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ORIGINAL ARTICLE

Abundance and ribotypes of phosphate-solubilizing bacteria in Argentinean agricultural soils under no-till management

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Abstract Phosphate-solubilizing bacteria (PSB) abundance and ribotypes were examined in the top soil (0–10 cm) of agricultural fields under no-till management sampled in two seasons, summer (February) and late winter (September), in 2010. No-till plots under sustainable agricultural practices (intense crop rotation) or under non-sustainable practices (soybean monocropping) were sampled at four different locations as replicates in a 400-km west–east transect in the most productive agricultural region of Argentina. Natural grasslands were selected close to the cultivated fields for comparative purposes. Culturable heterotrophic bacteria (CHB) were enumerated on nutrient agar plates, and PSB were counted on NBRIP agar plates containing $Ca_3(PO_4)_2$. The PSB community structure was explored by ribotyping (16S rDNA PCR-restriction fragment length polymorphism). Quantitatively, data showed that the number of CHB and PSB per gram of dry soil was not statistically different among sampled sites, soil management programs or seasons. Qualitatively, ribotyping showed that the most abundant PSB species differed in their fingerprinting patterns among geographical sites, which suggests that local soil conditions impose strong selective constraints. The comparative analysis of PSB ribotypes revealed seasonal differences among February and September isolates for all sampling sites.

Keywords Phosphate-solubilizing bacteria (PSB) · Abundance of PSB · Ribotyping · No-tillage soils

Introduction

Phosphorus (P) is an essential element for plant growth and cannot be substituted by any other nutrient. It stimulates several plant metabolic properties, such as carbohydrate content, total respiration rate, amino acid concentration and many important enzyme activities (Panhwar et al. 2012). Consequently, P deficiency limits plant growth in many agricultural soils. Although soils may have large reservoirs of total P, the amount of these P sources available for plant uptake usually only accounts for a small proportion of the total soil P content. The reason for this dichotomy is that most of the soil P is present in insoluble forms, whereas plants can only absorb soluble P species such as orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) (Bhattacharyya and Jha 2012). Thus, farmers usually try to compensate low soluble P content in soils by amendment with chemical fertilizers or sludge from wastewater treatments (Ravindra Naik et al. 2008). However, current environmental policies strongly recommend minimizing the use of such products to promote sustainable development of agriculture (Horrigan et al. 2002). As a consequence, there is a growing interest in identifying alternative strategies to increase P availability to crops while reducing the use of fertilizers (Bhattacharyya and Jha 2012). In this context, different microorganisms may contribute to plant P nutrition by increasing the availability of inorganic phosphate through separate and complementary mechanisms that are part of the P cycle in the soil: solubilization of inorganic phosphates or mineralization of organic phosphates. In this way,

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microorganisms displaying such activities can act as biofertilizers and represent a biotechnological tool that can contribute to sustainable agriculture (Lugtenberg and Kamilova 2009).

Phosphate-solubilizing bacteria (PSB) promote the release of soluble orthophosphates from inorganic phosphates (Richardson et al. 2009). The release of P from insoluble mineral phosphates occurs through the secretion of organic (usually gluconic) or mineral acids and siderophores (Puente et al. 2004; Rodríguez et al. 2006). PSB are readily detected and isolated on agar plates containing insoluble phosphates as the only source of P for growth as colonies that generate a solubilization halo (Nautiyal 1999). A large number of PSB have been isolated from the rhizosphere of several crops, and their taxonomical assignment indicates that PSB are a polyphyletic functional group. Relevant PSB genera include *Achromobacter*, *Aerobacter*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Xanthomonas* (Kundu et al. 2009). Although the ecology of these soil bacteria has been reported extensively (Kämpfer 2007), there are few reports on the impact that agricultural practices have on the abundance and diversity of PSB (Hu et al. 2009; Azziz et al. 2012; Ndung'u-Magiroi et al. 2012).

No-tillage (also referred to as no-till or zero tillage) is a farming system in which the seeds are directly deposited into untilled soil on top of the previous crop residues (Derpsch 2008). Pioneering no-till agricultural practices began in Argentina in the 1960s in Anguil (La Pampa province) and Pergamino (Buenos Aires province), with row crop production systems (Díaz-Zorita et al. 2002). Nowadays, about 25 million hectares are cultivated under no-till management, with soybean covering more than 15 million hectares, and maize and wheat together covering approximately five million hectares (Wall 2011). This conservative practice tends to increase soil organic matter content in the surface layer, improve soil aggregation, and preserve the soil resources to a greater extent than conventional till practices (Giuffré et al. 2006). It was also observed that under no-tillage farming the rate of macroaggregate formation and degradation leads to the formation of stable micro-aggregates in which carbon is stabilized and sequestered in the long term (Derpsch et al. 2010). Therefore, no-tillage crop production systems have the additional advantages of soil carbon sequestering, reduced CO_2 emissions from soil, and enhancement of sustainability (Horrigan et al. 2002). All these beneficial properties of no-till soil management systems are sustainable in the long term if no-till farming is combined with crop rotation. Otherwise, crop monoculture may result in the accumulation of soil borne pathogens due to continuous deposition of the same kind of crop residuals (Derpsch 2008).

The benefits of no-till farming in terms of physical and chemical soil properties have been well documented (Cantú et al. 2007; Ferreras et al. 2007; Picone et al. 2007; Álvarez et al. 2009; Campitelli et al. 2010), but data are either lacking or just emerging on the biological indicators of soil quality and/or soil health under this management regime (Figuerola et al. 2012). In this regard, a multidisciplinary public–private consortium named BIOSPAS (www.biospas.org) was created in 2009 to study soil biology in relation to crop productivity under sustainable agricultural management in no-till systems. One of the main goals of BIOSPAS was to identify quantitative and qualitative biological indicators of sustainable agricultural practices that promote soil health and long-term productivity under no-tillage management (Wall 2011; Figuerola et al. 2012). Within this consortium, we studied the influence of geographical location, season, and cropping practice on the abundance and community structure of PSB in agricultural soils under no-till management in the central region of Argentina.

Materials and methods

Site description

The agricultural fields were located across a west–east transect in the most productive region of the Argentinean Pampas, in the vicinity of Bengolea, Córdoba Province (33°01′31″ S; 63°37′53″ W); Monte Buey, Córdoba Province (32°58′14″ S; 62°27′06″ W); Pergamino, Buenos Aires Province (33°56′36″S; 60°33′57″ W), and Viale, Entre Ríos Province (31°52′59″ S; 59°40′07″ W) (Fig. 1a). The type and physicochemical properties of the soils, description of soil management, cover crops, rotation index, and fertilization regimes of the studied sites are summarized in Table 1. In each location, three cropping practices were defined: (1) "Good Agricultural Practices" (GAP): sustainable agricultural management under no-till management, subjected to intensive crop rotation, nutrient replacement, and minimal agrochemical use (herbicides, insecticides, and fungicides); (2) "Bad Agricultural Practices" (BAP): non-sustainable agricultural management under no-till management with crop monoculture (soybean), low nutrient replacement, high agrochemical use (herbicides, insecticides, and fungicides) and showing lower yields compared to GAP; (3) "Natural Environment" (NE): as reference, natural grassland was selected in an area of approximately 1 ha close to the cultivated plots (<5 km distant), where no cultivation has been practiced for (at least) the last 30 years.

Soil sampling

Top soil samples (depth 0–10 cm) were collected in triplicate for each treatment and site in 2010 in both the summer (February) and late winter (September), in three 5-m^2 sampling points separated by at least 50 m. Care was taken not to follow the sowing line in the field. Each replicate sample was

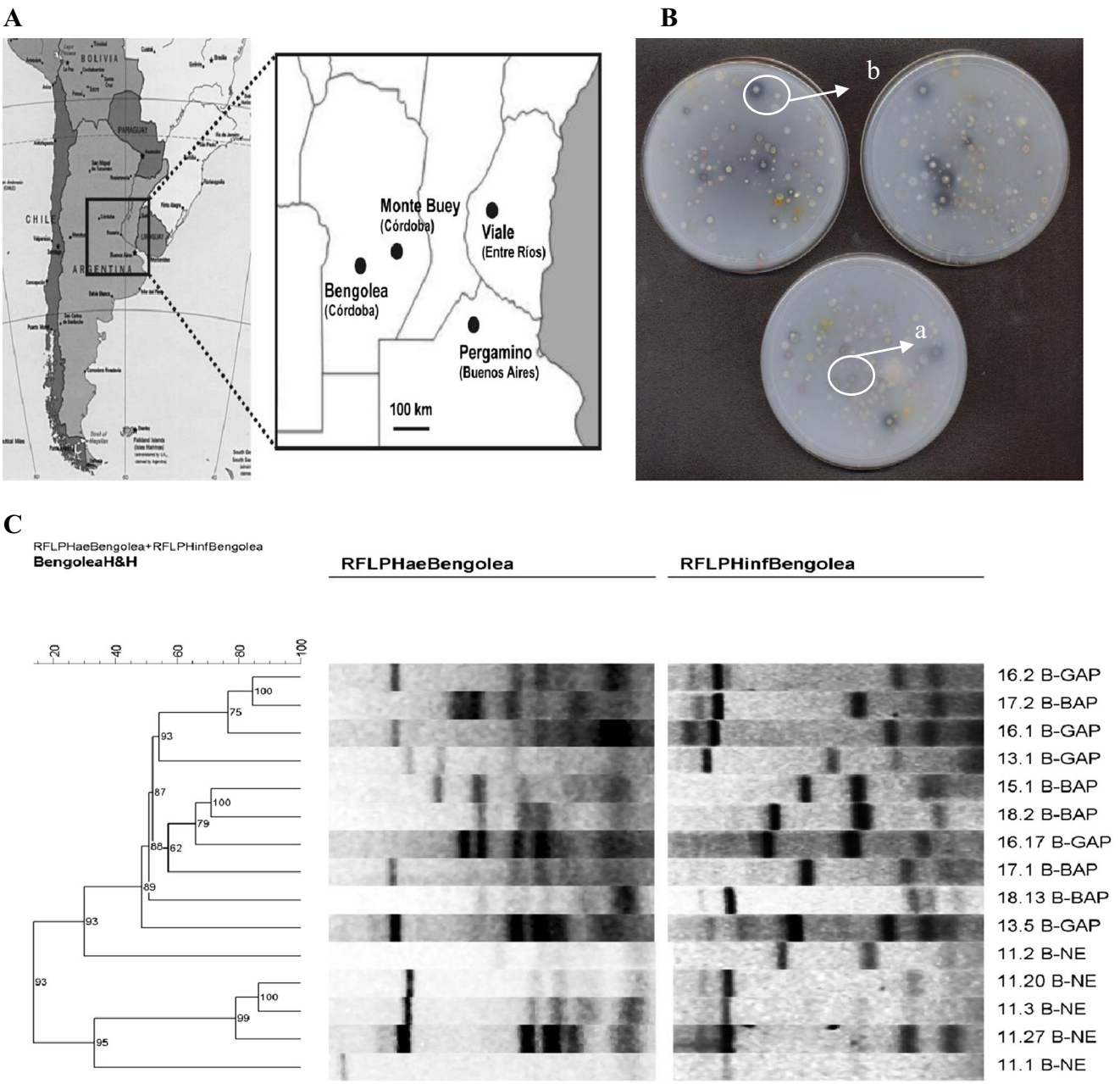


Fig. 1 **a** Geographical location of the soil sampling sites in Argentina. **b** Representative NBRIP plates containing $Ca_3(PO_4)_2$ as the sole source of phosphorus (P) after plating soil suspensions and subsequent incubation for 7 days at 28 °C. *a* Total bacteria in NBRIP medium (TBNBRIP), colonies able to develop under P-limiting conditions with and without $Ca_3(PO_4)_2$ (solubilization halo was enumerated as total bacteria in NBRIP medium), *b* phosphate-solubilizing bacteria (PSB), i.e., colonies with $Ca_3(PO_4)_2$ solubilization halo (enumerated as PSB). **c** Representative ribotype analysis of PSB isolates from Bengolea soils under different cropping practices. *NE* natural environment, *GAP* good agricultural practice, *BAP* bad agricultural practice (see section Site description for a full description of cropping practices and control). RFLP Restriction fragment length polymorphism, *Hae*, *Hinf* restriction enzymes *Hae*III and *Hinf*I, respectively

collected as a composite of 16–20 randomly selected subsamples. Soil subsamples were combined and homogenized in the field and transported to the laboratory at 4 °C. Within 3 days after collection, the soil samples were sieved through a 2-mm mesh to remove roots and plant detritus and stored at 4 °C until processing (not longer than 3 weeks), as recommended by Wallenius (2011).

Enumeration of culturable heterotrophic bacteria and PSB

Bulk soil suspensions were obtained by shaking 10 g of sieved soil in 90 ml of 0.85 % w/v NaCl for 30 min at 180 rpm at room temperature. Decimal serial dilutions were plated in triplicate onto nutrient agar (NA) (Britania, Argentina) to enumerate culturable heterotrophic bacteria (CHB) and in

Table 1 Soil characteristics according to site[a] and agricultural management practices[b] at all sampling locations[c]

Soil characteristic	Bengolea			Monte Buey			Pergamino			Viale		
	NE	GAP	BAP	NE	GAP	BAP	NE	GAP	PAP	NE	GAP	BAP
Soil classification	Entic Haplustoll			Typic Argiudoll			Typic Argiudoll			Argic Pelludert		
Texture	Sandy loam			Silt loam			Silt loam			Silty day/silty clay loam		
Carbon (%)	1.7	1.5	1.1	3.5	2.1	1.7	2.7	1.7	1.8	5	3.5	2.5
Nitrogen (%)	0.146	0.156	0.125	0.328	0.181	0.132	0.233	0.153	0.136	0.369	0.283	0.179
Extractable P (ppm)	44.3	53.1	17.8	296.5	126.5	20.6	10.5	18	11.9	20.2	40.4	41.8
pH	6.3	6.2	6.2	5.6	5.5	6.2	6.2	6	5.7	6.4	6.7	6.3

P, Phosphorus

[a] Study sites (Bengolea, Córdoba Province; Monte Buey, Córdoba Province; Pergamino, Buenos Aires Province; Viale, Entre Ríos Province) are described in detail in section Site description

[b] Soil management practices (NE, natural environment; GAP, good agricultural practice; BAP, bad agricultural practice) are described in section "Site description"

[c] Data are adapted from Figuerola et al. (2012)

triplicate onto NBRIP agar (Nautiyal 1999), a minimal medium with insoluble tricalcium phosphate $Ca_3(PO_4)_2$ as the sole P source. NBRIP plates were used to enumerate total bacteria that are able to grow under P-limiting conditions. All colonies which developed on NBRIP plates, either with or without a $Ca_3(PO_4)_2$ solubilization halo, were enumerated as total bacteria in NBRIP medium (TBNBRIP) (Fig. 1b, a). The bacterial colonies having a surrounding halo on NBRIP plates were counted differentially as PSB (Fig. 1b, b). Both media contained cycloheximide (100 μg/ml) to inhibit fungal growth. CHB were counted 48 h after plating, whereas TBNBRIP were counted after 7 days of incubation at 28 °C.

The moisture content of soil samples was estimated as weight loss upon drying at 100 °C for 48 h and then used to express the soil bacterial abundance as colony forming units (CFU) per gram of dry soil.

Ribotyping of PSB isolates

Between 30 and 100 colonies with a P-solubilizing halo grew on each replicate NBRIP plate for every site and treatment. Ten of these colonies were selected on the basis of their distinctive morphological features (color, border, mucosity), with the aim to to maximize diversity. Colonies were streaked onto fresh NBRIP plates to check their purity. Purified isolates were resuspended in 1 ml of deionized water and treated at 100 °C in boiling water for 15 min to lyse the cells. Treated suspensions were centrifuged at 14,000 rpm for 2 min. The supernatants were recovered in new clean 1.5-ml tubes and stored at −20 °C until used as a source of DNA for the PCR reactions (Agaras et al. 2012). Due to the lysing resistance of some of the isolates, a maximum of four or five ribotypes could be recovered from each set of ten selected colonies.

The 16S rDNA gene was amplified by PCR with primers P0 (5′-GAGAGTTTGATCCTGGCTCAG-3′) and P6 (5′-CTACGGCTACCTTGTTACGA-3′) (Picard et al. 2000). PCR conditions were optimized to obtain a single amplified fragment of approximately 1.5 kb. The total reaction volume of 25 μl contained 1–5 μl of DNA template 1 U of Taq DNA polymerase (PB-L, UNQ, Argentina), 1 μM of each primer, 1×polymerase buffer, 1.5 mM $MgCl_2$, and 0.2 mM of dNTPs. The thermal cycler (Biometra, USA) was set up with an initial denaturation step of 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min, and a final elongation step of 72 °C for 10 min. Aliquots of 5 μl from the PCR reactions were subjected to 1 % agarose gel electrophoresis (Ultrapure™ Agarose; Invitrogen, USA), run in 0.5× TBE at 5 V cm^{-1} for 40 min to check yield and purity. A 100 bp-ladder (PB-L, Argentina) was used as a size standard.

PCR products were split into two restriction fragment length polymorphism (RFLP) reactions containing 10 μl of PCR products and 2 U of the restriction enzymes *Hae*III (Promega, USA) or *Hinf*I (Fermentas, Lithuania) in a final volume of 20 μl. Reactions were incubated at 37 °C for 3 h. The restriction products were separated by electrophoresis in 2 % (w/v) agarose gels (Ultrapure™ Agarose; Invitrogen), in 0.5× TBE at 5 V/cm for 2 h. Gels were stained with ethidium bromide, and DNA banding patterns (ribotypes) were visualized under UV light and photographed. Electrophoretic band patterns were analyzed using Bionumerics 4.0 (Applied Maths, Belgium). For every sample location, the calculation of similarities was done on the basis of the Pearson correlation coefficient. The concatenated unweighted pair group method (clustering algorithm) with arithmetic mean (UPGMA) was used to calculate dendrograms of each *Hae*III and *Hinf*I RFLP gel and of a combination of all gels by cropping practice and by location (Fig. 1c). Cophenetic correlation values of >80

indicate that the resulting dendograms correspond to the original matrix (Van Verseveld and Röling 2008).

Statistical analysis

For every geographical site (four) and every treatment (three) within each geographical site, the number of biological replicates was three (n=3). Therefore, the sampling consisted of a total of four sites × 3 treatments × 3 replicates = 36 soil samples. Each sample was processed and plated oin triplicate plates, and a mean CFU was obtained for each sample. Thus, the data shown in Table 2 correspond to averages of three samples (biological replicates) per treatment and per site. CFU values were $\log_{10}$ transformed prior to statistical analyses. An analysis of variance was carried out using Statistix 7.0 software (Softonic International S.L., Spain) while the comparison of mean effects was based on Fisher's least significant difference (Fisher LSD) procedure. Differences were considered to be significant when the P value was ≤0.05.

Results

Abundance of PSB in agricultural soils under no-till management

The abundance of CHB detected on NA was similar for both sampling seasons. CHB counts in February and September ranged from 5.80 to 6.21 and from 5.98 to 6.14 $\log_{10}$ CFU/g dry soil, respectively (Table 2) $\log_{10}$ CFU/g dry soil. Total CHB counts had an overall average of 6.03 CFU/g dry soil, and there were no statistically meaningful differences among all sites and treatments.

The total number of bacterial cells that were able to develop colonies on NBRIP agar under P-limiting conditions (TBNBRIP) ranged in the February samples from 5.42 to 6.02 log10 CFU/g dry soil and was significantly higher in Pergamino than in the other three locations, within each cropping practice. Soil samples collected northeast of Bengolea also showed major levels of TBNBRIP. TBNBRIP abundance in the September samples ranged from 5.68 to 6.03 log10 CFU/g dry soil (Table 2) and there was no statistical difference among treatments and geographical locations. It is interesting to note that although there was no statistical differences between treatments in terms of TBNBRIP counts, there was a tendency of BAP samples to contain lower numbers of TBNBRIP than GAP samples for every site (6/8 GAP samples), except for the Monte Buey and Viale soils sampled in February (Table 2).

The number of bacteria that were able to grow on NBRIP plates and also to form a clearing zone around the colony was recorded as the number of PSB (Fig. 1b, b). Overall, the abundance of PSB ranged from 3.47 to 3.89 log10 CFU/g dry soil, with a general average of 3.78 log10 CFU/g dry soil (Table 2). It is important to note that the relative abundance of PSB to TNBRIP in cultivated soils (BAP or GAP) was higher (13/16 cases) or not less (3/16 cases) than the corresponding relative abundance in NE soil samples (Table 2). The influence of rotation intensity was analyzed for each site separately. In the February samplings, GAP plots from Monte Buey had significantly higher PSB counts than the corresponding BAP sampling sites. The same effect was observed in Pergamino plots sampled in September. In the other two sampled sites, there were no significant differences in the PSB abundance among cropping practices. The influence of geographical location on PSB abundance was analyzed for each cropping management (NE, GAP, or BAP). In this case, no significant statistical differences were revealed in PSB abundance for each management samples.

Taken together, our results show overall average CHB counts ranging from 0.6 to 1.4×10^6 CFU/g dry soil and average PSB counts ranging from $3–8\times10^3$ CFU/g dry soil (Table 2). Thus, in the sampled agricultural soils of central Argentina under no-till farming management, culturable PSB represented approximately 0.30–0.98 % of the CHB, independently of cropping management and season.

PSB ribotypes in agricultural soil under no-till management

As the abundance of culturable PSB among NBRIP did not show major differences among treatments or sampled sites (Table 2), we decided to explore whether the genetic community structure of the most abundant PSB isolates from the study sites was affected by the cropping practice, location, or sampling season. To this end, we determined the ribotype of PSB colonies developed on NBRIP medium at the highest plating dilution from each sampled soil.

Selected colonies with diverse morphotypes were subjected to hypotonic heat shock to obtain DNA for ribotyping. It must be stressed that this lysing method may have introduced a bias towards easily breakable cells (e.g., most Gram-negative and some Gram-positive cells). Interestingly, we found that the ribotypes of the most abundant PSB isolates for each cropping practice were clustered by geographical site of origin. As an example, Fig. 2 is a dendogram obtained for NE [for the same observations for the GAP and BAP treatments, see Electronic Supplementary Material (ESM) Fig. 1a, and b, respectively].

Multiple comparisons of all ribotypes from a single sampling site revealed a seasonal clustering of isolates into February and September subgroups. For example, PSB sampled in Pergamino in September clustered into a group with a cophenetic correlation value of 90, whereas February isolates clustered separately with a cophenetic correlation value of 95

Table 2 Abundance of phosphate solubilizing bacteria in soil samples from agricultural plots under no-till management in Argentina

Geographic site	Month of sampling in study year (2010)	Agricultural treatment								
		NE			GAP			BAP		
		CHB	TBNBRIP[a]	PSB[b]	CHB	TBNBRIP[a]	PSB[b]	CHB	TBNBRIP[a]	PSB[b]
Bengolea	February	6.02±0.12	5.95±0.23 (75.56) a	3.62±0.23 (0.59)	6.01±0.08	5.72±0.03 (53.63) b	3.64±0.07 (0.83)	5.80±0.13	5.70±0.15(83.48) b	3.71±0.18 (1.03)
	September	6.14±0.07	5.92±0.01 (61.23)	3.69±0.10 (0.60)	5.98±0.01	5.89±0.04 (52.42)	3.67±0.18 (0.62)	6.13±0.00	5.68±0.05 (50.99)	3.72±0.11 (1.18)
Monte Buey	February	6.21±0.29	5.75±0.06 (58.50) b	3.69±0.17 (0.70) d,e	6.00±0.13	5.65±0.07 (43.51) b	3.89±0.14 (1.76) d	6.03±0.14	5.70±0.06 (54.99) b	3.58±0.03 (1.47) e
	September	6.09±0.03	5.88±0.04 (62.89)	3.77±0.13 (0.81)	6.05±0.02	5.88±0.05 (67.82)	3.90±0.10 (1.03)	5.99±0.01	5.70±0.11 (52.91)	3.94±0.04 (1.52)
Pergamino	February	5.96±0.11	6.02±0.05 (80.50) a	3.82±0.10 (0.06)	6.07±0.13	5.94±0.04 (69.31) a	3.74±0.15 (0.65)	6.16±0.02	5.89±0.14 (51.14) a	3.68±0.30 (0.77)
	September	6.12±0.07	6.01±0.01 (80.57)	3.79±0.12 (0.60) d,e	6.15±0.00	5.89±0.04 (56.13)	3.91±0.08 (1.04) d	6.04±0.13	5.83±0.04 (65.14)	3.62±0.14 (0.63) e
Viale	February	6.07±0.10	5.57±0.08 (36.50) c	3.82±0.18 (0.13)	5.88±0.10	5.42±0.11 (35.42) c	3.47±0.19 (1.30)	6.00±0.14	5.55±0.02 (42.00) c	3.62±0.04 (0.1)
	September	6.12±0.02	6.01±0.01 (77.77)	3.73±0.11 (0.52)	6.08±0.06	6.03±0.22 (94.64)	3.86±0.09 (0.75)	6.16±0.07	5.87±0.07 (53.17)	3.83±0.10 (0.95)

Values are expressed in log CFU/g dry soil and correspond to averages (n =3 replicate platings per site and per treatment) ± standard deviation (SD). Values followed by different lowercase letters are significantly different statistically (P <0.05) in terms of average CFUs for the comparison of TBNBRIP counts from February samples among sampling sites (a, b, c) and for the comparison of PSB counts among cropping practice for Monte Buey in February and Pergamino on September (d, e). Otherwise, there were no significant statistical differences

CHB, Culturable heterotrophic bacteria; TBNBRIP, total bacteria in NBRIP medium; PSB, phosphate-solubilizing bacteria

[a] The percentage of TBNBRIP in relation to CHB is given in parenthesis

[b] The percentage of PSB in relation to TBNBRIP is given in parenthesis

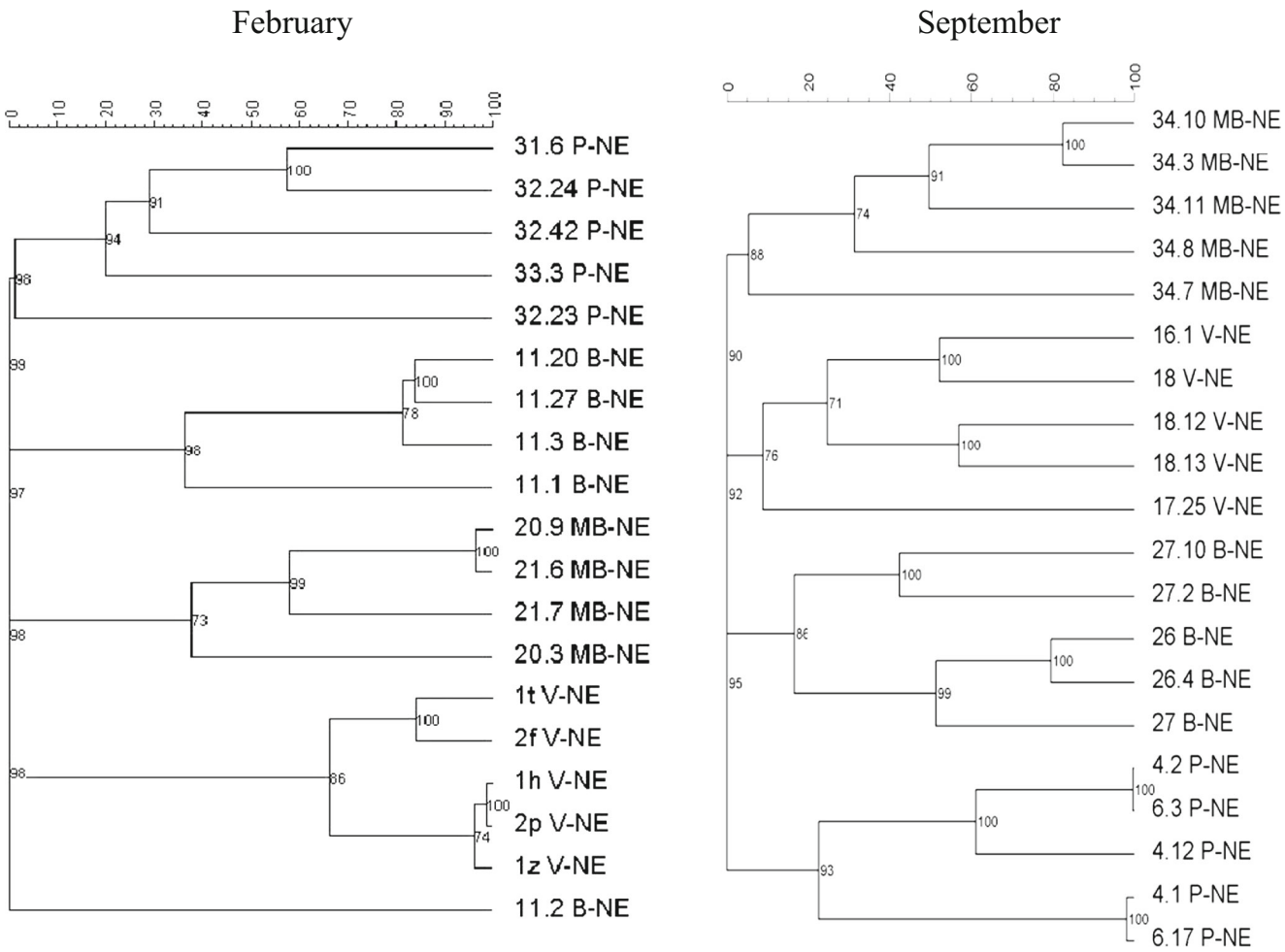


Fig. 2 Geographical clustering of PSB ribotypes from natural environment (*NE*) soil samples collected from agricultural plots under no-till farming practices in Argentina. *B* Bengolea, *MB* Monte Buey, *V* Viale, *P* Pergamino. Dendogram was obtained from the Pearson correlation coefficient, and clustering was done with the concatenated unweighted pair group method with arithmetic mean algorithm using Bionumerics 4.0 (Applied Maths) software. The cophenetic correlation value is indicated

(Fig. 3). Similar seasonal clustering was observed for the other the geographical locations (ESM Fig. 2a, b, c).

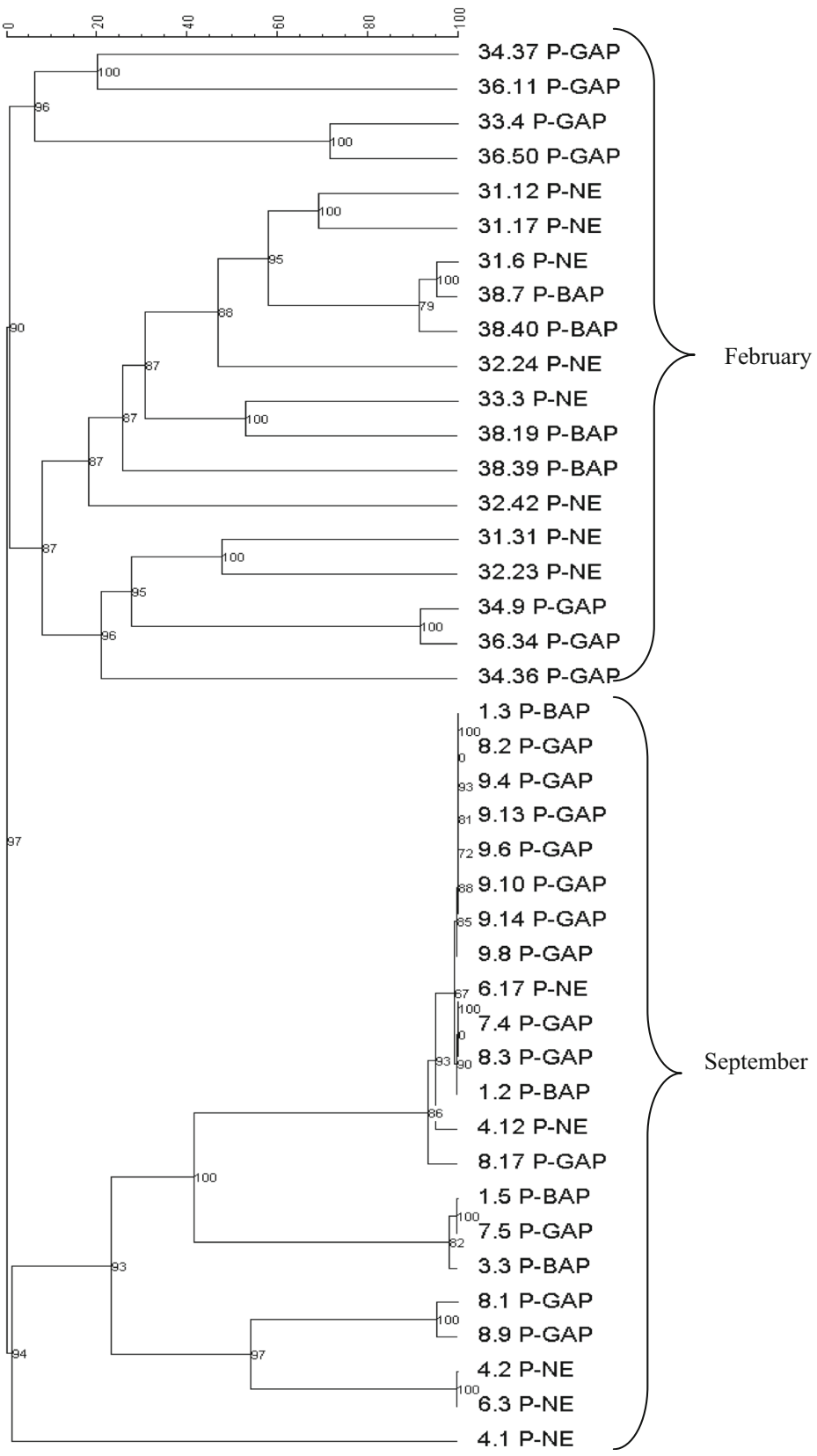
In the February samples, the analyses clearly showed that the ribotypes of Pergamino and Monte Buey isolates of the NE treatment clustered together with isolates from BAP in groups with >50 % similarity (Fig. 4a, b). However, in the case of Viale and Bengolea, the cluster analysis resulted into two distinct ribotype clusters, with the first clustering isolates from GAP and BAP treatments and the second clustering isolates from the NE treatment (Fig. 4c, d).

September samples showed different groupings compared to February at each location, suggesting a seasonal dynamics of populations. The cluster analysis of isolates from Bengolea was not able to separate ribotype groups (Fig. 5a), whereas in Monte Buey and Viale samples, isolates belonging to the same management group clustered together and differed from each other (Fig. 5b, c). Ribotypes of Pergamino isolates collected from soil subjected to GAP clustered together with >50 % similarity (Fig. 5d).

Discussion

Agricultural management practices impact soil quality, crop health, and crop yield (Kumar and Goh 2000). In particular, cropping practices influence soil microbial diversity because the latter is more sensitive than other soil properties to natural and anthropogenic effects (Mittal and Johri 2008). In this study, we evaluated the abundance of PSB in soil samples collected from agricultural plots under no-till management taking into account the effect of cropping practices with different rotation intensity, season, and geographical location.

Our results on the abundance of CHB were similar to those reported recently for soils under crop–pasture rotations in a no-tillage regime in Uruguay (Azziz et al. 2012), with culturable PSB representing a fraction of approximately 0.30–0.98 % of CHB, independently of cropping management and season. We also observed that PSB abundance was not statistically different among sampling seasons. However, there was a slight tendency for the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PergaminoFeb&Sep
0
20
40
60
80
100
34.37 P-GAP
36.11 P-GAP
33.4 P-GAP
36.50 P-GAP
31.12 P-NE
31.17 P-NE
31.6 P-NE
38.7 P-BAP
38.40 P-BAP
32.24 P-NE
33.3 P-NE
38.19 P-BAP
38.39 P-BAP
32.42 P-NE
31.31 P-NE
32.23 P-NE
34.9 P-GAP
36.34 P-GAP
34.36 P-GAP
1.3 P-BAP
8.2 P-GAP
9.4 P-GAP
9.13 P-GAP
9.6 P-GAP
9.10 P-GAP
9.14 P-GAP
9.8 P-GAP
6.17 P-NE
7.4 P-GAP
8.3 P-GAP
1.2 P-BAP
4.12 P-NE
8.17 P-GAP
1.5 P-BAP
7.5 P-GAP
3.3 P-BAP
8.1 P-GAP
8.9 P-GAP
4.2 P-NE
6.3 P-NE
4.1 P-NE
February
September

◀ **Fig. 3** Ribotype groups of representative PSB isolates from Pergamino (*P*) sampled in February and September 2010. For details, see caption to Fig. 2. *NE* natural environment, *GAP* good agricultural practice, *BAP* bad agricultural practice

number of PSB to increase from February to September. Similarly, Santa Regina et al. (2007) found that PSB abundance increased from the autumn to the spring, with a large fall in abundance in the summer, in soil samples of ex-arable lands from northern Spain.

It is noteworthy that the relative abundance of PSB to TBNBRIP ratio in agricultural soils was in most cases (81 %) higher than in samples from the NE (Table 2). One possible explanation is that crop plant roots provide specific nutrients for differential microbial growth and thus directly influence the composition and density of the soil microbial community (Dezhong 1997; Berg et al. 2005; Costa et al. 2006). In this context, it has been reported that soil PSB abundance depends on plant species, soil microbial composition, and soil conditions (Katiyar and Goel 2003). The carbon source of the plating medium may also influence, the counts of PSB (Grönemeyer et al. 2012). We were able to count PSB in all of the sampled sites and under different farming practices (detection limit = 1×10^2 CFU/g dry soil). The abundance of PSB in soils may vary from <100 CFU/g dry soil, as in the soils of northern Iran (Fallah and Kargar 2006) and northern Spain (Peix et al. 2001), to several orders of magnitude higher than our counts, as those found in an abandoned rock phosphate mine in Táchira (Venezuela), where the PSB ranged from 0.8×10^6 to 2.8×10^6 CFU/g dry soil (Reyes et al. 2007), in Kenyan soils, with PSB counts ranging from 3.8×10^4 to 9.1×10^5 CFU/g (Ndung'u-Magiroi et al. 2012), and in Chinese soils, with an abundance of PSB which varied between 7×10^5 and 2.0×10^6 CFU/g dry soil (Hu et al. 2009).

It has been demonstrated that high concentrations of soluble P in soils downregulate the establishment of arbuscular

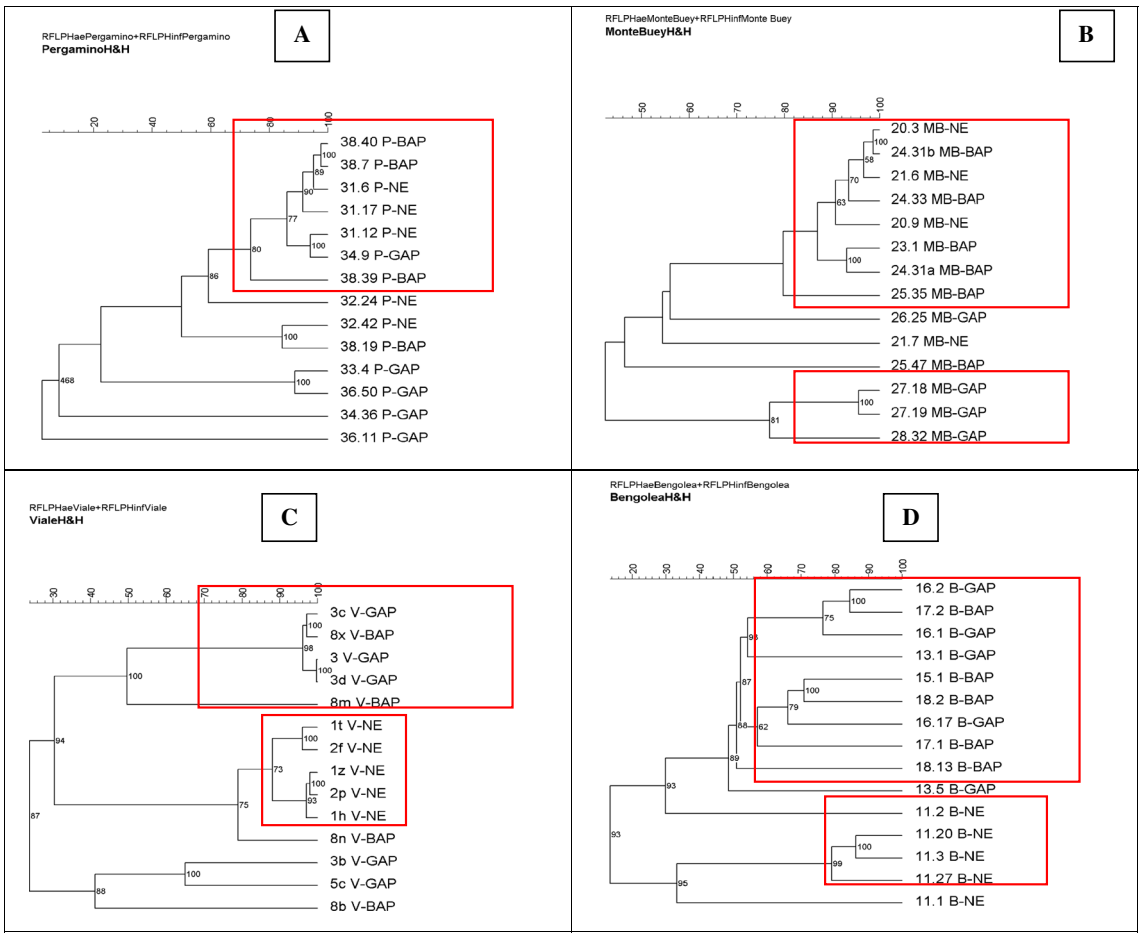

Fig. 4 Ribotype groups of PSB isolated from soil collected in February 2010 from agricultural plots under no-till management subjected to different cropping practices (NE, GAP, BAP) in four locations in Argentina. **a** Pergamino (*P*), **b** Monte Buey (*MB*), **c** Viale (*V*), **d** Bengolea (*B*). *Box* Isolates that formed clusters with ≥50 % of similarity (cophenetic correlation value is indicated).

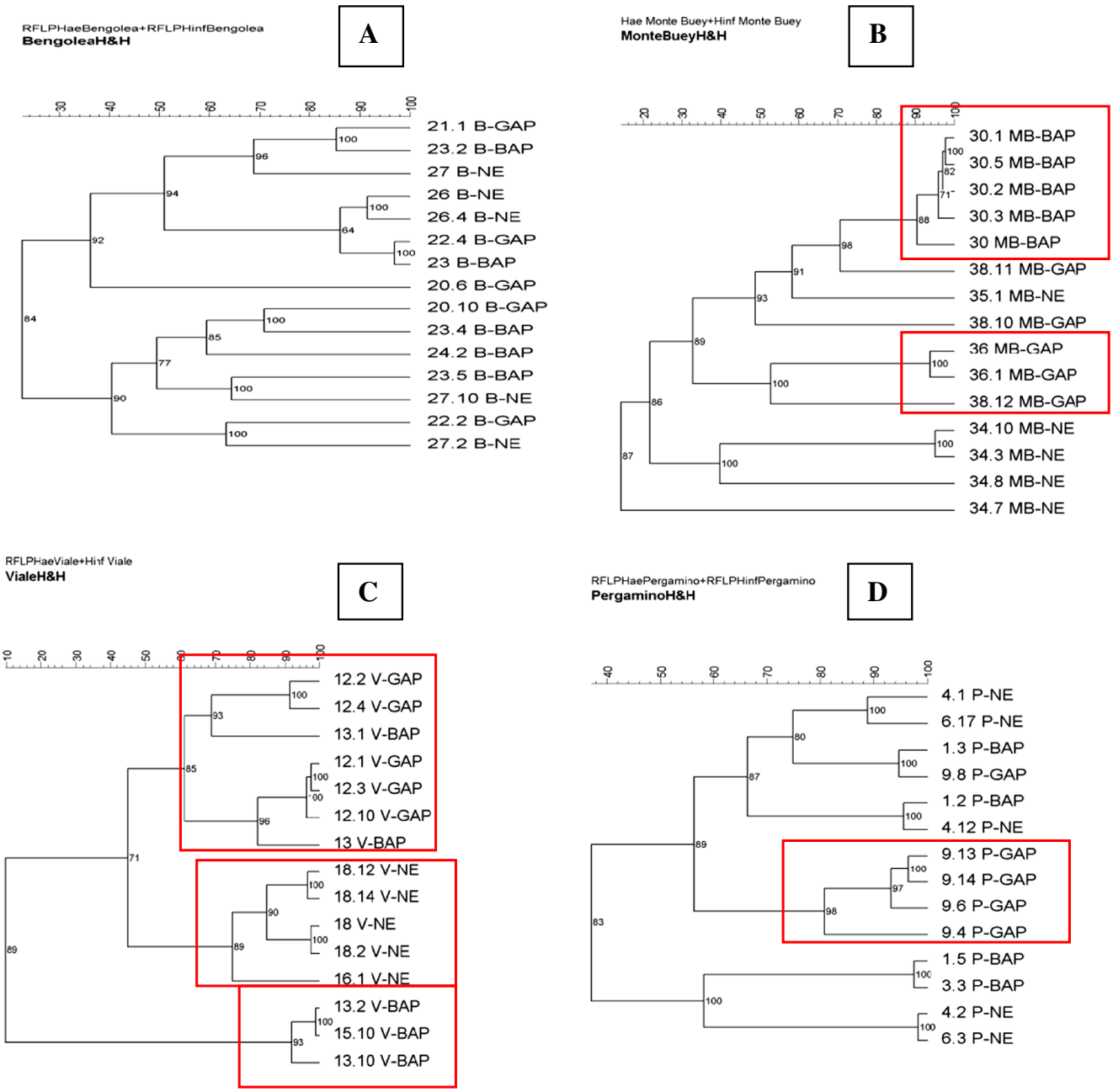

Fig. 5 Ribotype groups of PSB isolates from soils under different cropping practices from samples collected in September 2010. **a** Bengolea, **b** Monte Buey, **c** Viale, **d** Pergamino. For details, see caption to Fig. 4

mycorrhizal fungal symbioses with different plant partners (Martensson and Carigren 1994). A lower alkaline phosphatase activity has also been observed in soil subjected to higher chemical P fertilizer input (Tan et al. 2013). Such a feedback regulatory effect has not been well established for PSB abundance in soils (Kucey 1983; Nahas 2007; Ndung'u-Magiroi et al. 2012). Our results rule out such an effect for the PSB functional community in our study, as the PSB counts barely differed (Table 2) among sites and practices that contained extractable P levels ranging widely from 11.9 ppm (lowest soil P content; BAP, Pergamino) to 296.5 ppm (highest soil P content; NE, Monte Buey) (Table 1). Thus, in our study, a 25-fold change in the content of extractable P was associated with a <1.2-fold change in PSB abundance.

Distinct and diverse PSB in the soil and plant rhizosphere have been isolated and characterized (Rodríguez et al. 2006; Collavino et al. 2010). However, most reports related to PSB diversity have focused on specific taxa rather than considering

the whole community. In the present study, we explored the PSB community structure of the most abundant PSB isolated from four locations across a West–East transect in the most productive region of the Argentinean Pampas under no-till management. We observed that the ribotypes of the most abundant PSB species with easily extractable DNA differed among geographical sites for every treatment. Thus, considering all data together we can conclude that the abundance of culturable PSB is seemingly rather independent of the cropping practice and much more influenced by geographical location, following a general pattern for diversity of bacterial communities studied in the same soil samples (Figuerola et al. 2012). This may suggest that there are PSB locally adapted to soil conditions.

In conclusion, both CFU counts in NBRIP as well as the ribotype profiles of PSB obtained in no-till management agricultural soils of the central Pampas in Argentina revealed that: (1) culturable PSB represented approximately 0.30–0.98 % of CHB, with the proportion seemingly independent of cropping management and season; (2) the PSB:TBNBRIP ratio seemed to be higher in cropped soils than in the NE, suggesting that cropping introduces a selection towards PSB; (3) the most abundant PSB obtained in February and September showed seasonal differences in terms of ribotype in all of the studied sites, suggesting a seasonal population dynamic; (4) the most abundant PSB species differed in their ribotypes among geographical sites and, therefore, there may be local soil conditions which impose strong selective constraints; (5) there was a slight tendency of the PSB community structure to differentiate GAP and BAP from the natural environment (NE), an effect which was much more distinct in the September samples. It should be emphasized that in this work, PSB were studied both quantitatively and qualitatively as a functional group defined by the ability to solubilize mineral phosphates in vitro; we did not assess the impact of these microorganisms in planta, as recently proposed by Bashan et al. (2013) as a general screening procedure to select P biofertilizer bacteria. The impact of the P biofertilizer community may be assessed by testing the effect on plant P nutrition in the presence of insoluble mineral phosphates following the addition of a bacterial community preparation from different soil samples.

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