

Biochemical and microbiological properties of Argentinean Patagonia soil with implanted forest species

R.L. Defrieri, G. Sarti, M.F. Tortarolo, J. Escobar-Ortega, I. García de Salamone, F. D'Auria, D. Efron

Facultad de Agronomía. UBA. Av. San Martín 4453. Buenos Aires. Argentina. E mail: defrieri@agro.uba.ar

Abstract

The aim of the present study was to determine the effect of two implanted forest species with different vegetal residue chemical compositions on the biological and biochemical properties of soil. The study site was located in the INTA Forestal Station of Trevelin, Chubut, Argentina. Samples were extracted from soil in forest plots dominated by Radiata Pine (*Pinus radiata* D. Don.) or European Ash (*Fraxinus excelsior* L.). Microbial respiration, dehydrogenase activity, community-level physiological profile (CLPP) and index of microbial community functional diversity were used as biological parameters; and acid phosphatase, protease and β -glucosidase activities were employed as biochemical parameters. Microbial respiration, dehydrogenase and acid phosphatase activities showed greater values in soil under European Ash trees; however, significant differences in protease and β -glucosidase activities were not observed among species. The higher organic C content of soil under European Ash trees and lower lignin and greater N and P content of the leaves of European Ash trees could be responsible for the greater biological and biochemical activities observed in the soil. Principal component analysis (for PC1) showed significant differences in the physiology of microbial communities associated with these forest species. The Shannon-Weaver Index (H) of the functional diversity of microflora did not present significant differences among forest species.

Keywords: forest soil, implanted tree species, microbial respiration, dehydrogenase activity, microbial functional diversity, enzymatic activity

1. Introduction

The type of vegetation cover and dominant tree species of forest ecosystems affects the chemical, biological and biochemical properties of soils (Priha *et al.*, 2001). Due to the accumulation of organic residue, a significant proportion of the nutrients extracted from soil are retained by trees, and the decomposition of plant residue releases nutrients, which can be reused by vegetation and microorganisms (Smolander and Kitunen, 2002, Ayres *et al.*, 2006). Moreover, tree roots influence the development and activity of microflora. Previous studies have shown that the composition of the soil microbial community may be influenced by the diversity of plant species or the type of forest vegetation/afforestation (Balsler and Firestone, 2005). Therefore, the characteristics of microflora are dependent on the biomass and quality of materials produced by different tree species (Smolander and Kitunen, 2002). Microorganisms play a fundamental role in many essential processes and functions carried out in the soil and affect metabolism and plant growth (Paul and Clark, 1996).

Dehydrogenase, an intracellular enzyme, and carbon microbial respiration are parameters of soil biological activity. In contrast, the activities of extracellular enzymes are a measure of the potential of soil to carry out biochemical processes responsible for the release of nutrients to plants and microorganisms through the transformation of organic matter. Microbial communities and soil biological activity are closely linked to nutrient cycling (Ushio *et al.*, 2008). However, soil microbial diversity also affects soil biological activity. To determine microbial diversity, microorganisms must be quantified and identified, and the functional role of microorganisms must be investigated (Kjoller *et al.*, 2000). Several studies have shown that functional diversity, especially the catabolic versatility of

microorganisms and degradation potential of a soil, is more important than the taxonomic diversity of the community (Balsler and Firestone, 2005). In previous studies, microplates with different carbon substrates were used to identify functional differences in soil bacterial communities. Using this methodology, differences in carbon source use profiles were associated with different types of vegetation and water samples (Garland and Mills, 1991, Semmartin *et al.*, 2010).

Because biological and biochemical properties are closely associated with many functions carried out in the soil, these parameters are excellent indicators of soil quality. Long-term productivity is based on the development of management strategies for the maintenance and protection of soil quality, which can be evaluated through sensitive and appropriate indicators.

The aim of the present study was to evaluate the influence of two implanted tree species with different plant residue chemical compositions on microbiological parameters such as microbial respiration, dehydrogenase activity, microbial functional diversity and biochemical properties of soil, including various enzyme activities (β -glucosidase, acid phosphatase and proteases).

2. Materials and methods

2.1. Study site and sample collection

The research site is located in the Forest Station of the Argentinian Agricultural Technology Institute (INTA) of Trevelin, Province of Chubut, Argentina, at 43° south latitude, 71° 31' west longitude (altitude = 470 m). The surface station is approximately 3.020 ha in size, and large areas of native forest and numerous experimental plots implanted with different species are present. The soils are classified as Andisols, and

the original material consisted of volcanic ash mixed with colluvium material. In addition, the soils can be distinguished by the presence of amorphous components (allophane and imogolite). The climate is characterised by an average rainfall of 942 mm per year, of which 80.6% occurs between April and September. The annual maximum mean temperature is 15.7°C, and the annual minimum mean temperature is 3.4°C. In December and February of each year, temporary droughts occur, which affect the growth and development of several forest species.

In total, ten trees of each species in good sanitary stages with similar diameters were selected. A composite sample of soil was obtained from four subsamples taken from underneath each of the selected trees. Soil samples were collected from the soil surface (at a depth of 10 cm) at the foothill of two 2-ha plots forested with Radiata Pine (*Pinus radiata* D. Don.) and European Ash (*Fraxinus excelsior* L.). The plots had identical implantation times (50 years). Soil samples were taken after removing the litter from the soil surface and were obtained at the same distance from the trunk of the selected tree. Wet samples were taken to the laboratory, stored in plastic bags and refrigerated at 4°C until analysis. Samples were sieved on a 4-mm or 2-mm mesh sieve, depending on the analysis. The results were expressed based on dry soil analysed at 105°C.

In addition, five samples of fresh fallen leaves of each species were air-dried, milled (<0.5 mm) and analysed in duplicate to determine the content of nitrogen, phosphorus, lignin and cellulose.

2.2. Soil analysis

Microbial Carbon respiration (Anderson, 1982): carbon due to microbial respiration was determined by measuring CO₂ released during soil incubation.

Dehydrogenase activity (Gong, 1996): soil samples were incubated with a solution of a quaternary ammonium salt (chloride of 2-p-iodofenil-3-p-nitrophenyl-5-phenyl tetrazolium or INT), which functions as an artificial electron acceptor. Formazan (INTF) produced during incubation was extracted and determined colourimetrically.

Activity of hydrolytic enzymes:

Activity of acid phosphatase (EC 3.1.3.2) (Dick *et al.*, 1996): soil samples were incubated with p-nitrophenyl phosphate as a substrate, and p-nitrophenol released from the soil was determined colourimetrically (without toluene).

Protease activity (Ladd and Butler, 1972, modified by Dilly and Munch, 1996): soil samples were incubated with casein as a substrate, and tyrosine released from the soil was determined colourimetrically using Folin-Ciocalteu reagent.

β-glucosidase activity (EC 3.2.1.2) (Dick *et al.*, 1996): soil samples were incubated with p-nitrophenyl β D-glucoside as a substrate, and p-nitrophenol released from the soil was determined colourimetrically.

Microbial functional diversity was measured by determining the use of various carbon sources, according to the technique described by Garland and Mills (1991) and adapted by García de Salamone (2007). The analysis of community level physiological profiles (CLPP) of carbon use was performed using sterile microplates. In the present study, only four composite samples from each tree species were considered. Soil suspensions and dilutions were made for each sample. A 10⁻⁴-dilution was used to inoculate the microplates, which were incubated at 30°C for 4 days. Absorbance records at 590 nm were obtained using an automated microplate reader Multiskan EX™, (Thermo Corporation, Vantaa, Finland). Absorbance data were ana-

lyzed and the indexes of Shannon–Weaver (H) were calculated to characterize functional diversity as described by Gómez *et al.* (2004).

Chemical parameters: The organic carbon content was determined according to the method described by Walkley and Black (Nelson and Sommers, 1982), and the soluble carbon content was determined using a volumetric technique (Tinsley, 1950). The total N content was determined by the Kjeldahl method (Page *et al.*, 1982), and extractable P was measured using Bray–Kurtz No. 1 (Olsen and Sommers, 1982)

2.3. Foliar analysis

The N content was determined according to the semi-micro Kjeldahl method (Page *et al.*, 1982), and the available P content was measured according to the Murphy–Riley method (1962). The lignin–cellulose content was determined using the method described by Anderson and Ingram (1993).

2.4. Statistical analysis

Carbon respiration, dehydrogenase and extracellular activities and chemical soil variables were statistically analysed using a one-way analysis of variance, and a completely randomised design was employed. Prior to the ANOVA, the normality of the data and homogeneity of the variance were verified. Mean comparisons were performed using Tukey's test ($p < 0.05$). CLPP were obtained using multivariate principal component analysis. The statistical software INFOSAT/PROFESIONAL® Versión 1.1 (University of Rio Cuarto, Córdoba, Argentina) was used for the analyses.

3. Results

Microbial carbon respiration and dehydrogenase activity (microbial activity) showed significant differences ($p < 0.05$) among soils between the two species (Figure 1 and 2). The highest values for these two variables corresponded to soil under European Ash trees.

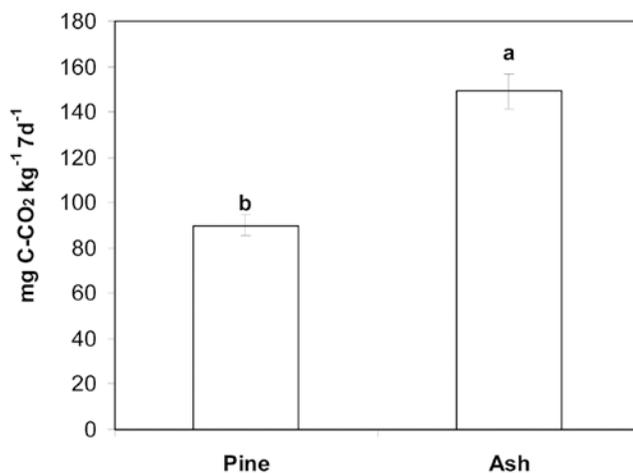


Figure 1. Mean microbial respiration in soil under Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees. Different letters indicate significant differences among species, according to Tukey's test ($p < 0.05$).

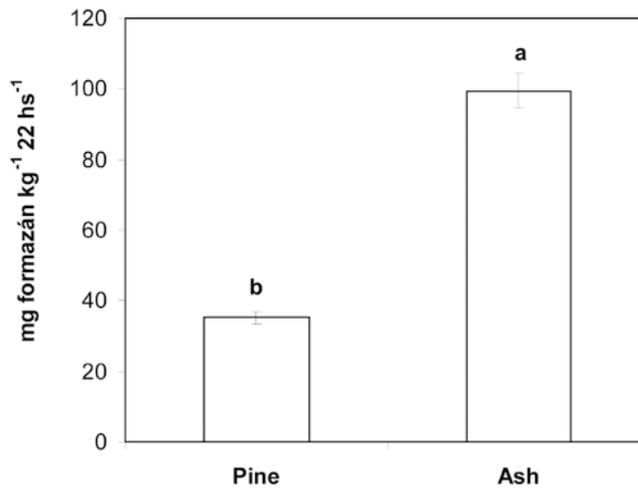


Figure 2. Mean dehydrogenase activity (1b) of soil under Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees. Different letters indicate significant differences among species, according to Tukey's test ($p < 0.05$).

The mean acid phosphatase activity was significantly ($p < 0.05$) higher in soil under European Ash trees (Figure 3). Although the protease activity (Figure 4) did not show significant differences among species ($p < 0.05$), higher activities were observed under Euro-

pean Ash trees. The b-glucosidase activity (Figure 5) of soil did not show significant differences ($p < 0.05$) among species. Similarly, significant differences in the soluble C content (Table 1) of soil were not observed among species.

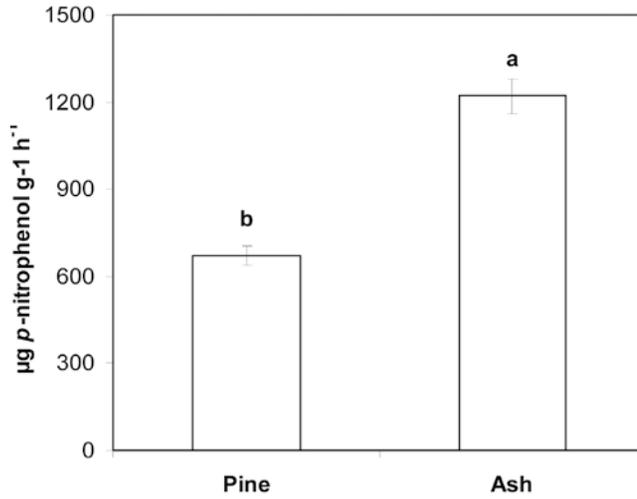


Figure 3. Mean acid phosphatase activity of soil under Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees. Different letters indicate significant differences among species, according to Tukey's test ($p < 0.05$).

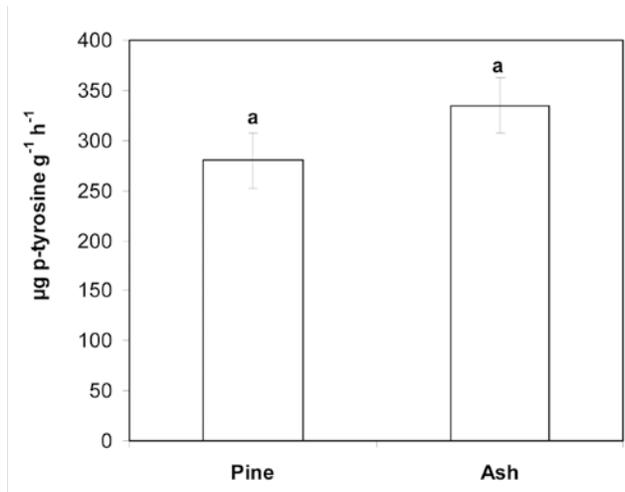


Figure 4. Mean protease activity of soil under Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees. Different letters indicate significant differences among species, according to Tukey's test ($p < 0.05$).

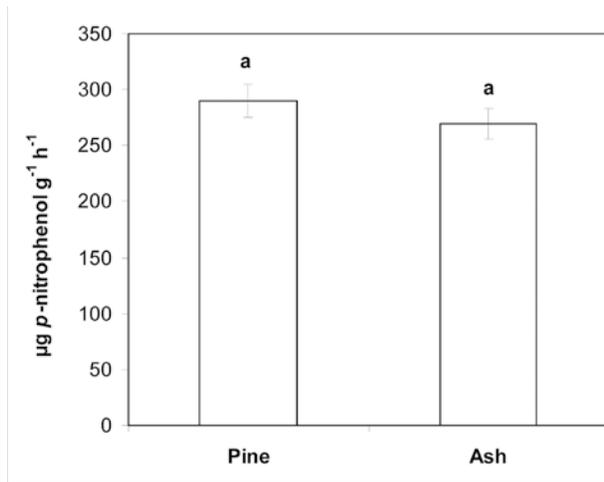


Figure 5. Mean β-glucosidase activity of soil under Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees. Different letters indicate significant differences among species, according to Tukey’s test ($p < 0.05$).

Table 1. Mean organic carbon, soluble carbon, total N and available P content of soil under Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees. Different letters indicate significant differences among species, according to Tukey’s test ($p < 0.05$).

Species	Organic C (%)	Soluble C (mg C kg ⁻¹)	Total N (%)	Available P (mg P kg ⁻¹)
Pine	2.3 b	167 a	0.33 a	24 a
Ash	3.5 a	180 a	0.30 a	26 a

The N and P content of senescent leaves showed the highest values in European Ash trees; however, the lignin and cellulose content showed the lowest values in this species (Table 2).

Table 2. Lignin, cellulose, N and P content of senescent leaves of Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees.

Species	Lignin (%)	Cellulose (%)	Leaf N (g kg ⁻¹)	Leaf P (g kg ⁻¹)
Pine	14.3	17.6	10.8	1.2
Ash	7.22	10.52	28.6	4.7

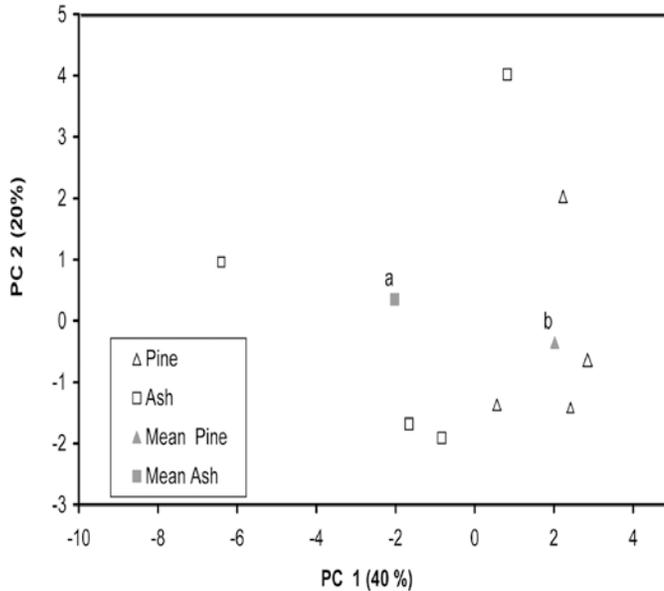


Figure 6. Principal component analysis of community-level physiological profiles of microflora associated with both tree species. PC1 and PC2: Principal component 1 and 2. Individual and mean PC1 values for samples affected by Radiata Pine (*Pinus radiata* D. Don) and European Ash trees (*Fraxinus excelsior* L.). Different letters indicate significant differences among species, according to Tukey's test ($p < 0.05$).

In the principal component analysis (PCA), the carbon substrate use patterns explained 40% and 20% of the total variance for PC1 and PC2, respectively (Figure 6). The physiologies of microbial communities associated with both forest species were significantly different, as indicated by the variance in the PC1 values. The Pearson correlation coefficients, which are shown in brackets for each of the carbon sources, were the highest for dextrose (0.28), malic acid (0.27), salicylic acid (0.27) and putrescine (0.27). The group formed by glutamine, proline, arginine, histidine and cellobiose showed intermediate values (0.26 to 0.23). The other carbon sources

(lactic acid, mannitol, Tween 20, itaconic acid, citric acid, glycine, maltose, rhamnose, glycerol, phenylalanine, xylose, fructose, benzoic acid and oxalic acid) showed values less than 0.22. The absorbance values of the four carbon sources indicated that the substrates differentially promoted the activity of microbial communities associated with both tree species (Table 3). Thus, phenylalanine and oxalic acid were the predominant carbon source of the microbial community associated with European Ash trees, while dextrose and arginine were the predominant carbon source of microflora in the soil under Radiata Pine trees.

Table 3. Absorbance values corrected by time for four carbon sources with high Pearson correlation coefficients with respect to PC1. Different letters indicate significant differences among species, according to Tukey’s test ($p < 0.05$).

Species	Absorbance values at 590 nm			
	Dextrose	Arginine	Pheny lalanine	Oxalic Acid
Pine	0.17 b	0.07 b	0.34 a	0.02a
Ash	0.11 a	0.05 a	0.38 b	0.03b

The Shannon Weaver (H) index of microbial functional diversity did not show significant differences among microbial communities associated with European Ash and Radiata Pine trees (Figure 7).

4. Discussion

Microbial respiration and dehydrogenase activity in the soil under European Ash trees were greater than that of Radiata Pine trees due to differences in the leaf N, lignin and cellulose contents of the residues (Table 2). A lower ratio lignin/cellulose and higher foliar N

content could be associated with a higher rate of plant material decomposition and greater biological and biochemical activity in the soil (Osono and Takeda, 2005). Similar results have been observed by Paul *et al.* (2002). In the aforementioned study, soil CO₂ production in Eucalyptus forest was compared to that of a plantation of Radiata Pine trees. The lower production of CO₂ in the latter was attributed to the relatively low quality of the litter. Recalcitrant phenolic compounds such as lignin and tannins inhibit microbial activity, slowing the rate of decomposition and mineralisation of soil organic matter (Satti *et al.* 2003).

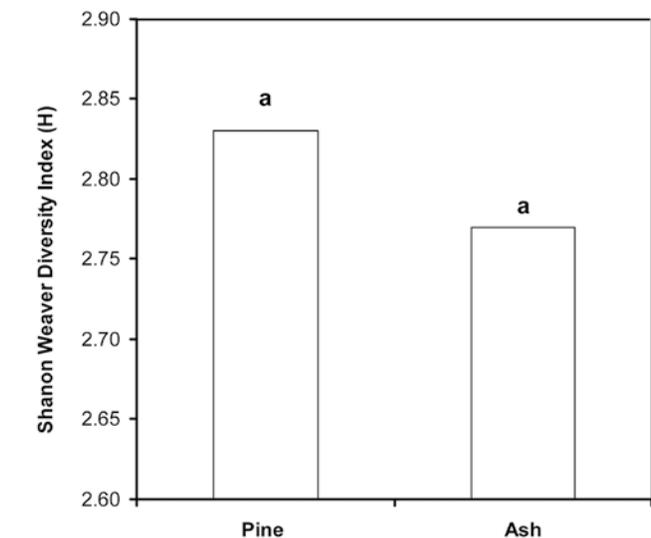


Figure 7. Shannon index of the microbial functional diversity of soil under Radiata Pine (*Pinus radiata* D. Don) and European Ash (*Fraxinus excelsior* L.) trees. Different letters indicate significant differences among species, according to Tukey’s test ($p < 0.05$).

In contrast, the higher acid phosphatase activity under European Ash cover could be related to the high leaf P content of this species, which is associated with a high rate of plant residue decomposition.

Although significant differences in the protease activity of soil under the species ($p < 0.05$) were not observed, a tendency toward higher values was detected in soil under European Ash trees. In accordance with our results, protease activities recorded by Rivas *et al.* (2009), which were obtained from soil under *Nothofagus obliqua* (Mirb) Oerst., were higher than those of Radiata Pine. However, the authors also demonstrated that the protease activity presented seasonal variations. Thus, protease activity levels in the forest soil of *Nothofagus obliqua* were significantly higher than those observed in the soil of Radiata Pine in several months of the year.

The β -glucosidase activity of soil may be related to the soluble carbon content (a measure of the amount of labile carbon) (Table 1), which did not show significant differences among species. The association between these two variables may be attributed to the involvement of β -glucosidase in the final pathways of the C cycle when soluble carbon compounds with low molecular weight are produced (Jiménez *et al.*, 2004).

The higher organic carbon content (Table 1) could partially explain the higher microbial and extracellular enzyme activities. Additionally, our results could be explained by differences in the composition of plant material produced by each species. High percentages of lignin and high ratios of lignin/cellulose materials indicate the presence of recalcitrant substances. The percentage of lignin and the lignin/cellulose and lignin/N ratio observed in the leaves of European Ash are 7.2%, 0.68 and 0.25, respectively, while those of Radiata pine are 14.3%, 0.81 and 1.32. These results show that European Ash trees present lower lignin contents and lignin/cellulose and lignin/N ratios than

Radiata Pine, which is reasonable considering that European Ash is a broadleaf species and Radiata Pine is a conifer.

In accordance with our results, Priha *et al.* (2001) concluded that forest species such as birch (*Betula pendula* and *Betula pubescens*) show higher carbon respiration values and enzyme activities than those in soil under Spruce (*Picea mariana*). The authors suggested that the differences in carbon respiration rates and enzyme activities may be due to differences in the amount of labile carbon released by each species and the impact on soil microorganisms. Chodak and Niklinska (2010) found that tree species such as Alder (*Alnus glutinosa*) accumulate greater amounts of carbon and nitrogen in the soil and have larger and more active microbial communities than do plantations with Pines (*Pinus sylvestris*) and Larches (*Larix decidua*), indicating that broadleaf species stimulate more growth in microbial communities than conifers.

The carbon sources that showed differential microbial activities among tested soils (Table 3) are often present in the rhizosphere of plants (Bolton *et al.*, 1993). The CLPP technique allows the growth of culturable microflora to be compared. The significantly lower utilisation of dextrose by the microbial community associated with soil beneath European Ash trees could indicate that the relatively high values of microbial respiration (Figure 1), dehydrogenase activity (Figure 2) and acid phosphatase activity (Figure 3) of this tree species are related to the use of carbon sources that are less accessible, such as phenylalanine and oxalic acid. Because the assay allows the growth of microflora over a short period of time, the microbial community associated with Radiata Pine was more adapted to the availability of dextrose and arginine. Similar results have been obtained for several different crops, including rice (García de Salamone *et al.*, 2010), wheat (Naiman *et al.*, 2009) and pasture

(Semmartin *et al.*, 2010). Moreover, microbial communities in the rhizosphere can respond to changes produced by applied management conditions.

Differences in the physiology of microflora were observed in the CLPP; however, significant differences in the Shannon Weaver (H) index of microbial functional diversity were not detected among microbial communities associated with both tree species (Figure 7) due to the overlapping and redundancy of functions among members of the microbial community (Naeem *et al.*, 1995). In accordance with our results, several studies have shown that differences among plant species do not necessarily involve changes in soil processes (Semmartin *et al.* 2008).

5. Conclusions

Microbial respiration, dehydrogenase and acid phosphatase activities were significantly higher in soil associated with European Ash species. Protease and β -glucosidase activities did not show significant differences among species. Microbial communities associated with these two forest species showed different CLPP values and similar H functional diversity indices. In soils under European Ash trees, the higher content of labile C, which was associated with a low lignin content and higher nitrogen and phosphorus levels in leaves, enhanced soil biological and biochemical activity.

The influence of other parameters such as root activity and seasonal factors should be evaluated in future studies. Additionally, other tree species should be studied, and complete comparative and correlation analyses among soil variables and plant species attributes should be conducted.

Acknowledgements

We thank Eng. Agr. Pedro E. Guerra for advice and collaboration with the fieldwork. We are grateful for funding from UBACyT (G413).

References

- Anderson, J. P. E. 1982. Soil respiration. En: Page A L et al. (Eds.) *Methods of Soil Analysis*. Agronomy. ASA y SSSA. Madison, Wisconsin, USA., pp: 841-845.
- Anderson, J.M., Ingram, J.S.I. 1993. *Tropical soil biology and fertility. A handbook of methods*. 2da. Edición. CAB International Oxford, pp: 221.
- Ayres, E., Dromph, K. M., Bardgett, R. D. 2006. Do plant species encourage soil biota that specialize in the rapid decomposition of their litter? *Soil Biol. Biochem.* 38, 183-186.
- Balsler, T.C., Firestone, M. K. 2005. Linking microbial community composition and soil processes in a california annual grassland and mixed conifer forest. *Biogeochemistry* 73, 395-415.
- Bolton, H., Fredrickson, J.K., Elliot, L.F. 1993. Microbial ecology of the rhizosphere. Microbial production of plant growth regulators. In: F.B Metting Jr. (ed.). *Soil microbial ecology. Applications in agricultural and environmental management*. Marcel Dekker, Inc., New York, pp: 27-63.
- Chodak, M., Niklinska, M. 2010. The effect of different tree species on the chemical and microbial properties of reclaimed mine soils. *Biol Fertild Soils*: 46, 555-566.
- Dick, R.P, Rakwell, D.P, Turco, R.F. 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In J. Doran y A. Jones (eds) *Methods for Assessing Soil Quality*. SSSA Spec. Publ. N° 49, pp: 247-271.

- Dilly, O., Munch, J.C. 1996. Microbial biomass content, basal respiration and enzyme activities during the course of decomposition of leaf litter in a Black Alder (*Alnus glutinosa* (L.) Gaertn). *Forest. Soil Biol. Biochem.* 28, 1073-1081.
- Garcia de Salamone, I.E., Di Salvo, L.P., Escobar Ortega, J.S., Tovagliari, A.E. 2007. Respuesta al cultivo de arroz a la inoculación con *Azospirillum* y fisiología de las comunidades bacterianas rizosféricas. *Actas VI Reunión Nacional Científico técnica de Biología de suelos y VI Encuentro sobre Fijación Biológica de N. Córdoba. Argentina.*
- García de Salamone, I.E.; Di Salvo, L.P.; Escobar Ortega, J.S.; Boa Sorte, M.P.; Urquiaga, S.; Dos Santos Teixeira, K.R. 2010. Field response of rice paddy crop to inoculation with *Azospirillum*: physiology of rhizosphere bacterial communities and the genetic diversity of endophytic bacteria in different parts of the plants. *Plant and Soil* 336, 351- 362.
- Garland, J., Mills, A. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community level soil carbon source utilization. *Appl. Environ. Microbiol.* 57, 2351-2359.
- Gong, P. 1996. Dehydrogenase activity in soil: a comparison between the TTC and INT assay under their optimum conditions. *Soil Biol. Biochem.* 29, 211-214.
- Gómez, E., Garland, J., Conti, M. 2004. Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient. *Applied soil Ecology.* 26, 21-30.
- Jimenez, MP; Effron, DN; Defrieri, RL; Tortarolo, MF and de la Horra, AM. 2004. Vinculación de las actividades de las enzimas β -glucosidasa y proteasa con distintas formas de C y N en un suelo nativo de bosque. *Biología del Suelo. Ed. Fac. de Agronomía. UBA*, pp: 105-110.
- Kjøller, A., Miller, M., Struwe, S., Wolters V., Pflug, A. 2000. Diversity and role of microorganisms. En: Schulze ED (ed) *Carbon and nitrogen cycling in European forest ecosystems*, Springer-Verlag, Berlin, Germany, pp: 382-402.
- Ladd, JN, Butler, JHA. 1972. Short-term assay of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol. Biochem.* 4, 19-30.
- Murphy, J., Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta*, 27, 31-36.
- Naeem, S., Thompson, L., Lawler S., Lawton, J.H., Woodfin R.M. 1995. Empirical evidence that declining species diversity may alter the performance of terrestrial ecosystems. *Phil. Trans. R. soc. Lond. B.* 345: 249-262.
- Naiman, A.D., Latronico, A.E., García de Salamone, I.E. 2009. Inoculation of Wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact on the production and rhizospheric microflora. *Eur. J. Soil Biol.* 45, 44-51.
- Nelson, D.W., Sommers, L.E. 1982. Total carbon, organic carbon and organic matter. En: AL Page; RH Miller; DR Keeney (eds.). *Methods of Soil Analysis, Part. 2.* 2da. edn. Agronomy 9. American Society of Agronomy, Madison, Wisconsin, USA., pp: 539-579
- Olsen, S.R., Sommers, L.E. 1982. Phosphorus. En: Page, AL; RH Miller; DR Keeney (eds). *Methods of soil analysis. Part 2.* Madison, Wisconsin, USA, pp: 403-430.
- Osono, T., Takeda, H. 2005. Decomposition of organic chemical components in relation to nitrogen dynamics in leaf litter of 14 tree species in cool temperate forest. *Ecol. Res.* 20, 41-49.
- Page, A.L., Miller, R.H., Keeney, D.R. 1982. *Methods of soil analysis. Part 2.* Second edition. Madison, Wisconsin, USA. 425 p

- Paul, E. A., Clark, F. E. 1996. *Soil Microbiology and Biochemistry* Academic Press, Inc., San Diego, 340 p.
- Paul, K., Polglase, P., Nyakuengama, J., Khanna, P. 2002. Change in soil carbon following afforestation. *Forest Ecology & Management* 168, 241-257.
- Priha, O., Grayston, S.J., Hiukka, R., Pennanen, Smolander, A. 2001. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. *Biol. Fertil. Soils*. 33, 17-24.
- Rivas, Y., Oyarzún, C., Godoy, R., Valenzuela, E. 2009. Nitrogen and carbon mineralization and enzyme activity in soils of *Nothofagus obliqua* (Mirb) Oerst stands and *Pinus radiata* D. Don plantation in south-central Chile. *Revista Chilena de Historia Natural* 82, 119-134.
- Satti, P., Mazzarino, M., Gobbi, M., Funes, F., Roselli, L. 2003. Soil N dynamics in relation to leaf litter quality and soil fertility in north-western Patagonian forests. *Journal of Ecology* 91, 173-181
- Semmartin, M., Di Bella, C., García de Salamone, I.E. 2010. Grazing-induced changes in plant species composition affect plant and soil properties of grassland mesocosms. *Plant and Soil*. 328, 471-481.
- Semmartin, M., Garibaldi, L.A., Chaneton, E.J. 2008. Grazing history effects on above- and below-ground litter decomposition and nutrient cycling in two co-occurring grasses. *Plant and Soil* 303, 177-189.
- Smolander, A., Kitunen, V. 2002. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. *Soil Biology & Biochemistry*. 34, 651 – 660.
- Tinsley, J. 1950. The determination of organic carbon in soils by dichromate mixture. *Trans. 4th. Int. Cong. Soil Sci. (Amsterdam)* 1, 292- 298.
- Ushio, M., Wagai, R., Balsler, T.C., Kitayama, K. 2008. Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? *Soil Biol. Biochem.* 40, 2699-2702.

