

MYCORRHIZAL DIVERSITY IN NATIVE AND EXOTIC WILLOWS (*SALIX HUMBOLDTIANA* AND *S. ALBA*) IN ARGENTINA

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ABSTRACT

Mycorrhizal morphology and diversity vary not only within each particular host family of plants, but also with soil characteristics, nutrients availability, spatial-temporal conditions, micro-habitats, and host's age. Ectomycorrhizae (ECM) and arbuscular mycorrhizae (AM) provide nutritional benefits to their hosts, besides their effects in soil aggregation, soil pollutants sequestration and host's interconnection by hyphal network that allows nutrients transport, seedlings establishment and conservation of forest ecosystems. Furthermore, certain hosts species such as *Salix* spp. in the Salicaceae are involved in dual associations with ECM and Glomeromycota fungal symbionts. *Salix*, presents a wide distribution in South America, native *Salix humboldtiana* populations as well as the introduced species are mostly located in riparian ecosystems, or in temporarily flooded areas. Riparian zones have an important role regulating the movement of material and water between soil-river systems. Mycorrhizal diversity and colonization was studied in riparian populations of native *S. humboldtiana* Willd. and of exotic *S. alba* L. in semiarid riparian environments. Differences in ECM morphotypes and mycorrhizal colonization were found in both species. Effects of soil physical-chemical features on ECM diversity are discussed and ECM morphotypes are illustrated.

Keywords: Argentina, mycorrhizal symbiosis, Riparian habitat, *Salix*, semi-arid ecosystems

INTRODUCTION

Mycorrhizal colonization of plants roots produces permanent or temporary changes, depending on the symbionts that define each particular mycorrhizal type (ECM, AM,

ectendo- arbutoid-, ericoid-, monotropoid and orchid-mycorrhizas, and dark septate endophyte (DSE) in a broad sense), each one characterized by its root-fungi-anatomy and morphology (Brundrett 2004, Peterson et al. 2004).

Arbuscular mycorrhizal fungi (AMF) are functionally important in riparian areas, but little is known about the role of AMF within these areas. It's also unknown how they are affected by water fluctuations and other associated factors such as changes in the plant community and edaphic conditions. AMF are non host specific (Smith and Read 2008), meaning that several species of fungi can associate with many different plant species; however, some host plants show a better fungal growth and sporulation for some AMF species than others (Bever 2002, van der Heijden et al. 1998, Streitwolf-Engel et al. 2001). As suggested above, changes in the composition of the plant community and soil conditions caused by flow regulation, could also affect the composition of the AM fungal community (Bever et al. 1996, van der Heijden et al. 1998), given that fungal species differ in their tolerances for soil moisture (Miller 2000, Miller and Bever 1999), pH (Abbot and Robson 1977, Porter et al 1987), or nutrient levels (Johnson et al. 1991, He et al. 2002).

Salicaceae is worldwide distributed, most species occurring in the Northern Hemisphere. The genus *Salix* comprises ca. 450 species mainly distributed in China with about 270 species, the former Soviet Union with ca. 120 species, North America with 103 species and 65 species occurring in Europe. *Salix* also occurs in Japan, Africa, the Middle East, India, and Central and South America, and it has been introduced in Australasia and Oceania (Argus 1997, 1999).

Only two species are native from South America, *S. humboldtiana* Willd. ("sauce criollo", "sauce colorado" or red willow) that is widely distributed in Argentina (Parodi 1978) and *S. martiana* Willd. distributed in Amazonia and other regions of Brazil. *Salix* species are common along water courses, lakes or ponds and has strong preference for temporarily flooded riparian zones; therefore, their roots survive wholly or partially submerged. In central Argentina native *S. humboldtiana* form small populations or co-exists with introduced species such as *Salix alba* L. ("white willow"). Both, native and exotic willows are used for their medicinal properties and their timber in the production of firewood and poles (Demaio et al. 2002).

In the Northern Hemisphere, Salicaceae was reported to form dual ECM-AM (Smith and Read 2008) or triple ECM-AM-DSE (dark-septate fungi) associations (Trowbridge and Jumpponen 2004). Among the reported *Salix* species forming this type of association, *S. viminalis* L., *S. babylonica* Kunth, *S. nigra* Marshall, *S. gooddingii* Ball, *S. alba* L., *S. dasyclados* Wimm, *S. repens* L., *S. herbacea* L., *S. barrattiana* Hook., *S. caprea* L., *S. commutata* Bebb. and *S. planifolia* Pursh., among others, mainly occurs in temperate and boreal forests (Lodge 1989, Khan 1993, Dhillon 1994, Graf and Brunner 1996, van der Heijden and Vosatka 1999, Kernaghan 2001, van der Heijden 2001, Baum et al. 2002, Püttsepp et al. 2004, Trowbridge and Jumpponen 2004, Watling 2005, Beauchamp et al. 2006, Parádi and Baar 2006, Hryniewicz et al. 2008).

In Argentina, only few studies have reported the mycorrhizal status (ECM-AM-DSE) of *Salix humboldtiana* (Silva 2004, Becerra et al. 2009). It is worth mentioning that *S. humboldtiana* besides the fact of being a native species occurring in South America, occupies an enormous gradient of habitats in which diverse biotic and abiotic factors might intensely affect not only the diversity of fungi associated but also their symbiotic contribution. These features would facilitate *Salix* populations establishment in extreme habitat conditions.

Salix species can settle as a pioneer community colonizing disturbed sites, accelerating the recovery of damaged ecosystems and re-establishment of natural ecological complexity (Kuzovkina and Quigley 2005). Thus, it can be expected that mycorrhizal infection in riparian areas will prove an important aspect of their ecology and of the restoration of forest as occurred in *Chamaesiparis* wetland trees (Cantelmo and Ehrenfeld 1999). Furthermore, *Salix* species had been successfully employed in phytoremediation programs where their biomass production overlapped waste handling in disturbed sites (Mirck et al. 2005), and in rhizosphere remediation due to the capacity of *Salix* species of self-seeding in polluted areas while its ECM symbiosis remains functional and cleaning-up the soil from persistent organic pollutants (Meharg and Cairney 2000).

The aim of this chapter is to characterize the mycorrhizal diversity in *S. humboldtiana* and the introduced *S. alba* and analyze the information on some environmental factors affecting the ECM diversity (soil gravimetric water, pH) in riparian habitats of Central Argentina.

ENVIRONMENTAL FACTORS AFFECTING ECM DIVERSITY

Considering community diversity composed by the species richness and relative abundance (Magurran 1988), two main groups of environmental factors can affect ECM fungal community diversity: natural habitat features such as soil moisture, nutrients levels, pH, temperature, soil structure, microsites characteristics, wildfires, etc., and anthropogenic perturbations like soil disturbances, heavy metal contamination, acidification, fertilization, CO₂ levels, among others. ECM fungal community diversity in terms of morphotypes diversity is the result of morpho-physiological interactions between ECM fungal species and their host's roots. Thereby, environmental factors also have influence on morphotypes diversity and host physiology. In this chapter, we only considered natural environmental factors that might be affecting *Salix* populations in sites without anthropogenic disturbances.

Soil Moisture

The lost of soil moisture generally is related with a reduction of the fungal community diversity. However, ECM fungi showed a controversial behavior about reduced soil moisture and water excess in wet soils. For example *Cenococcum geophilum* Moug. & Fr. seems to increase the amount of colonized root tips under soil drought conditions as well as in wetland environments; other ECM fungi such as *Tuber* prefer mesic sites. ECM fungi have different and specific soil moisture requirements; this preferential response to soil water content by specific ECM morphotypes was considered as the ecological support to individual fungal species variation of relative "niche width" (Erland and Taylor 2002 and reference therein).

Soil pH

Soil pH is a well known complex factor because of its relation to many soil properties that includes nutrients availability; particularly N and P, mostly involved in mycorrhizal functioning. Soil pH also interacts with other soil factors and changes widely between soil microsites. It is also a key factor in fungal ecology affecting ionic forms of CO₂ within the

soil; it also modifies the availability and ionic forms of nutrients and heavy metal ions solubility. Among this Al^{3+} , Cu^{2+} and Fe^{3+} are highly available at high hydrogen concentrations, that as well as Cd^+ , Zn^{2+} and heavy metals, can be toxic to fungi (Cooke and Whipps 1993).

Although most fungal species prefer acidic conditions to grow, they can generally tolerate a wide pH range; even, some Basidiomycetes species will develop above pH 7.5, such is the case of ECM fungi (Hung and Trappe 1983). Hyphae have a very effective fungal buffering system that includes an external concentration of hydrogen ions that affects fungal metabolism and indirect effects on growth. Hydrogen ions contribute to soil regulation of soluble salts, facilitating the availability of necessary ions for the fungus; furthermore, pH affects plasmalemma permeability and enzyme activity. Therefore, changes in fungal extracellular pH that are out of optimal value can reduce extracellular enzyme catalysis (Neville and Webster 1995).

As mentioned earlier fungal species differed in their optimal pH level for growth and ECM fungi are not exceptions. pH can alter the potential for ECM colonization, influencing ECM enzymatic and competition capabilities, species and morphotypes richness, sporocarps production and ECM community composition (Erland and Taylor 2002).

Soil Nutrients Levels

Ectomycorrhizal diversity effects on plant productivity and nutrient capture by host plant is a major benefit when nutrient conditions are poor. In terrestrial ecosystems and particularly in arid environments, plant growth is limited by deficient nutrition of N and P. ECM provide these elements to their host from organic sources such as protein and amino acids; instead, non mycorrhizal plants mainly access to inorganic N supplies. However in some boreal forest, plants are capable of amino acid uptake without ECM assistance (Nordin et al. 2001, Wiemken and Boller 2002). Wiemken and Boller (2002) pointed out that the structure and function of ECM is highly dependant of N sources in soil, and in situations with low N concentration, ECM fungal fruit bodies production had a clustered distribution, suggesting a nutrient patchiness effect (Wiemken and Boller 2006).

In forest soils, only a portion of the organic P is available for ECM fungi (Häussling and Marschner 1989). The hyphae of ECM fungi stored phosphates probably within vacuoles, which are later mobilized to the host plant. In general low levels of available soil nutrients, particularly N and P, affect the internal nutritional status of the host leading to increase ECM infection (Alexander 1983). Bruns (1995) suggested that the high ECM fungal diversity in Northern Hemisphere forests could be the result of resource partitioning, disturbance and interactions with other soil microorganisms.

ECM fungi can display a wide range from strictly symbiotic to hemi-saprobies (Högberg et al. 1999), based on their high capability to produce a diverse range of extra-cellular and cell wall-bound hydrolytic enzymes to break down soil organic molecules that contain N and P such as amino acids, protein and chitin (Leake and Read 1990, Leake et al. 2002, Smith and Read 2008). Furthermore, ECM fungi are able to produce extra-cellular enzymes for cellulose and lignin breakdown (Courty et al. 2005, 2006, 2007). Nevertheless, this theoretical postulated saprophytic role of ECM fungi (Tarkka et al. 2005; Weiken and Boller 2002) in the acquisition of carbon from soil, based on the increase of enzymatic activity during periods of low photosynthate supply from tree hosts, is actually questioned. Recently, Baldrian (2009)

argued that still there is not enough evidence to confirm the saprophytic functioning of ECM fungi.

Soil Structure

Forests soils are a well known heterogeneous environment. The spatial heterogeneity of forest soil affects the vertical distribution of habitats for microbial and fungal communities and the physicochemical soil properties along the gradient.

In temperate and boreal forests the upper soil layer with major litter content houses saprophytic fungi, whereas ECM fungi can colonize deeper soil horizons with low availability of carbon compounds and positive energetic value (Baldrian 2009). Vertical distribution of ECM fungi has been reported previously (Dickie et al. 2002, Neville et al. 2002, Rosling et al. 2003), for example, in a French oak forest it was found that the ECM richness was higher in the undisturbed A1 horizon and in the A2 sieved soil, than undisturbed A2 horizon and in the dead woody debris niches, indicating how the ECM community structure can strongly vary depending on the nature of soil horizon or habitat patches (Buée et al. 2007).

New results considering fine-scale spatial structure of ECM (Amend et al. 2009), distances between host plant and seedlings (Teste et al. 2009) and plot-level effects (Izzo et al. 2005), provided a high support to the earlier Bruns (1995) proposal about the maintenance of the high ECM fungal diversity in Northern Hemisphere forests by the resource partitioning, disturbance and interactions with other microorganisms.

Microsites Effect

ECM fungi are relatively host selective, while AM tend to be generalists. Ectomycorrhizas have been mainly studied in forest systems due to its preference to tree hosts, where a high number of fungal species usually associate to a low number of plant species (Read 1991, Allen et al. 1995). In this type of ecosystems, habitat conditions change with tree age and soil's spatial heterogeneity, usually showing a mosaic of diverse physical-chemical and biological properties along short distances. Microorganisms living within soils microsites, such as mycorrhizal fungi are highly influenced by environmental characteristics that modify their development and functioning (Bledsoe 1992). In mature Douglas-fir old-growth strands, ECM diversity and distribution were related to soil characteristics and microhabitats; ECM density and richness were higher in forest floor over the mineral soil than in mineral soil or logs (Goodman and Trofymow 1998). In addition, ECM systems present a spatially clumped distribution widespread in the forest soil, facilitated by wide variation range of root tips available to be colonized by fungal symbionts. Examples of clumped distribution are those occurring with ECM mats and ECM fungal clones (Dahlberg and Stenlid 1990, 1994, Griffiths et al. 1996). Also, environmental factors as soil depth have shown effects on dual ECM-AM in Salicaceae. In *Populus tremuloides* Michx. (Salicaceae) populations, the overall ECM colonization reached the 86%, of the roots, while AM colonization only reached a 6%, with a negative correlation between ECM and AM fungal colonization among all depths with "ECM colonization more abundant in the shallow organic

soils and AM colonization more abundant in deeper mineral soils” (Neville et al. 2002). This also suggests that ECM and AM are preferentially partitioned at different soil depths.

ECM FUNCTIONING AMONG AQUATIC ENVIRONMENTS

The presence of mycorrhizas in aquatic plants is controversial. Few years ago, scientists argued that the low concentration of oxygen in waterlogged or swamped soils, were a determining factor that inhibited the formation of mycorrhizal associations. ECM were reported for trees species of *Casuarina cunninghamiana* Miq., *Melaleuca quinquenervia* (Cav.) Blake and *Salix babylonica* Kunth when growth in drained and aerated soils but not in swamps, water or sediments (Khan 1993) and dual associations were confirmed in these trees in aquatic and terrestrial environments (Lodge 1989). Absence of AM were reported in hydrophytes and members of the families Urticaceae, Casuarinaceae, Nyctaginaceae, Portulacaceae, Caryophyllaceae, Zygophyllaceae, Tamarinaceae, Euphorbiaceae and Palmaceae growing in sandy swamp soils (Khan 1974). However, recent studies in terrestrial and aquatic plants with whole or part of the root systems submerge, determined the presence of mycorrhizas (Stenlund and Charvat 1994).

There are no studies that examine the relationship between the concentration of soil water and ECM colonization and diversity, neither the presence of mycorrhizas in submerged roots of *Salix* spp.

SALIX ALBA L. ECM DIVERSITY ALONG AN ARID TO RIPARIAN GRADIENT IN CENTRAL ARGENTINA

Salix alba is an introduced species in South America, it presents a wide distribution in Argentina, mostly growing in riparian ecosystems or in areas temporarily flooded. The riparian zone is the interface area between land and a river or stream. It has an important role regulating the movement of material and water between the ecotonal soil-river systems.

ECM diversity and colonization was studied during autumn-winter in a riparian population of *S. alba* in Potrero de los Funes, San Luis (Central Argentine), a semi-arid environment. Potrero de los Funes is located at 952 m of altitude at 33° 13' 0S and 66° 13' 60W. Average summer temperature is 21-23 °C and 8-9 °C, in autumn-winter. Rains occur mainly in summer. Potrero River is a permanent mountain river, characterized by an area of 2.41 km², a total length of 2987 m, a runoff density of 4.45 km/ km² and a flood flow of 31.6 m³/s. This river is located on a recent geological unit with alluvial sediment (silt, sand and gravel) that partially constitutes the river courses. Riparian soil is sandy, with gravel and rocks (Ojeda et al. 2007). During late autumn of 2006 three transects along Potrero riverside were established and soil samples were collected across riparian zone every 1.5 m from water to land (0, 1.5, 3, 4.5 and 6 m). At each point of distance within the transect, *S. alba* root samples were dug out. At the laboratory the gravimetric soil moisture, soil pH, ECM colonization, ECM relative frequency and ECM diversity and richness, were measured. Duplicate samples of soil and roots were passed through sieves of different diameter to separate roots from soil and debris. After sieving samples were weighed to obtain the initial weight (Wi); subsequently placed in a forced air oven at 65-70 °C, during 72 hours until

constant weight to estimate the final dry weight (Wf) considered as the last constant measurement.

A fraction of the roots were clarified and stained (Grace and Stribley 1991) to record AM associations, whereas another root fraction was used to characterize the ECM morphotypes (Agerer 1991, Baum et al. 2002) and estimate their colonization (Gehring and Whitham 1994, Giovannetti and Mosse 1980). ECM diversity was estimated based on the relative frequencies of the ECM morphotypes, using Simpson index (Helm et al. 1999).

Salix alba roots presented AM, ECM and DSE structures in their roots. The DSE structures were also present in the submerged roots. Previously, single ECM or AM or dual AM-ECM associations were reported for this species (Harley and Harley 1987, Lodge 1989, Khan 1993, Parádi and Baar 2006, Sumorok et al. 2008). The tripartite AM-ECM-DSE association had been previously reported only for shrub willows such as *Salix commutata* and *S. planifolia* (Trowbridge and Jumpponen 2004).

Soil water percentage was uniformly low along the distances gradient, except for the highest value at the extreme of the gradient where the roots were submerged. pH did also not differed along the transect (Table 1). We did expect gravimetric soil moisture and pH to diminish while distance from riverside increased but these correlations were not found.

Along the gradient 12 ECM morphotypes were found associated to *Salix alba* roots (Figures 1, 2). In general, the ECM morphotypes richness associated to *Salix* species is low. In native locations, *S. alba*, among Pilica River (Poland) riparian areas, presented 15 ECM morphotypes (Sumorok et al. 2008) and in the The Netherlands up to 20 morphotypes (Parádi and Baar 2006). In this sense, the morphotypes richness of *S. alba* seems to be similar in both, Northern and Southern Hemispheres locations. Another interesting aspect observed is that the introduced *S. alba* in Argentina, presented a relatively higher morphotypes richness than the native *S. humboldtiana* in Central Argentina (Becerra et al. 2009).

Table 1. Soil gravimetric water percentage (%H₂O) and pH along Potrero de los Funes riparian zone. Data are mean value ± SE

Distance from river (m)	H ₂ O (%)	pH
0	100 ± 0	6.5-7
1.5	9.69 ± 5.79	6.5-7
3	6.73 ± 2.48	6.5-7
4.4	5.70 ± 2.23	6.5-7
6	6.37 ± 4.23	6.5-7



Figure 1. General ECM Morphotypes view of *Salix alba* from San Luis. a. Morphotype 1 b. Morphotype 2. c. Morphotype 3, cystidia surrounding the root tip (→). d. Morphotype 4. e. Morphotype 5 rizomorph (→). f. Morphotype 6 emanating hyphae (→). g. Morphotype 8. h. Morphotype 9 (hyphae that appear in the photograph are loose hyphae that do not have connection to the mantle). Bars a, b, e: 225 μm ; c, f: 800 μm ; d: 300 μm ; g: 400 μm ; h: 145 μm .

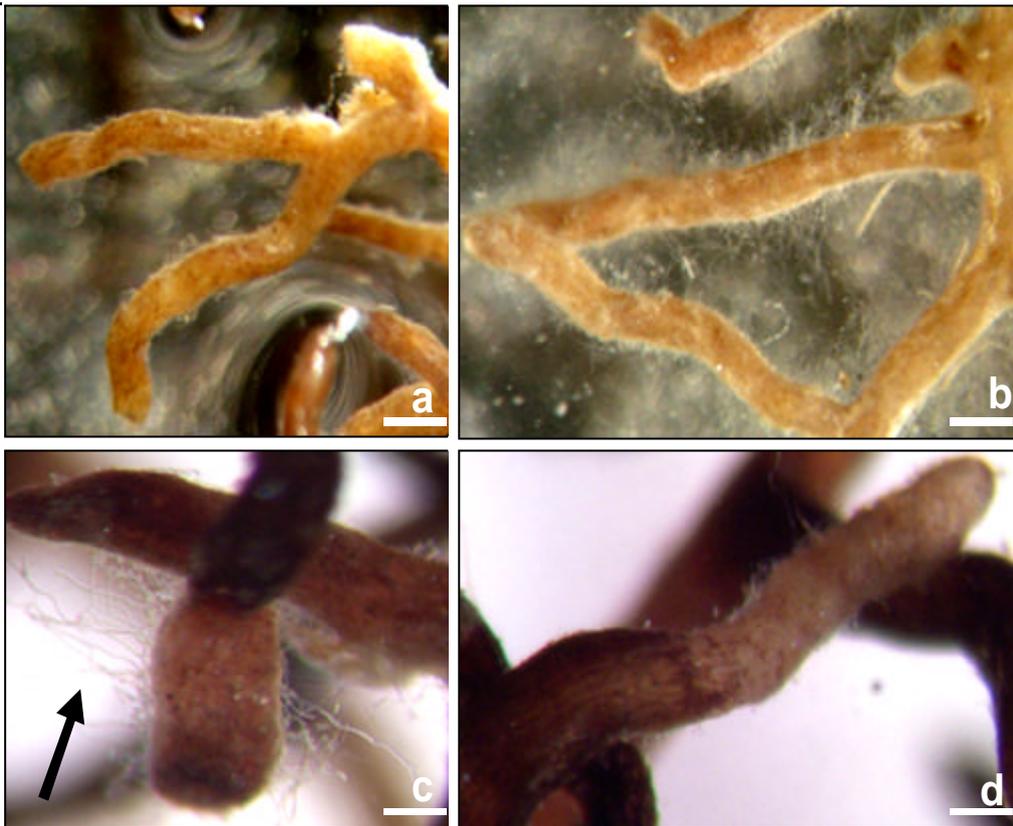


Figure 2. General ECM Morphotypes view of *Salix alba* from San Luis. a. Morphotype 10. b. Morphotype 11. c. Morphotype 12 points out emanating hyphae (→). d. Root tip dark bottom and light top. Bars a: 300 μ m; b: 400 μ m; c: 145 μ m; d: 225 μ m.

No ECM fungal sporocarps were found at the *Salix alba* riparian sites. The lack of fruiting bodies also occurs in the other *S. alba* locations, for example, in Poland only *Inocybe* and *Hebeloma* sporocarps were found at Pilica River (Sumorok et al. 2008); however, ca. 40 ECM fungi were found under *Salix* spp., *Betula pendula* and *Pinus sylvestris* in dry habitats of this floodplain. In other regions, only *Tricholoma cingulatum* (Almfelt) Jacobasch has been reported fructifying with *S. caprea* (Hryniewicz et al. 2008) and *Inocybe glabripes* Ricken, with *S. viminalis* in Germany (Baum et al. 2002). Surprisingly, in non riparian habitats, the ECM fungal diversity supported by sporocarps records is much higher, van der Heijden et al. (1999), registered up to 78 fungal species associated to *Salix repens* in a natural sand dunes system, and 53 ECM sporocarps taxa associated to *Salix herbacea* in alpine habitats (Graf and Brunner 1996). However, the riparian zones of Central Argentina, associated to both *S. alba* and *S. humboldtiana* despite their exotic or native status, sporocarps were uncommon and only one species of *Inocybe* was recorded under *S. humboldtiana* (unpublished data). In the semiarid sites associated to *Salix alba*, sporocarps of saprophytic fungi were also absent but several species were found under *S. humboldtiana* riparian areas (Becerra et al. 2009).

Salix alba presented a low AM colonization (15%, data not shown), whereas overall ECM mean frequency of colonization was higher and ranged from 45% to 100% (Figure 3).

None of the variables studied were significantly correlated by the percentage of gravimetric water, and the ECM morphotypes richness and diversity were negatively

correlated with the gravimetric water percentage of soil (Spearman $\rho = -0.11$, $p = 0.5$; Spearman $\rho = -0.02$, $p = 0.92$, respectively).

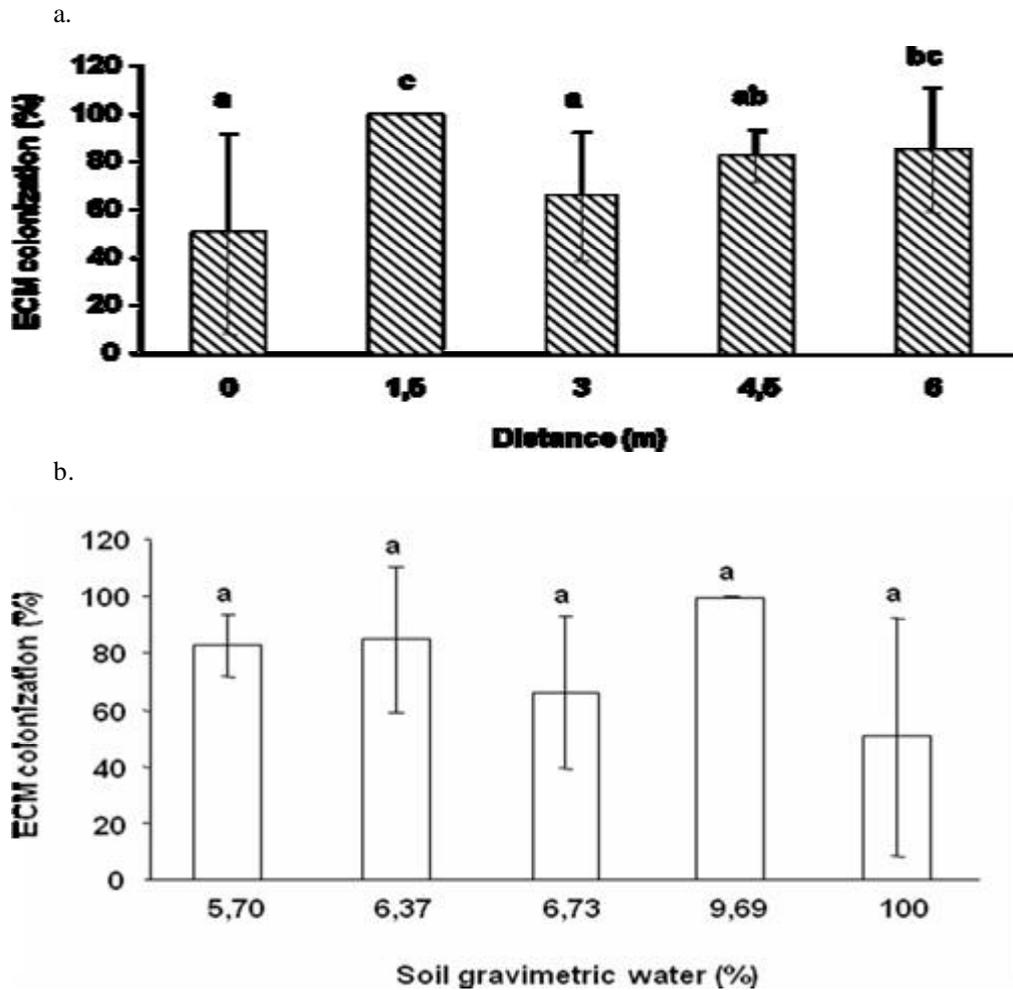


Figure 3. *Salix alba* ECM colonization along Potrero de los Funes riparian zone. a. ECM colonization versus distances from riverside. b. ECM colonization versus soil gravimetric water percentage. Data showed are mean value \pm SE. Complete data set were analyzed by Kruskal Wallis non-parametric test. Different letters represent the results of *a posteriori* test of multiple comparisons.

High frequency of ECM means colonization in roots of *S. alba* (Figure 3) was similarly observed in riparian habitats from Poland (Sumorok et al. 2008). However, *S. alba* ECM colonization was lower in flooded areas of Waal River than in dry riparian edges like those of Potrero in Argentina and Pilica in Poland.

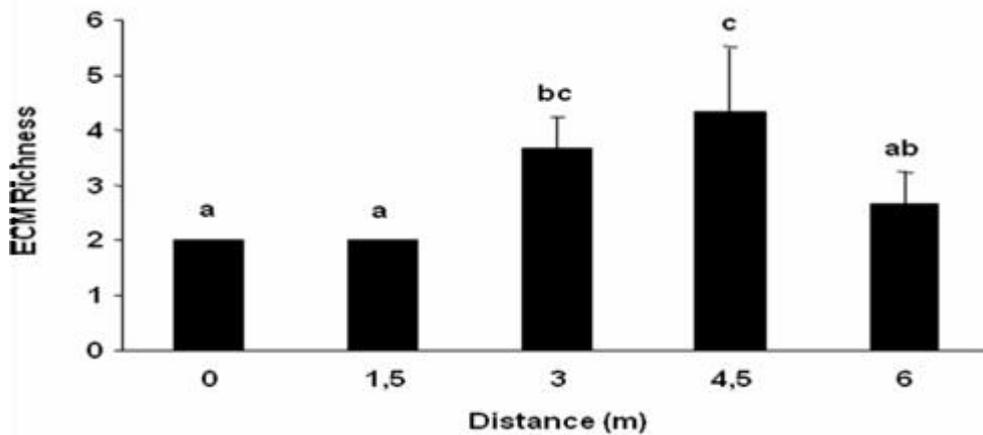


Figure 4. *Salix alba* ECM richness versus distances from riverside. Data showed are mean value \pm SE. Complete data set were analyzed by Kruskal Wallis non-parametric test. Different letters represent the results of *a posteriori* test of multiple comparisons.

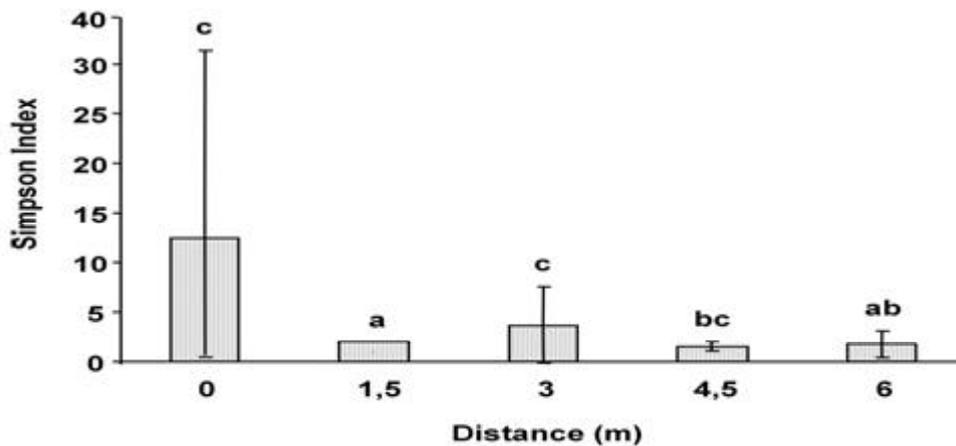
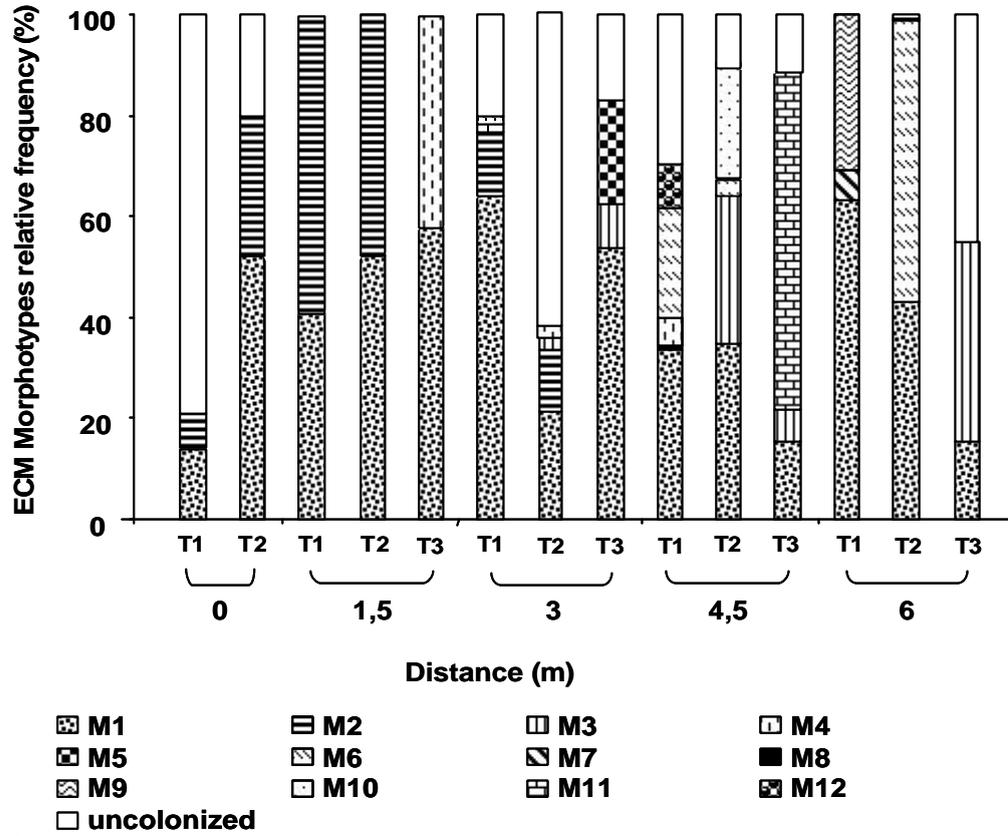


Figure 5. *Salix alba* ECM diversity versus distances from riverside. Data showed are Simpson index mean value \pm SE. Complete data set were analyzed by Kruskal Wallis non-parametric test. Different letters represent the results of *a posteriori* test of multiple comparisons.

Within the riparian zone, ECM root colonization (Figure 3), richness (Figure 4) and diversity (Figure 5) varied significantly with increasing distance from the river edge (Kruskal Wallis test $H=21.34$, $p<0.001$; $H=29.85$, $p<0.0001$; $H=21.04$, $p<0.001$, respectively). Furthermore, ECM richness was significantly correlated to distance from shore (Spearman $\rho=0.44$, $p<0.01$). Only morphotypes M1 and M2 were associated with flooded roots, representing the 17% of total ECM morphotypes richness. The remaining 83% were associated to land roots and within these morphotypes, 50% were recorded in only one distance from the river edge. Here, the distance with the highest richness was 4.5 m, in which 9 different morphotypes were recorded; followed by 3 and 6 m distances, each with 6 morphotypes; 1.5 m. distance with 3 morphotypes, and the submerged roots showed the lowest richness with only 2 morphotypes.

a.



b.

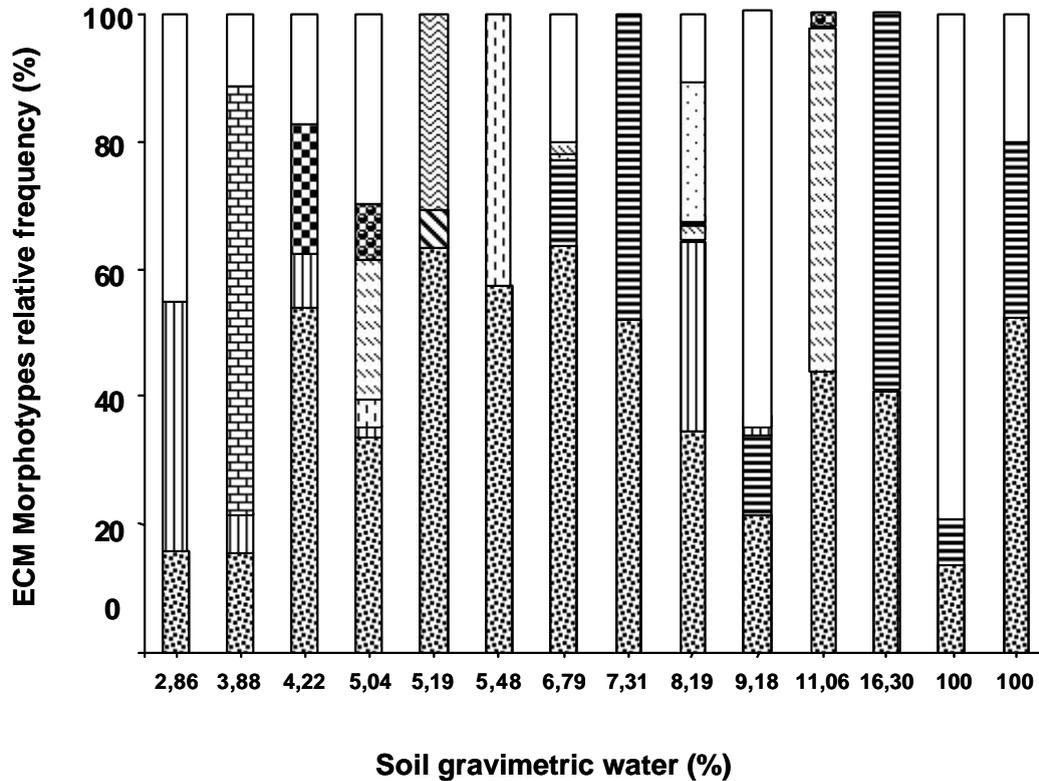


Figure 6. *Salix alba* ECM relative morphotypes frequency along riparian zone. a. ECM relative morphotypes frequency *versus* distance from riverside. Data showed are values from each transect sampled per site. Complete data set were analyzed by Kruskal Wallis non-parametric test. Significant differences were not found by *a posteriori* test of multiple comparisons. References: M: morphotype, followed by successive designation numbers.

ECM morphotypes relative frequency did not change significantly with the distances from the shore (Figure 6a) neither with the gravimetric water percentage (Figure 6b). However, all ECM morphotypes (M1 to M12) presented four differential patterns of distribution or guilds with water soil content and distance along the riparian zone: i) morphotypes distributed along all gravimetric water percentages and distances (M1); ii) morphotypes presented in most distances and in submerged roots (M2); iii) morphotypes presented in most distances, except submerged roots (M3, M4, M6) and iv) morphotypes distributed in only one or two distances or gravimetric water percentages excluding the submerged roots (M5, M7, M8, M9, M10, M11, M12). No ECM morphotypes were found exclusively in submerged roots.

Although, some morphotypes showed weakly significant correlations with soil gravimetric water percentage and distance to the river edge. Thus, only M2 frequency was positively correlated to gravimetric water percentage in soil (Spearman $\rho=0.61$, $p<0.05$) but it was negatively correlated with distance from river edge (Spearman $\rho=-0.62$, $p<0.05$). M6, M7, M9 and M12 frequencies were positively correlated with distance from river edge (Spearman $\rho=0.53$, $p<0.05$; $\rho=0.59$, $p<0.05$; $\rho=0.59$, $p<0.05$ and $\rho=0.57$, $p<0.05$, respectively).

Thus, morphotypes M1 (guild *i*) showed a wide distribution range, from dry soil to the submerged roots. Morphotype M2 (guild *ii*) was found almost in all gravimetric water percentages but shows a preference for high gravimetric water percentage in order to colonize *S. alba* roots; however, soil condition changes along the increased distance to the Potrero River affecting M2 negatively; for instance we could predict that M2 could be better adapted to wet and flooded habitats. M3, M4 and M6 (guild *iii*), could be considered as an intermediate guild, forming ECM evenly in dry soil conditions rather than in the water flow. M5, M7, M8, M9, M10, M11, M12 (guild *iv*) which were never found in the submerged roots, showed restricted distribution along the distance gradient, and could be terrestrial specialists that are absent in flooded environments as indicated in other studies (Khan 1993).

Among Potrero riverside gradient *S. alba* ECM colonization did not vary with soil gravimetric water percentage, although ECM richness and diversity were correlated in opposite senses. These results together with the four morphotypes guilds observed within the *S. alba* populations transects, suggest that gravimetric water percentage in the soil could not affect ECM colonization but could influence the species composition of the ECM fungal community. Thus, in Potrero riparian environment an increase in drought could "cause a shift in plant/fungus communities" as has been proposed by Tarkka et al. (2005).

SALIX HUMBOLDTIANA ECM DIVERSITY IN CENTRAL ARGENTINA

Salix humboldtiana Willd. (Sauce criollo) is the only native species of *Salix* and is one of these so-called dual mycorrhizal plant species. Their populations are distributed along streams, rivers and lakeshores and it occurs in habitats in which soils are heavily influenced

by water level fluctuations (Ragonese 1987). As a dual mycorrhizal plant, *S. humboldtiana* may have a selective advantage in these highly dynamic ecosystems.

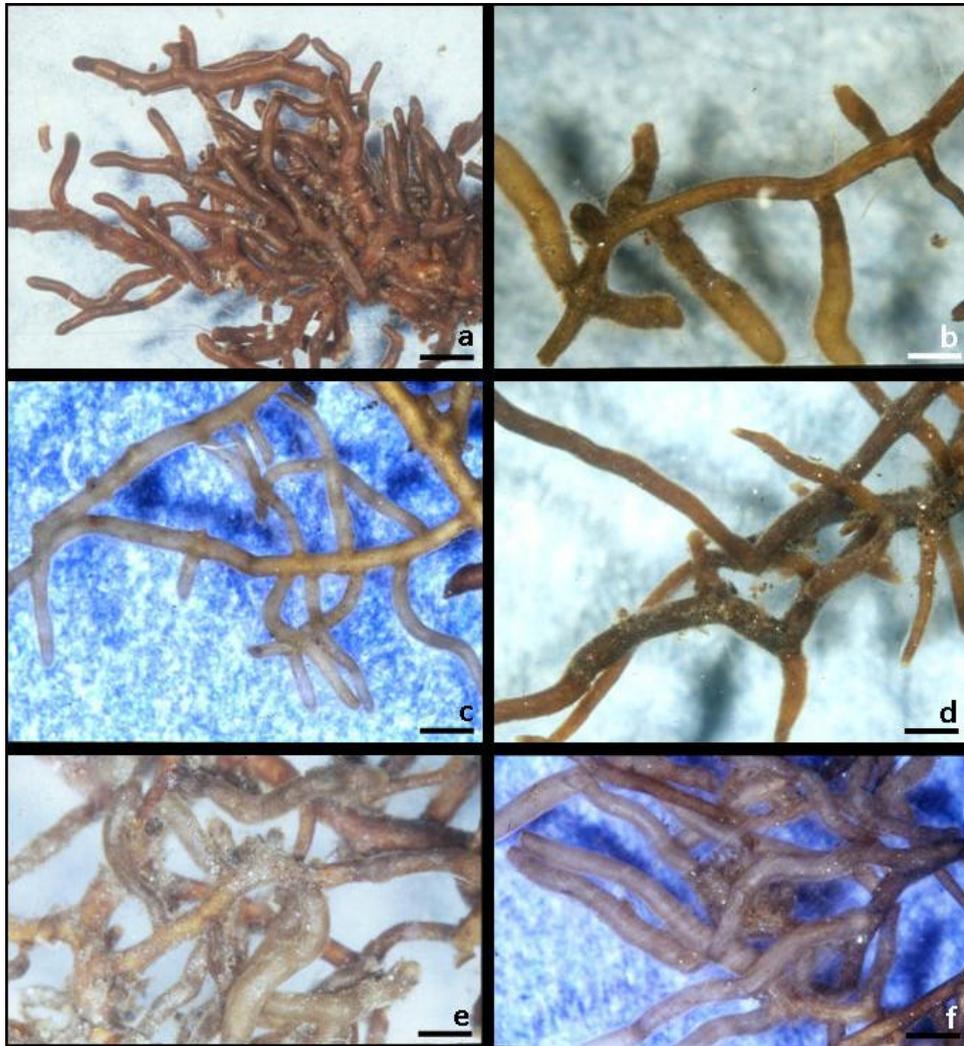


Figure 7. General view patterns of ECM of *Salix humboldtiana* from Central Argentina. a. Morfotype 1, *Inocybe* sp. b. Morfotype 2 *Tomentella* sp. c. Morfotype 3. d. Morfotype 4. e Morfotype 5. f. Morfotype 6. Bars a-f: 0.5 mm.

In Argentina, two field sites located in Córdoba province (Central Argentina) were studied (Punilla department and Colón department). Both soils presents a neutral soil pH, but differed in texture and in nutrient content. Soils from Colón Department site had higher contents of organic matter and total N, a higher electrical conductivity and higher levels in P than soils from Punilla department site. *S. humboldtiana* populations (height 6-15 m, age 20-30 years) are located along riverbanks. In both sites, the areas remained water-saturated during the rainy seasons (summer–autumn), having the trees partially submerged the root system during this time. *S. humboldtiana* root samples were taken during autumn, sampling monthly from April to June. Five square plots (10 m x 10 m) were established randomly within a homogenous area (100 x 50 m). The percentage of root tips colonized by ECM fungi

was determined as described by Gehring and Whitham (1994). Seven ECM morphotypes were found to be associated with *Salix humboldtiana* (Figures 7, 8). All of them appeared mostly turgid and active, with thin mantle layers and without rhizomorphs. For detailed description of the ECM morphotypes, see Becerra et al. (2009). In few cases, root tips appeared senescent and mycorrhizae were dark colored, probably indicating their inactive stage. Two morphotypes matched ECM taxa when blasted to the respective resulting consensus sequence in the NCBI database. The fungal symbionts in both matches are basidiomycetes, belonging to the genus *Tomentella* in the Thelephoraceae and *Inocybe* sp. within the Cortinariaceae. Amplification failures of the remaining morphotypes were probably due to old and inactive mycorrhizae, or to mycorrhizae morphotypes with poorly or discontinuously developed mantle layers, features that seem common in some species of *Salix* (Jones et al. 1990; Graf and Brunner 1996; Trowbridge and Jumpponen 2004; Püttsepp et al. 2004). Besides, the DNA amplification from *Salix* can be difficult, attributable to the co-extracting inhibitors (possibly salicylic acid) (Herrera Medina et al. 2003).

The ECM colonization of *S. humboldtiana* differed between the two sites ($P < 0.001$) and sampling dates ($P < 0.001$). ECM colonization was 71.13 % (S.E.= 24.7) at the Hayke site, and 86.31 % (S.E.=17.2) at the La Calera site, varying from 33 to 99 % at both sites. The ECM colonization significantly differed among sampling dates (from April to June), being higher in June and May than April. This variation could be mediated by differences in temperature, soil moisture and soil nutrient status, physiological and phenological changes in the host plant, ultimately affecting the symbiosis development (Harvey et al. 1978; Jones et al. 1990; Swaty et al. 1998; van der Heijden et al. 1999; Baum and Makeschin 2000; Baum et al. 2002; Püttsepp et al. 2004). Indeed, important changes on humidity and temperature usually occurred during the fall months (April to June), as it was previously registered by Luti et al. (1979).

Roots submerged in water (Figure 8) present a complete or incomplete thin mantle when they were observed under microscope. Mainly those roots present a simple ramification and brown color, 4.5-7.5 mm long and 0.1-0.3 mm diameter. No rhizomorphs and emanating hyphae were observed. A transitional type mantle between the plectenchymatous and pseudoparenchymatous mantle, in which irregularly shaped hyphae form a coarse net, 2.5-6 μm diameter, hyphae without clamps. These roots do not present AM colonization. Unique mycorrhizal associations were found in submerged roots of *S. alba* and *S. humboldtiana*. Other studies have shown similar mycorrhizal development in aerobic environments associated to *Alnus glutinosa* L. Gaertner in wet habitats (Baar et al. 2000).

To observe AM colonization, roots were clarified and stained (Grace and Stribley 1991), and the structures described in detail (Becerra et al. 2009), and photographed (Figure 8). AM colonization of *S. humboldtiana* differed between sampling dates ($P < 0.001$), showing a significant interaction effect between sites and sampling dates ($P < 0.01$). AM colonization at the Hayke site was 4.05 % (S.E.= 4.49), and 3.12 % (S.E.= 2.98) at the La Calera site, the colonization ranged between 0 to 17 % for both sites. AM colonization significantly differed among sampling dates being higher in April at Hayke and May at La Calera site. *S. humboldtiana* showed low AM colonization that differed between sampling dates. Our colonization rates resemble those observed by Trowbridge and Jumpponen (2004) on *S. commutata* and *S. planifolia* and van der Heijden and Vosatka (1999) on *S. repens*. Although we observed low levels of infection, AM fungi might still provide benefits to *S. humboldtiana*, as van der Heijden and Vosatka (1999) suggested.



Figure 8. a. General view pattern of morphotype 7 in *Salix humboldtiana* from Central Argentina. b. Coils of arbuscular mycorrhizal colonization. c. Roots submerged in water. d. Incomplete thin mantle of submerged roots. Bars a: 0.5 mm; b: 10 μm ; c: 1cm.

In both Argentinian sites, *S. humboldtiana* are colonized by ECM- AM-DSE. The occurrence of ECM and AM colonization in *S. humboldtiana* concurred with the observations of Lodge (1989), Khan (1993), van der Heijden and Kuyper (2003) and Hashimoto and Higuchi (2003).

CONCLUSION

In Central Argentina, *S. alba* and *S. humboldtiana* presented multiple fungal symbiosis (AM, DSE and ECM), which might be indicating alternative or synergistic fungal association that would be more efficient for *Salix* spp. within these semiarid ecosystems. Another interesting observation is the scarcity of fungal sporocarps in populations of both *Salix* spp.

The environmental conditions could greatly influence the host species plasticity to form alternative associations, particularly in riparian ecosystems. The ECM frequency, richness and diversity were not affected by soil gravimetric water percentage in *S. alba* but were influenced by distance to the water edge. The absence of moisture effect on ECM could be due to the very low soil gravimetric water percentage and the scarce differences of water values between each distance along the gradient, except the river point. The distance effects on ECM could be related to negligible and subtle changes in microhabitats along the gradient.

ECM morphotypes richness was higher in the exotic *S. alba* than in native *S. humboldtiana* (Silva 2004, Becerra et al. 2009), but similar to *S. alba* populations in European ecosystems. Therefore, dry-riparian condition could benefit some ECM fungal species well adapted to drought which would colonize successfully the roots of *S. alba*.

The four guilds of *S. alba* ECM morphotypes along the Potrero River riparian area could mirror differential responses of the fungal component in this community by microhabitat effects, that probably selects fungi capable of persisting under particular environmental conditions (Jumpponen et al., 1999; Trappe and Luoma, 1992; Trowbridge and Jumpponen, 2004). Thus, in this arid riparian system, *S. alba* ECM association could be more influenced by differential microhabitat features than by water content in soil because ECM fungal species would be resistant to dry environmental conditions.

Salix species pioneer capabilities, phytoremediation and rhizoremediation properties in addition to *S. alba* and *S. humboldtiana* high ECM colonization and multiple symbiosis are valuable traits to consider their potential use in restoration of arid systems. Further research could lead to understand the variation in colonization level among sites and microsites from stream to land in the arid riparian systems.

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