Rhizobacterial volatiles influence root system architecture, biomass production and allocation of the model grass *Brachypodium distachyon* (L.) P. Beauv.

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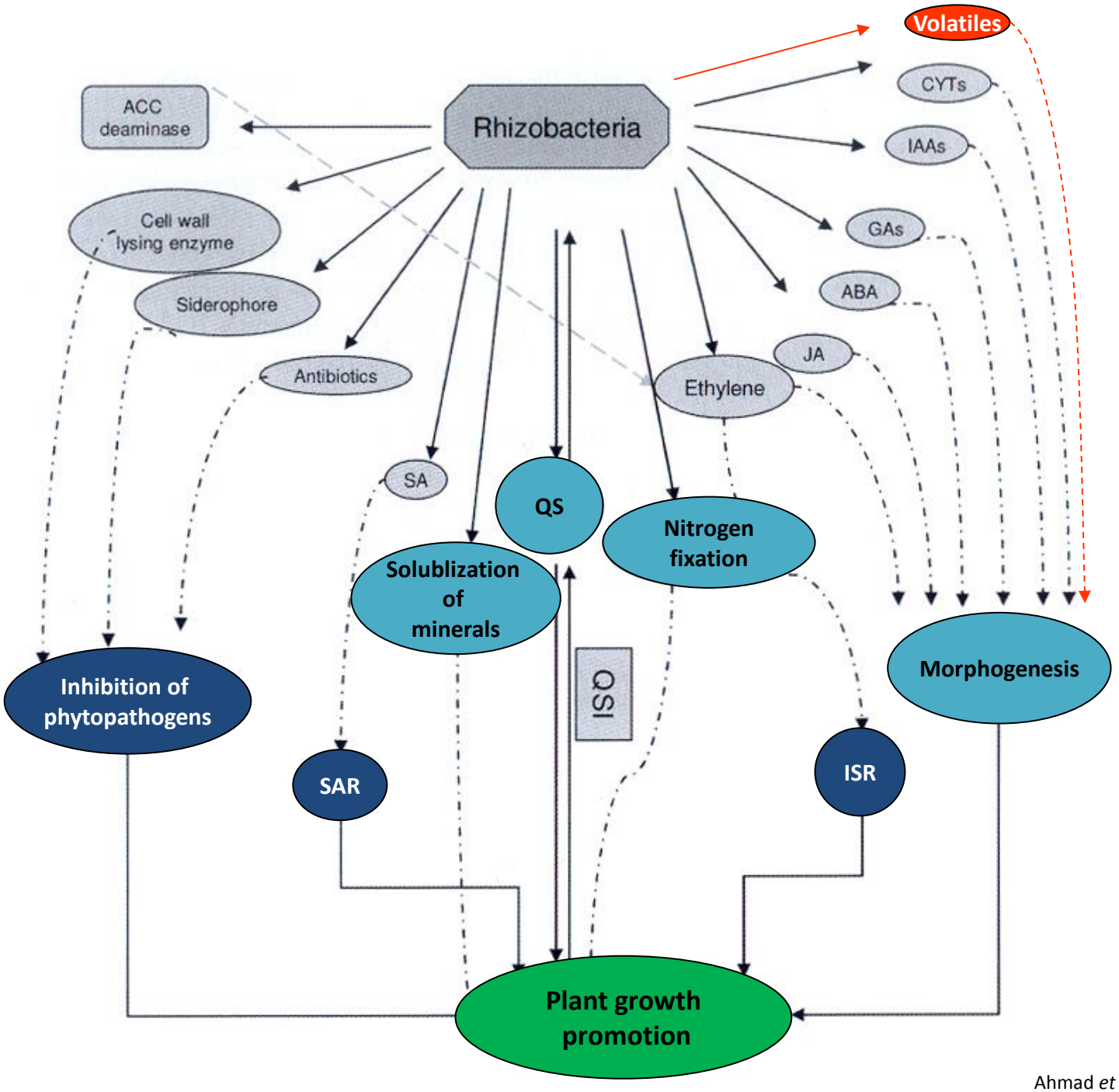
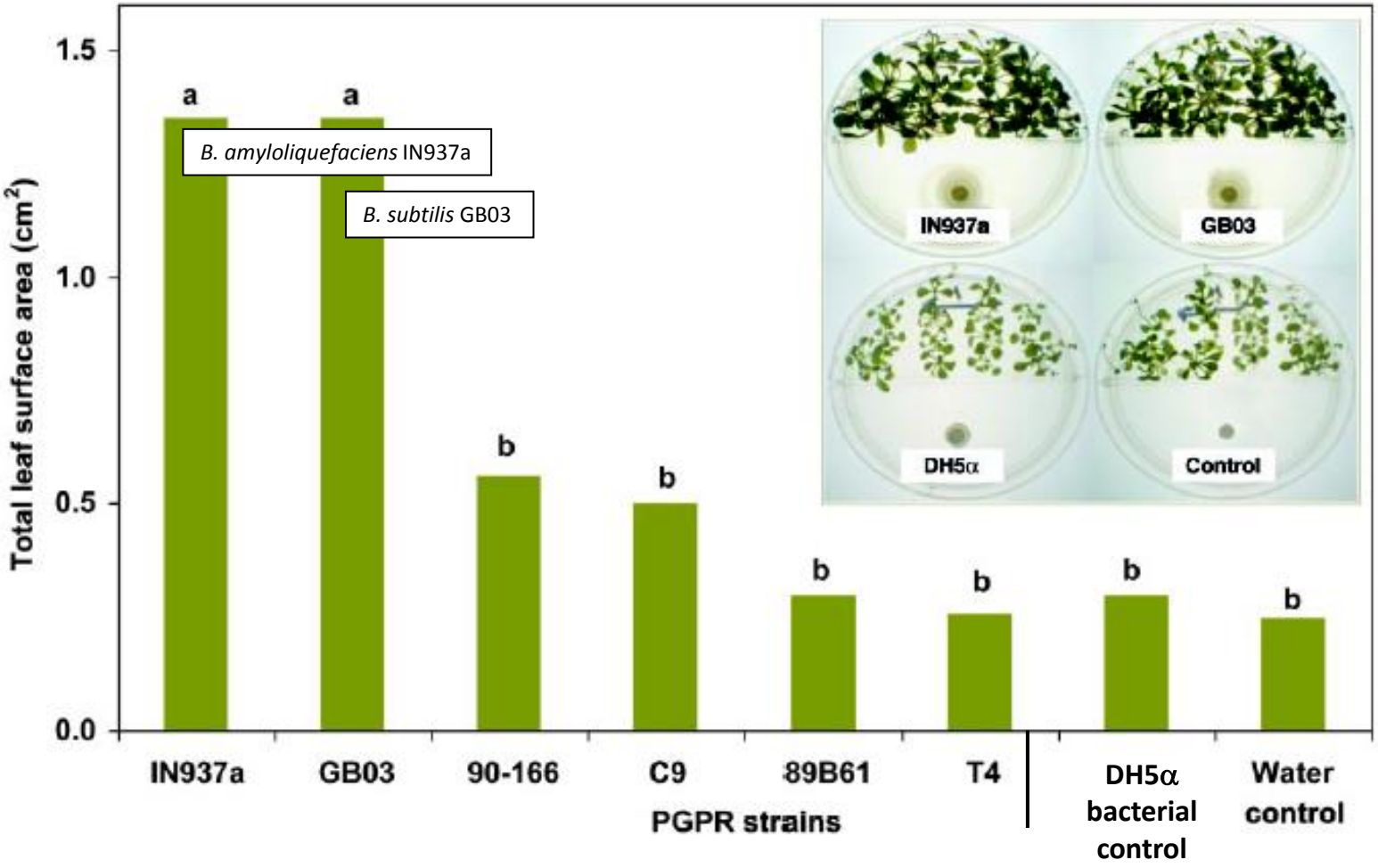


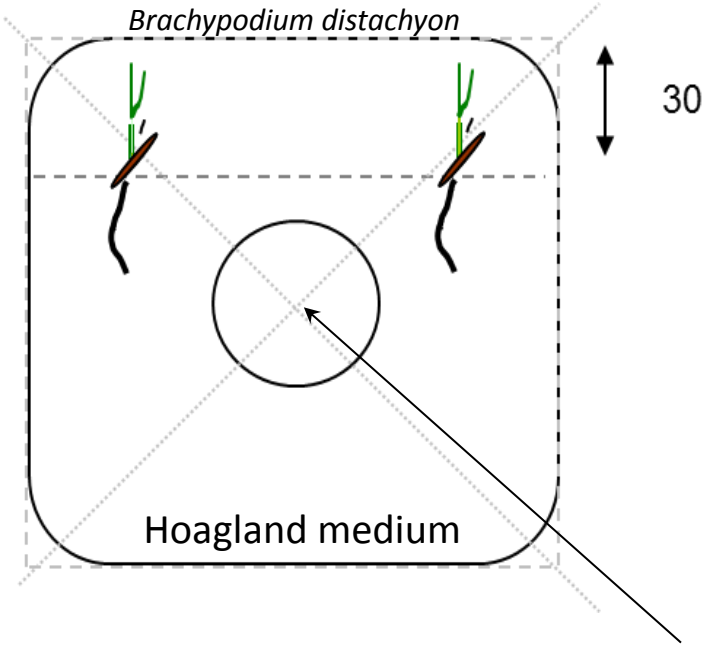
Ahmad *et al.*, 2008

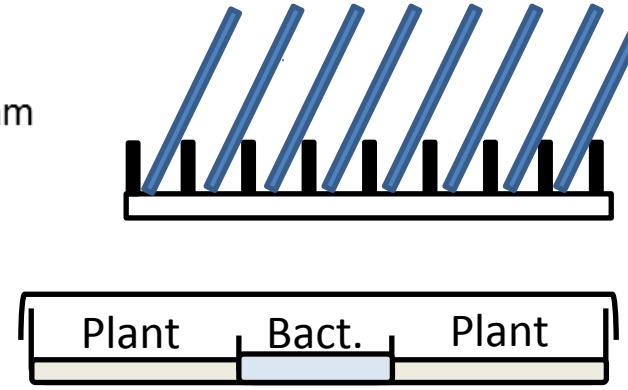
Some Volatile Organic Compounds emitted by rhizobacteria can promote plant growth.



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

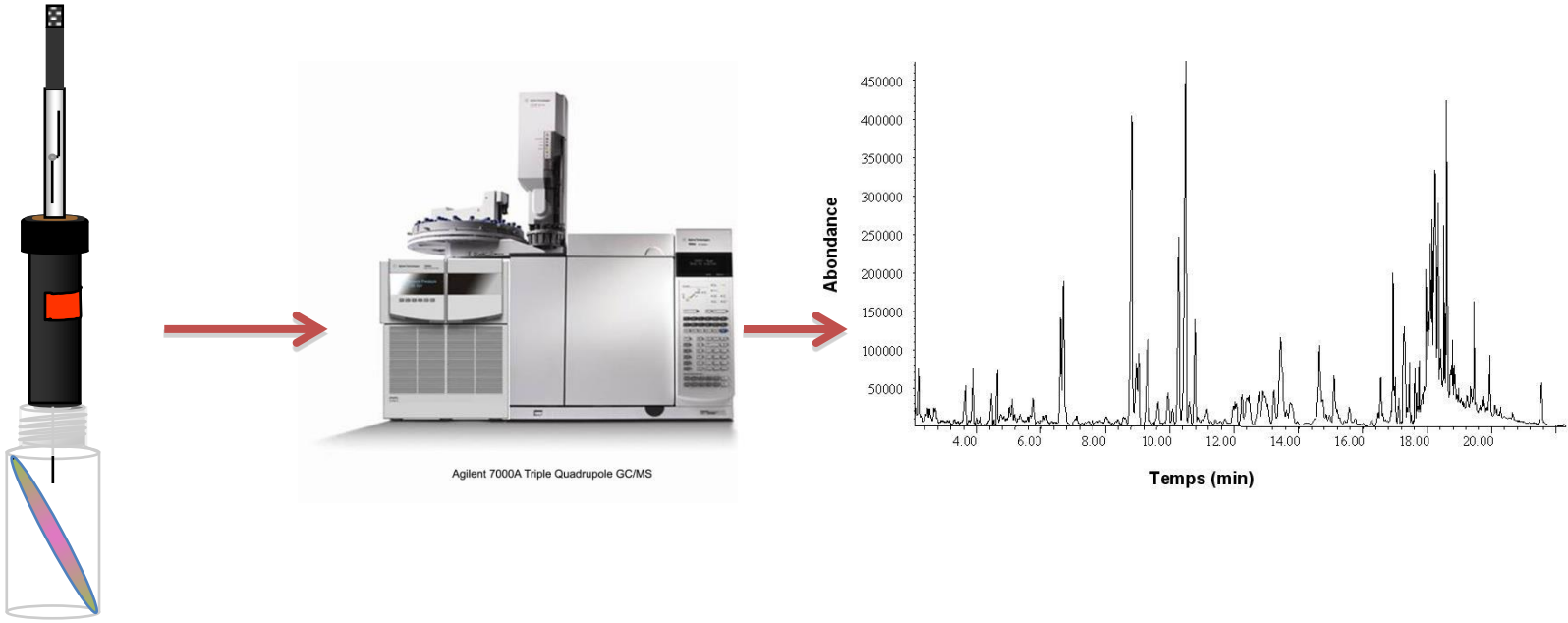
- Surface-sterilization of caryopses
- Vernalization
- Pre-germination
- Cocultivation for 10 days with bacteria in a shared atmosphere

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)

Rhizobacterial VOC analysis by SPME-GC-MS

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

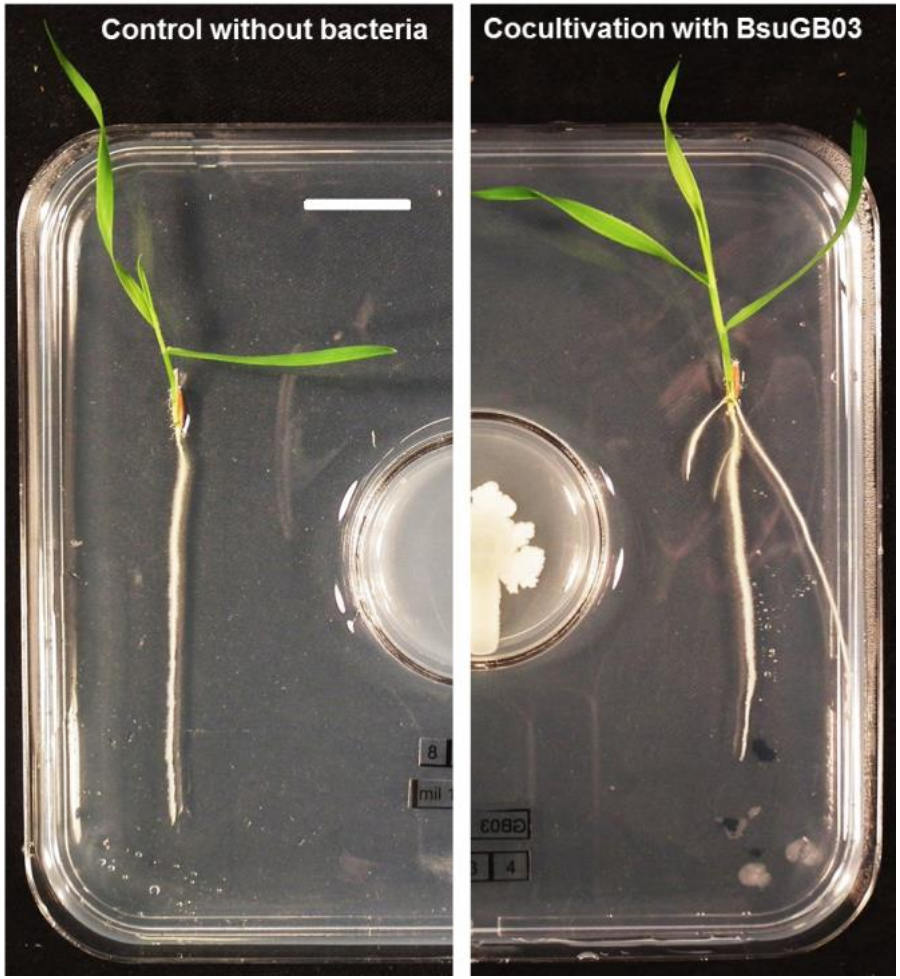
→ identification and quantitation based on retention time of commercial standards, mass spectra and peak area relative to internal standard



Linking five biomass-related variables and nine RSA traits…

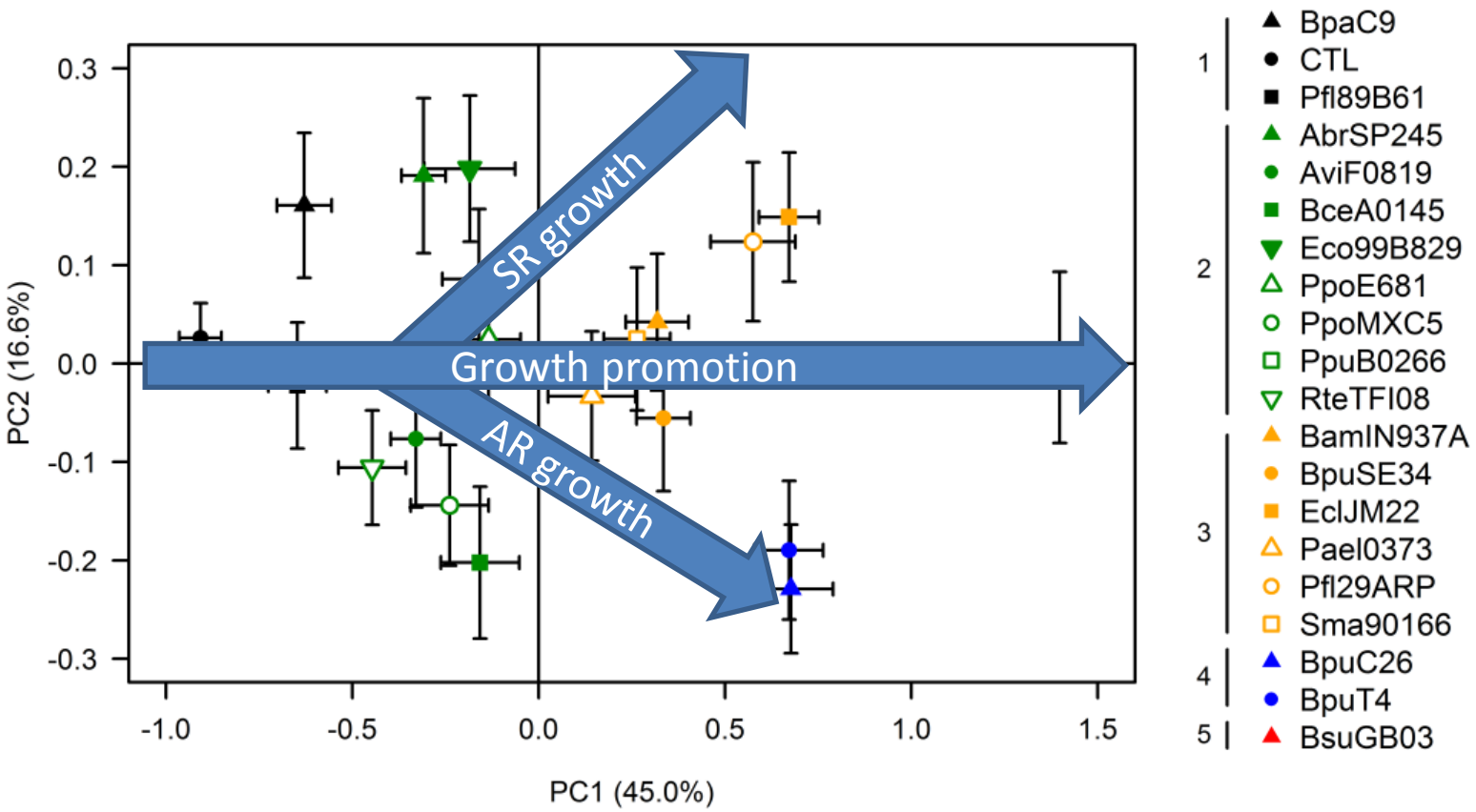
- Fourteen measured variables
- Four independent experimental replicates
- Principal Component analysis on weighted and reduced variables
- Hierarchical clustering based on the principal components
- Two-way ANOVA and Dunnett's test

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

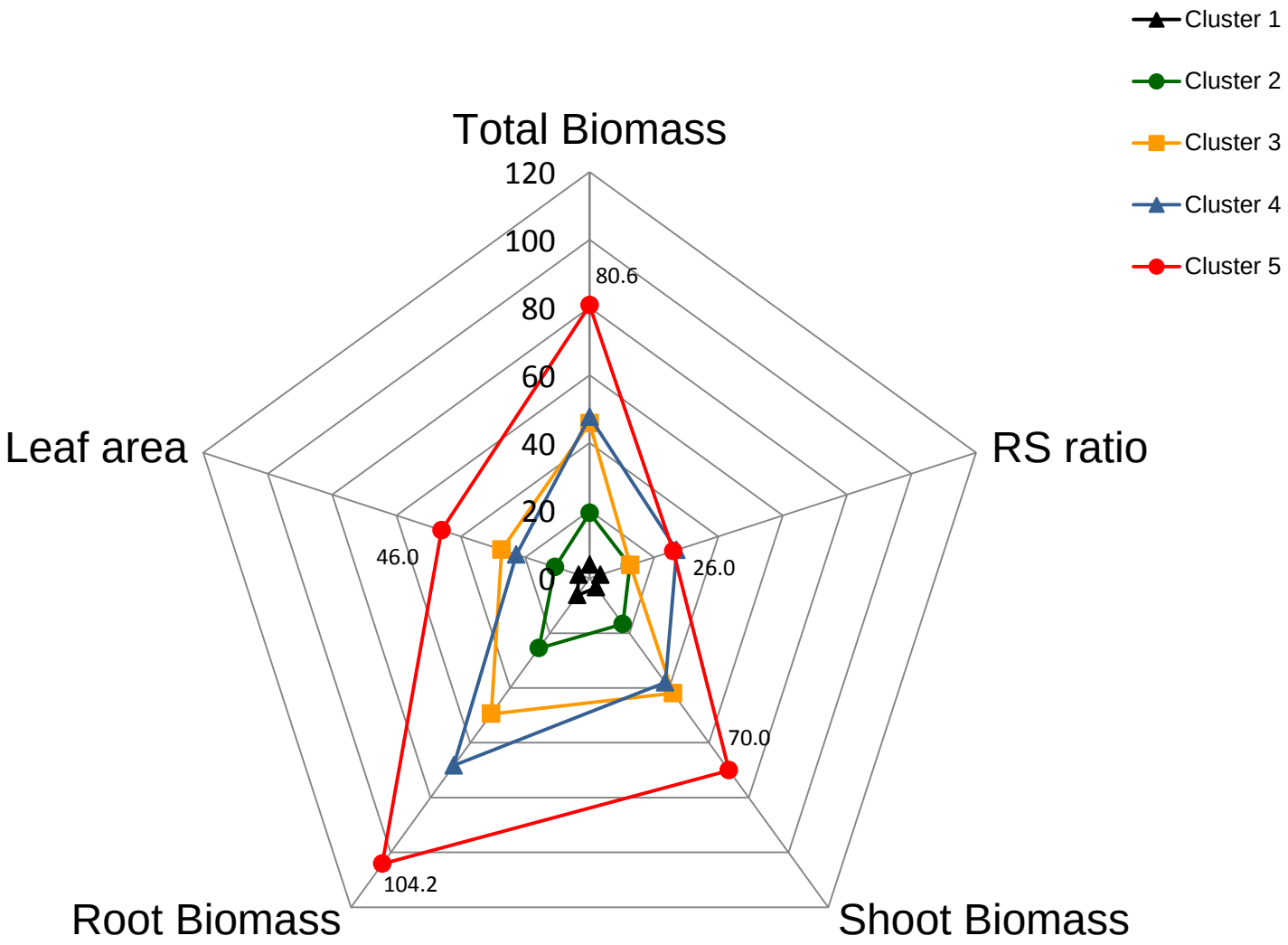


- Stage 12 vs 13 after 10 days
- Roots on top of the agar
- Strong correlation between biomass production and root branching traits
- Weak correlation with primary root length (PRL)
- PRL not correlated with other RSA traits

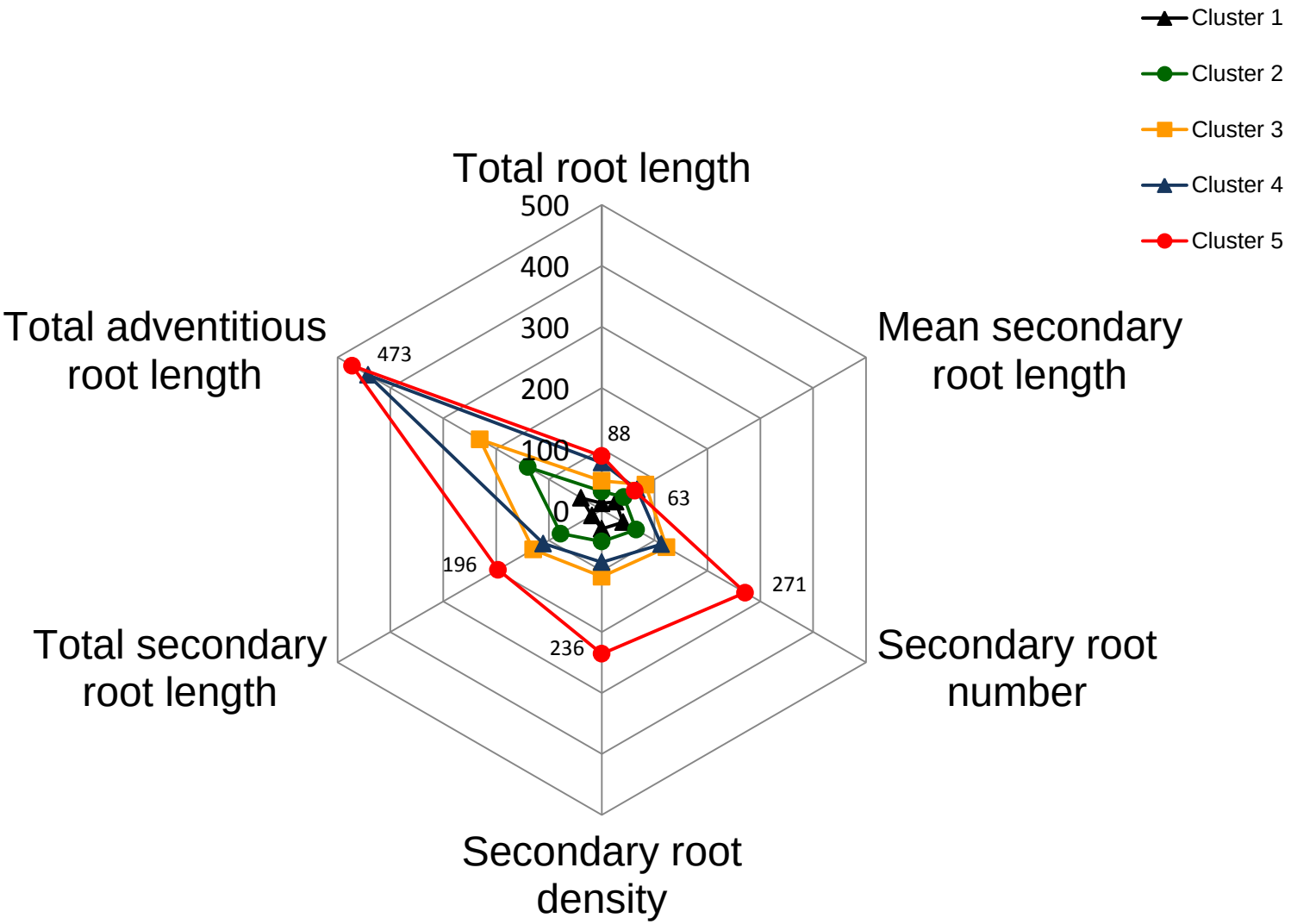
Contrasting biomass and RSA modulations define five groups of bacterial strains



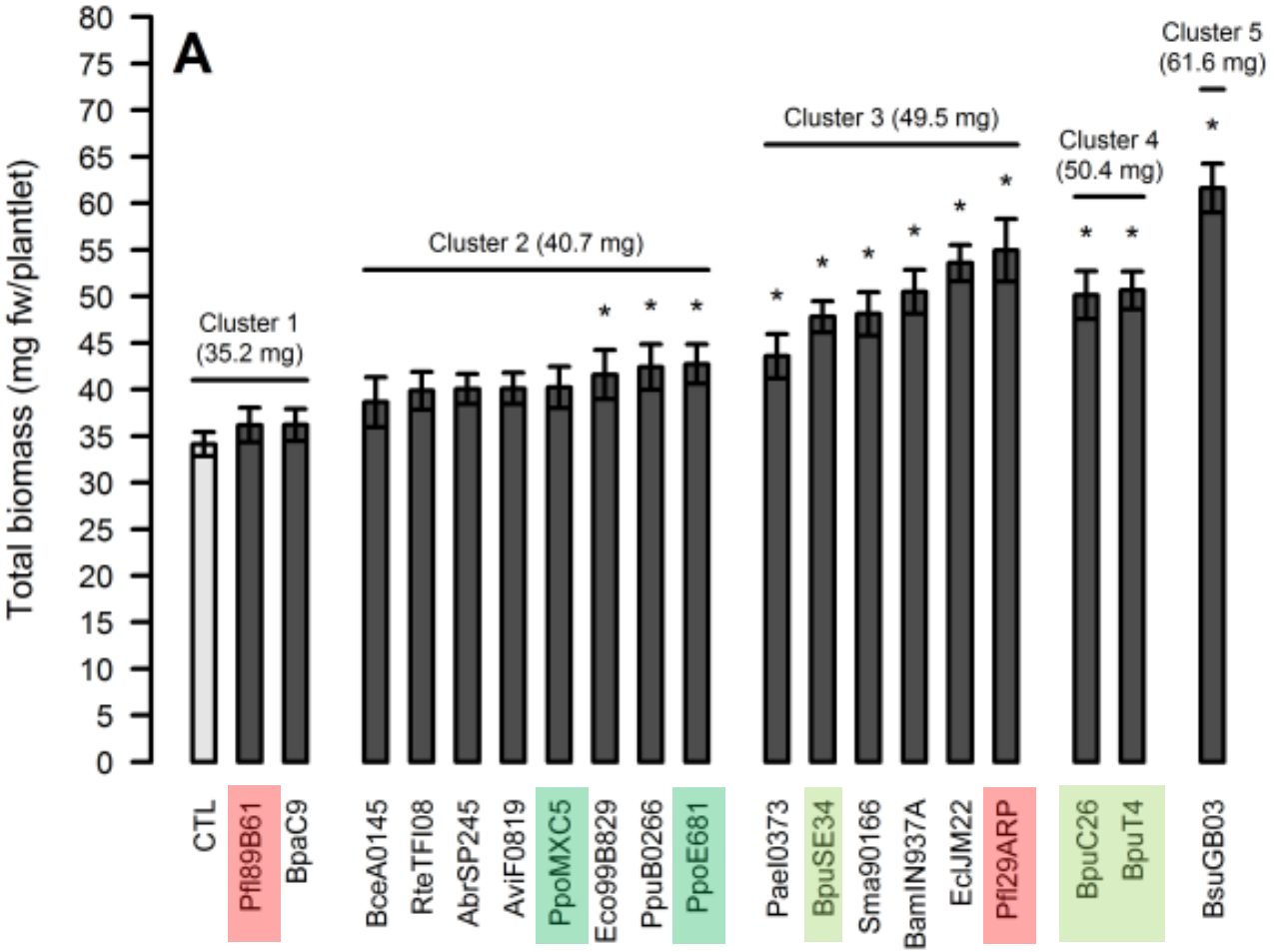
From non-significant to very high enhancement of biomass production



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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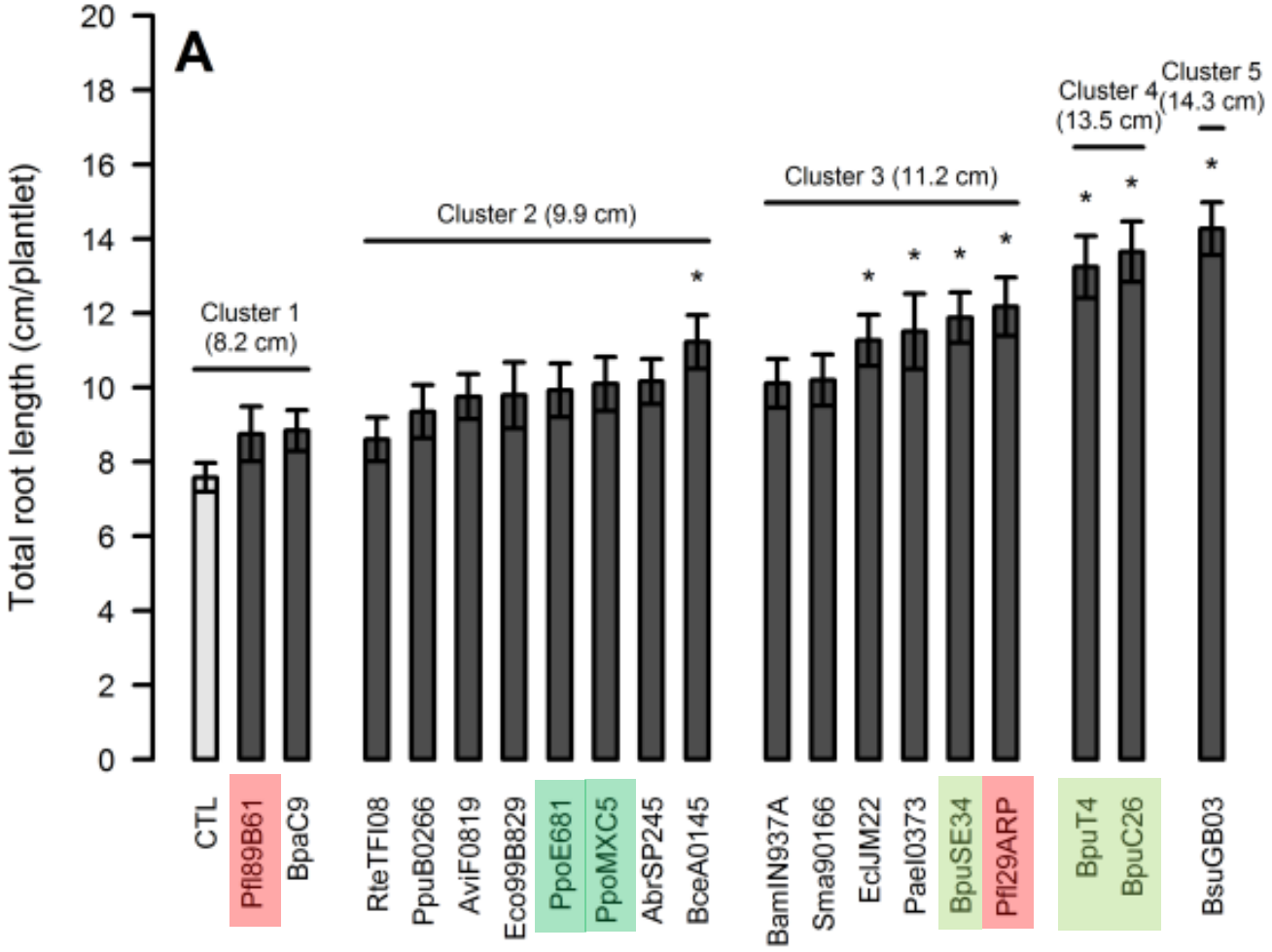
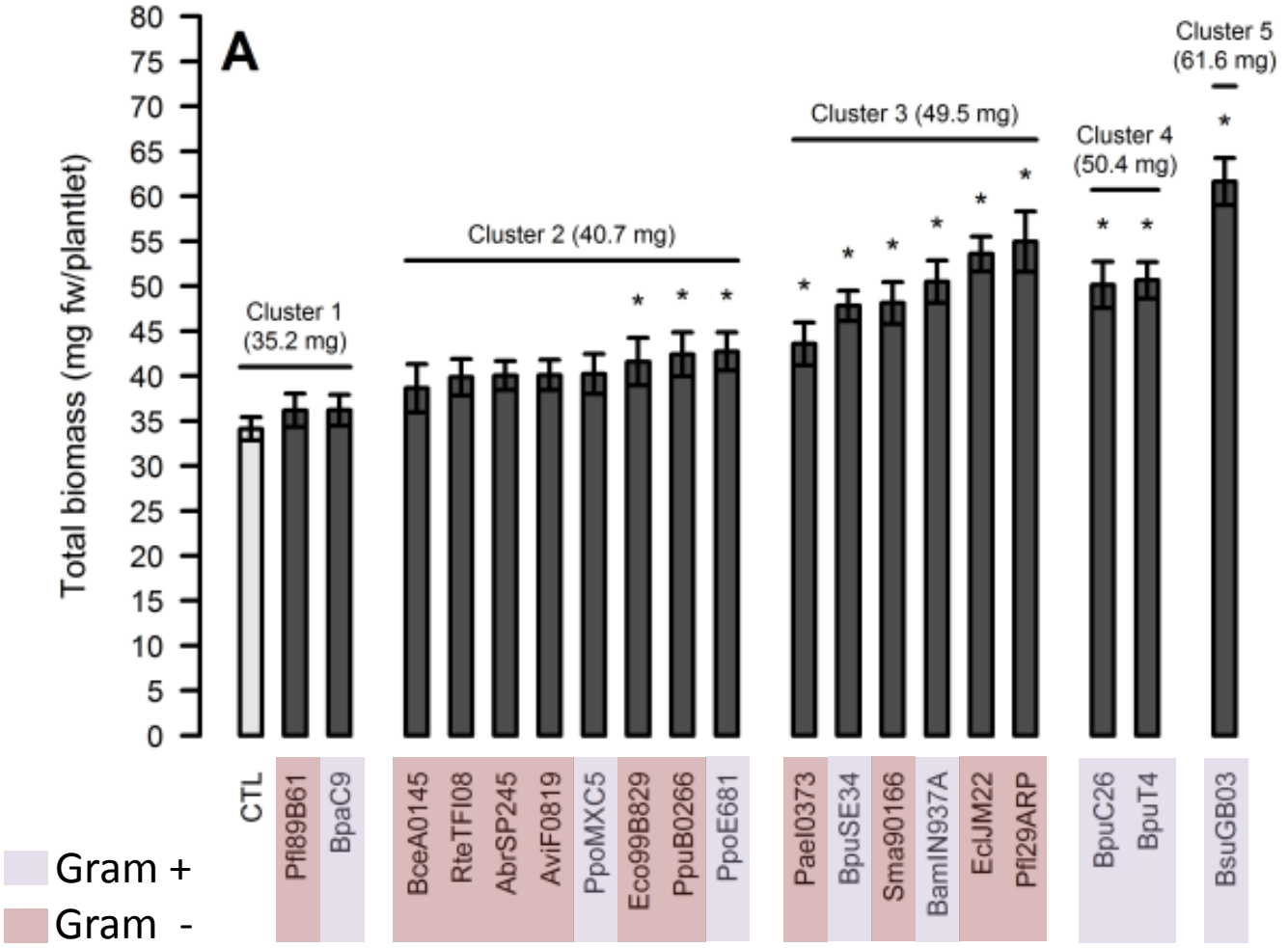


Significant changes compared with the control without bacteria are marked with an asterisk (*).

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



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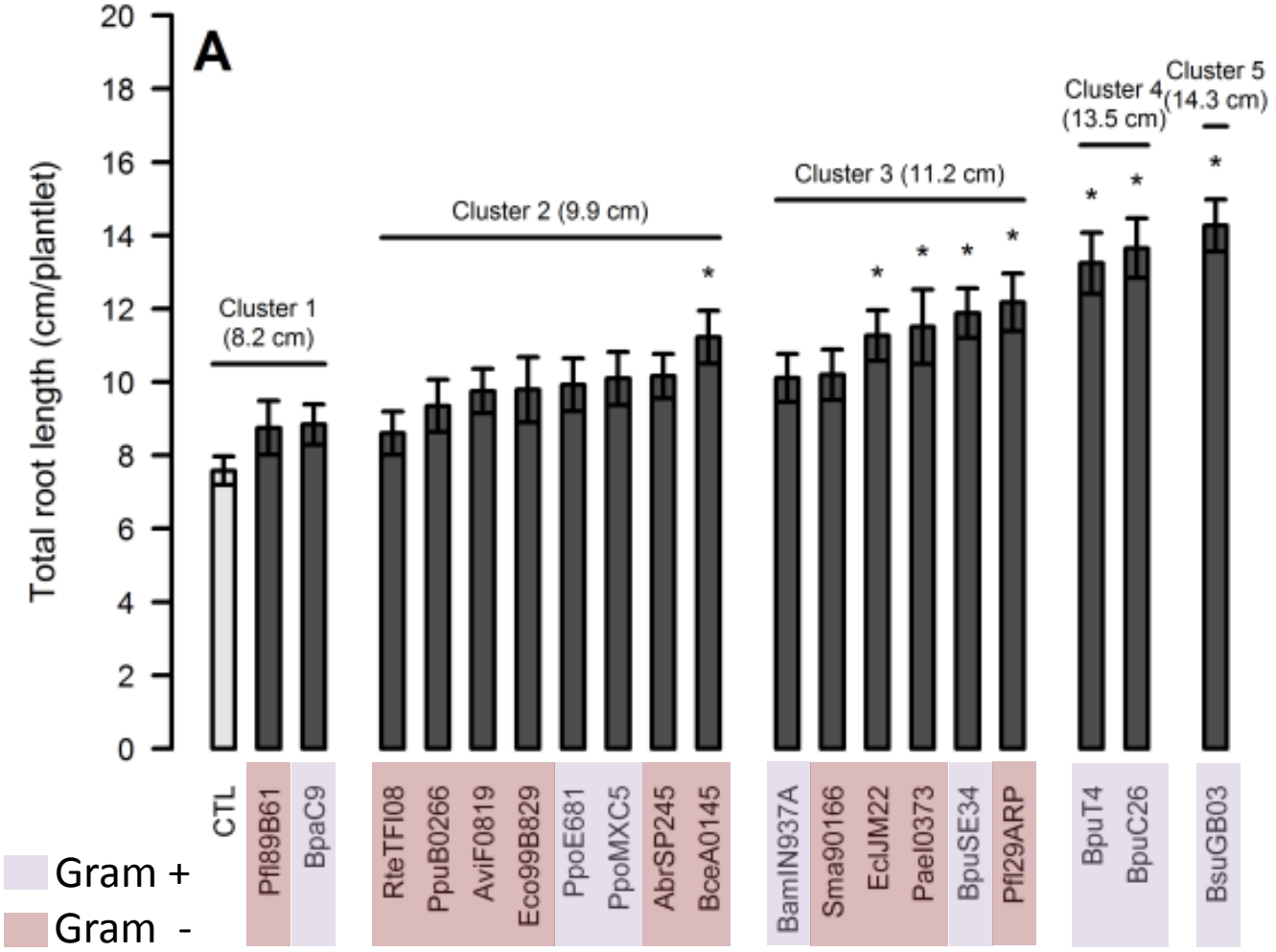
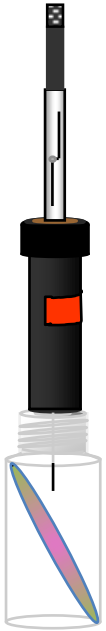
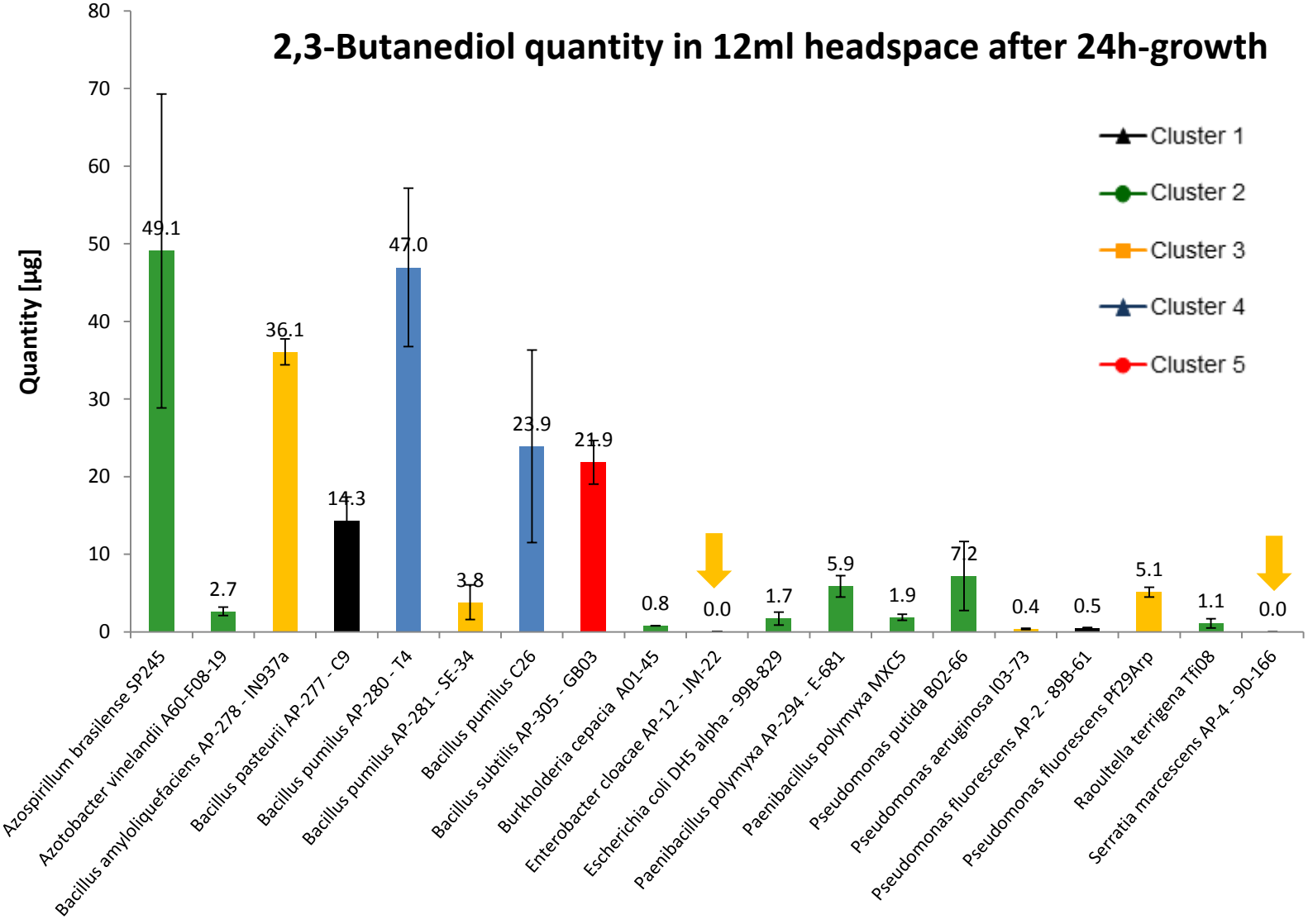


Contrasting effects indicate some heterogeneity in bacterial volatile production



Identified compounds after 24 hours of growth	
CO2	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

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



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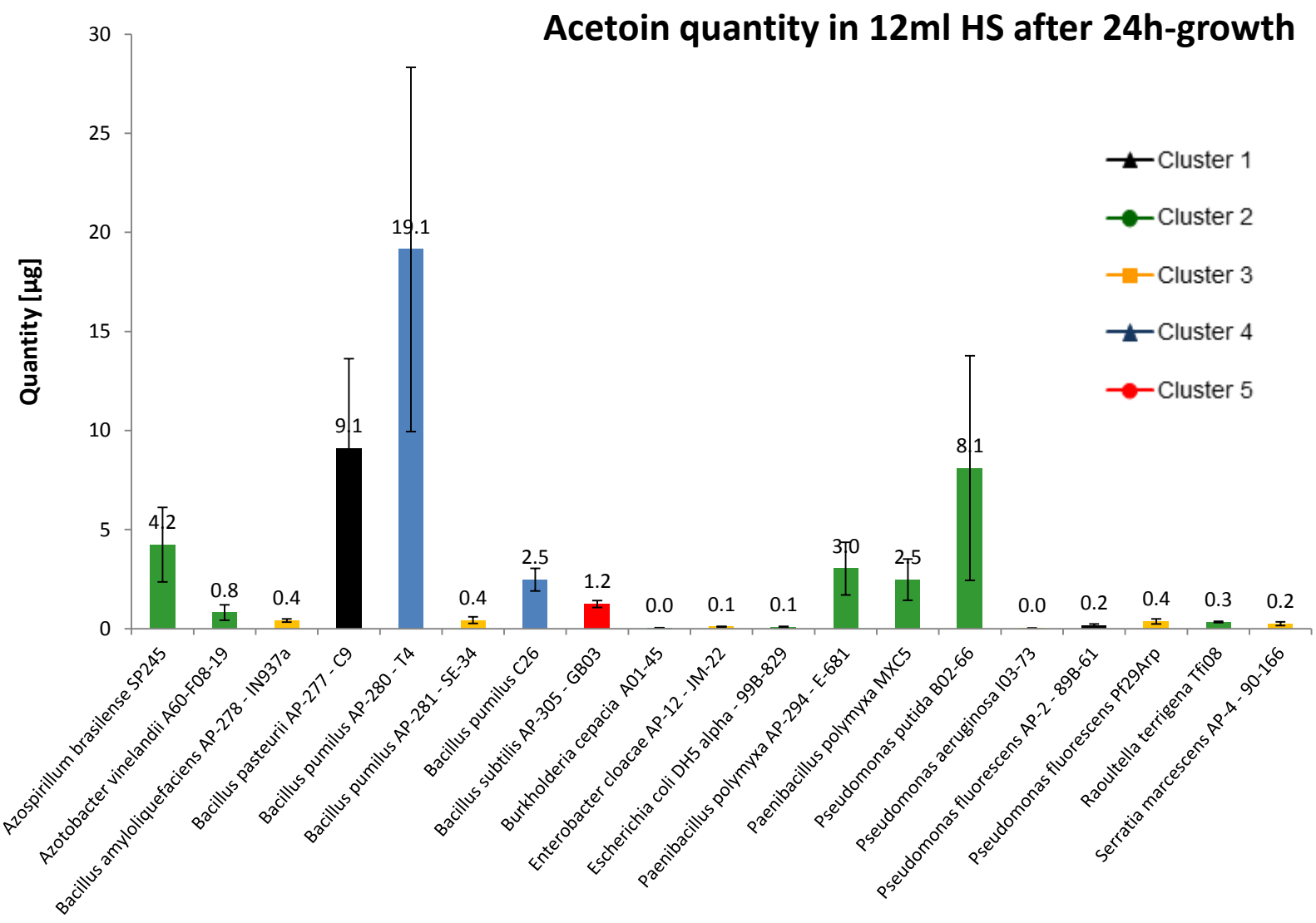

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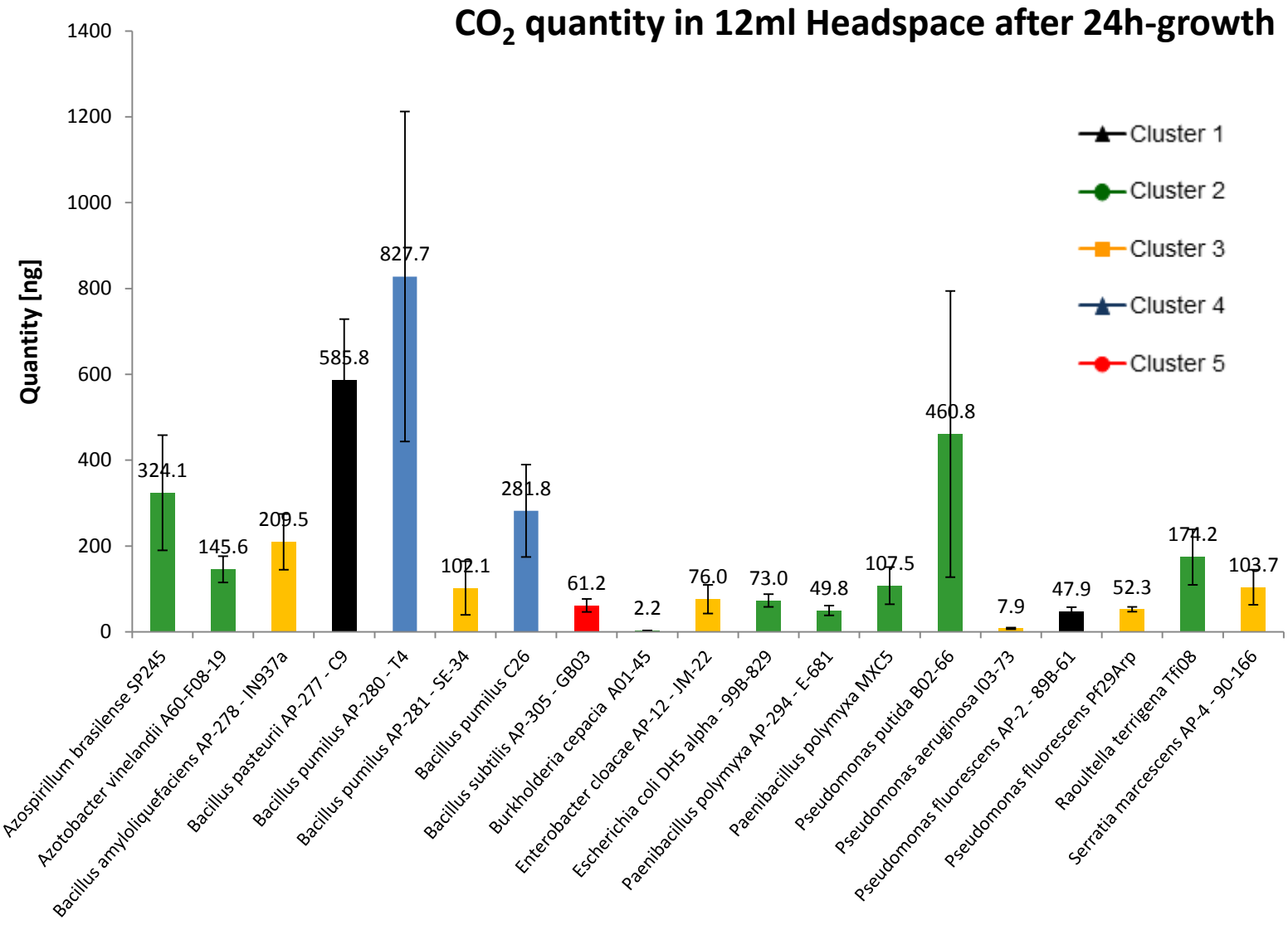

Take-home messages and future prospects!

- **First report** of bacterial volatile-mediated growth promotion of a grass plant (submitted to *BMC Plant Biology*)
- **A screening tool** 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 !

Baudson C., Delory B.M., du Jardin P., Delaplace P. (2015). **Can plant-growth promoting rhizobacteria mitigate P-starvation stress in *Brachypodium distachyon*?** Rhizosphere 4: Below and above ground interactions (Tuesday 23rd of June)

Delory B.M., Baudson C., Brostaux Y., Lobet G., du Jardin P., Pagès L., Delaplace P. (2015). **archiDART: an R package for the automated 2D computation of root architectural traits.** Rhizosphere 4: New tools and concepts in the rhizosphere (Wednesday 24th of June)

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