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Effect of *Pinus ponderosa* afforestation on soilborne *Frankia* and saprophytic Actinobacteria in Northwest Patagonia, Argentina

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Abstract
Large areas in the extra-Andean region in the forest - steppe ecotone in "Northwestern Argentinean Patagonia" have been replaced by plantations of the exotic conifer *Pinus ponderosa* which modify soils physical and chemical factors and alter the biodiversity. Considering that in the region occur saprophytic soilborne actinobacteria that play important role as the fixation of atmospheric nitrogen (N_2) in symbiosis with native plant species and the production of bioactive molecules in plants rhizosphere, we aimed to study the effect of the plantation on the abundance of the N_2 fixer *Frankia* and on the genus diversity of cultivable rhizospheric actinobacteria. The study was performed with soils of six paired sites with pine plantations and natural neighbor areas (including steppes or shrublands). Abundance of infective *Frankia* was estimated by evaluating the nodulation capacity of soils, through a plant bioassay using *Ochetophila trinervis* as trap plant. Isolation trials for saprophytic actinobacteria were performed by applying chemotactic and successive soils dilutions methods. We concluded that *P. ponderosa* afforestation affect soil actinobacteria. This was mainly evidenced by a decrease in the *Frankia* nodulation capacity in *O. trinervis*, which was related to plantation age, to lower soil carbon and nitrogen content, higher available phosphorus, and to a slight decrease in soils pH. Pine plantation influence on the cultivable saprophytic actinobacteria was less clear. The study highlights the importance of soils as source of *Frankia* and rhizospheric actinobacteria in relation to disturbance caused by pine plantation in natural environments with native actinorhizal plant species.

Keywords *Actinoplanes* · *Discaria* · *Frankia* population · Nodulation capacity · *Ochetophila trinervis*

1 Introduction

Soil microbial communities include different actinobacteria populations, which play important role in the ecosystem. They are saprophytic and capable of decomposing different litter compounds, of producing bioactive molecules (Goodfellow and Williams 1983; McCarthy and Williams 1992; Van der Meij et al. 2017) and of interacting with plant roots either in symbiosis in or the rhizosphere (Huss-Danell 1997; Solans et al. 2011). In Northwest Patagonia, Argentina, commonly occur the nitrogen fixer *Frankia,* a facultative symbiont of native plants and various rhizospheric genera such as *Streptomyces, Micromonopora, Actinoplanes, Nocardia* and *Actinomadura* (Cusato and Tortosa 1998; Chaia et al. 2006, Solans and Vobis 2003). Soilborne *Frankia* is widely distributed along the steppe and the shrublands in the ecotone with the xeric forests, including soils lacking host plants (Cardoso et al. 2010) and have long infectious retention time to nodulate the native species *Ochetophila trinervis* (Fam. Rhamnaceae) (Chaia et al. 2005, 2007). Some of the rhizospheric strains display multiple enzymatic activities, produce phytohormones and are considered helper bacteria that enhance the host plant-*Frankia* infection process and root-nodule formation (Solans 2007; Solans et al. 2011).

Northwest Patagonia has been the target of large conifer afforestation plans, mostly with *Pinus ponderosa.* Conifer plantations produced disturbances in the steppe ecosystem that affect the native plant and animal communities, water resources and fire regimes (Nuñez and Raffaele 2007; Gyenge et al. 2010; Richardson et al. 2014), and enhanced the risk of plant invasion due to the natural seedling

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recruitment outside plantations (Simberloff et al. 2010; Raffaele et al. 2015). Nutrient cycling also seemed to be affected by *P. ponderosa* plantation, as evidenced by a lower overall N turnover that was attributed to reduction in enzymatic microbial activity in rhizospheric and bulk soils (Hess and Austin 2017). Defrieri et al. (2011) also found a reduction in physiological microbial activity and suggested that it was due to changes in the litter composition (higher lignin, lower N and P content) and to reduction of soil organic C. Altered microbial activity could be a consequence of changes in the composition of soil microbial communities, as the occurrence of exotic mycorrhizal fungi (Nuñez et al. 2009; Salgado Salomón et al. 2011; Nuñez and Dickie 2014).

In conifer forests in the northern hemisphere, where actinobacteria represent a significant portion of the soil microflora (Davies and Williams 1970; Maunuksela et al. 1999; Cho et al. 2006), specific environmental conditions, such as higher C/N ratios, have been shown to favor actinobacteria in the microbial community's composition (Hackl et al. 2004). Consequences of *P. ponderosa* afforestation in soils actinobacteria in the ecotone between xeric forest and steppe in northwest Patagonia are less known. Therefore, we aimed to study the effect of *P. ponderosa* afforestation on soilborne actinobacteria, with regard to the abundance of infective *Frankia* on *Ochetophila trinervis* and the genus diversity of cultivable saprophytic strains.

2 Material and methods

2.1 The study area

The study was performed in the steppe and shrublands in the ecotone with the xeric forests belonging to the extra-Andean region of Northwest Patagonia, Argentina, with *Pinus ponderosa* plantations. Climate is temperate-cool, with strong westerly winds coming from the Pacific Ocean; mean annual temperature is 6–8 °C and precipitation (MAP) is mainly concentrated in winter, ranging westward from 500 to 1400 mm $yr.^{-1}$ (Paruelo et al. 1998). Soils are Andisols and Mollisols (Mazzarino et al. 1998). Vegetation is dominated by the bunchgrasses *Stipa* spp., *Acaena* spp. and *Festuca pallescens*, as well as shrubs as *Mulinum spinosum, Ochetophila trinervis, Discaria chacaye* and *Discaria articulata* (Boelcke et al. 1985). *Pinus ponderosa* plantations were about 15–35 yrs. old, with a tree density in a range of 7–15 trees per hectare (Tables 1 and 2).

2.2 Sampling and laboratory analysis

Sampling was performed in middle February 2011. We chose a set of six paired sites. Each sampling site comprised a pine plantation and the natural neighboring steppe or shrubland, at a maximum distance of 100 m. Within each site, a 50 m transect was established in the pine plantation and in the corresponding neighbor steppe or shrubland, where the dominant vegetation was recorded (Table 1). At 5 m intervals along the transect, superficial litter was removed, soil temperature was measured with a digital thermometer and 10 soil sub-samples (about 50 g) at 0 to 15 cm depth were aseptically collected with a shovel disinfected with 1% sodium hypochlorite and then with 70% ethanol. Sub-samples collected along each transect were placed in a sterile plastic bag and mixed thoroughly to compose each sample. The litter thickness of pine plantations was measured for each sampling point. Roots of at least one actinorhizal plant found in the pine neighbor areas were excavated up to a depth of 30 cm from around the base of the stem to register the occurrence of actinorhizal nodules.

The samples were immediately transported to the laboratory and divided into four parts. A part was stored at 4 °C to further determine soil moisture content by dehydration at 106 °C for 48 hs. Three parts were exposed to the air inside a paper bag for 2–5 d and stored at room temperature for further physico-chemical soil analyses, microbial isolation and plant bioassay, respectively.

Different soil properties were determined following methods described in Sparks et al. (1996): pH, electrical conductivity, organic carbon by the Walkley Black method, total N by the Kjeldahl method, extractable phosphorous by the Olsen test (extraction rate 1:20), exchangeable cations by extraction with ammonium acetate and determination by atomic absorption spectrometry. Soil water holding capacity was estimated according to Wilke (2005), and texture was analyzed by the pipette method and classified according USDA system (Gee and Or 2002).

2.3 Isolation and identification of saprophytic actinobacteria

The dilution - plate technique and the chemotactic method were used to isolate saprophytic actinobacteria, as described by Solans and Vobis (2003). The preliminary determination of taxonomic standing was based mainly on morphological criteria, and the strains were identified to genus level, as reported by Solans and Vobis (2003). All strains were subcultured in YpSs slants and stored at the culture collection of the Herbarium BCRU, Department of Botany, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Bariloche, Argentina.

2.4 Plant bioassays

A quantitative plant bioassay to establish *Frankia* nodulation units per cm^3 of soil (NU) was performed using the methods of nodulation capacity (NC; Van Dijk 1984) and the most probable number (MPN; Woomer 1994). Seeds of

Table 1 Location and description of sampling sites in Northwest Patagonia (P, *Pinus ponderosa* afforestation, N, neighbor steppe or shrubland sites, MAP, mean annual precipitation)

Site	Site code	Location	Altitude (m)	MAP[a] (mm)	Vegetation, main plant species and actinorhizal plants
Challhuaco	P1	41° 11' S 71° 19' W	936	1120	*P. ponderosa, Maytenus boaria, Schinus patagonicus, Berberis* spp., *Fabiana imbricata, Mutisia decurrens, Rosa rubiginosa, Acaena splendens, Alstroemeria aurea, Stipa speciosa*
	N1	41° 11' S 71° 19' W	913		*Nothofagus antarctica, Salix fragilis, Schinus patagonicus,* **Discaria chacaye**, *Fabiana imbricata, Mutisia decurrens*, *Acaena* spp., *Adesmia boronioides, Alstroemeria aurea, Matricaria* sp., *Senecio* sp.
Arroyo del Medio	P2	41° 10' S 71° 15' W	897	1000	*P. ponderosa, Maytenus boaria, Schinus patagonicus, Rosa rubiginosa*
	N2	41° 11' S 71° 15' W	885		*Embothrium coccineum*, *Schinus patagonicus*, *Berberis darwinii, Berberis microphylla,* **Discaria articulata**, *Fabiana imbricata, Mutisia decurrens*, *Acaena* spp., *Fragaria chiloensis, Mulinum spinosum, Senecio* sp., *Stipa speciosa*
Dina Huapi	P3	41° 05' S 71° 09' W	802	530	*P. ponderosa*, with no understorey plants
	N3	41° 05' S 71° 09' W	811		*Schinus patagonicus, P. ponderosa, Ephedra* sp., *Mulinum spinosum*, *Acaena splendens,* **Discaria articulata**, *Euphorbia* sp., *Rosa rubiginosa, Rumex acetosella*, *Senecio* sp., *Stipa speciosa*
San Ramón	P4	41° 04' S 71° 03' W	966	580	*P. ponderosa, Mulinum spinosum, Senecio sp., Stipa speciosa*
	N4	41° 04' S 71° 03' W	970		*Berberis darwinii,* **Discaria articulata**, *Fabiana imbricata*, *Mutisia decurrens, Rosa rubiginosa*, *Acaena* spp., *Senecio* sp., *Stipa speciosa*
Fortín Chacabuco	P5	41° 01' S 71° 09' W	800	530	*P. ponderosa* , no understorey plants
	N5	41° 00' S 71° 09' W	798		*Carduus* sp., **Discaria spp**., *Mulinum spinosum*, *Senecio* sp., *Stipa speciosa, Taraxacum officinale*
Alicura	P6	40° 37' S 70° 56' W	873	520	*P. ponderosa, Stipa speciosa*
	N6	40° 37' S 70° 56' W	880		*Mulinum spinosum*, *Stipa speciosa, Verbascum* sp.

Sites N1 and N2, and N3 to N6, correspond to shrublands and steppes, respectively. Bold values denote actinorhizal plant species

[a] Precipitation data from nearest meteorological station BDHI (for Chalhuaco) and AIC (2017) (for Dina Huapi, Fortin Chacabuco and Alicura); or published data for Arroyo del Medio (Gyenge and Fernández 2014) and San Ramon (Ghermandi et al. 2013)

Ochetophila trinervis collected in Pampa de Huenuleo (Bariloche, March 2010) and dry-stored at −20 °C, were scarified and stratified (Chaia et al. 2006). Each soil sample, air-dried stored for ca. 25 days, was prepared externally kneading it by hand and then sieving it (2 mm mesh). Six successive 5-fold dilutions (5^{-1} to 5^{-6}) were prepared directly diluting and mixing thoroughly each soil sample (on a dry weight basis) with a sterile sand and vermiculite mixture (1:1 *v*/v). A 30 cm^3 sample, from each respective soil dilution, was placed in a sterile glass tube (200 mm length × 26 mm dia) and watered with the Evans solution diluted to one tenth of full strength and with N 0.71 mM as NH_4NO_3 (Huss-Danell 1978). Two *O. trinervis* seedlings at the cotyledon stage, previously germinated on sterile humid vermiculite, were transferred to each tube. Five replica were run for each tested dilution. Five additional tubes with plants, per soil sample were also inoculated with *Frankia* strain BCU110501 (Chaia 1998) as positive control. Forty non-inoculated plants were used as contamination control. After five weeks, seedlings were fertilized with Evans solution with N 0.071 mM and watered when necessary.

Plants were kept in a growth chamber for 9 weeks, with 16 h photoperiod provided by metal halogen lamps (Philips HPI-T 400 W and Philips SON-T Plus 400; photosynthetically active radiation was ca. 318 μM m^{-2} s^{-1}), at 20–22 °C temperature and 48% relative humidity.

The plants nodulation was recorded for each soil dilution. Lobes of 3 nodules per plant were excised in slices by hand, stained with cotton blue and were examined under an Olympus light microscope to determine the presence of *Frankia* vesicles.

2.5 Data analysis

Soils properties of sites comprising pine plantations and natural neighbor areas (including shrublands and steppes) were compared by paired t-tests, with the exception of humidity, water holding capacity, temperature, pH, and nutrient ratios data (which did not fulfill the normality assumption) and were analyzed by Wilcoxon Signed Rank test. Shannon diversity index (H′) was calculated for saprophytic actinobacteria. The abundances of the saprophytic actinobacteria (number of colony forming units, cfu g^{-1}) and the *Frankia* nodulation units per cm^3 of soil (NU), from sites comprising pine plantations and natural neighbor areas were compared using one way ANOVA. Pearson or Spearman correlations (unless normality assumption was not fulfilled) were performed between the

Table 2 Characteristics of *Pinus ponderosa* plantations in Northwest Patagonian sites

Site code	Pine litter thickness (cm)	Proportion of decomposing pine litter (%)	Density (trees/ Ha)	DBH[a] (cm)	Plantation age (yr)	Forest thinning[b] (yr)
P1	5.5	58	15	99	30	no
P2	11.2	50	15	84	30	3
P3	12.0	21	11	145	35	no
P4	7.2	51	7	107	30	0[c]
P5	14.6	41	9	102	30	10
P6	3.9	0	7.5	58	15	5

See site codes in Table 1

[a] Diameter at breath height

[b] Elapsed time since the last thinning

[c] Site management includes pruning

saprophytic actinobacteria cfu g^{-1} or the *Frankia* NU and the environmental variables. Unless otherwise stated, differences were regarded as being significant if $P < 0.05$ (Zar 1999). All tests were performed with the statistical program InfoStat (Student Version 2010, Universidad Nacional de Córdoba). The studied sites were classified by performing a Normed Principal Component Analysis (PCA) and hierarchical clustering (following Ward's aggregation criterion) using SPAD 5.5 software package. The analysis was performed using environmental variables (soils content of C, N, P, Mg and K, pH, electrical conductivity, water holding capacity, soils temperature, moisture content at sampling time and MAP) and biological variables (nodulation capacity and Shannon diversity index).

3 Results

3.1 Soils properties

Pine plantations soils had a different textural class than those of the neighboring shrublands, and they had the same class for sites in the steppe. Pine plantations had lower soil temperature (W = 23.00), C and N concentration ($T = -3.47$ and $T = -3.25$, respectively) and higher P content ($T = 2.91$) than the neighboring natural areas (shrublands and steppes). Other soil properties ($p > 0.05$, Table 3), and the C/N, C/P and N/P ratios were similar for both soil groups (W = 38.50; 29.00 and 31.00, respectively, $p > 0.05$).

3.2 Cultivable saprophytic actinobacteria

Saprophytic actinobacteria were isolated from soils of all sites. Colony forming units per gram of dry soil (cfu g^{-1}) and Shannon diversity index (H′) were similar for pine plantations and the corresponding neighboring natural areas ($T = 0.51$ and $T = 0.83$, respectively $p > 0.05$). Abundance ranged from 4×10^4 to 5×10^5 cfu g^{-1} and the mean H′ was 0.3 (±0.1 SD) for each soil group. Actinobacteria cfu g^{-1} were positively correlated with soil Mg content ($r = 0.7$ $p < 0.05$), and those of pine plantations were positively correlated with pine soils pH ($r = 0.9$, $p = 0.02$). Other soil properties were not correlated with actinobacteria cfu g^{-1}. Isolated strains growing in YpSs medium had the typical pigmented substrate and aerial mycelium. Isolated actinobacteria recovered from soils belonged to the genus *Streptomyces* (from all sites); *Actinoplanes* (from all sites except P5), *Actinomadura* (from of P5, P6, N1, N2 and N3), *Micromonospora* (from P1 and N2) and *Nocardiopsis* (from N2, P3 and P5).

3.3 The occurrence of soilborne *Frankia* and the symbiosis establishment

Nodulation occurred in *O. trinervis* plants naturally growing in steppe and shrubland soils. The nodulation capacity of *Frankia* from soils with *P. ponderosa* plantations was significantly lower than those of the corresponding neighboring natural areas (F = 6.69 $p = 0.03$, Fig. 1). The estimated nodulation capacity of soils by the different methods (MPN and NC) were positively correlated ($r = 0.8$, $p = 0.002$).

Frankia NU (MPN) were significantly correlated with the soils pH (r = 0.7, $p = 0.02$, Fig. 2) and temperature (r = 0.7, $p = 0.01$). Moreover, negative correlations were found for *Frankia* NU with soils P content from natural neighbor areas ($r = -0.9$, Fig. 2) and with pine plantations age ($r = -0.8$, $p = 0.03$). Other environmental variables were not significantly correlated with *Frankia* NU ($p > 0.05$).

Nodules of plants that were inoculated with the first dilution series of pine and neighbor soils had *Frankia* vesicles inside, suggesting the effectiveness of the nodulating strains. Plants used as control had no nodules.

Table 3 Soils properties of steppe and shrubland sites with *Pinus ponderosa* plantations in Northwest Patagonia (P, *P. ponderosa,* N, neighbor steppe or shrubland sites)

Site code	Soil physical properties								Soil chemical properties						
	Relative humidity (%)	Water holding capacity (%)	Temperature (°C)	EC (μ S cm^{-1})	Sand (%)	Silt (%)	Clay (%)	Textural class	pH $_{(H2O)}$	C (%)	N (%)	P (ppm)	Ca (m e / 100 g)	Mg (m e / 100 g)	K (m e / 100 g)
P1	5.1	47	15.1	0.03	80.8	13.4	5.8	LS	6.5	1.15	0.13	5.96	3.74	0.50	0.51
N1	14.3	65	18.3	0.06	73.7	20.0	6.3	SL	7.0	1.95	0.13	6.55	2.82	1.70	0.57
P2	5.5	58	15.1	0.04	79.2	16.1	4.7	LS	6.4	1.88	0.16	6.55	5.42	0.90	0.79
N2	3.6	55	24.5	0.04	73.8	20.7	5.5	SL	6.5	2.64	0.25	4.34	5.14	0.47	0.54
P3	2.5	53	13.5	0.05	78.8	15.2	6.0	LS	6.4	1.49	0.08	21.32	4.28	1.27	1.49
N3	2.0	51	16.8	0.04	77.9	17.5	4.6	LS	6.5	2.17	0.15	15.12	5.44	1.10	1.47
P4	7.0	58	18.2	0.05	73.3	19.1	7.6	SL	6.4	1.56	0.12	24.57	5.50	1.50	1.25
N4	3.0	39	25.9	0.04	75.4	17.5	7.1	SL	6.8	2.10	0.14	11.72	6.90	1.37	1.29
P5	3.3	52	14.2	0.05	85.2	10.7	4.1	LS	6.6	1.01	0.11	23.54	4.78	1.28	1.02
N5	1.4	48	23.7	0.04	86.2	9.6	4.2	LS	6.4	1.85	0.15	16.15	4.40	0.93	0.67
P6	2.1	46	18.2	0.06	83.0	9.0	8.0	LS	7.1	1.08	0.05	16.15	7.26	0.97	1.03
N6	2.2	50	18.5	0.03	82.2	10.1	7.7	LS	7.0	0.86	0.09	11.28	6.32	2.17	0.79

See site codes in Table 1

LS, loamy sand; SL, sandy loam

3.4 Sites categorization

Classification of sites with pine plantations in natural areas of Northwest Patagonia, shown an evident distinction according to MAP, where the two sites with the highest values (≥ 1100 mm $yr.^{-1}$) were clustered together. In this "high-precipitation's" cluster, both pine plantations were more similar between them than with their neighbor sites. The "low-precipitation's" cluster was characterized by a higher P, Ca, Mg and K content. Within this cluster, pine plantation associated to the higher value of P content and electrical conductivity, split off from the neighbor paired sites. In both clusters (high and low precipitation), soils P content was higher in pine plantations than in neighboring area, while nodulation capacity was higher in neighboring area than pine plantation (Fig. 3).

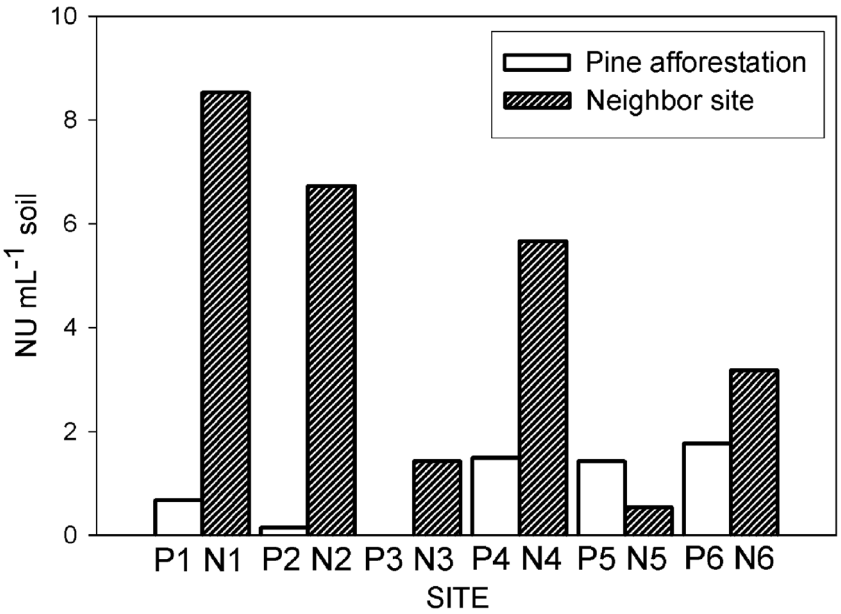

Fig. 1 Nodulation capacity (NU mL^{-1} soil) estimated by the most probable number method, of *Ochetophila trinervis*-infective *Frankia* in soils under *Pinus ponderosa* plantations and neighbor natural areas in Northwest Patagonia. NU of soils from pine plantations and neighbor sites are significantly different ($p < 0.05$)

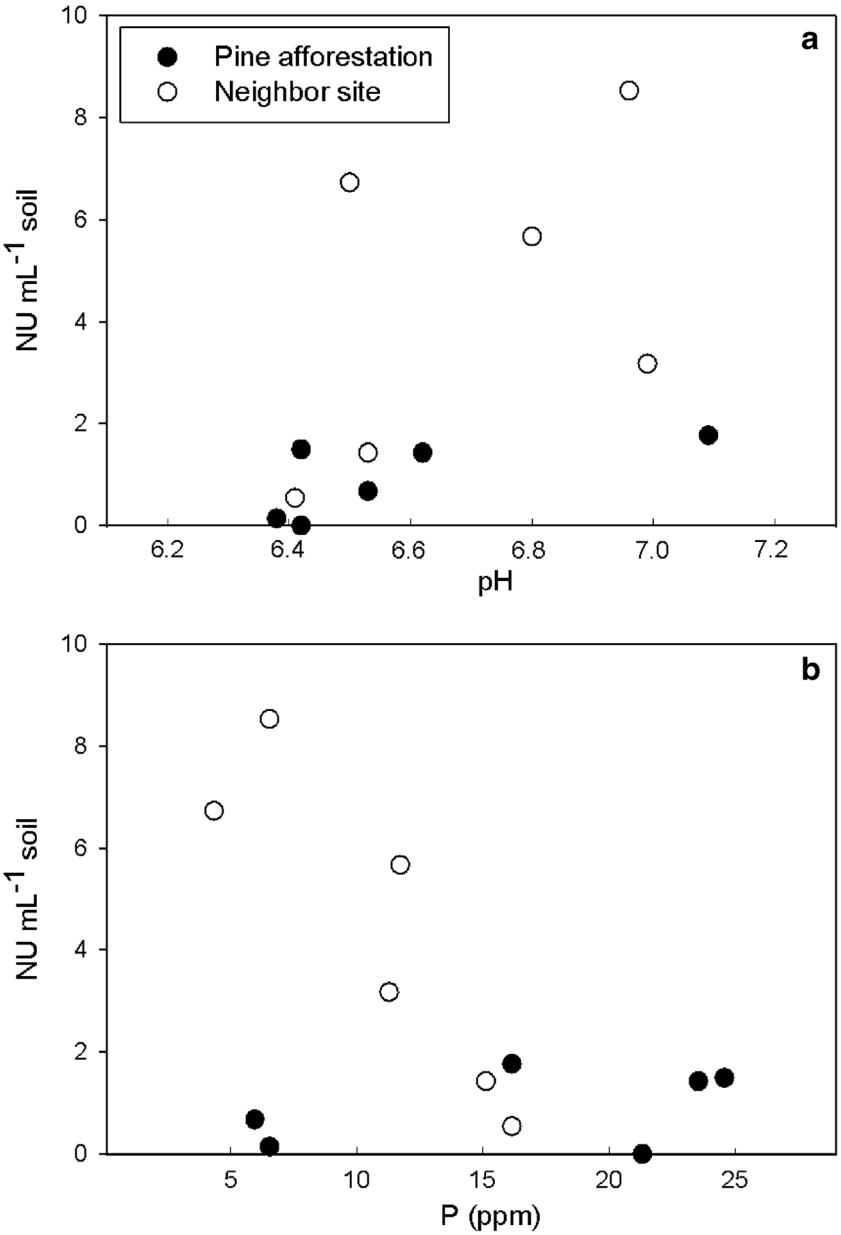

Fig. 2 Correlation between the properties of soils (**a**, pH content, **b**, available P) and the nodulation capacity (NU mL^{-1} soil), estimated by the most probable number method, of *Ochetophila trinervis*-infective *Frankia* inoculated with soils of the study area

4 Discussion

Pinus ponderosa plantations in the steppe and shrublands from the ecotone with the xeric forests in Northwest Patagonia produce an impact on soil actinobacteria, mainly evidenced by a decline in the *Frankia* nodulation capacity in *O. trinervis*, while the influence of the plantations on cultivable saprophytic actinobacteria was less clear under the scope of this study.

Unlike the results of this study, the capacity of soils under native *P. ponderosa* forests (in Cascade Range, Oregon) following wildfire and salvage logging, to nodulate invading *Ceanothus velutinus,* another rhamanceaous shrubs, seemed to be hardly affected (Youngberg and Wollum 1976). Nevertheless, other studies performed with soils under conifer forests in the northern hemisphere revealed a decline in *Frankia* infective populations in soils (Smolander and Sundman 1987; Maunuksela et al. 1999; Gauthier et al. 2000; Jeong and Myrold 2001) as in the pine afforestation in the Patagonian region (Fig. 1). Different factors could account this decline, as those affecting either *Frankia* populations in soils or the capacity of host plants to be nodulated (for a review see Dawson 2008, Chaia et al. 2010). In this study, two factors seemed to be involved: the absence of host plants and some of the environmental conditions. By one hand, the time elapsed since de lack of host plants in the afforested sites could be associated to the decline (Wollum et al. 1968) that is inferred from the negative correlation between the age of *P. ponderosa* plantations and the nodulation capacity.

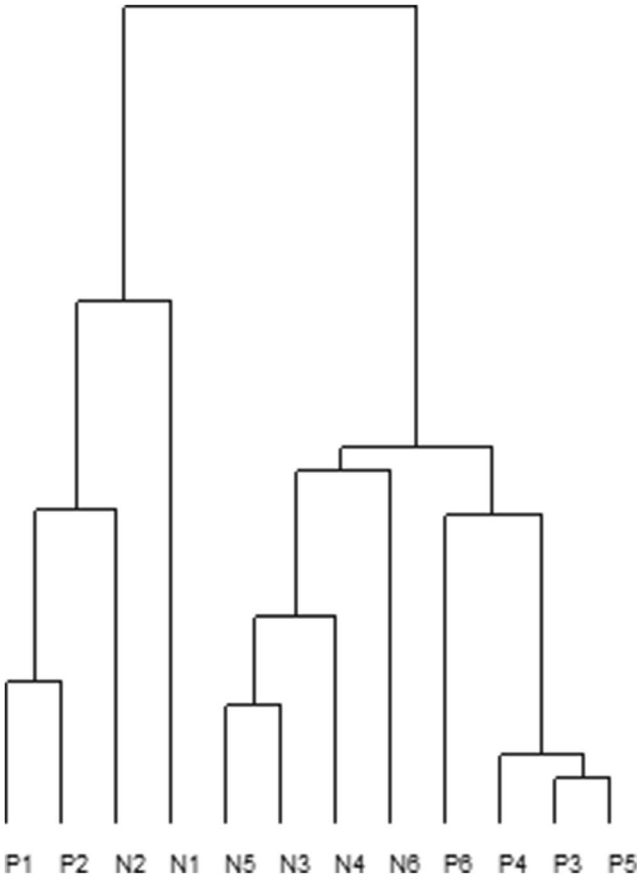

Fig. 3 Homogeneous site groups according to environmental variables (MAP and soil properties), the *Frankia* nodulation capacity and the diversity of cultivable saprophytic actinobacteria (Shannon index) in soils of sites in pine plantations (P) and neighbor natural areas (N) in Northwest Patagonia

On the other hand, environmental conditions appeared to clearly distinguish afforested sites (Fig. 3), being natural areas (shrubland and steppe) likewise affected. The dense plantations, with up to 35 yr. old defined a distinct environment inserted in the natural milieu with a reduced or absent understorey and soils covered by a thick pine litter layer. Otherwise, natural vegetation comprised grasses, forbs, dispersed shrubs or trees, and a scarce or discrete litter layer. The differences in vegetal cover and litter composition together, could account the lower temperature in soils. These factor coupled with the reduction in solar radiation under pine canopy (data not shown), with a consequent reduced photodegradation of litter could contribute to generate different conditions for nutrient cycling in soils (Austin and Vivanco 2006; Araujo et al. 2012). Changes in the studied afforested soils were evidenced by the lower carbon, lower nitrogen and higher phosphorus contents. Carbon decay, after replacing the natural vegetation with *P. ponderosa* afforestation in the region, could be due to a slower decomposition rate of pine litter than that of native species (Araujo and Austin 2015). The lower C content, probably limited saprophytic growth for soilborne *Frankia* populations and in consequence a decreased nodulation capacity of soils. This relationship was already found for *O. trinervis* infective *Frankia* in northwest Patagonian soils (Cardoso et al. 2010).

The negative correlation between *Frankia* NU and the available phosphorus for the steppe and shrubland areas suggested that this nutrient under moderate levels, might regulate the nodulation capacity of *O. trinervis* plants, while at high levels, as those found in pine soils (almost twice than the neighbor sites, Table 3), nodulation was impaired (Fig. 2). This was previously found under experimental conditions by (Valverde et al. 2002), who shown that high levels of P supply cause a decline in the nodulation (nodule biomass) of *O. trinervis*.

The decline of *Frankia* nodulation capacity could be also due to change in soils pH, as evidenced by the positive relationship with *Frankia* NU. pH has been already found as a factor that regulates the nodulating *Frankia* populations from soils, including those from the region (Smolander et al. 1988; Martin et al. 2003; Cardoso et al. 2010).

Other factors, not considered in this study, but that probably have contributed to limit growth of soilborne *Frankia,* therefore favoring a lower nodulation capacity of pine soils, are the loss of soil microsites due to the homogenizing effect of *P. ponderosa* plantations in the steppe (Raffaele and Schlichter 2000); and the impaired dispersal of *Frankia* through different vertebrates transporting viable propagules (Chaia et al. 2012), which could possibly use resources distinctly in natural and in afforested sites (Nuñez et al. 2008; Caballé et al. 2016).

With regard of cultivable saprophytic actinobacteria, we did not found major differences under the coarse taxonomic level used in this study, in pine soils relative to those under natural vegetation. Nevertheless, probably more accurate results might be found by performing rhizospheric soil analysis, taking in account its significant role in such microsites producing bioactive compounds with a growth promotion effects (Solans 2007; Solans et al. 2011; Van der Meij et al. 2017), and that greater rhizosphere effects, related to microbial biomass and C mineralization rates, were found for *P. ponderosa* as compared to native trees in the region (Hess and Austin 2017).

Bioassay method used in this study, allowed the detection of nodule-forming populations from soils in northwest Patagonia, on the specific host-plant *O trinervis* naturally occurring in the region. Unlike molecular qualitative and quantitative analysis, that are used for *Frankia* detection in soil and measuring environmental DNA, respectively (Hahn et al. 1999, Myrold and Huss-Danell 1994), or quantitative PCR (qPCR) analysis, for following dynamics of indigenous *Frankia* populations in soils as a function of environmental characteristics (Samant et al. 2015), the MPN method used (Woomer 1994) did not allow to know the identity of the

soilborne *Frankia* populations. Nevertheless the numbers of NU provided useful information about the amount of nodulating *Frankia* in interaction with other soil microorganisms (Chaia et al. 2010), as the actinobacteria which promote the actinorhizal symbiosis (Solans 2007), that were present in the same soil samples. Therefore, using soils as a source of inoculum, we tested the nodulation capacity of soils, which is directly related to the abundance of infective *Frankia* propagules, theoretically represented by a single spore, a hyphal fragment, or a colony (Myrold et al. 1994) but also to other soil factors affecting nodulation.

In conclusion, afforestation with exotic *P. ponderosa* pines in steppe and shrublands, in the ecotone with the xeric forests in Northwest Patagonia, Argentina, affect soil actinobacteria, mainly evidenced by a decline in the *Frankia* nodulation capacity in *O. trinervis*. This decline increased in relation to the age of the plantation, to a reduced content of the soil carbon and nitrogen, and to increased available phosphorus, in addition to slight decrease in soils pH. The influence of the plantations on cultivable saprophytic actinobacteria was less clear, but more detailed studies should be performed with soils from microsites such as rhizosphere, to detect any change in cultivable strains. This study shows a parallel finding in the Southern and Northern hemispheres and open new insights, in favor of the protection of soils of Extra Andean environments, as sources of actinobacteria developing important role in the soil ecosystem.

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