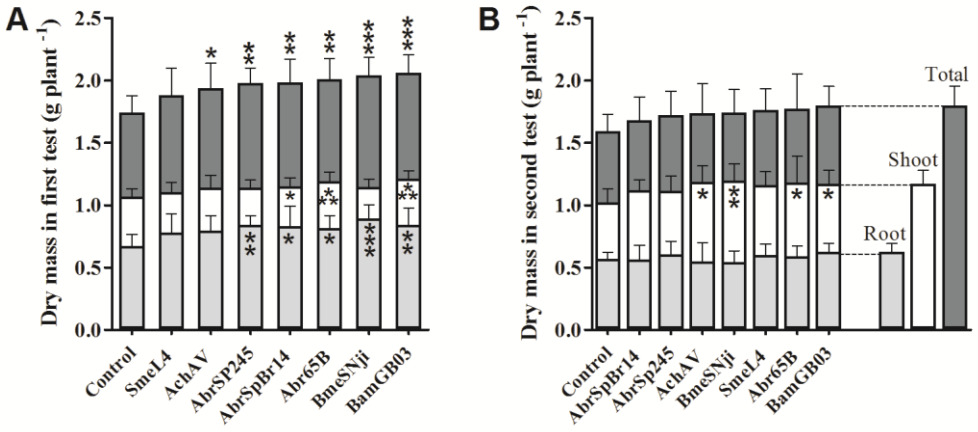
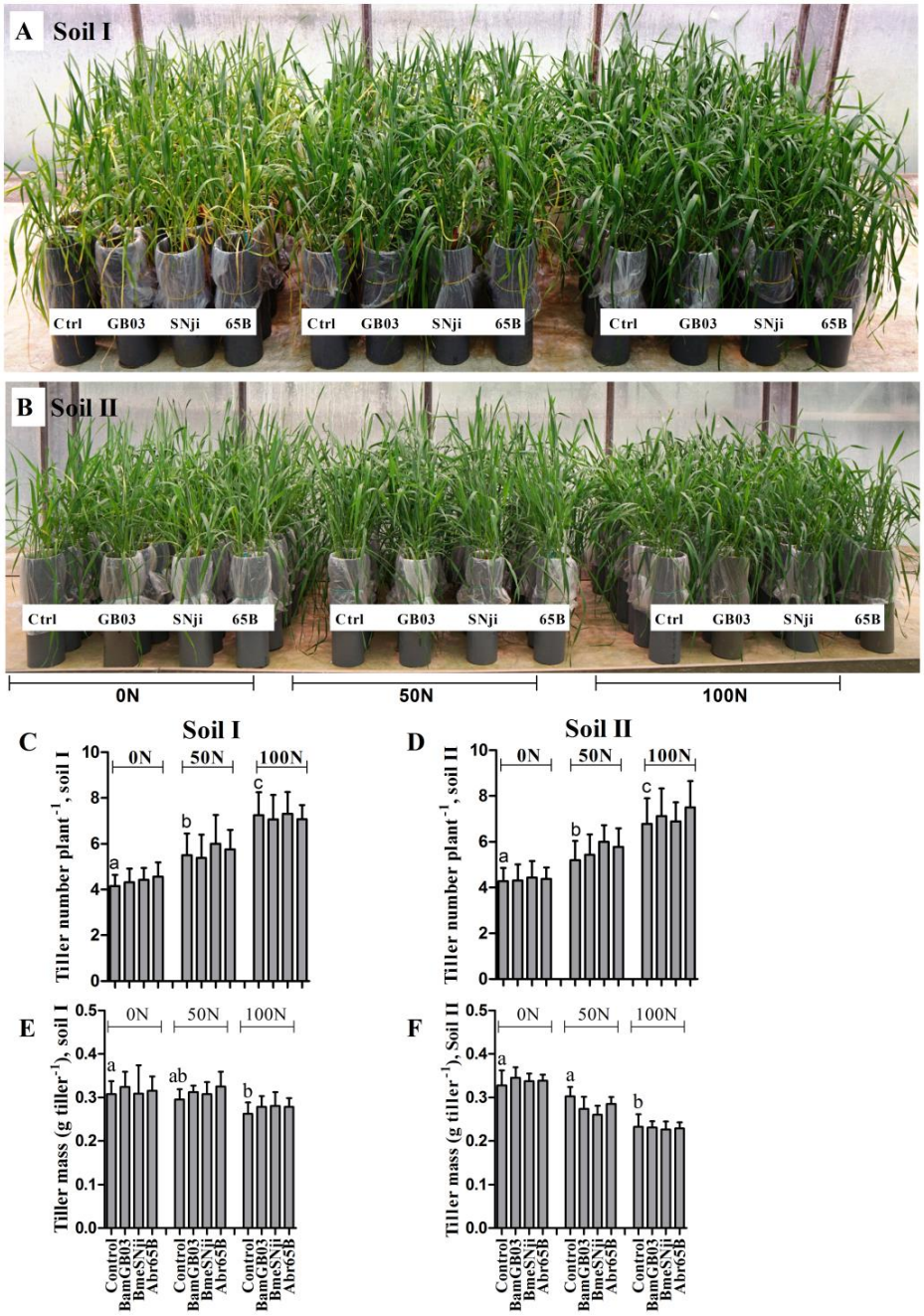
Biostimulant effects of rhizobacteria on wheat growth and nutrient uptake depend on nitrogen application and plant development

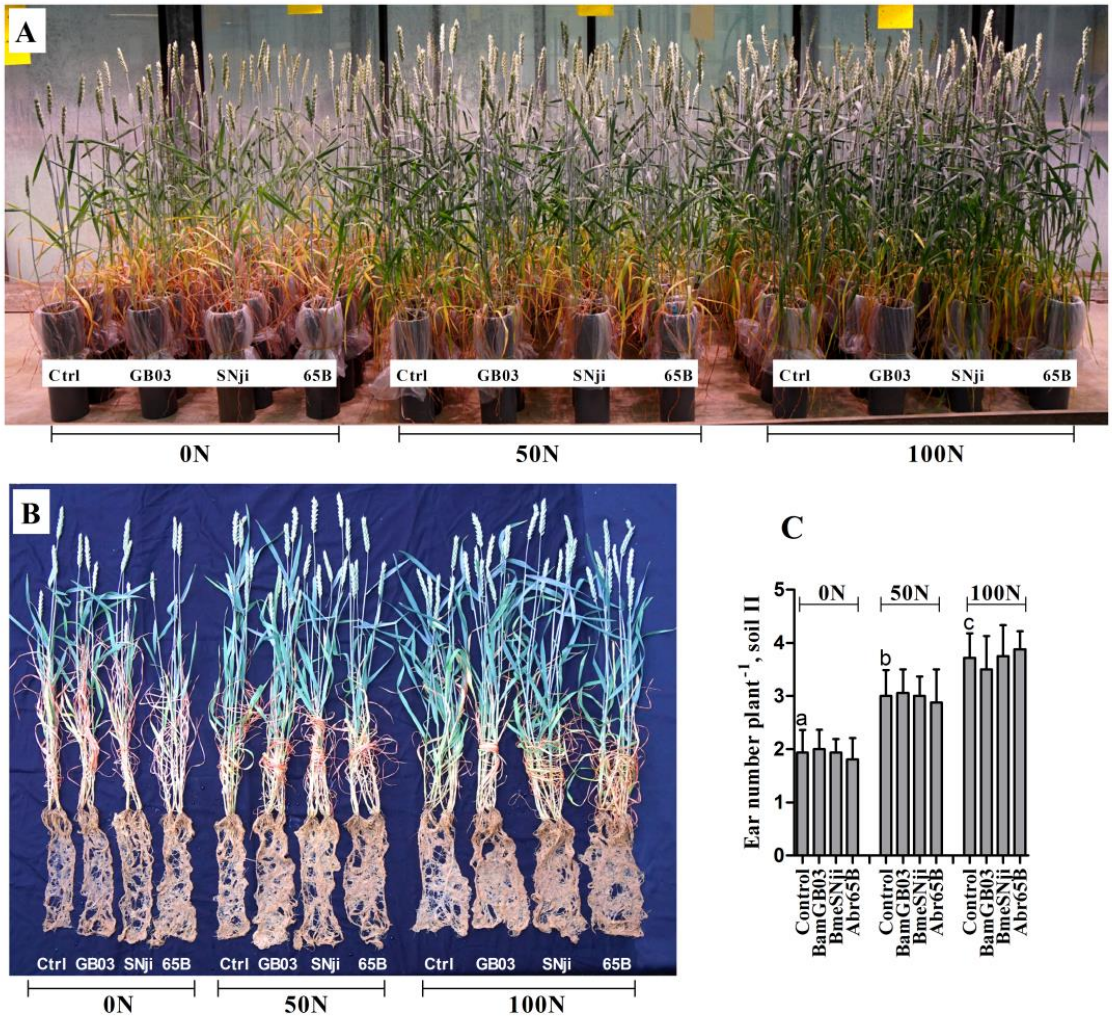
Archives of Agronomy and Soil Science



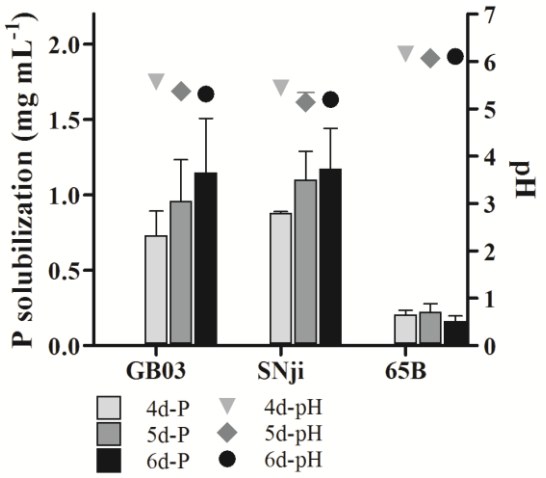
Supplementary Figure 1. Preliminary screening in the greenhouse to select the three best PGPR strains. Seven PGPR candidates, including *Sinorhizobium meliloti* L4, *Azotobacter chroococcum* AV, *Azospirillum brasilense* SP245, *Azospirillum brasilense* SpBr14, *Azospirillum brasilense* 65B, *Bacillus megaterium* SNji, and *Bacillus amyloliquefaciens* AP-305-GB03, were screened for their growth promotion capacity. Two experimental replicates were performed with the first test (A) in May 2014 and the second test (B) in October 2014. Seven PGPR strains were individually inoculated to spring wheat seeds sown in mixture of soil I without additional fertilizer and grown as described in the "Greenhouse experiments" section. The plants were harvested after 30d at Zadoks 30–32. Based on the most significant increases in root, shoot and total dry biomass, the three best strains were selected. Asterisks denote statistical significance between treatments with PGPR to their respective control in root, shoot, and total dry biomasses. One-way ANOVA test was followed by Dunnett's Multiple Comparison Test (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). The values are the means of 8–10 pots for PGPR treatments and 14–15 pots for controls ± SD. The overlapping bars presesenting root, shoot and total biomass have the same base line at 0 (g plant^{-1})



Supplementary Figure 2. Response of plants inoculated with *Bacillus amyloliquefaciens* GB03, *B. megaterium* SNji, and *Azospirillum brasilense* 65B to different N rates (0, 50, and 100N) 30 days after sowing. The control was treated with sterile phosphate buffer. Representative images of plant response to the supply of different N rates: (A) in soil I in Fig.2 and (B) in soil II in Fig.3. (C, D) Tiller number in soil I from A and soil II from B, respectively. (E, F) Tiller biomass in soil I from A and soil II from B, respectively. The values are the means of eight pots ± SD. Means that do not share a letter are significantly different in the control plants supplied with 0, 50, and 100N. In each N group, there was no significant impact of PGPRs on tiller number and tiller biomass compared with their respective non-inoculated control



Supplementary Figure 3. Response of plants inoculated with or without *Bacillus amyloliquefaciens* GB03, *B. megaterium* SNji, and *Azospirillum brasilense* 65B under different N rates (0, 50, and 100 N) at 60 days after sowing (Zadoks 65-70). The response of plants with different N rates at this stage were similar between soil I and soil II, so only images and ear number per plant from soil II were shown. (A) Plants grown in pots until flowering, (B) representative plants of each treatment after washing of roots, and (C) ear number per plant at 60d. The values are the means of eight pots ± standard deviations. Means that do not share a letter are significantly different in the control plants supplied with 0, 50, and 100N. In each N group, there was no significant impact of PGPR on ear number compared with the respective non-inoculated control



Supplementary Figure 4. Phosphate solubilization capacity of *Bacillus amyloliquefaciens* GB03, *B. megaterium* SNji, and *Azospirillum brasilense* 65B strains in liquid culture (NBRIP medium) containing 0.5% insoluble phosphate $Ca_3(PO_4)_2$. Bacteria were added to the medium with a final cell density of 10^7 CFU mL^{-1}. After 4, 5, and 6 days incubation, solubilized P and pH were measured. The values are the mean of three replicates.

Supplementary Table 1. IAA production capacity of *Bacillus amyloliquefaciens* GB03, *B. megaterium* SNji, and *Azospirillum brasilense* 65B strains in TSB liquid culture supplemented with or without tryptophan (1g L^{-1}). IAA concentrations are expressed in µg mL^{-1} or pg CFU^{-1}. The values are the mean of six replicates.

PGPR	- Tryptophan			+ Tryptophan		
	24 h	48 h	72 h	24 h	48 h	72 h
µg mL^{-1}						
BamGB03	9.7 ± 1.6	8.7 ± 1.0	15.3 ± 3.2	6.3 ± 1.8	11.3 ± 0.4	23.4 ± 0.6
BmeSNji	3.0 ± 0.5	4.6 ± 0.4	8.9 ± 0.7	3.7 ± 0.5	6.0 ± 2.1	18.5 ± 4.9
Abr65B	2.1 ± 0.5	3.5 ± 0.2	7.2 ± 0.4	5.2 ± 0.5	15.3 ± 1.8	24.4 ± 3.3
pg CFU^{-1}						
BamGB03	0.13 ± 0.02	0.12 ± 0.01	0.41 ± 0.08	0.08 ± 0.02	0.15 ± 0.02	0.46 ± 0.01
BmeSNji	0.30 ± 0.05	0.24 ± 0.02	0.30 ± 0.02	0.29 ± 0.04	0.28 ± 0.05	0.57 ± 0.15
Abr65B	0.07 ± 0.02	0.11 ± 0.01	0.14 ± 0.01	0.05 ± 0.01	0.11 ± 0.01	0.11 ± 0.02