). This suggested that neither strain had a competitive
572 advantage when greater concentrations of SA were present.

573 The simplest explanation of these results is that SA does not specifically attract
574 streptomycetes to the rhizosphere of *A. thaliana*. This does not necessarily contradict the
575 previously published observation (in Lebeis et al 2015) that SA is being used as a carbon source
576 by streptomycetes, as this has been demonstrated before in another streptomycete isolate
577 (Ishiyama et al 2004). However, we observe that the supplementary methods of this paper state
578 that the minimal medium agar with 0.5 mM SA that was used to grow *Streptomyces* sp. 303
579 also contained 1 g L⁻¹ (3.875 mM) sodium citrate, which is an additional candidate source of
580 carbon and energy. To test this, we grew all the genome-sequenced strains from our study on
581 minimal medium (MM) agar and MM agar containing 0.5mM SA, 3.875 mM sodium citrate,
582 or their preferred carbon source (either 5 gL⁻¹ mannitol, maltose or sucrose). All the strains
583 grew well on their preferred carbon source and most strains also grew on sodium-citrate-
584 containing plates, but we observed no growth on MM agar with or without SA (Fig. S9). Our
585 results suggest that SA is not used as a carbon source by the bacteria used in our study, and this
586 is additionally supported by the absence of known SA degradation genes in the genomes of all
587 the sequenced isolates (Table S3).

588

589 **Figure 7.** Number of colony forming units (CFU) of inoculated strains returned from soil
590 microcosms treated with either SA or dH₂O after 10 days. Microcosms were inoculated with
591 spores of either *S. coelicolor* (*S.co*), M3 or left uninoculated as a control. *N* = 9 microcosms
592 per treatment.



593

594 Taking these results together, we conclude that the observed increase in colonisation by
595 streptomycetes in *cpr5* mutant plants was likely due to a pleiotropic effect of plant genotype
596 rather than the presence of SA. This is consistent with the observation that there was no effect
597 of attenuated SA production in the *pad4* and *sid2-2* plants on colonisation, and the observation
598 that *cpr5* plants have been noted to undergo early senescence and overproduce reactive oxygen
599 species (Jing et al 2007, Jing et al 2008, Kirik et al 2001). Overall, we found no evidence to
600 support the hypothesis that SA specifically attracts streptomycetes to *A. thaliana* roots, but
601 rather they may have better access to the roots of senescing *cpr5* plants due to their saprotrophic
602 and filamentous lifestyle.

603

604 **Discussion.**

605 Here we used DNA-SIP to identify the bacteria feeding on exudates in the *A. thaliana*
606 rhizosphere and endosphere. To our knowledge, this is the first report of DNA-SIP on the
607 endosphere compartment of *A. thaliana*. The role of Proteobacteria and Firmicutes have
608 previously been investigated in the rhizosphere, however this was using DNA-SIP combined
609 with a Denaturing Gradient Gel Electrophoresis approach instead of with 16S rRNA amplicon

610 sequencing (Haichar et al 2012). Our results, combined with previous published reports on the
611 chemistry of *A. thaliana* exudates, suggest that root exudates are a rich and public food resource
612 (Badri and Vivanco 2009, Bais et al 2006, Haichar et al 2012). That is, many different genera
613 of bacteria appear to be able to use root exudates as a carbon source, as they contain a diverse
614 range of widely used metabolites including sugars, amino acids, and phenolic compounds
615 (Badri and Vivanco 2009, Chaparro et al 2013). In our study, bacteria that could metabolise
616 root exudates were those that have previously been noted for their positive influence on plant
617 fitness and were enriched in the endophytic compartment. For example, members of the genus
618 *Pseudomonas* are known to produce many bioactive and growth-promoting molecules (Glick
619 et al 1997, Hernández-León et al 2015, Mercado-Blanco et al 2016, Raza et al 2016), and
620 *Rhizobia* have been shown to have a positive effect on root development and plant biomass in
621 *Arabidopsis thaliana* (Zhao et al 2017). We hypothesise that this apparent mutualism between
622 plant and bacteria is evolutionarily stabilised via a Partner Fidelity Feedback mechanism
623 (Foster and Wenseleers 2006, Weyl et al 2010). Bacterial colonies that release growth-
624 promoting factors and/or antibiotics exploit a widespread pre-adaptation in plants to proliferate
625 root biomass in nutrient-rich or disease-free soils. In return, plant root masses release food
626 resources for bacteria which promote the local colony growth. Root-derived resources are also
627 likely to fuel interference competition amongst microbes, with the priority advantage given to
628 the most abundant strains. In our SIP experiment, these were the proteobacterial genera
629 (Scheuring and Yu 2012). Interference competition amongst bacteria typically manifests as
630 competitive exclusion by taking up niche space, plus the secretion of antibacterials and
631 antifungals, the latter of which may indirectly benefit the plant by inhibiting the growth of
632 pathogens.

633 However, such interactions can be dynamic over time since the composition of plant
634 root exudates is known to fluctuate considerably over the plant life cycle. The abundance of
635 particular microbial taxa has been shown to correlate with these changes, suggesting some
636 specificity in exudate usage (Chaparro et al 2013, Chaparro et al 2014, Haichar et al 2012,
637 Zhalnina et al 2018). Interestingly streptomycetes, the main focus of our study, did not feed off
638 root exudates in our SIP experiment despite being able to grow on purified root exudates *in*
639 *vitro*. *Streptomyces* were still present at low abundance in the endosphere and rhizosphere,
640 suggesting that their preferred substrates might have been absent from root exudates at the time
641 of ¹³C labelling, or that they were largely out-competed by the more abundant proteobacterial
642 genera. *Streptomyces* are spore-formers and thus may enter a dormant phase in their lifecycle
643 under unfavourable conditions (van der Meij et al 2017). As such, they may have been

644 detectable via their DNA, but dormant, under conditions in the root and rhizosphere at the time
645 of sampling. It should also be noted that *Streptomyces* are effective saprophytes, and so it is
646 possible that they feed off older, plant-originated organic material that had been sloughed off
647 before the ^{13}C labelling SIP experiment or on similar matter in the compost growth medium.

648 Our study shows that *Streptomyces* strains isolated from *A. thaliana* plant roots encode
649 an array of plant beneficial traits, including those involved in plant-growth promotion and
650 protection against infection. If such strains are to be used successfully as biocontrol and
651 growth-promoting agents, the next challenge is to determine how to ensure consistent
652 establishment in the rhizosphere and endosphere and how to promote the activity of these
653 strains within the plant root microbiome. Previous studies have suggested that phenolic
654 compounds, particularly SA, can directly affect the abundance of streptomycetes in the root
655 microbiome (Lebeis et al 2015). However, such studies have made use of *Arabidopsis* lines,
656 such as *cpr5* plants, that have mutations in key regulatory pathways. We suggest that such
657 mutations result in pleiotropic effects that make it difficult to determine causal relationships
658 between any given compound and bacterial colonisation. Thus, a more targeted approach is
659 required to determine if particular exudates, in addition to the initial abundance of strains in
660 the starting soil inoculum, are important for recruiting a higher abundance of *Streptomyces*
661 species to plant roots.

662 **Acknowledgments.** SFW was funded by a Natural Environment Research Council (NERC)
663 PhD studentship (NERC Doctoral Training Programme grant NE/L002582/1). MCM and JTN
664 were funded by Biotechnology and Biological Sciences Research Council (BBSRC) PhD
665 studentships (BBSRC Doctoral Training Program grant BB/M011216/1). Elaine Patrick was
666 supported by NERC grant NE/M015033/1 awarded to DWY, BW, JCM and MIH. This work
667 was also supported by the Norwich Research Park (NRP) through a Science Links Seed Corn
668 grant to MIH and JCM and by the NRP Earth and Life Systems Alliance (ELSA).

669 **Conflicts of interest.** The authors declare no conflicts of interest.

670 **References**

671 Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA *et al* (2008). The RAST
672 Server: rapid annotations using subsystems technology. *BMC Genomics* **9**: 75.

673
674 Badri DV, Vivanco JM (2009). Regulation and function of root exudates. *Plant, Cell &*
675 *Environment* **32**: 666-681.

676

- 677 Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013). Application of natural
678 blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that
679 phenolic-related compounds predominantly modulate the soil microbiome. *Journal of*
680 *Biological Chemistry* **288**: 4502-4512.
- 681
682 Bai Y, Muller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M *et al* (2015). Functional
683 overlap of the *Arabidopsis* leaf and root microbiota. *Nature* **528**: 364-369.
- 684
685 Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006). The role of root exudates in
686 rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* **57**: 233-266.
- 687
688 Berg G (2009). Plant-microbe interactions promoting plant growth and health: perspectives
689 for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol* **84**: 11-18.
- 690
691 Bodenhausen N, Horton MW, Bergelson J (2013). Bacterial communities associated with the
692 leaves and the roots of *Arabidopsis thaliana*. *PloS one* **8**: e56329.
- 693
694 Bowling SA, Guo A, Cao H, Gordon AS, Klessig DF, Dong X (1994). A mutation in
695 *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. *Plant Cell*
696 **6**: 1845-1857.
- 697
698 Bric JM, Bostock RM, Silverstone SE (1991). Rapid in situ assay for indoleacetic Acid
699 production by bacteria immobilized on a nitrocellulose membrane. *Applied and*
700 *environmental microbiology* **57**: 535-538.
- 701
702 Bulgarelli D, Rott M, Schlaeppi K, van Themaat EVL, Ahmadinejad N, Assenza F *et al*
703 (2012). Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial
704 microbiota. *Nature* **488**: 91-95.
- 705
706 Bulgarelli D, Garrido-Oter R, Munch PC, Weiman A, Droge J, Pan Y *et al* (2015). Structure
707 and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host*
708 *Microbe* **17**: 392-403.
- 709
710 Carvalhais LC, Dennis PG, Badri DV, Kidd BN, Vivanco JM, Schenk PM (2015). Linking
711 Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. *Mol Plant Microbe*
712 *Interact* **28**: 1049-1058.
- 713
714 Cha JY, Han S, Hong HJ, Cho H, Kim D, Kwon Y *et al* (2016). Microbial and biochemical
715 basis of a *Fusarium* wilt-suppressive soil. *ISME J* **10**: 119-129.
- 716
717 Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013). Root
718 exudation of phytochemicals in *Arabidopsis* follows specific patterns that are
719 developmentally programmed and correlate with soil microbial functions. *PLoS One* **8**:
720 e55731.
- 721
722 Chaparro JM, Badri DV, Vivanco JM (2014). Rhizosphere microbiome assemblage is
723 affected by plant development. *ISME J* **8**: 790-803.
- 724
725 Chater KF (2016). Recent advances in understanding *Streptomyces*. *F1000Res* **5**: 2795.
- 726

- 727 Chaurasia A, Meena BR, Tripathi AN, Pandey KK, Rai AB, Singh B (2018). Actinomycetes:
728 an unexplored microorganisms for plant growth promotion and biocontrol in vegetable crops.
729 *World J Microbiol Biotechnol* **34**: 132.
- 730
731 DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K *et al* (2006).
732 Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with
733 ARB. *Appl Environ Microbiol* **72**: 5069-5072.
- 734
735 Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeehan T, Hagevoort RG *et al* (2008a).
736 Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-
737 encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiol* **8**: 125.
- 738
739 Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D (2008b). Polymicrobial
740 nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag
741 encoded FLX amplicon pyrosequencing (bTEFAP). *PLoS One* **3**: e3326.
- 742
743 Dworkin M, Foster JW (1958). Experiments with some microorganisms which utilize ethane
744 and hydrogen. *J Bacteriol* **75**: 592-603.
- 745
746 Foster KR, Wenseleers T (2006). A general model for the evolution of mutualisms. *Journal*
747 *of Evolutionary Biology* **19**: 1283-1293.
- 748
749 Glick BR, Liu C, Ghosh S, Dumbroff EB (1997). Early development of canola seedlings in
750 the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2.
751 *Soil Biology and Biochemistry* **29**: 1233-1239.
- 752
753 Glick BR (2014). Bacteria with ACC deaminase can promote plant growth and help to feed
754 the world. *Microbiol Res* **169**: 30-39.
- 755
756 Haichar FE, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J *et al* (2008). Plant
757 host habitat and root exudates shape soil bacterial community structure. *Isme Journal* **2**:
758 1221-1230.
- 759
760 Haichar FEZ, Heulin T, Guyonnet JP, Achouak W (2016). Stable isotope probing of carbon
761 flow in the plant holobiont. *Curr Opin Biotechnol* **41**: 9-13.
- 762
763 Haichar FZ, Roncato MA, Achouak W (2012). Stable isotope probing of bacterial community
764 structure and gene expression in the rhizosphere of *Arabidopsis thaliana*. *FEMS Microbiol*
765 *Ecol* **81**: 291-302.
- 766
767 Hernández-León R, Rojas-Solís D, Contreras-Pérez M, Orozco-Mosqueda MdC, Macías-
768 Rodríguez LI, Reyes-de la Cruz H *et al* (2015). Characterization of the antifungal and plant
769 growth-promoting effects of diffusible and volatile organic compounds produced by
770 *Pseudomonas fluorescens* strains. *Biol Control* **81**: 83-92.
- 771
772 Holmes NA, Devine R, Qin Z, Seipke RF, Wilkinson B, Hutchings MI (2018). Complete
773 genome sequence of *Streptomyces formicae* KY5, the formicamycin producer. *J Biotechnol*
774 **265**: 116-118.
- 775

- 776 Inderbitzin P, Ward J, Barbella A, Solares N, Izyumin D, Burman P *et al* (2018). Soil
777 Microbiomes Associated with *Verticillium* Wilt-Suppressive Broccoli and Chitin
778 Amendments are Enriched with Potential Biocontrol Agents. *Phytopathology* **108**: 31-43.
779
- 780 Ishiyama D, Vujaklija D, Davies J (2004). Novel pathway of salicylate degradation by
781 *Streptomyces* sp. strain WA46. *Appl Environ Microbiol* **70**: 1297-1306.
782
- 783 Jing HC, Anderson L, Sturre MJ, Hille J, Dijkwel PP (2007). *Arabidopsis* CPR5 is a
784 senescence-regulatory gene with pleiotropic functions as predicted by the evolutionary theory
785 of senescence. *J Exp Bot* **58**: 3885-3894.
786
- 787 Jing HC, Hebel R, Oeljeklaus S, Sitek B, Stuhler K, Meyer HE *et al* (2008). Early leaf
788 senescence is associated with an altered cellular redox balance in *Arabidopsis* cpr5/old1
789 mutants. *Plant Biol (Stuttg)* **10 Suppl 1**: 85-98.
790
- 791 Jirage D, Tootle TL, Reuber TL, Frost LN, Feys BJ, Parker JE *et al* (1999). *Arabidopsis*
792 *thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. *Proc*
793 *Natl Acad Sci U S A* **96**: 13583-13588.
794
- 795 Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000). *Practical Streptomyces*
796 *genetics*. John Innes Foundation: Norwich.
797
- 798 Kirik V, Bouyer D, Schobinger U, Bechtold N, Herzog M, Bonneville JM *et al* (2001). CPR5
799 is involved in cell proliferation and cell death control and encodes a novel transmembrane
800 protein. *Curr Biol* **11**: 1891-1895.
801
- 802 Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M *et al* (2015).
803 Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa.
804 *Science* **349**: 860-864.
805
- 806 Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S *et al* (2012).
807 Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**: 86-90.
808
- 809 Mercado-Blanco J, Alós E, Rey MD, Prieto P (2016). *Pseudomonas fluorescens* PICF7
810 displays an endophytic lifestyle in cultivated cereals and enhances yield in barley. *FEMS*
811 *Microbiology Ecology* **92**: fiw092-fiw092.
812
- 813 Neufeld JD, Vohra J, Dumont MG, Lueders T, Manefield M, Friedrich MW *et al* (2007).
814 DNA stable-isotope probing. *Nat Protoc* **2**: 860-866.
815
- 816 Palaniyandi SA, Damodharan K, Yang SH, Suh JW (2014). *Streptomyces* sp. strain PGPA39
817 alleviates salt stress and promotes growth of 'Micro Tom' tomato plants. *J Appl Microbiol*
818 **117**: 766-773.
819
- 820 Qin Z, Munnoch JT, Devine R, Holmes NA, Seipke RF, Wilkinson KA *et al* (2017).
821 Formicamycins, antibacterial polyketides produced by *Streptomyces formicae* isolated from
822 African *Tetraponera* plant-ants. *Chemical Science*.
823

- 824 Raza W, Ling N, Liu D, Wei Z, Huang Q, Shen Q (2016). Volatile organic compounds
825 produced by *Pseudomonas fluorescens* WR-1 restrict the growth and virulence traits of
826 *Ralstonia solanacearum*. *Microbiol Res* **192**: 103-113.
- 827
828 Remans R, Spaepen S, Vanderleyden J (2006). Auxin signaling in plant defense. *Science*
829 **313**: 171.
- 830
831 Ren G, Ma Y, Guo D, Gentry TJ, Hu P, Pierson EA *et al* (2018). Soil Bacterial Community
832 Was Changed after Brassicaceous Seed Meal Application for Suppression of *Fusarium* Wilt
833 on Pepper. *Front Microbiol* **9**: 185.
- 834
835 Rey T, Dumas B (2017). Plenty Is No Plague: *Streptomyces* Symbiosis with Crops. *Trends*
836 *Plant Sci* **22**: 30-37.
- 837
838 Scheuring I, Yu DW (2012). How to assemble a beneficial microbiome in three easy steps.
839 *Ecology Letters* **15**: 1300-1307.
- 840
841 Schlaeppi K, Dombrowski N, Oter RG, Ver Loren van Themaat E, Schulze-Lefert P (2014).
842 Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives.
843 *Proc Natl Acad Sci U S A* **111**: 585-592.
- 844
845 Schrey SD, Tarkka MT (2008). Friends and foes: streptomycetes as modulators of plant
846 disease and symbiosis. *Antonie Van Leeuwenhoek* **94**: 11-19.
- 847
848 Sun J, Kelemen GH, Fernandez-Abalos JM, Bibb MJ (1999). Green fluorescent protein as a
849 reporter for spatial and temporal gene expression in *Streptomyces coelicolor* A3(2).
850 *Microbiology* **145** (Pt 9): 2221-2227.
- 851
852 Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA *et al* (2002). Novel
853 plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea
854 plant (*Pisum sativum*). *Appl Environ Microbiol* **68**: 2161-2171.
- 855
856 van der Meij A, Worsley SF, Hutchings MI, van Wezel GP (2017). Chemical ecology of
857 antibiotic production by actinomycetes. *FEMS Microbiol Rev* **41**: 392-416.
- 858
859 van der Meij A, Willemsse J, Schneijderberg MA, Geurts R, Raaijmakers JM, van Wezel GP
860 (2018). Inter- and intracellular colonization of *Arabidopsis* roots by endophytic actinobacteria
861 and the impact of plant hormones on their antimicrobial activity. *Antonie Van Leeuwenhoek*
862 **111**: 679-690.
- 863
864 Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016). *Streptomyces* as a
865 plant's best friend? *FEMS Microbiology Ecology*: fiw119.
- 866
867 Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucoleri R *et al* (2015). antiSMASH 3.0-a
868 comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids*
869 *Res* **43**: W237-243.
- 870
871 Wei Z, Jousset A (2017). Plant Breeding Goes Microbial. *Trends Plant Sci* **22**: 555-558.
- 872

- 873 Weyl EG, Frederickson ME, Yu DW, Pierce NE (2010). Economic contract theory tests
874 models of mutualism. *Proc Natl Acad Sci U S A* **107**: 15712-15716.
875
- 876 Whipps JM (1990). Carbon economy. In: Lynch JM (ed). *The Rhizosphere*. John Wiley &
877 Sons Ltd: Essex, UK. pp 59-97.
878
- 879 Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001). Isochorismate synthase is required
880 to synthesize salicylic acid for plant defence. *Nature* **414**: 562-565.
881
- 882 Yang J, Kloepper JW, Ryu CM (2009). Rhizosphere bacteria help plants tolerate abiotic
883 stress. *Trends Plant Sci* **14**: 1-4.
884
- 885 Yu Y, Lee C, Kim J, Hwang S (2005). Group-specific primer and probe sets to detect
886 methanogenic communities using quantitative real-time polymerase chain reaction.
887 *Biotechnol Bioeng* **89**: 670-679.
888
- 889 Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S *et al* (2018). Dynamic root
890 exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial
891 community assembly. *Nat Microbiol* **3**: 470-480.
892
- 893 Zhao CZ, Huang J, Gyaneshwar P, Zhao D (2017). *Rhizobium* sp. IRBG74 Alters
894 *Arabidopsis* Root Development by Affecting Auxin Signaling. *Front Microbiol* **8**: 2556.
895
- 896 Zhou N, Tootle TL, Tsui F, Klessig DF, Glazebrook J (1998). PAD4 functions upstream from
897 salicylic acid to control defense responses in *Arabidopsis*. *Plant Cell* **10**: 1021-1030.
898
- 899 Zhou X, Shen Y, Fu X, Wu F (2018). Application of Sodium Silicate Enhances Cucumber
900 Resistance to *Fusarium* Wilt and Alters Soil Microbial Communities. *Front Plant Sci* **9**: 624.
901
902