How do Rhizobacterial Volatiles Influence Root System Architecture, Biomass Production and Allocation of the Model Grass *Brachypodium distachyon*?

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International Plant & Animal Genome XXIV
Brachypodium Genomics Workshop

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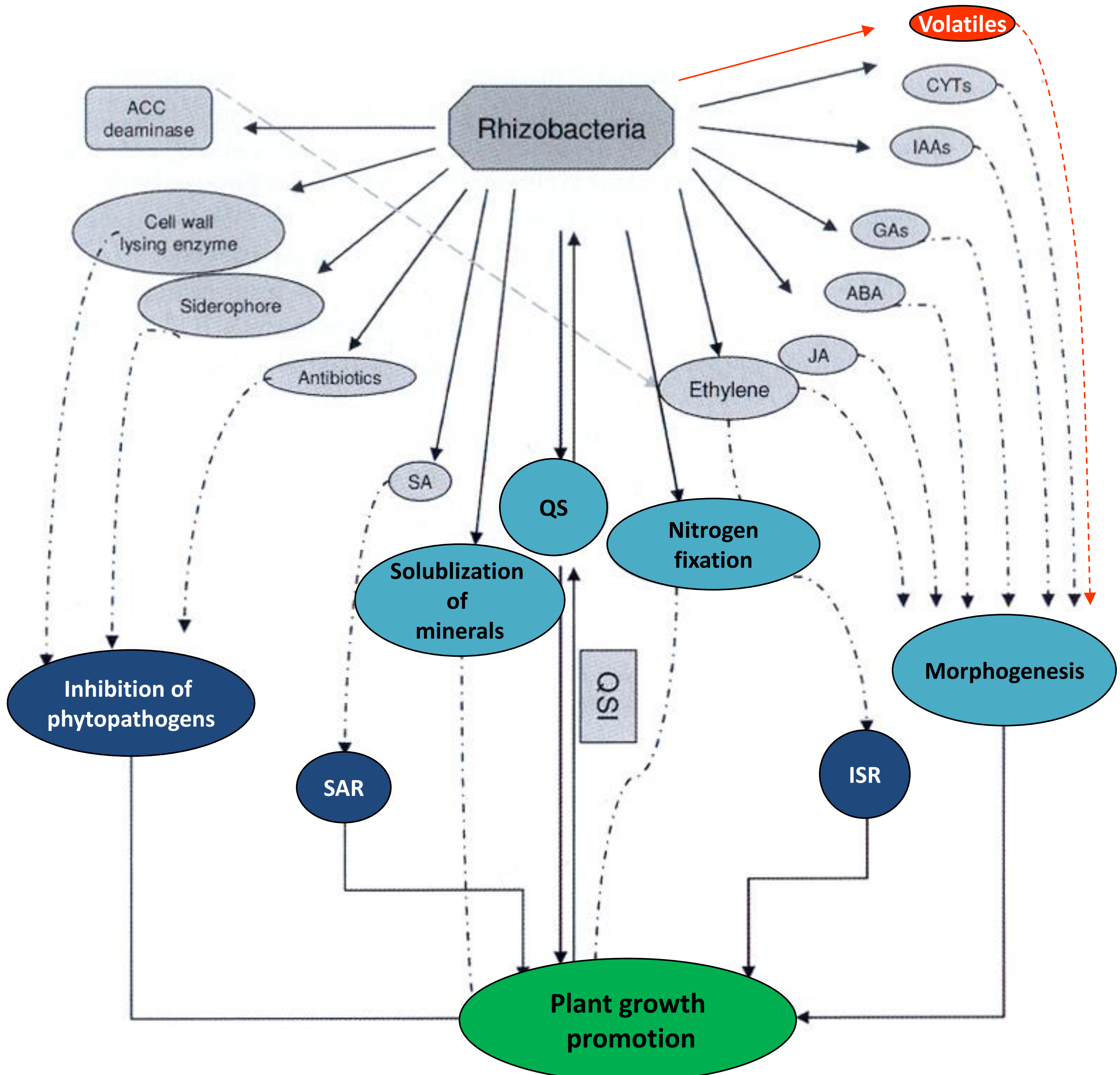
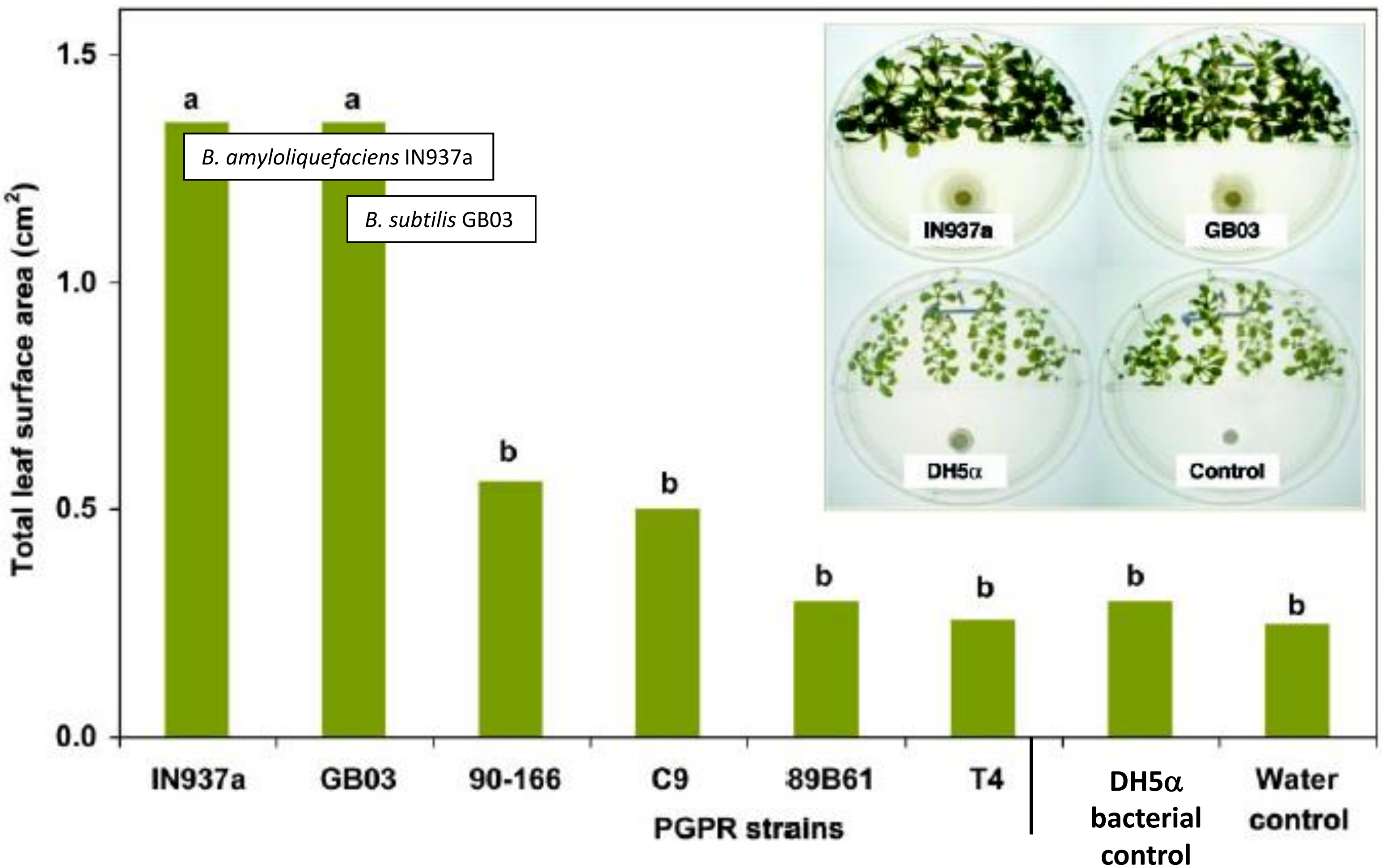


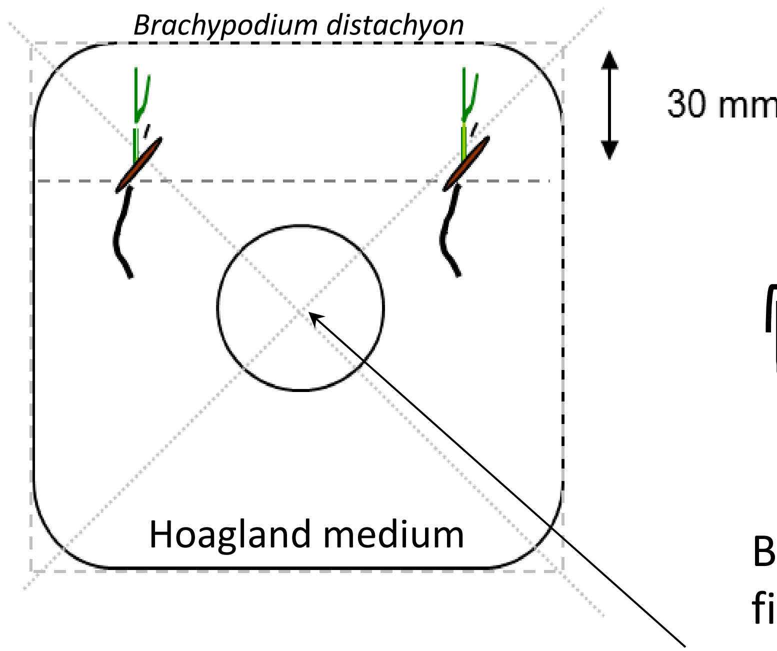
Ahmad *et al.*, 2008

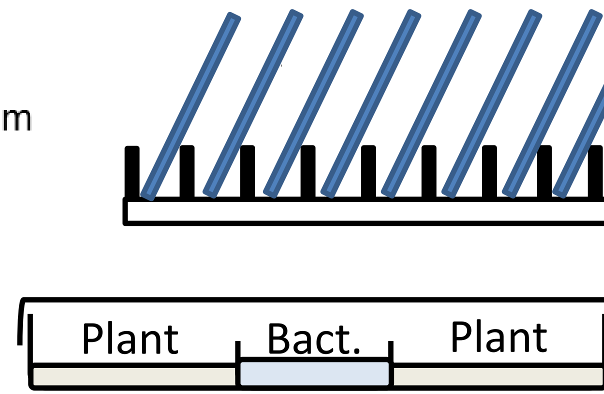
Some Volatile Organic Compounds emitted by rhizobacteria can promote plant growth.



Ryu *et al.* (2003) *PNAS* 100: 4927-4932

How to unravel plant response to rhizobacterial volatiles while studying root system architecture (RSA)?



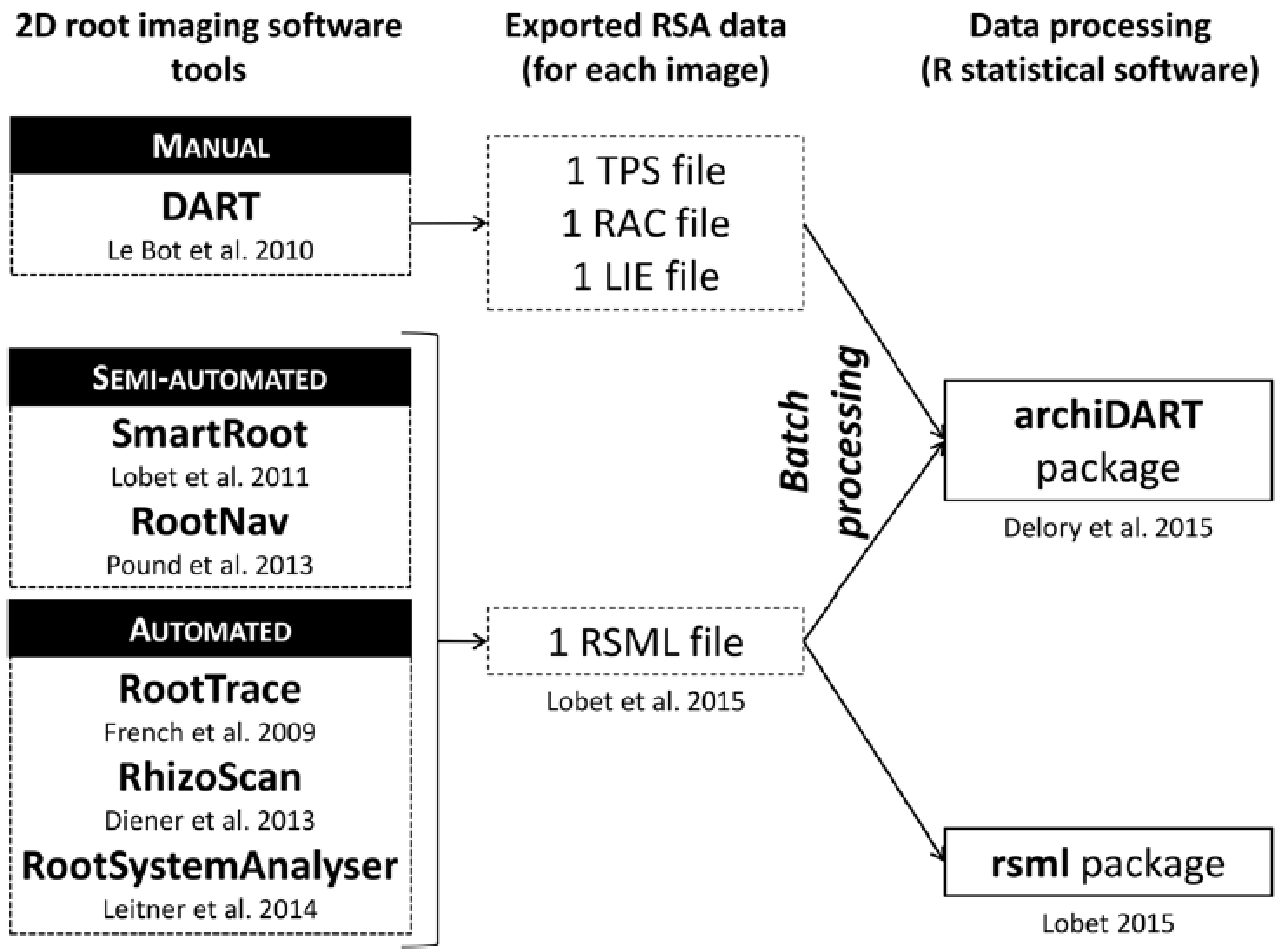


Bacterial compartment filled with Farag *et al.* (2006) medium

- Surface-sterilization of caryopses
- Vernalization
- Pre-germination
- Cocultivation for 10 days with bacteria in a shared atmosphere

Strain	Gram type	Family	Caracteristics
Azospirillum brasilense **SP245**	-	Rhodospirillaceae	Associative microaerophilic diazotroph (Kennedy et al., 2004)
Azotobacter vinelandii **A60 - F08 19**	-	Pseudomonadaceae	Free-living aerophilic diazotroph (de Freitas et al., 1990)
Bacillus amyloliquefaciens **AP278 - IN937a**	+	Bacillaceae	Some strains are diazotrophic, facultative microaerophilic ; many *Bacillus* produce antibiotics (Ryu et al., 2003 and 2005, Farag et al., 2006, Zhang et al., 2007 and 2008, *newly isolated strain)
Bacillus pasteurii **AP277 - C9**	+	Bacillaceae	
Bacillus pumilus **AP280 - T4**	+	Bacillaceae	
Bacillus pumilus **AP281 - SE34**	+	Bacillaceae	
Bacillus pumilus **C26***	+	Bacillaceae	
Bacillus subtilis **AP305 - GB03**	+	Bacillaceae	
Burkholderia cepacia **A01-45**	-	Burkholderiaceae	Rarely diazotrophic, associative endophytic nitrogen fixer, wheat PGPR (Walley and Germida, 1997)
Enterobacter cloaceae **AP12 - JM22**	-	Enterobacteriaceae	PGPR (Ryu et al., 2003)
Escherichia coli **DH5 alpha 99B829**	-	Enterobacteriaceae	Bacterial control (Ryu et al., 2003)
Paenibacillus polymyxa **AP294 - E681**	+	Paenibacillaceae	Facultative microaerophilic, can produce phytohormones, suppress pathogens and solubilize organic phosphate (Ryu et al., 2005, *newly isolated strain)
Paenibacillus polymyxa **MXC5***	+	Paenibacillaceae	
Pseudomonas aeruginosa **I03-73**	-	Pseudomonadaceae	Associative wheat PGPR (Walley and Germida, 1991)
Pseudomonas fluorescens **AP2 - 89B61**	-	Pseudomonadaceae	
Pseudomonas fluorescens **Pf29Arp**	-	Pseudomonadaceae	
Pseudomonas putida **KT2440 - B02 66**	-	Pseudomonadaceae	
Raoultella terrigena **Tfi08***	-	Enterobacteriaceae	Aerophilic or facultatively anaerophilic, newly isolated
Serratia marcescens **AP4 - 90 166**	-	Enterobacteriaceae	PGPR (Ryu et al., 2003 and 2005)

How to dig into the RSA traits of *Brachypodium* ?



ArchiDART: an R package for the automated computation of plant root architectural traits

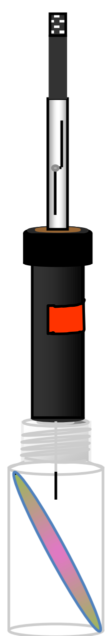
R functions	DART files			RSML files	Description
	TPS	RAC	LIE		
archidraw			X	X	Graphical representation of vectorized root systems
archigrow	X		X		Calculation of growth rates and their graphical representation
architect	X	X		X	Calculation of integrated RSA traits (overall description)
latdist		X		X	Calculation of the lateral root length and density distribution
trajectory	X	X	X	X	Calculation of root growth angle, orientation, tortuosity and curvature parameters

Delory B.M. *et al.* (2015). **Plant & Soil** 15pp. DOI 10.1007/s11104-015-2673-4

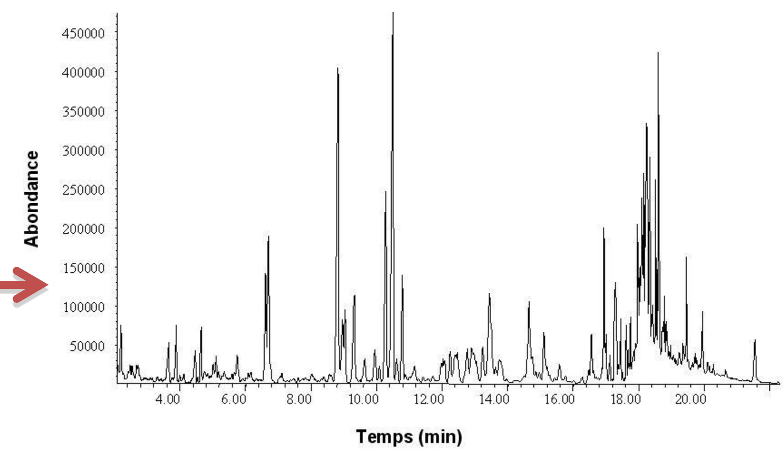
Rhizobacterial VOC analysis by SPME-GC-MS

- Solid Phase Micro-Extraction
- Gas Chromatography
- Mass Spectrometry

→ identification and quantitation based on retention time of commercial standards, mass spectra and peak area relative to internal standard



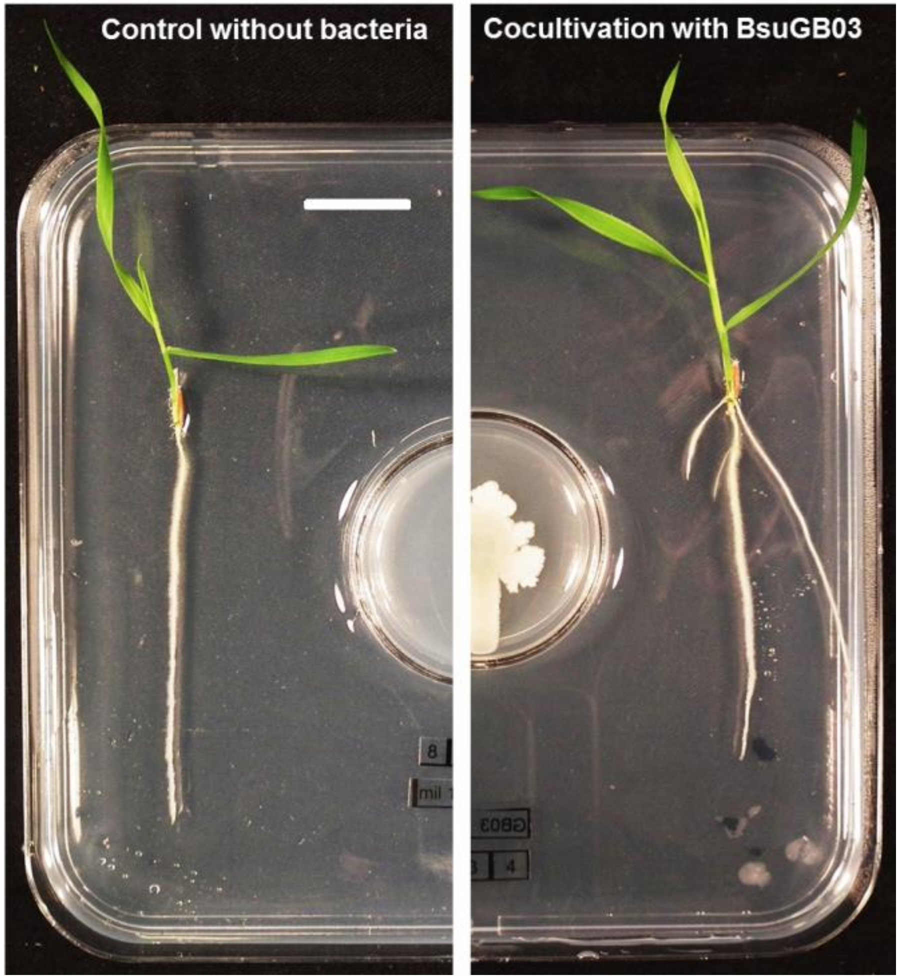
Agilent 7000A Triple Quadrupole GC/MS



Linking five biomass-related variables and nine RSA traits…

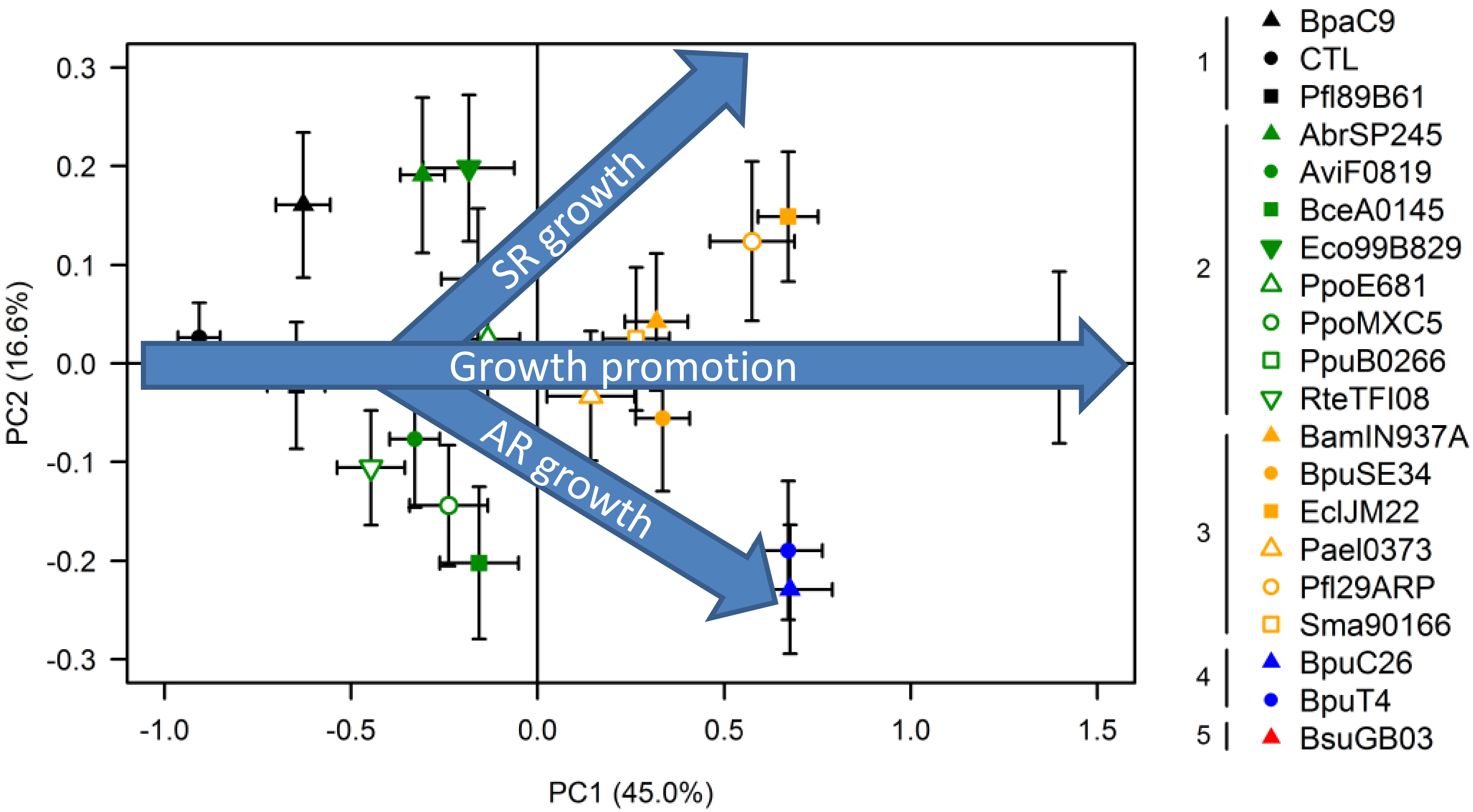
- Fourteen measured variables
- Four independent experimental replicates
- Principal Component analysis on weighted and reduced variables
- Hierarchical clustering based on the principal components
- Two-way ANOVA and Dunnett's test

Bacterial volatiles have a significant impact on the early developmental stages of a model grass

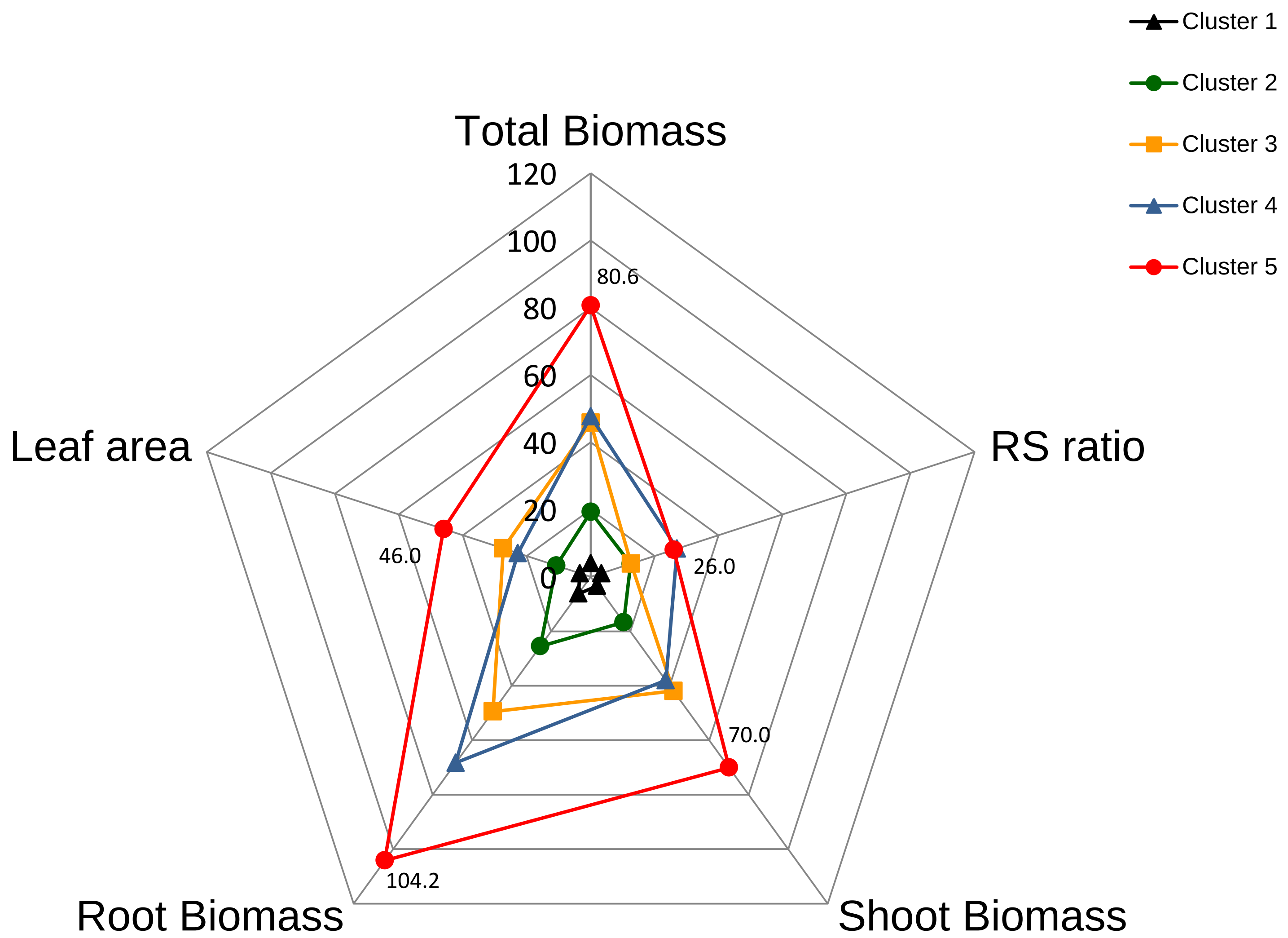


- Stage 12 vs 13 after 10 days
- Roots on top of the agar
- Strong correlation between biomass production and root branching traits
- Weak correlation with primary root length (PRL)
- PRL not correlated with other RSA traits

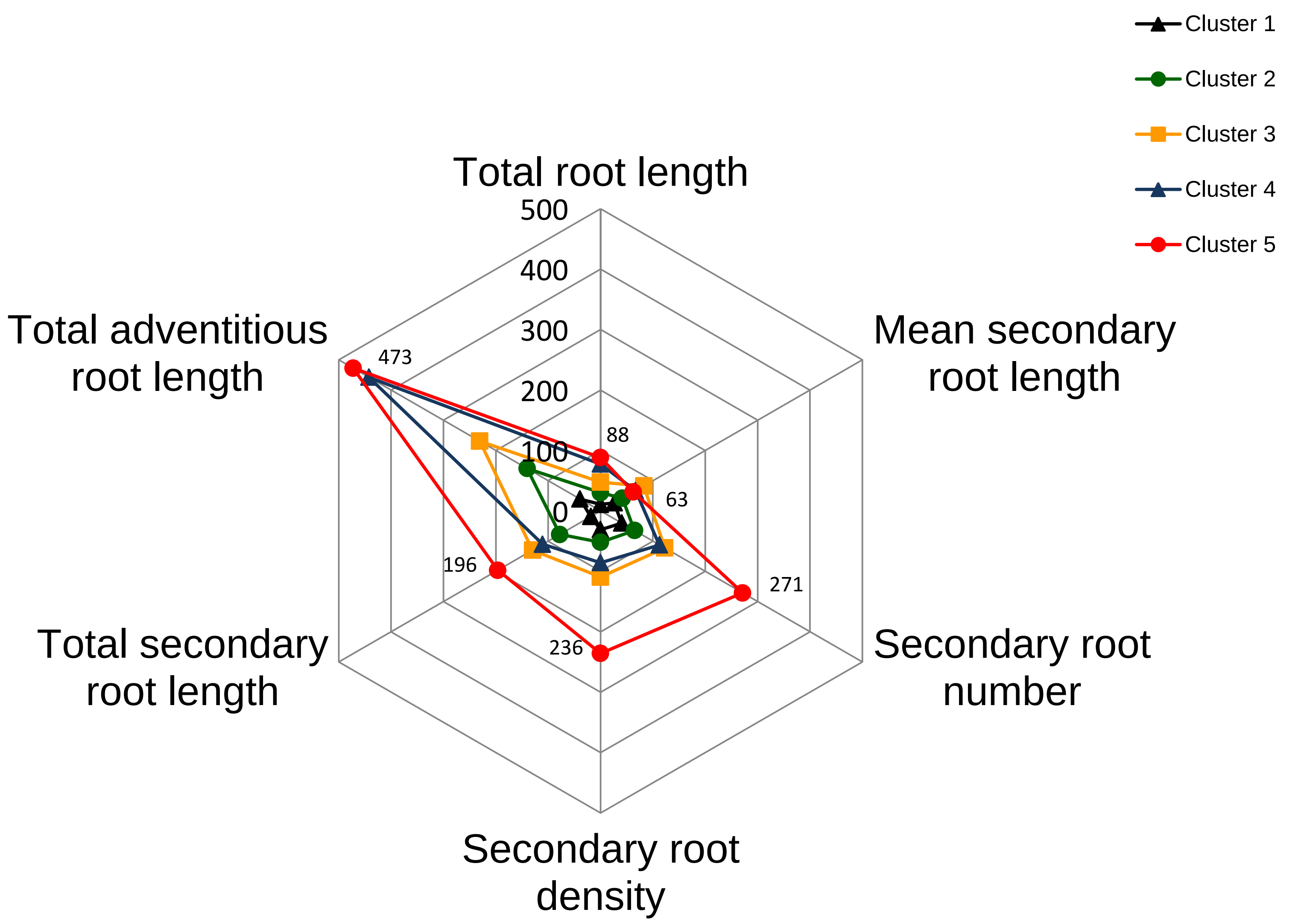
Contrasting biomass and RSA modulations define five groups of bacterial strains



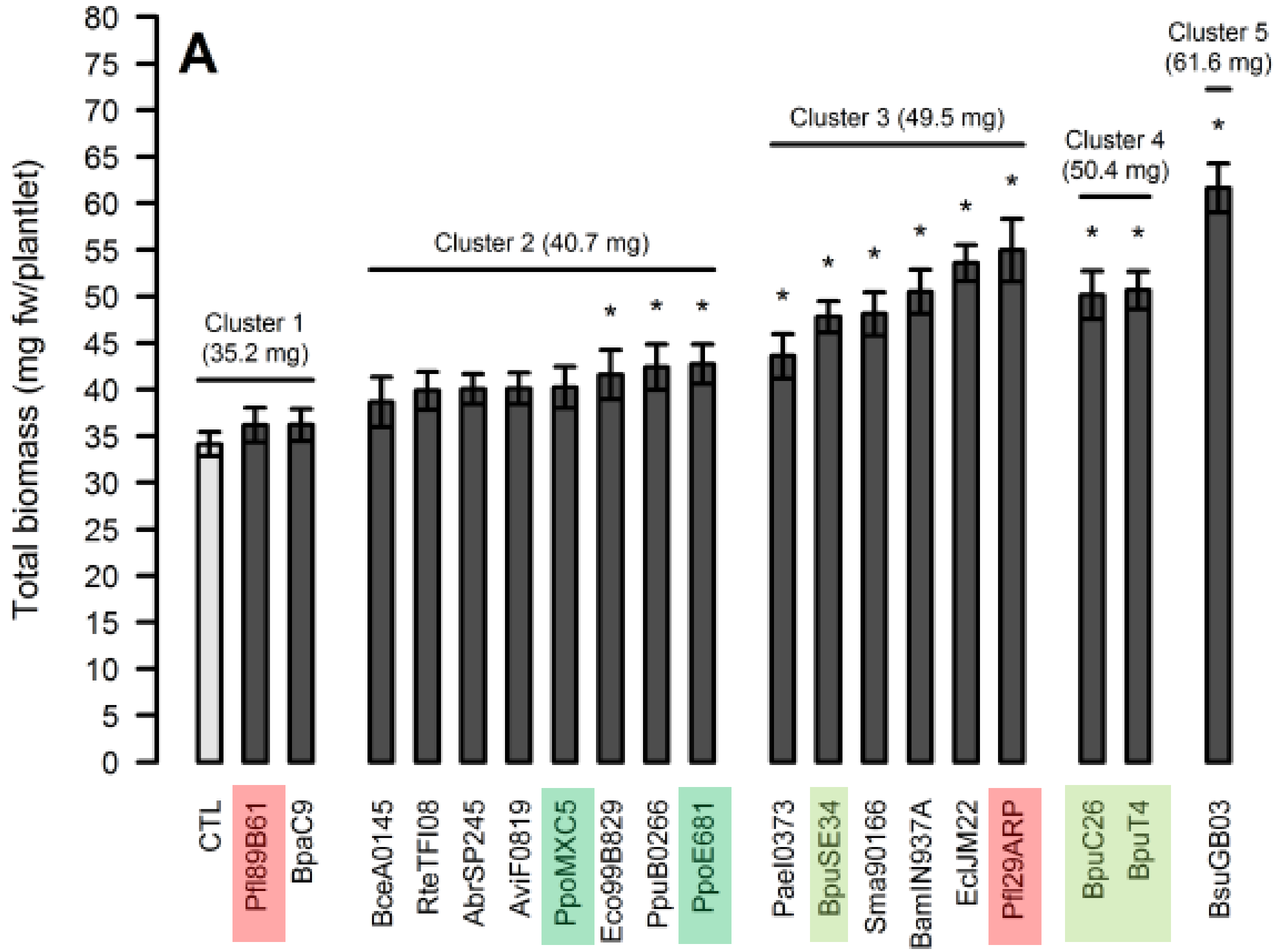
From non-significant to very high enhancement of biomass production



Relative growth promotion effects on RSA traits



Variability exists up to the intra-specific level and is not related to taxonomy



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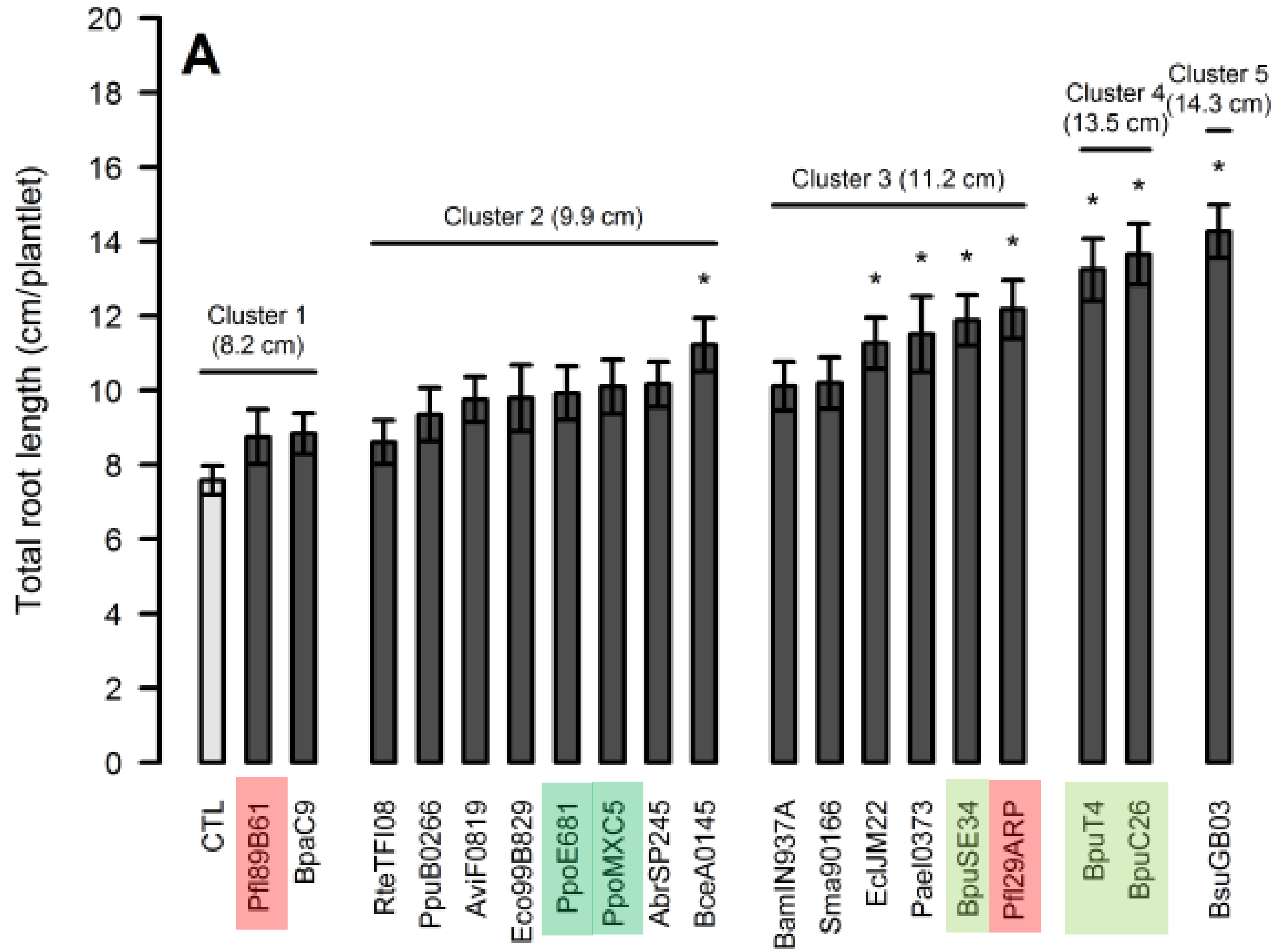

Significant changes compared with the control without bacteria are marked with an asterisk (*).

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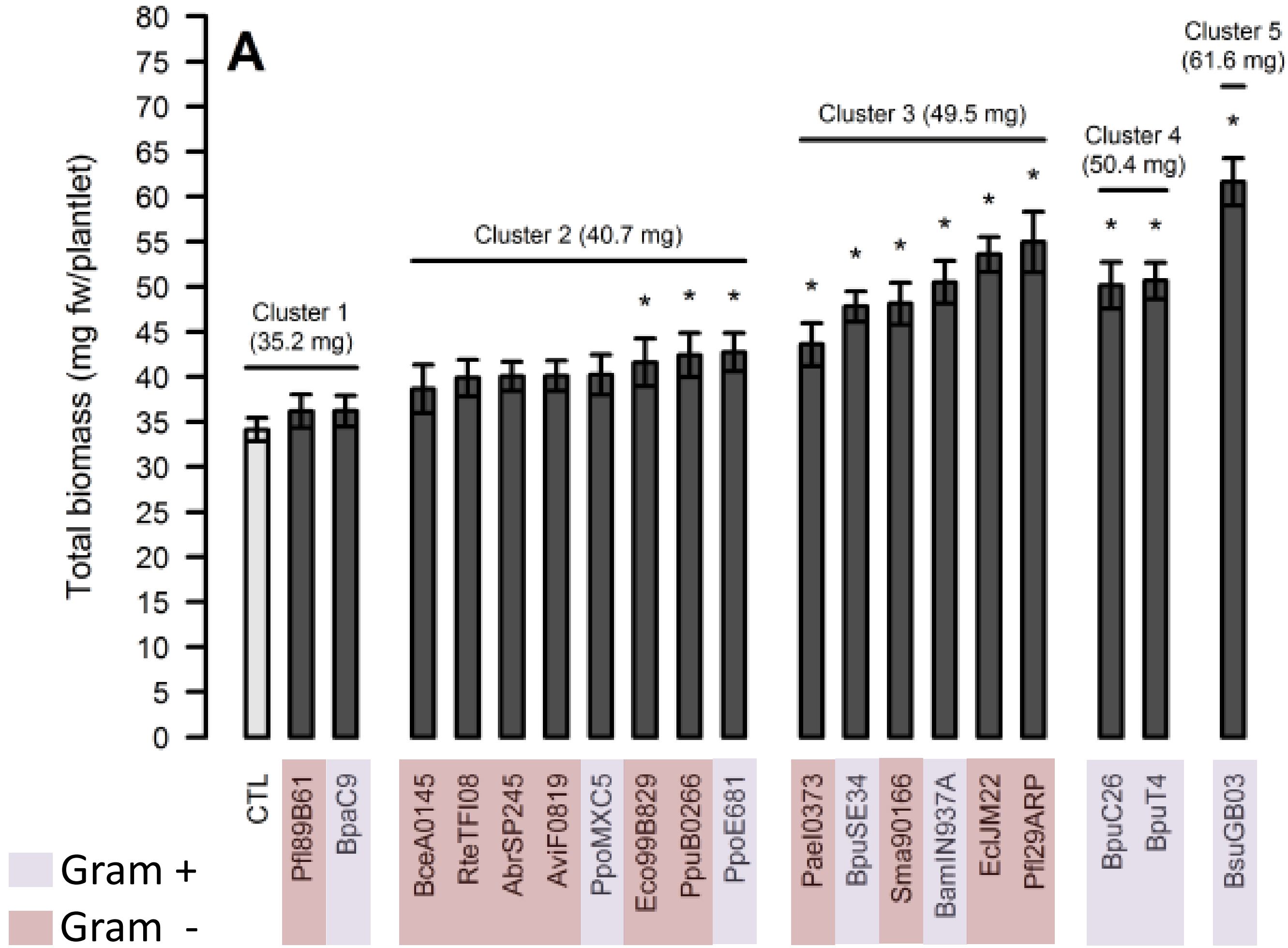
Variability exists up to the intra-specific level and is not related to Gram type



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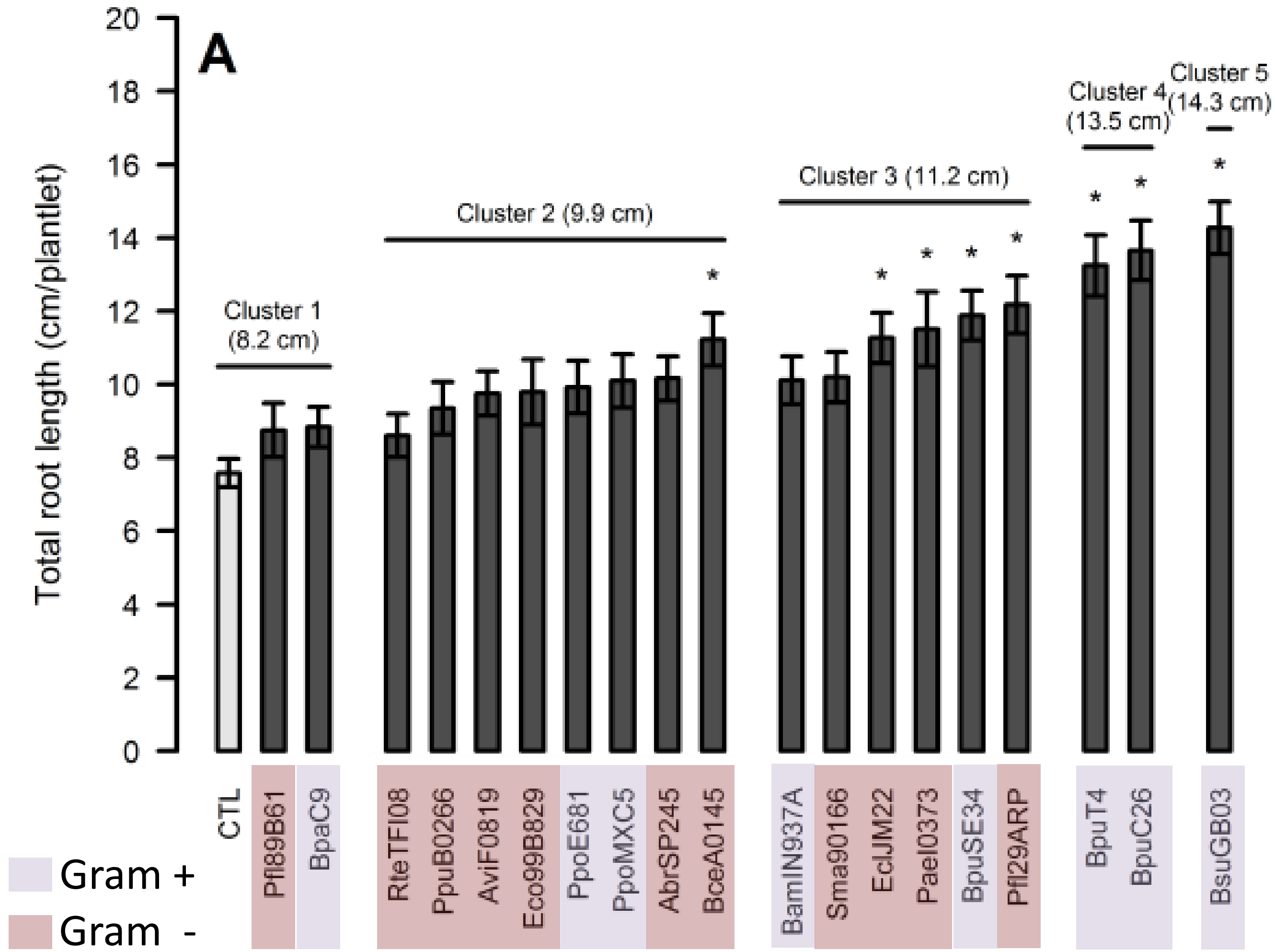
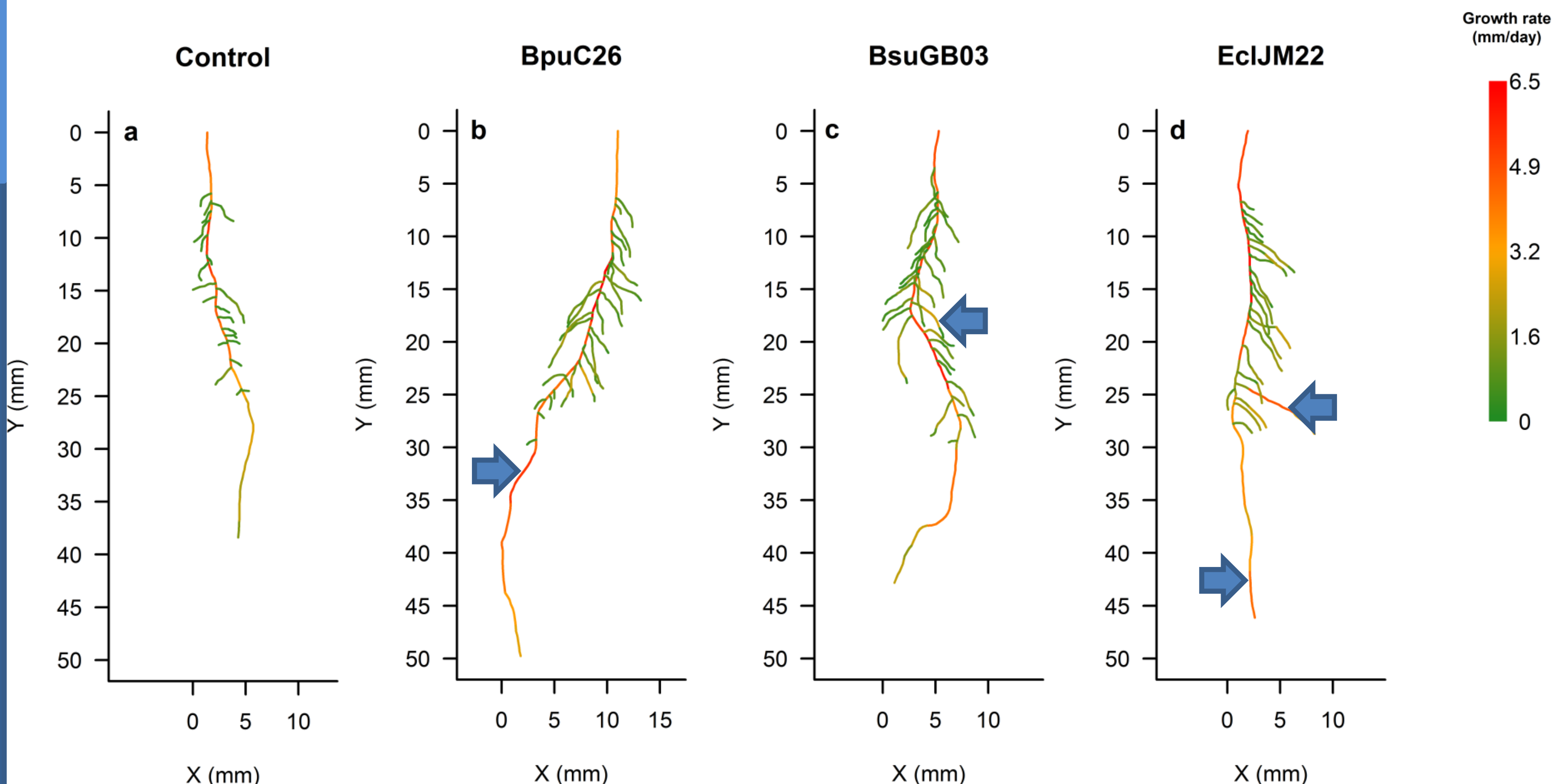
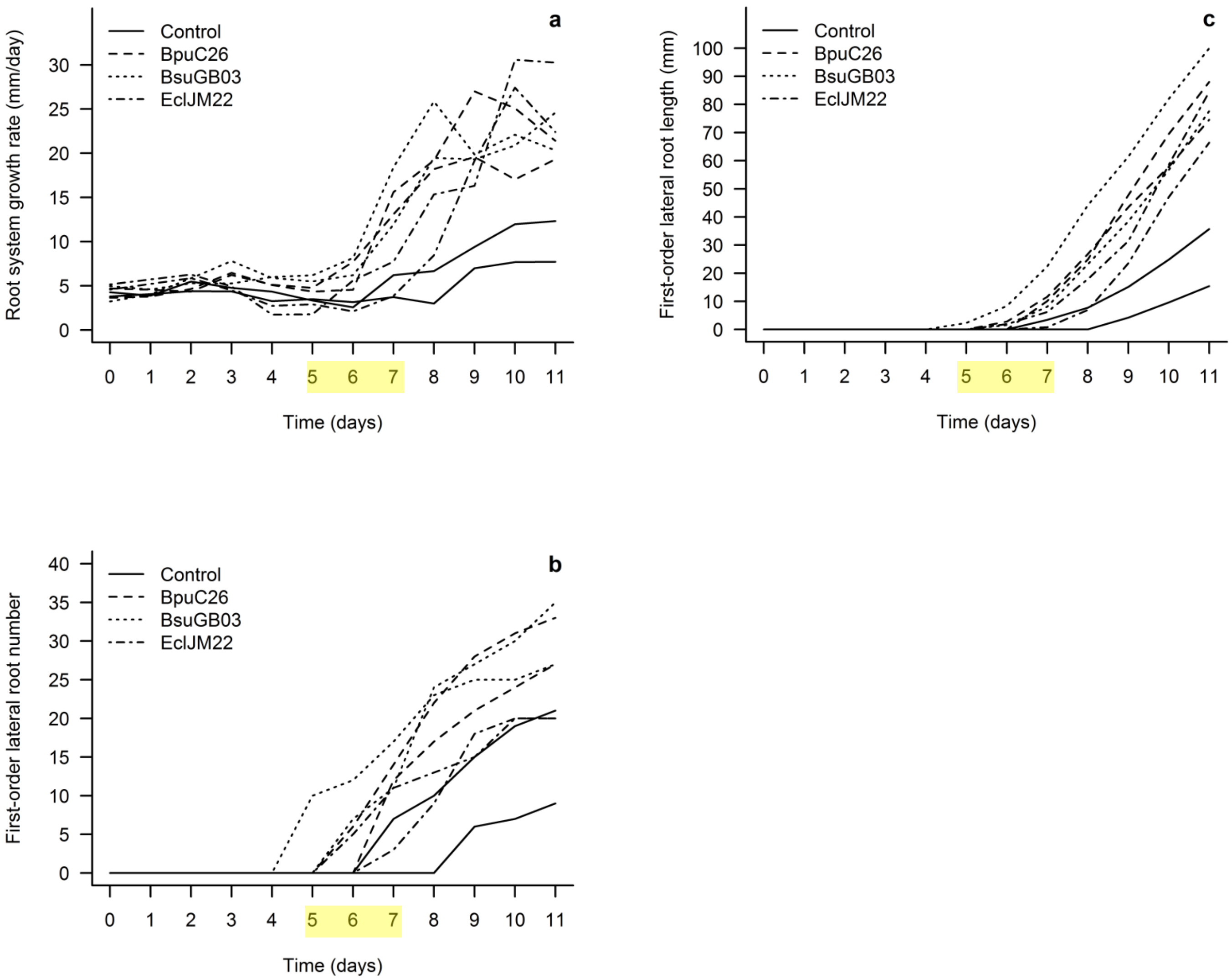


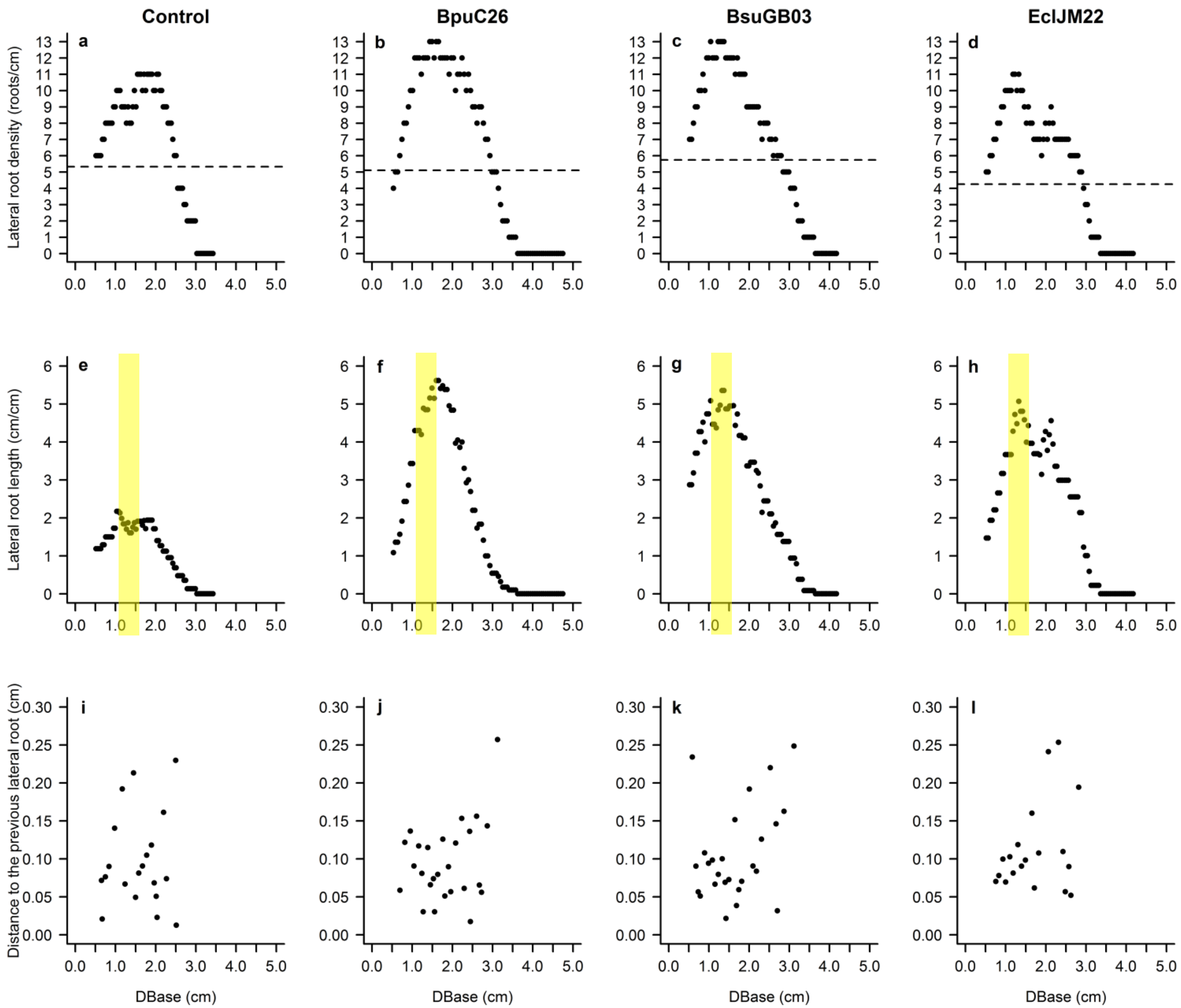
Bacterial volatiles increase the growth rates of primary and secondary roots of *B. distachyon* Bd21



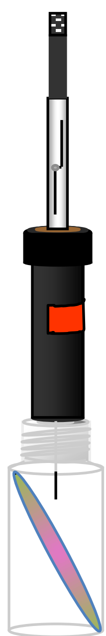
Increases in global growth rate and secondary root number and length are noticed after 5 to 7 days.



The secondary root length is increased locally between 1.1 and 1.6 cm from the primary root base.



Contrasting effects indicate some heterogeneity in bacterial volatile production



Identified compounds after 24 hours of growth	
CO_2	n-butylacetate
methanol	5-methyl-2-hexanone
ethanol	3-methyl-butanoic acid
propanone	2-methyl-butanoic acid
isoprene	3-methyl-acetate-1-butanol
dimethyl sulfide	4-penten-1-yl-acetate
3-methyl-butanal	1-nonene
2-methyl-butanal	2-heptanone
butane-1-methoxy-3-methyl	styrene
acetoin	heptanal
1-butanol,3-methyl	oxime metoxiphenyl
1-butanol,2-methyl	2-buten-1-ol,3methyl-acetate
dimethyl,-disulfide	6-methyl-2-heptanone
butanoic acid, 2-methyl, methyl ester	5-methyl-2-heptanone
butane-2,3-diol	benzaldehyde
hexanal	2-ethylhexanol

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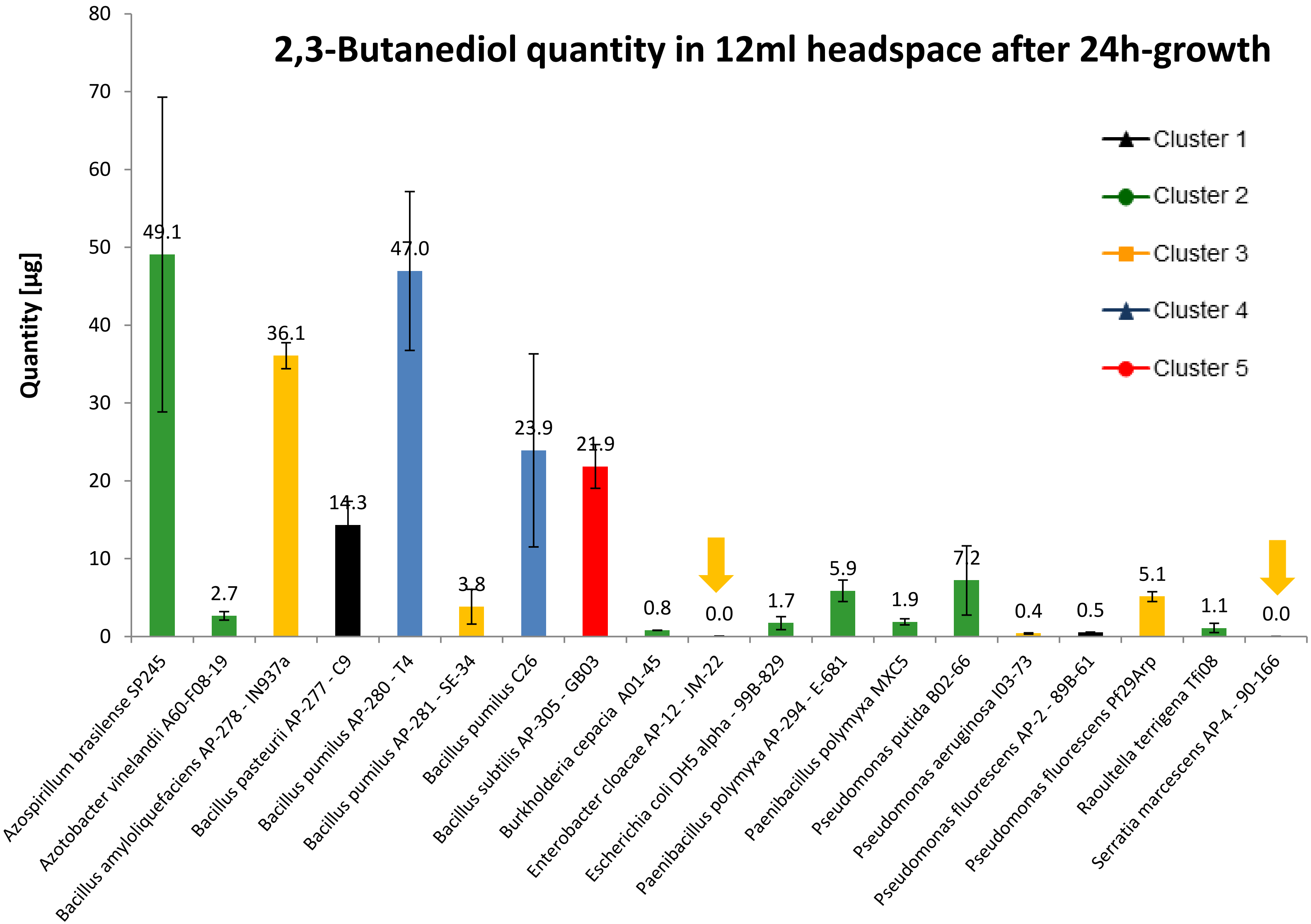
The observed effects can not be explained using previously published growth-promoting bacterial VOC.



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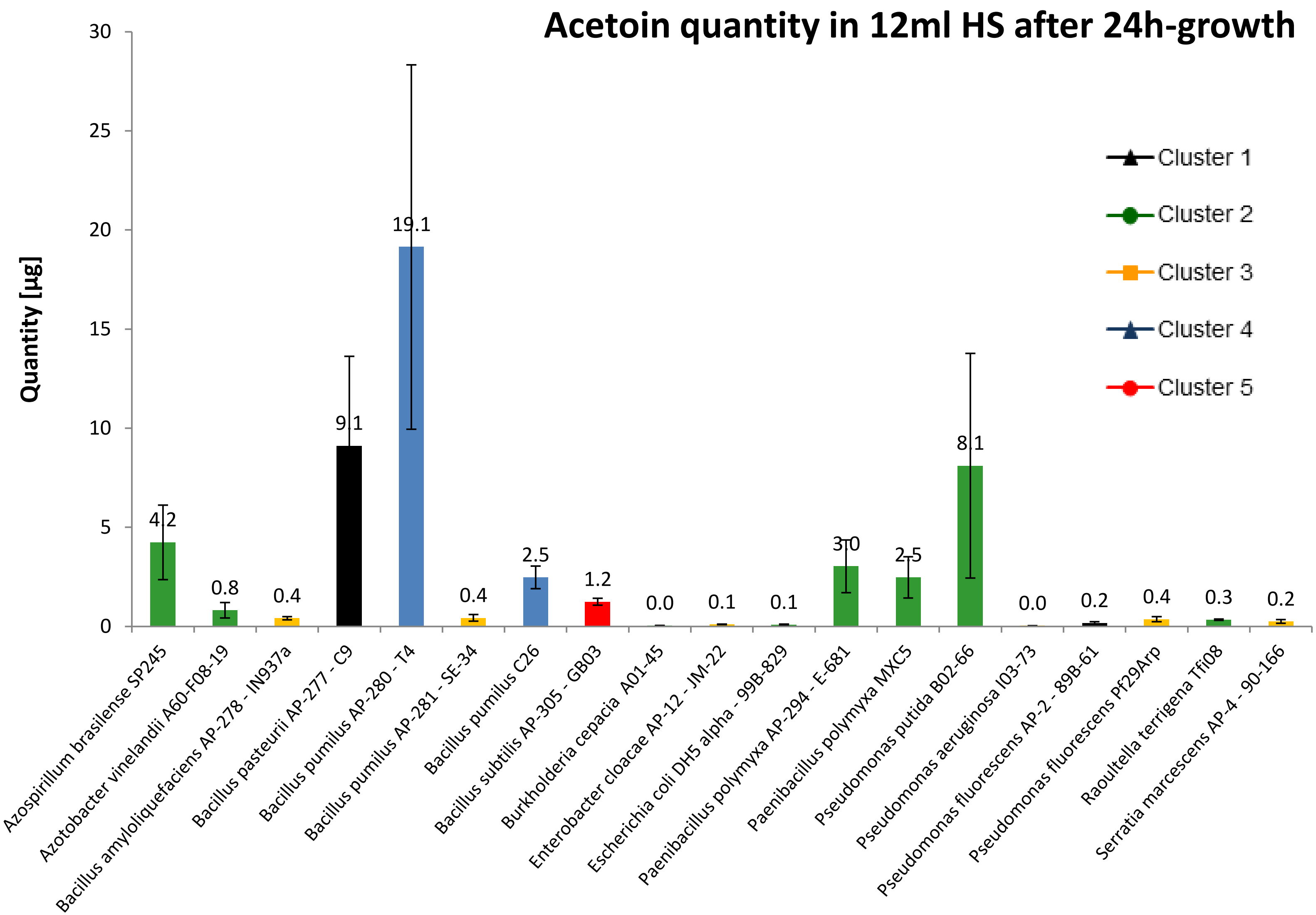

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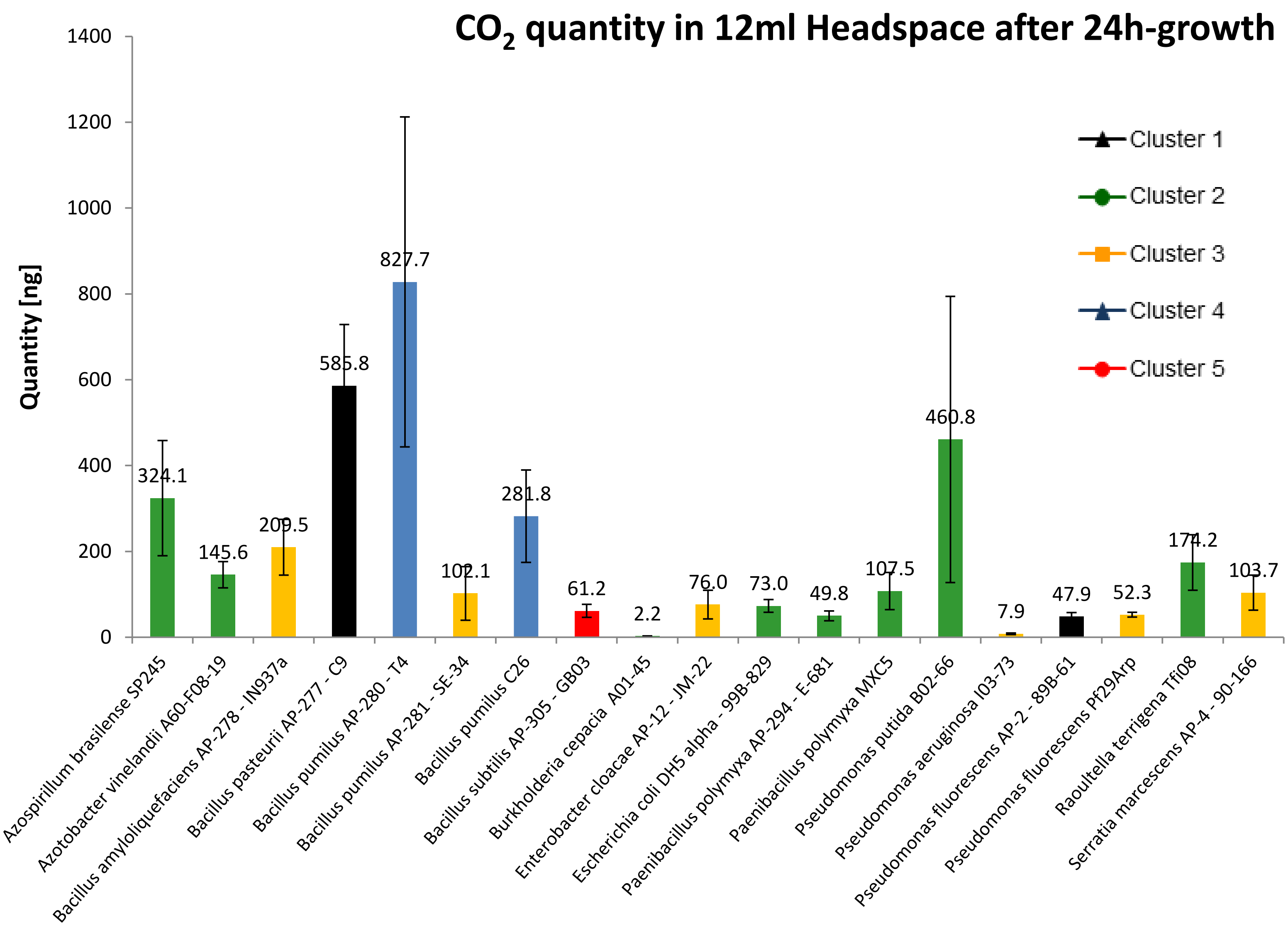

Take-home messages and future prospects!

- **First report** of bacterial volatile-mediated growth promotion of a grass plant (published in *BMC Plant Biology*)
- **Screening tools** for bacterial volatile-mediated growth promotion and RSA modulation
- **Five groups of bacterial strains can be identified** based on their contrasted effects on biomass production and RSA traits.
- The growth promotion effects can be linked to **modifications in shoot development and root architecture (length and branching)**
- Irrespective of the considered variables, ***Bacillus subtilis* GB03 volatile compounds induced the most significant changes**
- The plant growth-promoting strains emit **different volatile blends** that should be further investigated to be linked to their biological effects.
- **Bioactive compounds identification**: a prerequisite to assess effects on older developmental stages and focus the VOC exposure on the root system ?
- **Bioactive compounds identification**: a first step towards slow-release formulations of VOC candidates?
- **From in vitro to the field**: RSA modulations => drought stress tolerance, increased nutrient uptake ?

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Thank you for your attention !

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Delory B.M., Baudson C., Brostaux Y., Lobet G., du Jardin P., Pagès L. and Delaplace P. (2015). archiDART: an R package for the automated computation of plant root architectural traits. **Plant & Soil** 15pp. DOI 10.1007/s11104-015-2673-4

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