

## Baccharis incarum and fungus Arbuscular Mycorrhizal symbiotic relationship for land fallow in the Bolivian highland

*Relación simbiótica de Baccharis incarum y hongos Arbuscular Mycorrhizal en parcelas en descanso en el altiplano Boliviano*

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### Abstract

Arbuscular Mycorrhizal (AM) fungi were studied from a natural soil rhizosphere of *Baccharis incarum* sampled from fallow plots in the Bolivian highland (Pumani community in La Paz department). In these ecosystems, farmers had developed a system of communal crop rotation which includes a land fallow that goes till 30 years. The host specie in their crown diameters, age, and fallow effects over VAM fungal diversity and colonization were analyzed. Among the genus of fungus VAM that were identified (*Glomus*, *Gigaspora* and *Scutellospora*) in the rhizosferic soil, the highest population in these soils was for *Glomus*. Density of mycorrhizal fungi was found to be strongly influenced with fallow years, although plant age, initial fallow period of the plots and fungus genus had a variable effect on the mycorrhizal colonization. Whereas land degradation produced by crops and grassing is closely regulated by this symbiotic relationship and farmers had the habit to rest the soils to restore its fertility to an agreed standard, generally among farmers such labours of land fallow are mandatory for the members of the community to continuous the agriculture works.

**Key words:** Mycorrhiza, fallow plots, *Baccharis incarum*, rhizosferic soil, hyphae infection, land recuperation.

### Resumen

Se estudiaron los hongos Micorrícicos Arbusculares (MA) en suelos rizosféricos naturales de *Baccharis incarum*, muestreados de parcelas de barbecho en el altiplano boliviano (comunidad de Pumani, del departamento de La Paz). En estos ecosistemas, los agricultores han desarrollado un sistema de rotación de cultivos comunales que incluye un barbecho de la tierra que va hasta los 30 años. En la especie estudiada se analizaron parámetros como el diámetro de la copa, edad, y los efectos de descanso sobre la población de la diversidad fúngica y la colonización de los hongos VAM. Entre los géneros de hongos VAM que se identificaron (*Glomus*, *Gigaspora* y *Scutellospora*). Se encontró que la densidad de hongos micorrícicos está fuertemente influenciado con los años de descanso, aunque la edad de la planta, el período de barbecho inicial de las parcelas y los hongos del género tuvieron efectos variables sobre la colonización de micorrizas. Mientras que la degradación del suelo producida por los cultivos y el pastoreo están estrechamente reguladas por esta relación simbiótica, los agricultores acostumbran descansar los suelos para restaurar su fertilidad a un nivel en el que es posible utilizarlos. Por lo general, entre los agricultores tales labores de descanso de la tierra son obligatorias para los miembros de la comunidad.

**Palabras clave:** Micorrizas, parcelas de barbecho, *Baccharis incarum*, suelo rizosférico, infección, hifas, recuperación de suelos.

## INTRODUCTION

Land degradation has generally been defined from the functional perspective of agricultural, horticultural or forestry uses of land. These definitions emphasize soil properties rather than landscapes. Lal and Stewart (1992) suggest that soil degradation implies diminution of productive capacity through intensive use leading to changes in soil physical, chemical and biological processes. Blum (1998) takes a different approach and defines soil degradation as a loss or reduction of soil energy. Conacher and Conacher (1995) defined land degradation more broadly as alteration to all aspects of the natural (or biophysical) environment by human actions, to the detriment of vegetation, soils, landforms, water and ecosystems. Their definition whilst broadly encompassing and overlooks the significant cultural or archaeological heritage contained within land and landscapes which can be degraded or compromised by inappropriate land use, such as the intensive use of the land. Notwithstanding these differences, all definitions of land degradation include the notion of detrimental change in land or soil conditions and the actions of humans.

Most authors recognize that soils are a finite and non-renewable resource (Lal and Stewart, 1992) and this is central to the notion of land degradation. Hence, the challenge for sustainable use of existing land resources embraces both the avoidance of degrading processes, and the restoration of previously degraded land. The formerly it is used normally in the highland for restoring the soil fertility. For instance, the term restoration in this study is used as a generic term after the usage of Hobbs and Jasper (1996) whose suggest that restoration occurs along a continuum and that different activities are simply different forms of restoration. Restoration will usually focus on restoring ecosystem functions such as nutrient cycling, hydrological balance, and ecosystem resilience (Hobbs, 2002), although restoring the original flora may on occasions be a realistic and appropriate goal.

In the Bolivian highland there are three different ecosystems: the north, central and south, so called "altiplano". Part of the central highland is characterized to have high climatic risks (freeze periods that produce, sometimes, loss of whole crop, hail that cause loss of the vegetation, variable and scarce precipitations) and intensive land use causing over grassing and over extraction of plants for firewood. Over grazing and intensive use for crops lead the soils to be left without vegetation covering, consequently ready for eolic and hydrological erosion in particular in plateau plains and

slopes, respectively. In the former, it happens in windy periods a huge dust transportation in a similar way of large storm. For the last, in rainy periods, due to the lack of vegetation covering, a hydrological erosion occurs because the dragging of arable upper layer, leaving the soils poor in nutrients, and therefore, making difficult its fertility recovering. For this reason, farmers seated in these places conserve in their cultivation plots a system of communal rotation that includes a rest of the land from 3 to 23 years. In this sense, the land rest seems to allow the recovering of soil degradation, but supported by a natural fungal colonization on the plant rhizosphere.

In the first years of fallow plots, the soils begin to be colonized by seeds of native plants such as the *Baccharis incarum* that it is well adapted to the climatic and soil conditions of the highland. The development of these plants avoids the eolic and hydrological soil erosion, which in mature conditions work as a windy break, making the freeze effects low and gives to the soil a contribution of organic matter.

The *Baccharis incarum* develop at root level a mutual symbiosis with the arbuscular mycorrhizal fungi. In which processes the fungus by means of their fungal hyphae (Graham *et al.* 1982) increase the exploration surface and absorption of all the mineral elements. Those absorptions occurs mainly to the less soluble ions such as the copper, zinc and phosphorus, making possible the nutrition of plants in poor soils; in this symbiosis the plant provides to the fungus an ecological niche (Mosse, 1973; Ferrera, 1978).

There is scarcity of mycorrhiza fungus studies for the highland ecosystems. The most refers the presence of arbuscular mycorrhiza in crop rotations (Sivila, 1993) and the relationship of the presence of spores AV with the fallow time (Sivila and Herve, 1995). Other Latin American studies on arbuscular mycorrhiza in the mountain range is the Venezuelan studies (Montilla *et al.* 1992) where they highlighted the importance of these fungus in the development and nutrition of plants. Until nowadays it has not been carried out studies of soil recuperation in the highland taking into account the symbiotic relationship of the *Baccharis incarum* native specie and the arbuscular mycorrhizal fungus present in the fallow lands. Because these considerations, the aim of the present work was to evaluate the percentage of spores of arbuscular mycorrhizal fungus in the rhizosferic fallow soil of *Baccharis incarum* and to evaluate the grade of endophytic arbuscular mycorrhization present in this host plant.

## METHODOLOGY

### *Location and description of the area study*

The Pumani community is located in the Central Highland of the Aroma Province, La Paz department, in Bolivia. This community was chosen because the plots with 4, 7, 9, 17, 20 and 23 fallow years. This place has a tropical cold weather type, the texture of the soil is sandy-loamy with stone presence in the hillsides and foot of hillsides (Brugioni, 1992) with low and moderate organic matter content according to the texture characteristics, topography, soil colour and predominant vegetation; most of arable soil are between 3800 and 3950 m above level of sea.

### *Plot selection and sampling*

Taking into account previous works carried out by Sivila and Herve (1995) after observations in each plot, we selected those plants that had the same height (18 cm) and the same diameter crown (20 cm) approximately, taking a total of 10 plants for each plot. All of them were marked with red tapes, making a total of 60 plants.

### *Rhizosferic soil and root host-plant sampling*

The rhizosferic soil and root host-plant sampling were carried out according the method of Bohn (1979). The sampled rhizosferic soils were kept properly in labelled plastic bags and roots conserved in flasks glass previously labelled and containing fixation solution of formol-acid acetic-alcohol (FAA) until their analysis in laboratory.

### *Soil analysis*

To know the physical and chemical soil characteristics an analysis in the laboratory of the Centro de Investigación Agrícola Tropical (CIAT) located in Santa Cruz city (Bolivia) were carried out. We analyzed the following parameters: pH, electric conductivity, free carbonates, interchangeable cations (Ca, Mg, Na, K), total interchangeable bases, cation effective exchange capacity, saturation of bases, acidity, aluminium, phosphorus, organic matter, total nitrogen and texture.

### *Spore evaluation in soil sample*

To know the number of spores in the soil of the fallow plots, we had mixed all samples obtained from all 10 plots to obtain a mixed sample. A portion of 25 g of combined sample underwent through the separation of spores using in the first stage of the method of Gerdeman and Nicholson (1963) which uses a sieve and decantation in humid; and for the second stage, the employment of pre-fractions of sieves on filtering membranes to facilitate the counting of spores.

Once obtained the samples in the filtering membranes, this was taken to the stereo microscope for their observation and quantification. The evaluation of spores of the soil samples was carried out with ten repetitions.

### *Taxonomic identification of the spores*

To identify the spores we used the arbuscular mycorrhiza fungi manual of Schenck and Perez (1990), which indicates the separation of the fungus according its genus. To identify the fungus we took into account the spore colour, hyphae insertion into the spore, bulb presence or bulb absence, bulb form and spore size.

### *Evaluation of the mycorrhization in root samples*

To determine the percentage of mycorrhiza infection in the plant root, we used two stages: 1) to observe the infection, we tint the roots and afterwards a clear up of them; and 2) the mycorrhization grade in the *Baccharis incarum* specie was carried out using the method of Mosse (1973) denominated intersection of quadrants. This evaluation was carried out with ten repetitions.

### *Statistical analysis*

For the statistical analyses we used the standard deviation, mean standard error, correlation, regression, and the Tukey analysis. The hypothesis was that the difference between the fallow plots is not different from 0.

## RESULTS

### *Spore evaluation from the rizospheric soil in the fallow plots*

The spore evaluation from the rizospheric soils of *Baccharis incarum* it is shown in the Fig. 1. This evaluation shows that there was a positive increment in the average number of spores while the time increases. The highest number of spores was for the 23 year fallow plots with  $7.96 \pm 6.65$  spores in average. The lowest value was  $1.37 \pm 2.49$  spores found at 4 years fallow plots. The correlation between the average number of spores and the fallow years showed that for each year we can expect an increase of 320 spores ( $R^2=0.928$ ,  $p<1.27$ ) although the tendency of the spore

population could stabilize from 25 year ahead. In these years we can find a high density native plant population, showing the recuperation of the soil.

The statistical analysis of the Tukey test shows that there were significant differences between 4 and 23 years, 7 and 23 years, 9 and 23 years, and between 4 and 20 years without cropping.

### *Evaluation of root samples of extracted plants from fallow lands*

The VA evaluation at level of the root allows us to obtain the percentage of mycorrhizal infection in *Baccharis incarum* (Fig. 2).

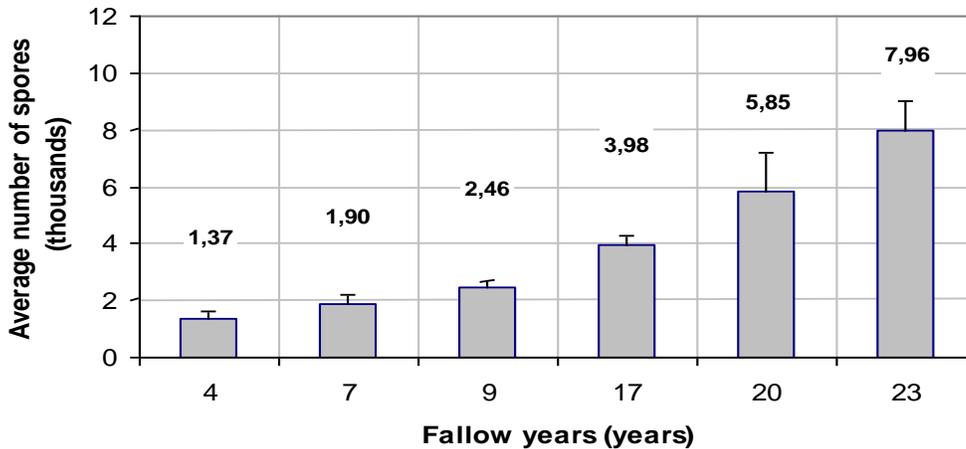


Fig. 1. Spore average number obtained from 100 g of soil sampled from fallow plots of the Pumani community.

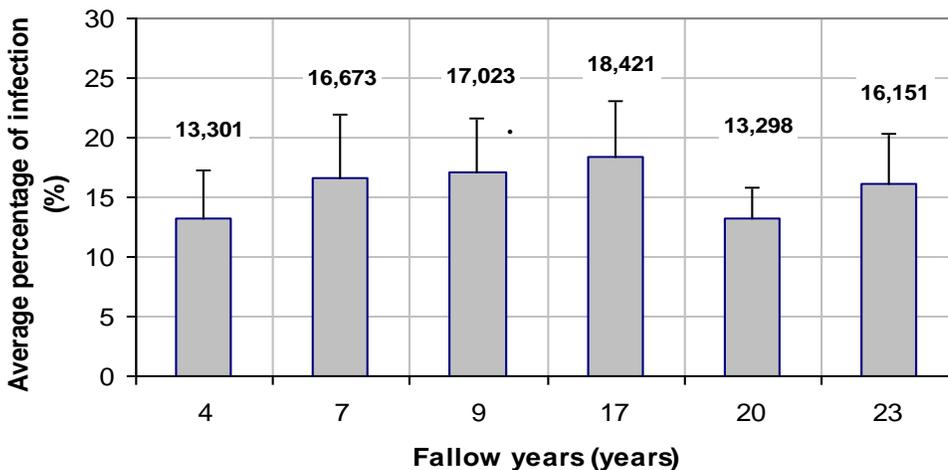


Fig. 2. Average percentage of root infection in *Baccharis incarum* obtained from fallow plots of the Pumani communi

**Table 1. Predominance of goods of spores in 100 grams of floor of the community of Pumani.**

Study areas	Average of spores/100 g of soil		
	<i>Glomus</i>	<i>Gygaspora</i>	<i>Scutello-spora</i>
Fallow plots of Pumani	904	720	384

We observed that there were small differences among the root infections. The Tukey test shows that it does not show significant differences.

*Spore gender VA identification in fallow soils of the community of Pumani.*

Once it was identified the spores by gender, we find what shows us the Table 1.

## Physical-chemical analysis of soil

The physical-chemical analyses were carried out in the laboratories of the Center of Agricultural tropical Investigation, the results show that they are floors sour, strong salinity, Mg and Na in low quantities, present match in low quantity, poor in organic matter and total nitrogen.

## DISCUSSION

According to sustainable plant-soil ecosystems occur when utilization of mineral resources by the plant is balanced by efficient biogeochemical cycling, such that nutrients are not rapidly exhausted and plant communities can exist in a stable form for prolonged periods. According to these authors, the concept of sustainability in agriculture aims to conserve the productive capacity of the soil, minimizing energy and resource use, and optimising the rate of turnover and cycling of matter and nutrients.

The importance of AM fungi is based on their role as a link between plant and soil (Bethlenfalvay and Linderman, 1992). With this link, AM fungi can help the plants to exploit more nutrients and water from the soil, which would not be exploited by the roots with no AM association. In this case AM symbiosis can increase the opportunity the exploration of P which will be used by the plant (Miller *et al.* 1995,

Arihara and Karasawa 2001, Miyasaka and Habte 2001).

Vesicular Arbuscular Mycorrhizal (VAM) fungi are important in sustainable agriculture because they improve plant water relations and thus increase the drought resistance of host plants (Allen and Allen, 1986; Nelsen, 1987), they improve disease control (Linderman, 1994), and they increase mineral uptake, which reduces the use of fertilizers (Ruiz-Lozano *et al.*, 1995). Improved plant water status and changes in water relations have been attributed to a wide variety of mechanisms, including some mechanisms not directly related to phosphorus nutrition or water uptake (Davies *et al.*, 1992, Smith and Gianinazzi-Pearson, 1988). Little is known about the physiological specialization and functioning of these soil microorganisms.

The figure 1 present the number of spores average of 10 repetitions of the community of Pumani in parcels of 4, 7, 9, 17, 20 and 23 years of rest, where the number of spores goes being increased as the fallow years goes increasing. We observe that in 4 year-old lands the number of spores is smaller with relationship to lands with 23 years of fallow.

The statistical analysis for repetitions indicates us, a positive and lineal correlation and that result is showing that the years of fallow plots have a proportional effect on the number of spores in the rhisopheric soil. Applying the test of Tukey for 1% level we show a high significant difference between the number of spores of 23 years fallow plots and the values of 4, 7 and 9 years fallow plots.

This result confirms what Sivila and Herve (1994) observed in the same area. The fallow plots from 1 years until 30 years is different because the lack of vegetation in first years of the fallow plots. The next years the spores of MVA do not germinate but rather they remain in latency. Afterwards, while the fungi do not find a host they finish perishing. Once occurs the establishment of the native flora, the mycorrhiza spores are stimulated by the root of these hosts, beginning the vital cycle that allows the reproduction of the spores in the soil.

According studies done for soil, the years of fallow plots and plant age affects the population of spores. Because those factors, the germination can generally be good or bad. In addition, one can say, *Baccharis incarum* and the fungi MVA have developed adaptations efficient ecophysiology as Sivila (1993) mentions. This is seeing on the native plots with *Baccharis incarum*, because in the beginning, the spores do not colonize all sites and as the fallow years happens the spores grows more in quantity.

Although the number of spores seems to be a possible indicator of the reestablishment of the biological fertility of the soil (Sivila and Herve, 1995), the present study do not show other correlation with climatic factors, which could be other parameter taken into account for next research.

The mycorrhiza colonization (Fig 2) shows that until 17 years of fallow the colonization percentage have an increment; afterwards it shows a decrement from 20 years.

Carrying out the analysis of the correlation between the infection and the fallow years, we observed a high association among the variables from 20 and 23. The statistical analysis of test Tukey at 5% shows that there is no significant differences among the fallow years. In this sense, one can show off that the fallow years do not influence on the percentage of colonization of the roots of *Baccharis incarum*. This behaviour of colonization in the fallow plots could be because the mycorrhiza activity it is not the same in all plots. The mycorrhization depends also by the soil characteristic, climatic, morphological and physiologic conditions of the host. Sivila (1993) indicates that the colonization of the plant roots can be affected by the climatic and soil characteristics.

We found no relationship between percentage of colonization and fallow years. That could be explained by the relationship between *Baccharis incarum* and the factors that stimulate the growth and mycelium ramification of the fungi MVA as indicates Vierheilig and Ocampo (1990).

The presence of *Glomus* is typical for cultivated and fallow soils (Azcón and Barea, 1980), on other words, soils intensively used. Several research show that under semiarid conditions, soil nutrients become less available due to water stress (Barber, 1984) and plant survival depends on the plant strategy to overcome the nutrient and water deficit. The differential capacities of plant types to access soil nutrients include differences in the surface area of contact between roots and soil and the composition and amount of root exudates (Jones *et al.*, 2004), rhizosphere microbiota (Rengel and Marschner, 2005) and the ability of plants to interact and select symbiotic microorganisms (Jones *et al.*, 2004; Van der Heijden *et al.*, 1998).

Arbuscular mycorrhizal fungi are able to improve nutrients and water acquisition ability assuring the survival and plant growth (Augé *et al.*, 1987) and also play an important role in nutrient cycling improving structural soil characteristics (Hodge *et al.*, 2001).

## CONCLUSIONS

- a) There is a high association between the number of spores and the fallow years in the plots of Pomani.
- b) The *Glomus* genus were dependent of plant type (mainly *Baccharis*) and soil management, which allow it to be used as soil quality indicator under semiarid conditions.
- c) Based on MVA populations as indicator the recuperation procedure appears to be developing towards the fallow plots.
- d) A low mycorrhizal colonization it is observed in the first fallow years and the beginning of growth plants.
- e) There is a good relationship between *Baccharis incarum* and the MVA fungi that shows a role for soil recuperation in the highland.

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