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# An appraisal on saprophytic and functional microbial communities associated to the carnivorous plant *Drosera latifolia* (Eichler) Gonella & Rivadavia (Droseraceae)

# Avaliação da comunidade microbiana saprofítica e funcional associada à planta carnívora *Drosera latifolia* (Eichler) Gonella & Rivadavia (Droseraceae)

Dennis Panayotis Saridakis[1]; Marco Antônio Nogueira[2]; Galdino Andrade Filho[3]; Franklin Galvão[4]

## Abstract

The carnivorous plant *Drosera latifolia* (Eichler) Gonella & Rivadavia (Droseraceae) colonizes humid, acidic, and oligotrophic soils, relying on several strategies to get nutrients for growth. Besides predation, microbial functional groups are critical for carbon and nutrient cycling in the rhizosphere, but there is no information on how they interact in the (endo) rhizosphere of *Drosera*. We assessed the occurrence of heterotrophic bacteria, saprophytic fungi, actinobacteria and pseudomonads and correlated them with microbial functional groups (cellulolytic, amylolytic, nitrogen-fixing and proteolytic bacteria) in the rhizosphere and endorhizosphere of *Drosera* collected during the winter and summer in two sites of natural occurrence (Rio Verde, SP and Joaquim Murtinho, PR). In each site, five samples of plants occurring on three substrates were taken: moss, peat, and sand. The population of heterotrophic bacteria correlated positively with saprophytic fungi, nitrogen-fixing bacteria that use malate as C source and amylolytic populations. The fungi showed a positive correlation with amylolytics, cellulolytics and both nitrogen-fixing bacterial groups. The correlation between the *Pseudomonas fluorescens* and amylolytic populations was negative, as well as between *P. fluorescens* and nitrogen-fixing bacteria that use malate as carbon source. Although *Drosera* nutrition partially relies on the capture of small animals, mainly insects, plants also interact synergistically with functional microorganisms associated to their rhizosphere, which could therefore contribute to the nutritional requirements under oligotrophic environments, helping the plant to cope with survival in low-fertility soils.

**Key words**: Soil Ecology. Carnivorous Plants. Oligotrophic Environment. Sundew.

## Resumo

A planta carnivora *Drosera latifolia* (Eichler) Gonella & Rivadavia (Droseraceae) coloniza solos úmidos, ácidos e de baixa fertilidade, e por isso depende de diversas estratégias para obter os nutrientes de que necessita. Além da predação, grupos funcionais de microrganismos atuam na ciclagem de carbono e nutrientes na rizosfera, mas há poucas informações sobre como essas interações ocorrem na rizosfera de *Drosera*. Avaliaram-se as ocorrências de bactérias heterotróficas, fungos saprofíticos, actinobactérias e pseudomonas, os quais foram correlacionados com grupos funcionais de microrganismos (celulolíticos, amilolíticos, fixadores de N e proteolíticos) na rizosfera e endorrizosfera de *Drosera*, coletada durante o inverno e verão, em duas localidades de ocorrência natural (Rio Verde, SP e Joaquim Murtinho,

[1] Doutorando em Conservação da Natureza, Universidade Federal do Paraná - UFPR. Email: dpsaridakis@gmail.com
[2] Doutor em Agronomia. Pesquisador da Embrapa Soja. Email: marco.nogueira@embrapa.br
[3] Doutor em Ecologia Microbiana. Professor Associado da Universidade Estadual de Londrina - UEL. Email: andradeg@uel.br
[4] Doutor em Engenharia Florestal. Professor Associado da Universidade Federal do Paraná - UFPR.

PR). Em cada local, foram amostradas cinco plantas ocorrendo sobre três tipos de substrato: musgo, solo orgânico e areia. A comunidade de bactérias heterotróficas correlacionou-se positivamente com fungos saprofíticos, fixadores de nitrogênio que usam malato como fonte de carbono e amilolíticos. Os fungos se correlacionaram positivamente com amilolíticos, celulolíticos e os dois grupos fixadores de nitrogênio (que usam malato e glicose como fonte de carbono). A correlação entre *Pseudomonas* e amilolíticos foi negativa, bem como entre *Pseudomnas* e as bactérias fixadoras de nitrogênio que usam malato como fonte de carbono. Embora a nutrição de *Drosera* dependa parcialmente da captura de pequenos animais, principalmente insetos, as plantas também interagem sinegisticamente com grupos funcionais de microrganismos associados à sua rizosfera, o que pode contribuir para o suprimento de nutrientes em ambientes oligotróficos, auxiliando a planta a se manter em solos de baixa fertilidade.
**Palavras-chave**: Ecologia do Solo. Plantas Carnívoras. Ambientes Oligotróficos. *Drosera*.

## Introduction

Microorganisms play important roles in the maintenance of soil fertility (ANDRADE, 1999). The solar power drives the soil microbial community as energy supply via photosynthesis that fix the carbon (C) that enters the soil as plant biomass and exudates through the rhizosphere (TATE, 1995). Many microbial groups that interact directly with plant nutrition, as rhizobia and mycorrhizal fungi, have been extensively studied (FITTER, GARBAYER, 1994; REQUEMA et al., 1997). However, little progress has been made on the relationships between other microbial groups also important for the maintenance of biological processes in soil. Understanding interactions between microbial communities according to their specific functional roles could better explain processes occurring in the soil, since this approach is more realistic than focusing on a particular species involved in a particular process. Although only a small percentage of the microbial community can grow in artificial conditions, considering that culturable microorganisms are a sample of the microbial community, they can be used in the monitoring of biological processes in soil (ANDRADE, 1999).

Carnivorous plants are so named for their capacity to attract, capture, digest and use substances from their preys for their own benefit (JUNIPER; ROBINS; JOEL, 1989), as adaptation to low-fertility soils, generally associated to flooding and anoxic conditions (JUNIPER; ROBINS; JOEL, 1989; BRUNDRETT; ABBOTT, 1991; ADAMEC, 1997). The capacity of capturing and digesting insects has been associated to nitrogen (N)-deficient habitats (ADAMEC, 1997). Raven et al. (2005) suggest that the low pH of substrates where these plants occur can hinder the occurrence and activity of N-fixing bacteria, but they can take advantage when associated to a host plant, resulting in mutual advantages.

The carnivorous plant *Drosera villosa* A. St.-Hil., recently reclassified as *Drosera latifolia* (Eichler) (GONELLA et al., 2014), occurs in habitats with high altitudinal ranges, wet, exposed to full solar radiation and established on low-fertility substrate (SARIDAKIS et al., 2004). Many authors have suggested interactions between carnivorous plants and microorganisms (JUNIPER; ROBINS; JOEL, 1989; SCHULZE; SCHULZE, 1997; KOOPMAN; CARSTENS, 2011), including free living N-fixing bacteria (JUNIPER; ROBINS; JOEL, 1989; SCHULZE; SCHULZE, 1997; SARIDAKIS, 2002; ALBINO et al., 2006) and culturable fungal root endophytes (QUILLIAM; JONES, 2010), arbuscular mycorrhizal fungi and dark septate endophytic fungi (FUCHS; HASELWANDTER, 2004; WEISHAMPEL; BEDFORD, 2006). Even when not capturing and digesting preys, the surfaces of *Drosera* traps are colonized by microorganisms (JUNIPER; ROBINS; JOEL, 1989) that also occur intercellularly (ANTHONY, 1992).

Despite several works conducted on the interactions between microorganisms and carnivorous plants, a unique article describing N-fixing microorganisms associated with *Drosera latifolia* has been published so far (ALBINO et al., 2006), but the interactions between C- and N-cycling

microbial functional groups remain unclear.

In a preliminary study (SARIDAKIS, 2002 - unpublished), found high occurrence of culturable microorganisms in *Drosera latifolia* roots, including bacteria able to grow in N-free culture medium and showing plant-growth promotion capacity (BATISTA-JUNIOR et al., 2002; MATSUMOTO et al., 2005; ALBINO et al., 2006; RAIMAM et al., 2007).

Based on these findings, we raised the following questions to be investigated in this work: *i)* How do these microbial populations behave in the rhizosphere and endorhizosphere of *D. latifolia*? *ii)* Which are the interactions that occur between the C- and N-cycling microbial functional groups in roots? *iii)* Do different substrates where the plant occurs affect the microbial populations?

## Material and Methods

*Experimental design and samplings*

The experimental design considered two seasons (winter and summer), three substrates of occurrence (moss, peat and sand), and isolations from rhizospheric soil and endorhizosphere. Samplings were carried out during winter (Wi) of 2000 and summer (Su) of 2001 at native wet grasslands (“Campos úmidos”) under full open sun sites, in Rio Verde locality, São Paulo State (24°05'00" S; 49°11'57" W), and Joaquim Murtinho locality, Paraná State (24°23'23" S; 49°51'21" W), to evaluate the microbial communities associated to *D. latifolia*. From each area, five plants with approximately the same size were collected from each substrate: moss (Mo), peat (Pe) and sand (Sa). Plants were collected with a monolith of substrate to assure the integrity of their roots. The soil adhered to roots was considered rhizosphere (Rhi), and the surface-sterilized root tissues were considered endorhizosphere (Er). In each season, 30 samples from rhizosphere and 30 from endorhizosphere were analysed, amounting to 60 samples per locality. Results of chemical analysis of the three different substrates in each locality are shown in Table 1.

*D. latifolia* samples were collected and kept in ice boxes (~ 7°C), in plastic bags, and processed within 12 h. Roots were separated from shoots and the rhizosphere soil was sampled. Roots were then washed under tap water, surface-disinfected (Sodium hipocloride 1% for 5 min), washed three times with sterile saline (0.85% NaCl) and cut in approximately 1 cm-long fragments. Each sample of roots or rhizosphere soil (1 g) was homogenized and suspended in 9 mL of sterile saline, following ten-fold dilutions up to $10^{-8}$ and kept at 5°C until plating in specific culture media (ZUBERER, 1994).

*Assessment of the microbial populations*

The populations of heterotrophic bacteria, saprophytic fungi, actinobacteria, fluorescent pseudomonads, and the microbial functional groups of C (cellulolytic and amylolytic) and N (free living N-fixing bacteria) were assessed using appropriate culture media (Table 2). For N-fixing bacteria, two groups were estimated: one that uses glucose as carbon source (GCS) and other that uses malate as carbon source (MCS). Aliquots (100 µL) of ten-fold dilutions ($10^{-4}$ to $10^{-7}$ for N-fixing bacteria, $10^{-3}$ to $10^{-6}$ for the other microbial groups) of the homogenized samples of rhizosphere soil and *D. latifolia* roots were spread on duplicate Petri’s dishes containing the appropriate respective culture medium.

**Table 1** - Chemical analysis of the three substrates where *Drosera latifolia* occurs naturally at Joaquim Murtinho – PR and Rio Verde – SP.

| LOCALITY | Attribute | Moss | Sand | Peat |
|---|---|---|---|---|
| Joaquim Murtinho | Organic matter (%) | 57.9 | 20.2 | 42.2 |
| | pH $CaCl_2$ | 6.1 | 6.0 | 4.1 |
| | Al ($cmol_c\ dm^{-3}$) | 0.60 | 0.80 | 1.60 |
| | N ($g\ dm^{-3}$) | 2.90 | 1.01 | 2.11 |
| | Ca ($cmol_c\ dm^{-3}$) | 0.50 | 0.02 | 0.02 |
| | Mg ($cmol_c\ dm^{-3}$) | 0.45 | 0.04 | 0.04 |
| | K ($cmol_c\ dm^{-3}$) | 0.41 | 0.05 | 0.15 |
| | P ($mg\ dm^{-3}$) | 6.35 | 1.46 | 2.25 |
| Rio Verde | Organic matter (%) | 47.9 | 20.2 | 83.2 |
| | pH $CaCl_2$ | 6.1 | 6.9 | 4.1 |
| | Al ($cmol_c\ dm^{-3}$) | 0.60 | n.d. | 1.30 |
| | N ($g\ dm^{-3}$) | 2.39 | 1.01 | 0.90 |
| | Ca ($cmol_c\ dm^{-3}$) | 0.34 | 0.78 | 0.31 |
| | Mg ($cmol_c\ dm^{-3}$) | 0.28 | 0.56 | 0.39 |
| | K ($cmol_c\ dm^{-3}$) | 0.31 | 0.15 | 0.28 |
| | P ($mg\ dm^{-3}$) | 2.25 | 2.12 | 3.57 |

**Table 2** - Culture media for enumerating heterotrophic bacteria (TSA), saprophytic fungi (PDA), actinobacteria (casein starch media), fluorescent pseudomonads (King's B) and the microbial functional groups of microorganisms from C (cellulolytic and amylolytic media) and N cycle (NFb and Burk media).

| Microbial Group | Culture medium origin/constitution |
|---|---|
| Heterotrophic bacteria | Tryptic Soy Agar - DIFCO order number 0369-17-6. |
| Saprophytic fungi | Potato Dextrose Agar - DIFCO order number 0013-17-6. |
| Actinobacteria (KUSTER; WILLIAMS, 1964) | Soluble Starch 10.0 g, Casein 0.3 g, $KNO_3$ 2.0 g, NaCl 2.0 g, $K_2HPO_4$ 2.0 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01 g, agar 15 g, distilled water 1000 mL, pH = 6.5 to 7.0. |
| Fluorescent pseudomonads (SCHER; BAKER, 1982) | King's B - Peptone 20 g, Glycerine 10 mL, $K_2HPO_4$ 1.5 g, $MgSO_4.7H_2O$ 1.5 g, agar 15 g, distilled water 1000 mL, pH = 6.5 to 7.0. |
| Cellulolytic (WOOD, 1980) | 5.0 g carboxymethyl cellulose, 1.0 g $NO_3NH_4$, 50 mL NaCl solution (0.85%), 950 mL soil extract (v:v), 15.0 g agar, pH = 7.0. Halo development: Pour 1 M NaCl to the medium for 5 min.; remove; add a 0.1% Red Congo solution for 30 min.; rinse with distilled water until the |
| Amylolytic(PONTECORVO et al., 1953) | 10 g soluble starch, 10 g casein, 1.0 g glucose, 3.0 g $Na_2HPO_4$, 0.1 g $MgSO_4.7H_2O$, 15 g agar. Degradation halo: add iodine solution to the surface of the medium, remove the excess and count the halo-forming colonies. |
| N-fixing bacteria using glucose as C source (Wilson and Knight, 1952) | Burk medium: Solution A: 6.4 g $K_2HPO_4$ 6.4 g, 1.6 g $KH_2PO_4$ and 1,000 mL distilled water; Solution B: 2.0 g NaCl, 2.0 g $MgSO_4.7H_2O$, 0.5 g $CaSO_4.2H_2O$ and 1,000 mL distilled water; Solution C: 0.01 g $NaMoO_4.2H_2O$, 0.03 g $FeSO_4$ and 1,000 mL distilled water; Medium compositions: 100 mL solution A, 100 mL solution B, 100 mL solution C, 5.0 g glucose, 15.0 g agar and 700 mL distilled water, pH = 7.0. |
| N-fixing bacteria using malate as C source (DÖBEREINER; Day, 1976) | NFb medium: 0.4 g $KH_2PO_4$, 0.1 g $K_2HPO_4$, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2$, 0.01 g $FeCl_3$,0.002 g $MoO_4Na.2H_2O$, 5.0 g sodium malate, 5.0 mL 0.5% bromotimol blue, 15.0 g agar and 1000 mL distilled water pH = 6.8. |

Plates were incubated at 28°C and the colonies counted after 3 and 5 days to allow the development of slower-growing microorganisms and the number of colony forming units (CFU) per gram of dry soil or fresh roots was evaluated. Cellulolytic and amylolytic populations were detected by the presence of a degradation halo around the colonies. The free living N-fixing growing media were maintained under microaerophilic conditions for the adequate growth. Any growth in the specific medium was considered as positive for this group.

*Statistical Analysis*

Data were submitted to analyses of variance (ANOVA), followed by Tukey's honest significant difference (HSD) test to compare means among factor levels. Linear regression was also considered to check for relationships between variables using a model II for regression analysis. The significance level for all analyses was at $p \leq 0.05$. For regression analysis, both variables (X and Y) were tested as independent and dependent. We considered the regression with the higher coefficient of determination ($r^2$) value, assuming X as the independent and Y as the dependent variables. The coefficient of determination establishes and estimates the dependence of a variable upon another, describing a functional relationship (SOKAL; ROHLF, 2000).

**Table 3 -** Colony forming units - CFU (log CFU g dry sample$^{-1}$) of different general and functional groups of microorganisms (total heterotrophic bacteria, saprophytic fungi, actinobacteria, fluorescent pseudomonads) obtained from the rhizospheric soil (RHI) or endorhizosphere (ER) of *D. latifolia.*

| | **Heterotrophic bacteria** | **Saprophytic fungi** | **Actinobacteria** | **Fluorescent pseudomonads** |
|---|---|---|---|---|
| **Season** | | | | |
| **WI** | 6.92 b | 4.69 b | 4.64 a | 5.36 a |
| **SU** | 7.37 a | 5.72 a | 4.07 b | 3.58 b |
| **ANOVA** | <0.01 | <0.01 | <0.01 | <0.01 |
| **Substrate** | | | | |
| **MO** | 7.22 a | 5.27 a | 4.51 a | 4.72 a |
| **PE** | 7.02 a | 4.95 a | 4.00 b | 4.37 a |
| **SA** | 7.32 a | 5.46 a | 4.74 a | 4.42 a |
| **ANOVA** | n.s. | n.s. | < 0.05 | n.s. |
| **Sample** | | | | |
| **RHI** | 7.04 a | 4.95 b | 4.48 a | 4.17 b |
| **ER** | 7.18 a | 5.42 a | 4.13 b | 4.77 a |
| **ANOVA** | n.s. | < 0.01 | < 0.01 | < 0.01 |

Means followed by the same letter (vertically) are not significantly different (Tukey's honest significant difference HSD test, $p \leq 0.05$). Season: winter (WI), summer (SU); substrate: moss (MO), peat (PE), sand (SA); sample: rhizosphere (RHI), endorhizosphere (ER).

## Results and Discussion

The population of culturable heterotrophic bacteria was smaller in winter than in summer, and no significant difference was observed between substrates and between rhizosphere and endorhizosphere (Table 3). A smaller population of culturable saprophytic fungi was observed in winter, with no significant differences among substrates, but were greater in the endorhizosphere (Table 3). The actinobacteria had greater occurrence in winter, in the sandy substrate, and in the rhizosphere (Table 3). The fluorescent pseudomonads also had more prevalence in winter, while no difference was observed among the substrates, but a higher CFU was found in the endorhizosphere than in the rhizosphere soil (Table 3).

The influence of different seasons was observed in the occurrence of the microbial functional groups, which increased in the summer (Table 4). In relation to substrates from where the samples were taken, only the cellulolytic presented a significant difference, showing lower CFU on moss and increased on peat and sand (Table 4). Considering the rhizosphere and the endorhizosphere, only the cellulolytics and the N-fixing malate users showed greater population in the rhizosphere. Preliminary data on the presence of free living N-fixing populations on the roots of *D. latifolia* were confirmed in this work. The number of CFU of free living N-fixing GCS in the rhizosphere was similar to the endorhizosphere, and the same happened with the population of amylolytic. However, free living N-fixing MCS and cellulolytic populations presented a higher population in the endorhizosphere (Table 4).

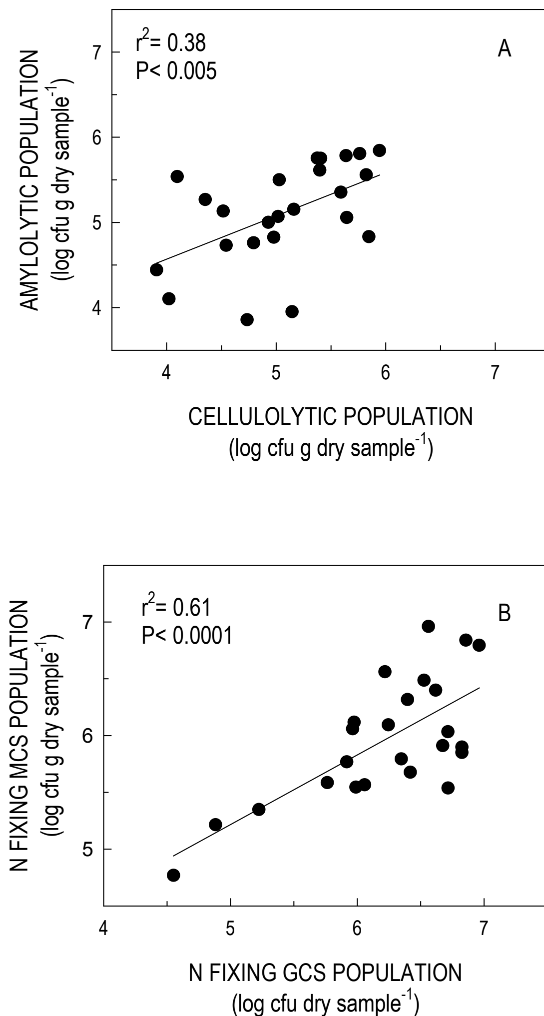
The determination coefficients obtained by the regressions between populations of functional microorganisms of C and N cycles indicate that cellulolytic population has positive correlation with the amylolytic ($r^2 = 0.38$ $p<0.005$) (Figure 1A), the same occurring between the two free living N fixing populations ($r^2 = 0.61$ $p<0.0001$) (Figure 1B).

**Table 4 -** CFU (log CFU g dry sample$^{-1}$) of functional groups of microorganisms (amylolytic; cellulolytic; free living N-fixing bacteria that use glucose as carbon source and free living N-fixing that use malate as carbon source) obtained from the rhizospheric soil (RHI) or endorhizosphere (ER) of *Drosera latifolia*.

| | Amilollytic | Cellulolytic | N-fixing glucose user | N-fixing malate user |
|---|---|---|---|---|
| **Season** | | | | |
| **WI** | 4.74 b | 5.02 b | 5.79 b | 5.88 b |
| **SU** | 5.37 a | 5.17 a | 6.08 a | 6.42 a |
| **ANOVA** | <0.0001 | <0.02 | <0.0001 | <0.0001 |
| **Substrate** | | | | |
| **MO** | 5.09 a | 4.87 b | 5.87 a | 6.12 a |
| **PE** | 5.10 a | 5.14 a | 6.04 a | 6.14 a |
| **SA** | 5.04 a | 5.31 a | 5.98 a | 6.39 a |
| **ANOVA** | n.s. | <0.02 | n.s. | n.s. |
| **Sample** | | | | |
| **RHI** | 5.10 a | 5.18 a | 5.91 a | 6.27 a |
| **ER** | 4.98 a | 4.98 b | 5.90 a | 5.94 b |
| **ANOVA** | n.s. | <0.08 | n.s. | <0.01 |

Means followed by the same letter (vertically) are not significantly different (Tukey's honest significant difference HSD test, p≤*0.05*). Season: winter (WI), summer (SU); substrate: moss (MO), peat (PE), sand (SA); sample: rhizosphere (RHI), endorhizosphere (ER).

**Figure 1** - Interactions between culturable populations of functional groups of microorganisms from C and N cycles isolated from the rhizosphere and endorhizosphere of *Drosera latifolia*. Linear regression ($p \leq 0.05$): (A) Culturable amylolytic and cellulolytic populations; (B) Culturable populations of free living N-fixing MCS (malate as carbon source) and free living N-fixing GCS (glucose as carbon source).




The determination coefficients among the functional groups of C cycle with those of N cycle were also highly significant. The amylolytic population, responsible for cellulose degradation, was positively correlated with both free living N-fixing populations. The correlation between amylolytic population and N-fixing GCS population presented a determination coefficient ($r^2 = 0.46$ p<*0.0001*) (Figure 2A) very similar to the N fixing MCS population ($r^2 = 0.45$ $p<0.0001$) (Figure 2B). The cellulolytic also showed positive determination coefficient with N-fixing GCS population ($r^2 = 0.44$ p<*0.0001*) (Figure 3A), as well as by N-fixing MCS population ($r^2 = 0.48$ $p<0.0001$) (Figure 3B).

**Figure 2** - Interactions between culturable amylolytic and different functional groups of free living N-fixing bacteria isolated from rhizosphere and endorhizosphere of *D. latifolia*. Linear regression ($p \leq 0.05$): (A) Culturable amylolytic and free living N-fixing GCS (glucose as carbon source) populations; (B) Culturable amylolytic and free living N-fixing MCS (malate as carbon source) population.

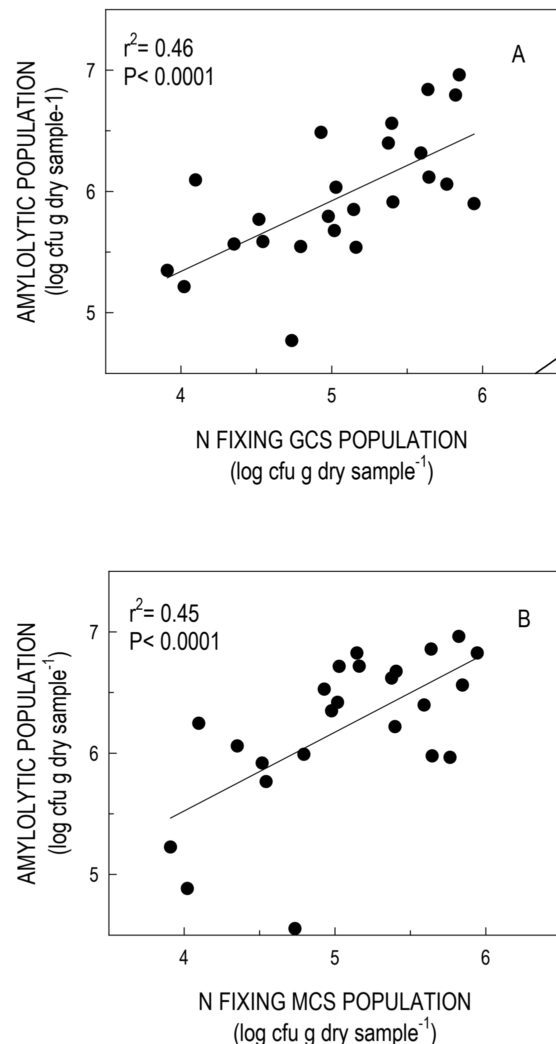
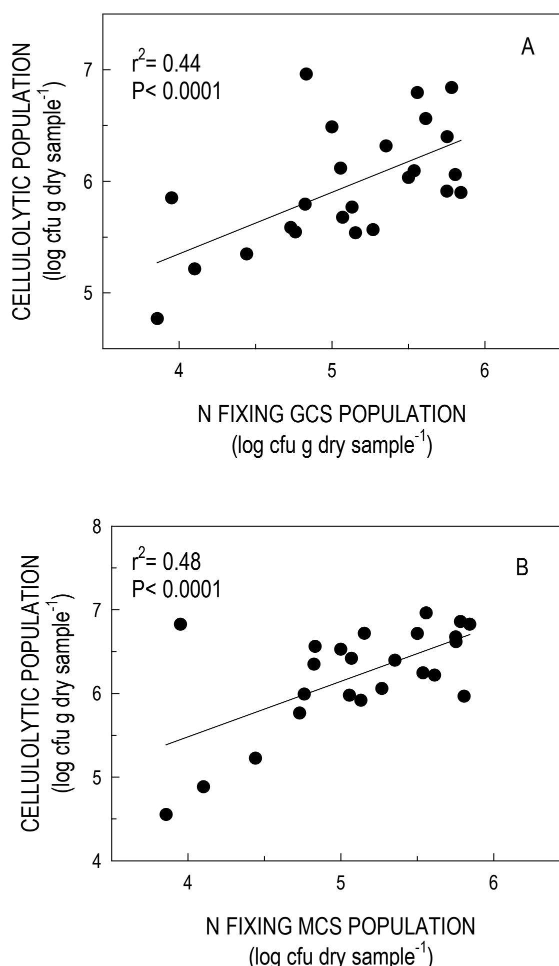


**Figure 3** - Interactions between culturable cellulolytic population and different functional groups of N-cycling bacteria isolated from rhizosphere and endorhizosphere of *D. latifolia.* Linear regression (p≤0.05): (A) cellulolytic and free living N-fixing GCS (glucose as carbon source) populations; (B) Culturable cellulolytic and free living N-fixing MCS (malato as carbon source) population.




This study on the interactions between functional groups of microorganisms of C and N cycles from the roots of *D. latifolia* was driven by the following factors: use of small arthropoda (mainly insects) as part of the nutrition of this plant species; the low soil fertility; the absence of mycorrhizal colonization in most of cases (QUILLIAM; JONES, 2010) and the presence of a high population of free living N-fixing bacteria associated with the rhizosphere and endorhizosphere.

Considering that summer is the warmer season, higher populations of all microorganisms associated with *D. latifolia* roots were expected, due to more intense metabolic activity of the plants as well as microorganisms. This higher activity induces the roots to release significant amounts of sugars, aminoacids, hormones and vitamins, increasing the rhizodeposition and the availability of nutrients in the rhizosphere (WHIPPS, 1990). However, during the winter, these plants have their metabolic activities diminished, decreasing the secretion of exudates and, consequently, the density of microorganisms associated to their roots. It is well known that the availability of plant-derived organic compounds regulate the microbial communities in soils (SPARLING, 1997; OLSSON; PERSSON, 1999).

In spite of higher cellulolytic population have occurred in summer, different factors influence their growth, since these microorganisms degrade cellulose, a compound not available in the exudates. However, the population of cellulolytic was the only one, among the four groups assessed, which presented different population densities in the different substrates. A possible explanation for the smaller population in moss is the higher mineralization rate of this substrate by microbial enzymes other than cellulases due to fewer occurrences of more complex carbon compounds like cellulose.

The amylolytic and N-fixing GCS populations did not show preference for a specific root zone; however, cellulolytics and N-fixing MCS predominated in the rhizosphere. The populations of amylolytic and N-fixing depend upon the nutrients released by the breakdown of cellulose by cellulolytics as well as the nutrients released by plant roots through lysates and exudates (HORNBY, 1990). The prevalence of cellulolytic population in the rhizosphere soil was expected, since more substrate available for these microorganisms are found outside the roots. However, the difference between rhizosphere and endorhizosphere populations is small, and there

is a high number of individuals living in both endorhizosphere and rhizosphere soil. Probably, the population of N-fixing microorganisms also occurs in the roots of *D. latifolia*, since availability of C source in other plants, as malic acid and glucose, are present in root cells and in their exudates (SYLVIA et al., 1998).

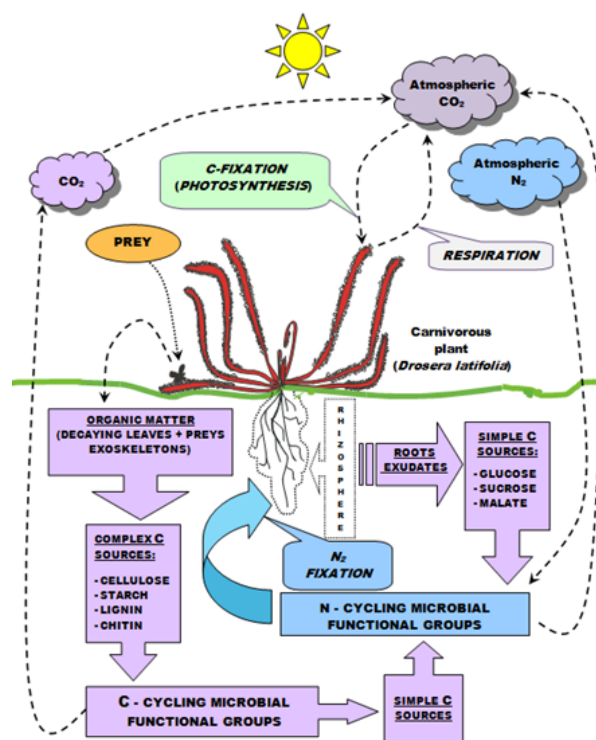
The highly significant positive coefficient of determination obtained between cellulolytic and amylolytic populations could be also due to the rhizodeposition along with the plant growth. Curl and Truelove (1986) demonstrated that the composition of organic matter deposited by roots varies with the plant species, age and growth conditions, which directly affects the microbial composition in the rhizosphere. These same factors can also be involved in the positive correlation between the two free living N-fixing populations (OLSSON; PERSSON, 1999).

The interactions between amylolytic and the two N-fixing populations showed a positive correlation due to the release of sugars and N. We can also deduce that the same happened between N-fixing and the cellulolytics since both populations of free living N-fixing presented a high positive correlation with the latter.

Sugar availability seems to be the regulator factor of the interaction between these populations. Cellulolytics make available the glucose from starch, while the N-fixation introduces N in the plant root, stimulating the rhizodeposition. The effect observed by the action of the two N-fixing populations on cellulolytic population can also be a result of plant growth, which could be influenced by various factors such as N-fixation.

The two free living N-fixing populations were also positively correlated with the amylolytic population. This effect could be involved with the acceleration of plant metabolism, thus increasing rhizodeposition and the amount of available starch in the plant-soil system. The interactions among these groups certainly influenced plant nutrition, contributing to increase the N input to the plant. Our results clearly show that *D. latifolia* interacts with the soil microbial communities in the rhizosphere and endorhizospere to increase the nutrient cycling in the soil-root interface at oligotrophic environments, in addition to nutrients obtained from preys (Figure 4). Saprophytic fungi, cellulolytic and amylolytic play essential role on C cycling in soil, whereas the N-fixing bacteria, once supplied with simpler C sources by means of these microorganisms can contribute with the plant requirements for N, showing clearly that, at least for the studied conditions, carnivory is not the only strategy that allows *Drosera latifolia* to grow at distrophic environments.

**Figure 4** - Schematic model representing the interactions between Carbon and Nitrogen microbial functional groups, C and N cycles and *Drosera latifolia* at its natural habitat.



## Conclusions

Besides having part of its nutrition from captured preys, *D. latifolia* interacts synergistically with amylolytic, cellulolytic and free living N-fixing microorganisms associated with their roots, which help this species to cope with nutrient-limiting conditions. In answer to the raised questions, we can state: *i)* The C and N microbial populations are generally more abundant in the rhizosphere than in the endorhizosphere of *D. latifolia*; *ii)* The C and N microbial functional groups are directly correlated, showing a close interaction each other; and *iii*) Except for cellulolytics, the substrate on which the plant grow have no effect on the microbial population, showing that the plant have more decisive role on the control of the microbial population in its rhizosphere than the substrate on which the plant grow.

To our knowledge this is the first report on the interactions between microbial functional groups of C and N cycles in the rhizosphere of the carnivorous plant *D. latifolia*.

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