HYPHOMYCETES ASSOCIATED WITH SUBMERGED BAMBOO LEAVES (*Bambusa vulgaris*) IN QUEBRADA DE ORO IN MAYAGÜEZ, PUERTO RICO

By

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ABSTRACT

Leaf litter is a major source of nutrients for heterotrophic organisms in aquatic ecosystems in which microorganisms contribute significantly to the breakdown process of leaf litter. Fungi as aquatic hyphomycetes are dominant decomposers in many aquatic environments. Common bamboo (*Bambusa vulgaris*), an introduced species, has reduced the diversity of the riparian vegetation; in Puerto Rico. The main goal of this study was to identify aquatic hyphomycetes associated with bamboo leaf litter by placing leaves of common bamboo inside mesh bags that were submerged at three stations along Quebrada de Oro. At each sampling site, physico-chemical parameters were measured, leaves bags were exposed to fungal colonization, and natural leaf litter and water samples were collected to monitor the fungal community and water quality. Leaf discs from samples of each bag were mounted on slides with lactophenol-cotton blue and examined under the microscope. Other discs were placed in aeration chambers to induce fungal growth and sporulation. Water samples from the stream were filtered through membrane filters and discs of the leaf litter were stained with lactophenol-cotton blue to observe conidia. The temperature, dissolved oxygen concentration, and pH registered during this study were within the standard values. Conidia of the genera *Anguillospora Campylospora*, *Helicomyces, and Pyramidospora* were found on bamboo leaf discs. Twenty-seven species were found on the bamboo leaf discs in the first phase of monitoring and twenty-eight in the second phase of monitoring. Conidia found in water samples from the stream belonged to the genera *Anguillospora, Campylospora*, *Clavariopsis, Clavatospora*, and *Pyramidospora.* Dissolved oxygen concentration was the factor with the highest

correlation with the species found, particularly in the first phase of monitoring (r = 0.67). Most of the species observed on the bamboo leaves were also found in the water column. About ten of the twenty-four species found on the water column were observed on bamboo leaves. However, the number of species counted in the water column was lower than in a previous report from the same stream. The aquatic fungal community in Quebrada de Oro used the bamboo leaves as substrates, especially *Campylospora* and *Helicomyces*. Apparently, bamboo leaves provide a good habitat for some aquatic hyphomycetes when the results are compared with other studies made with native and exotic vegetation in Puerto Rico. Although two different techniques were used to monitor the colonization of bamboo leaves, the same number of species and similar species composition were observed on these substrates.

RESUMEN

La hojarasca es una fuente importante de nutrientes para organismos heterótrofos en ecosistemas acuáticos. Los microorganismos contribuyen significativamente al proceso de descomposición de hojarasca. Los hongos, como los hifomicetos acuáticos, son descomponedores dominantes en muchos ambientes acuáticos. El objetivo de este estudio fue identificar los hifomicetos acuáticos asociados a la hojarasca de bambú. El bambú (*Bambusa vulgaris*) fue introducido a Puerto Rico y ha reducido la diversidad de la vegetación riparina. Se colocaron hojas de bambú común en bolsas de mallas que fueron sumergidas en tres estaciones a lo largo de la Quebrada de Oro. En cada área de muestreo, se midieron parámetros físico-químicos, las hojas de bambú se expusieron a la colonización por hongos y se colectó hojarasca y agua para monitorear la comunidad de hogos y la calidad del agua. Se montaron discos de hojas en laminillas con lactofenol con azul de algodón y se examinaron bajo el microscopio. También, algunos discos se colocaron en cámaras de aeración para inducir el crecimiento y esporulación de los hongos. Las muestras de agua fueron filtradas a través filtros de membrana y los discos de las hojarascas fueron teñidos con lactofenol con azul de algodón para observar las conidias. La temperatura, concentración de oxígeno disuelto y pH registrada durante este estudio fueron entre los valores estandares. Se encontraron conidias de los géneros *Anguillospora*, *Campylospora*, *Helicomyces* y *Pyramidospora* en las hojas del bambú. Veintisiete especies fueron encontradas en las hojas de bambú en la primera fase de muestreo y veintiocho en la segunda fase de muestreo. Las conidias encontradas en las muestras de agua pertenecen a los géneros *Anguillospora*, *Campylospora*, *Clavariopsis*, *Clavatospora* y *Pyramidospora*. El oxígeno disuelto fue el factor con mayor correlación

con las especies encontradas, particularmente en la primera fase de muestreo (r = 0.67). La mayoría de las especies observadas en las hojas de bambú fueron encontradas también en la columna de agua. Como diez especies de las veinticuatro especies encontradas en la columna de agua fueron observadas en las hojas de bambú. Aunque, el número de especies contadas en la columna de agua fue menor que en reportes previos en la mismo cuerpo de agua. La comunidad de hongos acuáticos en la Quebrada de Oro usa las hojas de bambú como substrato, especialmente *Campylospora* y *Helicomyces*. Aparentemente, las hojas de bambú proveen un buen hábitat para algunos hifomicetos acuáticos cuando éstas se comparan con los resultados obtenidos con otros estudios realizados con vegetación nativa y exótica en Puerto Rico. Aunque se utilizaron dos técnicas diferentes para monitorear la colonización de las hojas de bambú, el mismo número de especies y una composición similar de especies fueron observadas en este substrato.

DEDICATION

To Dad

Sometimes we do not value the wealth until we lose them. You were that invaluable treasure in my life. I never knew how much I missed you until you were gone. You were our model and our inspiration. You always admitted your mistakes and advised us. You left us the major bequest, to be thoughtful and honest. Thanks for leaving us the best of your inheritances, an education, which you did not have but wished that your children had. Thanks to you and to mom, I am who I am. Thanks for your love...

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Thank God for giving the opportunity to this soul to be here in your World…

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INTRODUCTION

Riparian ecosystems are influenced by and depend much of the time on allochtonous organic matter originating from riparian vegetation (Hershey and Lamberti, 2001). In the water, leaves are lixiviated and colonized by microorganisms that modify their composition, as a product of the leaf breakdown by biotic and abiotic factors (Suberkropp, 2001). The resulting detritus is disintegrated and converted into sources of energy (Hershey and Lamberti, 2001; Suberkropp, 2001) for aquatic microorganisms (e.g. filtering organisms, shedders) through the abrasive force of the water and the mechanical and enzymatic action of decomposers (e.g. bacteria, macroinvertebrates, and fungi).

Dominant microorganisms in the process of leaf litter decomposition are aquatic and aero-aquatic hyphomycetes (Suberkropp and Weyers, 1996; Suberkropp, 2001), collectively known as Ingoldian or freshwater hyphomycetes. The role of these heterotrophic organisms on submerged leaves and wood has been widely studied (Nilsson, 1964; Ingold, 1974; Suberkropp, 1991; Bärlocher, 1992). They can grow on the surface of the substrate and inside the vascular system of the leaves, forming a hyaline mycelium (Nilsson, 1964). Also, they can be found in the roots of riparian vegetation (Sridhar and Bärlocher, 1992), have a wide distribution (Bärlocher, 1992), and can be found in diverse habitats like wetlands (Hackney et al., 2000), streams (Chauvet and Suberkropp, 1998; Wong et al., 1998), and lakes (Tubaki, 1957).

Aquatic hyphomycetes are characterized by morphological and physiological adaptations that lead to the colonization of the substrate in the water (Bärlocher, 1992; Suberkropp, 2001). As a general feature, aquatic hyphomycetes are hyaline and can grow

and sporulate while attached to the submerged substrates. Some aquatic hyphomycetes produce sigmoid conidia, which attach to the substrate by one of their appendices, and produce mucilage that improves attachment. Most species, however, make tetraradial conidia, characterized by multiple appendages that provide a better chance for attachment to the substrate (Wong et al., 1998).

Aero-aquatic hyphomycetes can grow under submerged or exposed conditions, and produce helicoid or elaborate, multi-celled conidia that trap air-bubbles during their formation. These conidia are adapted for flotation and their development is induced by the exposure of the fungi-harboring substrates to atmospheric oxygen pressures. The ecological role of this component of the fungal community has been underestimated in tropical streams (Santos-Flores, pers. comm.).

In the leaf colonization process, a common pattern is observed. There is a period of fungal growth without sporulation followed by a sudden increase of sporulation, and ultimately a decrease in growth (Maharning and Bärlocher, 1996; Chauvet and Suberkropp, 1998). In addition, there are studies on the colonization of fungi in different types of leaves, using ergosterol as indicator of fungal biomass within the substrate. This compound was shown to increase with time, reaching a peak in concentration and then declining (Gessner and Chauvet, 1994).

There are some environmental factors, like temperature, that affect the sporulation of the aquatic fungi, resulting in a lineal relation between the factor and the sporulation of some species (Chauvet and Suberkropp, 1998). The optimal temperatures for growing and sporulation of common aquatic hyphomycetes are between 20 and 30^{o}C. Bärlocher (1992) indicated that some species grow better at temperatures between 15 and 25^{o}C in

temperate zones, but 25^{o}C seems the optimal temperature for common tropical species (Rajashekhar and Kaveriappa, 2000). Light is another factor that influences the reproductive capacity of fungi. Rajashekhar and Kaveriappa (op. cit.) found that continuous light exposure stimulated the sporulation in some tropical species. Precipitation and contamination can also affect the diversity, reproduction, and the biomass of the aquatic hyphomycetes (Raviraja et al., 1998).

The amount and diversity of the conidia in foam, the water column, and submerged leaves are indicators of the activity of the freshwater hyphomycetes in aquatic ecosystems (Suberkropp, 1991). However, few studies have considered the fungal community of polluted, urban streams. Many of the available studies on fungal colonization and degradation of submerged leaf litter have been conducted in temperate streams, and few included the examination of recalcitrant (i.e. hard to degrade) substrates or exotic plant material.

The main goal of this study was to provide information about the species of freshwater hyphomycetes associated with bamboo leaf litter in Quebrada de Oro, a subtropical stream in western Puerto Rico. Common bamboo (*Bambusa vulgaris*) is a non-indigenous plant, which was introduced into Puerto Rico to prevent erosion in the littoral zone of some freshwater ecosystems. The introduction of this species affected, and has displaced, some of the native plants, and the effect of this exotic species into the food web and the composition and diversity of aquatic organisms is unknown. This research also explores the correlation between some physico-chemical parameters and the fungal diversity in the water and on submerged of bamboo leaves. These data will update the information about the freshwater hyphomycetes in Quebrada de Oro an aquatic

ecosystem now studied for over twenty years (Betancourt and Justiniano, 1989; Santos-Flores, 1996; Santos-Flores and Betancourt, 1997; present study).

OBJECTIVES

The objectives of this study were to:

(1) identify freshwater (aquatic and aero-aquatic) hyphomycetes associated with submerged bamboo leaves using the leaf-pack method;

(2) study the hyphomycetes community in the Quebrada de Oro stream by monitoring naturally occurring leaf litter and sampling water from the stream to determine if changes on fungal diversity on bamboo leaves were correlated with changes in the conidial population of the stream;

(3) correlate traditional limnological parameters (i.e. temperature, dissolved oxygen concentration, pH, nitrate, and phosphate) of the stream water with the aquatic fungal diversity at three different stations in Quebrada de Oro.

LITERATURE REVIEW

Common Bamboo

Bambusa vulgaris (Poaceae, Bambusoideae) was described by Wendland in 1810, and is a member of the tribe Bambuseae and subtribe Bambusinae. The subfamily Bambusoideae has a wide distribution, but the majority of the species occur in the tropics (Soderstrom and Calderón, 1979b). Categorized as a non-native species in the New World, *B. vulgaris* is found from Argentina to the United States, including the Caribbean region, and is usually cultivated (Judziewicz et al., 2000). It is the most widely distributed bamboo species in the world (Mc Clure, 1967). The native home of the common bamboo is unknown and some conjectures of its origin are Madagascar, Java, and Sri Lanka (Farrelly, 1984).

The plant produces green erect or suberect culms about 20-50 feet long. However, some culms can be yellow to green to brown with or without green stripes. Several branches come from each node, and leaves are narrowly to broadly lanceolate. The common bamboo generates abundant leaves, and is monocarpic, flowering once and dying after this period. Usually, *B. vulgaris* does not flower for decades. As a consequence, this species relies on vegetative reproduction for its dispersal. It has the ability to grow under a wide range of soil and moisture conditions for easy propagation.

Bambusa vulgaris is a remarkable plant for its adaptation to a variety of habitats, but susceptibility to a beetle of the genus *Dinoderus* and termites limits its utilization (McClure, 1967). The chemical and physical properties of bamboo are not different among genera and species. Bamboo culms content about 0.5 to 5% of silica depend to species (Liese, 1992). In some countries, bamboo is useful in the production of cellulose

pulp for paper. In Latin America, *B. vulgaris* and related species are used in fences, houses, little pipes for irrigation, basketry, and containers for liquids (Farrelly, 1984). The leaves are used as food for cattle and horses because they are rich in nitrogenous materials. The leaves contain vitamin A and hydrocyanic acid, which is toxic. The composition of the leaves varies during the year and the digestibility decreases with age.

In Puerto Rico, the common bamboo can be found in areas with an annual precipitation of 150 to 380 cm, but it can also grow in dry zones (Francis, 2000). The common bamboo can grow on moist, well-drained soils and is often associated with secondary forest vegetation (Francis, 2000). In Puerto Rico, the Department of the Interior used bamboo to control stabilize the soil in fills and on steep road embankments because it develops large clumps and grows dense. It is also good for controlling erosion of stream riparian zones because can resist eroding flows. Some bamboos produce an extensive rhizome and root system in the top 30 cm of soil, thus contributing to erosion control (Soderstrom and Calderón, 1979a). Although *B. vulgaris* and other bamboo species are mostly of Asiatic origin, they have established wild colonies; for instance *Phyllostachys* have spread in Brazil.

Fungi on bamboo

Francis (2000) categorized the common bamboo as a plant with few natural enemies. However, there are two insects reported in Puerto Rico that affect the common bamboo, but there is little information about the susceptibility of bamboo to microorganisms, especially when referring to fungi. Francis (op. cit.) commented that *Chaetominum globosum* and *Coniophora puteana* affect some bamboo species. The

concentration of lignin in the culms is a determinant factor for its degradation by *Chaetominum globosum* and *Coriolus versicolor* (Murphy et al., 1997). In a study about decomposition of young and mature culms of *Phyllostachys viridi-glaucescens* by three fungi, Murphy et al. (1997) found that the susceptibility of the bamboo differed among fungi.

There are several studies relating to fungi that can degrade bamboo; most of them are centered on sexual stage, of Ascomycota (Hyde, 2001) and most studies consider bamboo under terrestrial conditions. Hyde et al. (2002) provided a review about the bambusicolous fungi, where they recorded about 1,100 species of fungi associated with bamboo.

Spirodecospora bambusicola was described from dead culms of *Bambusa* sp. and compared with other genera in the Xylariaceae (Ascomycota) (Lu et al., 1998). Hyde and Wong (1999) described the ascomycete *Didymella aptrootii* from bamboo submerged in a lake and rivers in Hong Kong, Malaysia, and the Philippines. Later, *Sunmersisphaeria bambusicola* (Ascomycota) was isolated from dead culms of *Arundinaria hindsii* in Hong Kong (Zhou and Hyde, 2000). The previous species can be found on decaying bamboo culms in terrestrial and freshwater habitats.

Hyde et al. (2001) studied the fungi associated with bamboo culms in Philippines and Hong Kong. Fifty-eight species were observed in submerged bamboo in the Philippines. Lei Cai et al. (2003) identified 58 taxa from submerged bamboo in the Liput River in the Philippines, 18 of them were terrestrial bambusicolous fungi.

Tanaka and Harada (2004) reported four species of *Phaeosphaeria* (Ascomycota) on leaves and culms of some bamboo species in Japan. *Phaeosphaeria oryzae* was found

on culms of *Bambusa multiplex* and *Chusquea serrulata*. Also, they described a *Phaeosphaeria* species similar to *Leptosphaeria sasae* on leaves of the bamboo *Sasa kurilensis*. *Phaeosphaeria brevispora* occurred on culms of *Sasa* species and *P. bambusae* on leaves of *Pleioblastus simony*, as well as on leaves of various other bamboos such as members of *Phyllostachys*, *Pseudosasa*, *Sasaella*, and *Semiarundinaria*. More recently, *Cataractispora receptaculorum* (Ascomycota) was described from bamboo submerged in a freshwater ecosystem of Hong Kong (Wai Hong et al., 2004).

Freshwater (aquatic and aero-aquatic) Hyphomycetes

Freshwater hyphomycetes are a genetically diverse, ecological group (Chan et.al., 2000) conformed by deuteromycetes, (i.e. the anamorphic phases of ascomycotes and, to a lesser extent, basidiomycetes). They are usually treated together during mycological surveys because they share the same habitat and many morphological adaptations (Ingold, 1975). These fungi are further classified into aquatic or aero-aquatic according to their conidial morphology and capabilities to sporulate while still attached to a submerged substratum. Wong et al. (1998) categorized aquatic hyphomycetes as one of the main group of freshwater fungi. Chan et al. (2000), Schoenlein-Crusius and Piccolo Grandi (2003), and Tsui and Hyde (2003) referred to aquatic hyphomycetes as Ingoldian fungi in honor to C.T. Ingold. Aquatic hyphomycetes, per se, comprise fungi that produce conidia under submerged condition. However, Kendrick (2003) classified some aquatic hyphomycetes species as amphibian fungi, due to their ability to grow and sporulate in terrestrial and aquatic environments.

Studies related to aquatic hyphomycetes in lotic systems have identified the conidia in foam (Ingold, 1974; Burgos and Shearer, 1983; Betancourt and Justiniano, 1989), in the water column (Bärlocher and Graça, 2002), associated with leaf litter (Betancourt and Caballero, 1983; Burgos and Shearer, 1983) or have monitored the leaf litter (Bärlocher and Oertli, 1978; Gessner et al., 1993).

Some aquatic hyphomycetes produce tetraradial conidia and other species form sigmoid conidia (Bärlocher, 1992). The tetraradial morphology helps in the aquatic dispersion, but Gulis (2001) observed more sigmoid conidia in streams of the temperate zone. The morphology of the conidia, the capacity to germinate, the production of mucilage and the ability to make appresoria, provide a species a better possibility to colonize the substrate (Read et al., 1992a; Sridhar and Bärlocher, 1994).

Some species of aquatic hyphomycetes can be found associated with the riparian plant roots, where the roots provide a habitat to the organisms. Sati and Belwal (2005) reported eighteen species of aquatic hyphomycetes as root endophytes in living grasses and ferns. Also, these fungi can be found in wood, leaves of monocotyledons (Gulis, 2001) and in leaves of aquatic plants (Schlickeisen et al., 2003).

Findlay and Arsuffi (1989) explained the decomposition of leaf litter in freshwater habitats as a transformation process of leaf's carbon to CO_2, fine particulate organic matter (FPOM), dissolve organic carbon (DOC) and biomass. The degradation of the leaf is dependent on the leaf's composition (Bärlocher and Oertli, 1978; Findlay and Arsuffi, 1989; Suberkropp, 1991), physical-chemical parameters of the water (Rosset and Bärlocher, 1985; Betancourt et al., 1987; Justiniano and Betancourt, 1989b; Suberkropp, 1991, 1995), the location of the organic matter in the stream (Bärlocher, 1992), the solar

radiation (Mans et al., 1998), and even the stream biota (Justiniano and Betancourt, 1989a). Photochemical reactions produced by the solar radiation also promote mineralization of the organic matter; thus, increasing the fine particulate in streams (Mans et al., 1998).

Bärlocher et al. (1979) suggested that the decomposition of the leaves may depend on some compounds, like inhibitors. These substances prevent the colonization of the leaves by fungi. Gulis (2001) expressed that the chemical composition of the leaf can influence in the preference of the substrate by the aquatic hyphomycetes, observing a high coefficient of colonization and number of species in some types of substrates. In the tropics, aquatic hyphomycetes sometimes have been shown to be substrate specific (Padgett, 1976), but the colonization of the leaf litter also depends on the mycoflora or conidial pool of the stream (Justiniano and Betancourt, 1989a).

The decomposition of leaves in freshwater occurs by a succession of organisms (Suberkropp and Klug, 1976; Gessner et al., 1993). During the decomposition process, the leaf is macerated by the microbial activity. Pioneer species initiate the process providing conditions to establish a mature community, then the diversity of species declines, as well as the conidial production (Gessner et al., 1993). Fungi are the predominant organisms on the coarse particulate organic material because they have the capacity to degrade plant matter and use it as an energy source (Findlay et al., 1989). Then, the bacteria substitute fungi in the final stages of degradation (Suberkropp and Klug, 1976). Bacterial biomass predominates in the fine particulate (Findlay et al., 2002). Hackney et al. (2000) suggested that fungi are important in the decomposition of complex matter like wood or substrates with lignified walls. Schlickeisen et al. (2003) found more

bacteria associated with macrophyte leaves of *Sagittaria platyphylla* than of *Populus deltoids*. However, they found fungi associated with both types of leaves.

Padgett (1976) indicated: "a gradual mycelial development in decomposing litter would result in sequential increase in overall leaf protein content". Therefore, the microorganisms associated with the leaf litter are nutritional components for other aquatic organisms (Fidlay and Arsuffi, 1989). Some studies make reference about the selection of detritus colonized by fungi by detritivores because fungi alter the chemical and physical composition of the leaf, changing the palatability of the detritus (Suberkropp et al., 1983).

The physico-chemical parameters have been show to influence fungal development in submerged substrates. Hardwater streams have higher numbers, more biomass, and enzymatic activity of aquatic fungi associated with leaves (Rosset and Bärlocher, 1985; Suberkropp, 1991). Rosset and Bärlocher (op. cit.) found that calcium promoted changes in the pH of the water and stimulated the production of enzymes in the decomposition process. Sridhar and Bärlocher (1997) noticed that small quantities of nitrate, phosphate, sodium chloride, and calcium chloride stimulate sporulation, but that leaf extracts and yeast extracts suppressed sporulation. This last observation suggests that the lower the concentration of organic molecules, the higher the fungal sporulation. Also, the sporulation rate seems related to the increase of fungal biomass and respiration in the habitat (Suberkropp, 1991).

High temperatures promote the decomposition of leaves (Findlay and Arsuffi, 1989; Chauvet and Suberkropp, 1998). Suberkropp and Weyers (1996) considered that temperature affects fungal and bacterial production in a similar manner. However,

Sridhar and Bärlocher (1994) found greater number of viable conidia in foam samples taken when the water temperature was low in a temperate stream.

Nutrient concentration can affect the growth and sporulation of aquatic hyphomycetes (Suberkropp and Klug, 1976; Rosset et al., 1982; Suberkropp, 1991, 1995). Sridhar and Bärlocher (2000) observed an increase in conidial production and mycelial growth when inorganic nitrogen and phosphate levels were high, concluding that nutrient levels stimulate fungal metabolism and the degradation of leaf litter. Some studies suggest that aquatic hyphomycetes associated with decomposing leaves can obtain nitrogen directly from the water flowing over the leaves (Suberkropp, 1995).

In tropical streams, the decomposition of leaf litter is faster than in temperate zones, with aquatic hyphomycetes playing a key role in this process (Padgett, 1976). Sridhar and Kaveriappa (1989) reported a peak in the production of conidia when rainfall increases. The number of species found in foam samples also increases when pluvial precipitation increases (Tan and Koh, 1995). Also, the input of leaves promotes the increment of aquatic hyphomycetes in a stream (Justiniano and Betancourt, 1989a; Tan and Koh, 1995).

Studies in the Caribbean

The study of aquatic hyphomycetes in the Caribbean started in the 1960's, with Hudson and Ingold in Jamaica, Nilsson in Venezuela, and Maranová and Marvan in Cuba (all referenced in Betancourt et al., 1987). About twenty-six species of aquatic hyphomycetes were reported from leaf litter and foam samples collected from eight rivers

in the Dominican Republic, which were all new records for this country (Betancourt et al., 1986).

Hamilton (1973) and Padgett (1976) studied the aquatic hyphomycetes in two aquatic ecosystems localized in the Caribbean National Forest of Puerto Rico (now El Yunque). These two studies were the first reports of aquatic hyphomycetes from Puerto Rico. Hamilton (1973) established three study sites in the Sonadora River and Quebrada Jiménez in the Luquillo Experimental Forest, where she found 27 aquatic species of fungi. The other research was conducted in a small stream adjacent to El Verde Field Station, where sixteen species of aquatic hyphomycetes were found (Padgett, 1976).

Betancourt and Caballero (1983) identified fifteen species from decomposing leaves in Los Chorros waterfall in Utuado. Betancourt et al. (1987) reported fifty-two species of aquatic hyphomycetes associated with leaf litter samples from Quebrada Doña Juana in the Toro Negro Forest Reserve. Justiniano and Betancourt (1989a) reported twelve species from decomposing leaves of *Syzygium jambos* from the Maricao River. The same authors recorded thirty-five species of aquatic hyphomycetes from leaf litter and foam in Quebrada de Oro (Mayagüez), with three new records for Puerto Rico (Betancourt and Justiniano, 1989). Also, twenty-nine species were found in foam and leaf samples from Loco River at Susúa Forest Reserve, nine of which were new records for Puerto Rico (Santos-Flores and Betancourt, 1994).

Cardona and Rivera (1986) in Río Cañas, Mayagüez, found thirty-one species of aquatic hyphomycetes. Twelve species of aquatic hyphomycetes were identified by Vale (1995) from Chuco Ramos River in Aguada. Jiménez (1996) in El Guamá stream, in the municipality of San Sebastián, identified thirty-six species from leaf and foam samples.

In 1997, a compendium on the aquatic hyphomycetes found in water, foam, and submerged litter from streams of Puerto Rico was prepared by Santos-Flores and Betancourt. In this publication 143 fungal species were reported including, 41 new records for the Neotropics.

MATERIALS AND METHODS

Study Site: Quebrada de Oro

The study was conducted in Quebrada de Oro, a small stream located northeast of Mayagüez, Puerto Rico. The stream is composed of two first- and second-order tributaries of that join at east of the Road 108 to make a second-order stream. West of the Road 2, a tributary of first order and another of second order connect to the stream, making a third order stream. Quebrada de Oro is situated in an area of high demographic density and anthropogenic activity, with high foci of pollution. This creek flows into the Mayagüez Bay, close to Punta Boca Morena. In addition, runoff and drainage from the principal roads (e.g. Road 108, Road 2, and Concordia Street), urban roads, and the streets of UPR-Mayagüez Campus contribute to the stream's flow (Fig. 1).

The study area lies within the subtropical moist forest zone (*sensu* Holdridge), with a mean annual rainfall from about 100 to about 220 cm, with temperatures from 18 to 24ºC (Ewel and Whitmore, 1973). Some portions of the creek run through a secondary forest. The riparian vegetation was disturbed by anthropogenic activity and the introduction of exotic plants. Moreover, some areas have high erosion in their littoral zones and are still deforested. The general area of the watershed was primarily used for shade coffee plantations and for pasture. Most of the agricultural activities in the area have disappeared and forest recovery is taking place. However, the dominant vegetation is composed of exotic species such as *Castilla elastica*, *Swietenia macrophylla*, *Bambusa* spp., *Magnifera indica*, *Syzigium jambos*, and *Panicum aquaticum,* among others (Table 1).

The study was performed in the tributaries localized at the east of the Road 108 N and in the area canalized to the west of the same road. Three stations were established, two of them situated upstream of Road 108 (Site 1 and Site 2) and a third site was selected downstream (Fig. 1). Site 1 was established at the latitude 18°12'55" N, longitude 67°08'19" W, site 2 was located at the latitude 18°12'30" N, longitude 67°08'29" W, and site 3 was sited at the latitude 18°12'56" N, longitude 67°07'30" W. The location of the stations was calculated using a topographic map of the Mayagüez quadrangle from the U.S. Geological Survey and Department of Public Works (1964).

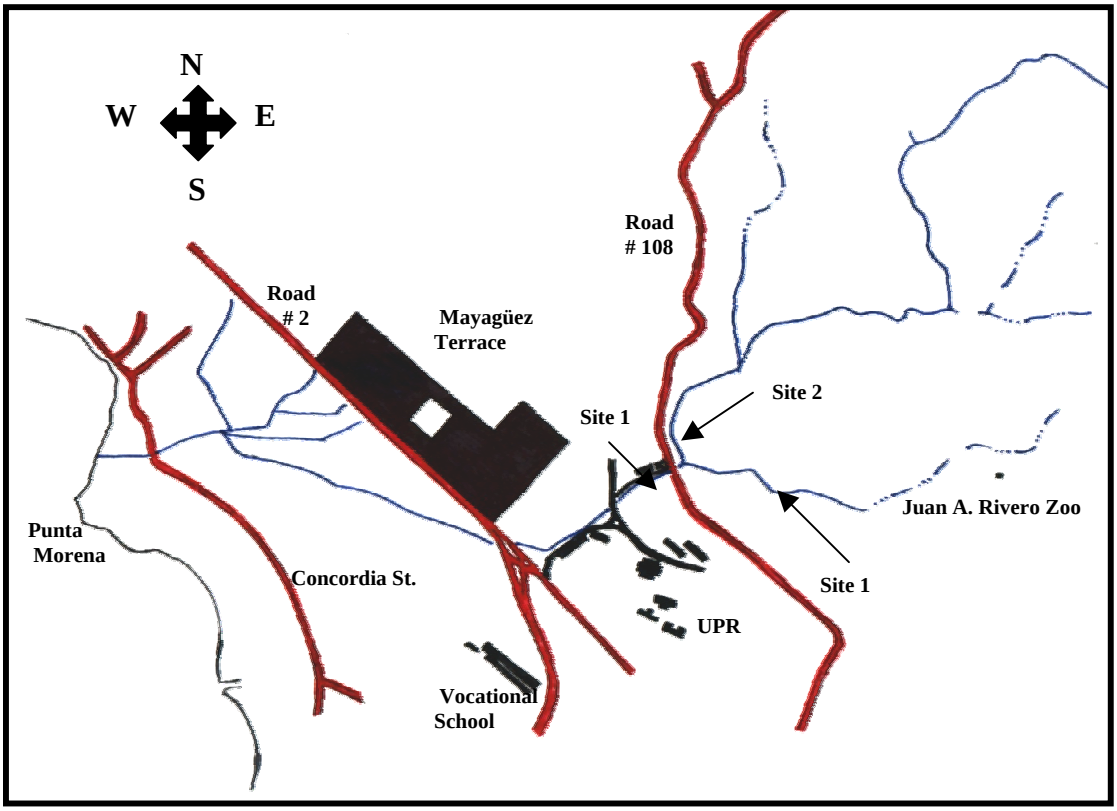

Figure 1. Map of the study area at Quebrada de Oro in Mayagüez, Puerto Rico.

The soils of the area where the creek is located are classified as Consumo-Humatas association, which are strongly leached, loamy, clayey, sticky and plastic soils underlain by thick layers of weathered rock, in a humid climate (U.S.D.A. and University

of Puerto Rico, 1969). The stations selected along Quebrada de Oro are located on soils classified as Dagüey clay (site 1), Consumo clay (site 2), and lever land (site 3).

Soils of the Dagüey series are well drained, strongly acidic and moderately permeable. The surface layer is reddish-brown and strongly acid. Usually, these soils have been used for agriculture for many years, have a moderate runoff with a high water capacity, and a medium fertility.

Soils of the Consumo series are well drained, strongly acidic, and moderately permeable, and are composed of residuals from volcanic rocks. The surface layer is about 6 inches thick, reddish-brown and strongly acidic. These types of soils are used in subsistence crop. Runoff is rapid and erosion is a hazard (U.S.D.A. and University of Puerto Rico, 1969).

The third station has a lever land soil (i.e. leveled), which is frequently flooded and typical of flood plains along rivers. It consists of disturbed soils, neutral to slightly acid, with poor to moderate permeability.

Table 1. Typical vegetation associated with the three study sites.

Site 1	Site 2	Site 3
Castilla elastica Ceru.	*Bambusa* sp. Schreber	*Bambusa vulgaris* Schrad. Ex. J.C. Wendl.
Inga laurina (Sw.) Wild	*Cissus verticilata* (L.) Nicolson & Jarvis	*Inga laurina* (Sw.) Wild
Guarea guidonia (L.) Sleumer	*Urochloa maxima* (Jacq.) R.D. Webster	*Cyperus* sp. L.
Roystonea borinquena O.F. Cook	*Terminalia catappa* L.	
Coffea arabica L.	*Inga vera* Willd. (Guaba)	
Epipremnum pinnatum (L.) Engl. "Aureum" Nicolson		
Theobroma cacao L.		
Syngonium podophyllum Schott		

Physico-Chemical Factors

Physical and chemical factors were measured fourth times at the sampling sites where the leaves bags were submerged (Santos-Flores, 1996). The temperature and dissolved oxygen (Abdel-Raheem, 1997, Raviraja et al., 1998; Sridhar and Bärlocher, 2000; Bärlocher and Graca, 2002) were measured with an YSI 55® oxygen meter, and the pH was determined with an Oaklon® Acorn Series pH 5 meter. Water samples were collected in 500 ml jars (I-Chem Nalgene®), previously cleaned and sterilized. These samples were analyzed for concentration of nitrate, nitrite and phosphate (Suberkropp, 1995, Baldy and Gessner, 1997) using a La Motte Smart 2® colorimeter. For each nutrient determination and each physical-chemical parameter, four repetitions were measured at each study site.

Sampling using leaf discs in mesh bags

Dry senescent bamboo leaves were collected directly from a single plant to avoid the contact with soil. The following cleaning process was used to minimize viable epiphytic microflora of the leaves: leaves were washed with a solution of 70% alcohol for 1 minute, submerged in a solution of 0.05% sodium hypochlorite for 1 minute, rinsed with sterile distilled water, and dried overnight in a microbiological hood under UV light.

The leaves were cut into four 10-mm diameter discs with a sterilized cork borer. One disc was cut from the base, one from the apex, and two other discs from the middle of the leaf blade (Newell and Fallon, 1988) (Fig. 2). The disc method has been recommended over whole-leaves exposure because it reduces contamination and the density of conidia in the aeration systems.

Four discs of each leaf were placed in litter bags (6 cm wide x 6 cm length), made with plastic mesh of 2 mm^2 (Suberkropp, 1995; Sridhar and Bärlocher, 1997; Sridhar and Bärlocher, 2000; Bärlocher and Graca, 2002), and used to monitor the degradation of bamboo leaves in the stream (Fig. 2 and Fig. 3A). Each mesh bag contained four leaf discs cut from the same leaf. The mesh bags were cleaned before placing the discs inside, using a solution of hypochlorite and sterile distilled water. The mesh bag method was used assuming that: (1) the condition inside the bag resembled outside conditions, (2) the growth of benthic organisms in the mesh had minor effect, and (3) the plant material did not escape through the mesh (Boulton and Boon, 1991). Also, mesh bags limit access to large shredders and other aquatic invertebrates.

Sixty bags with bamboo discs, which represented sixty different bamboo leaves, were attached to a cage (15 cm high x 57 cm wide x 93 cm length) made with 7-mm^2 metallic mesh The field study was initiated on November 2004 by introducing two cages in the creek and concluded in January 2005. The cages were submerged in two sampling sites (Site 1 and Site 3), located at approximately 0.66 m from the littoral zone. Every 14 days, ten bags were collected randomly from each site (Suberkropp and Klug, 1976) and used to determine the aquatic hyphomycetes associated with the decomposing leaf discs. Therefore, in this procedure, the numbers of samples were ten per site, equivalent to ten bamboo leaves. Intervals of 14 days are considered a reasonable time to detect changes in leaves with rapid degradation rates (Benfield, 1996). The collected bags were washed with water from the creek to remove adhered sediments, invertebrates, and detritus.

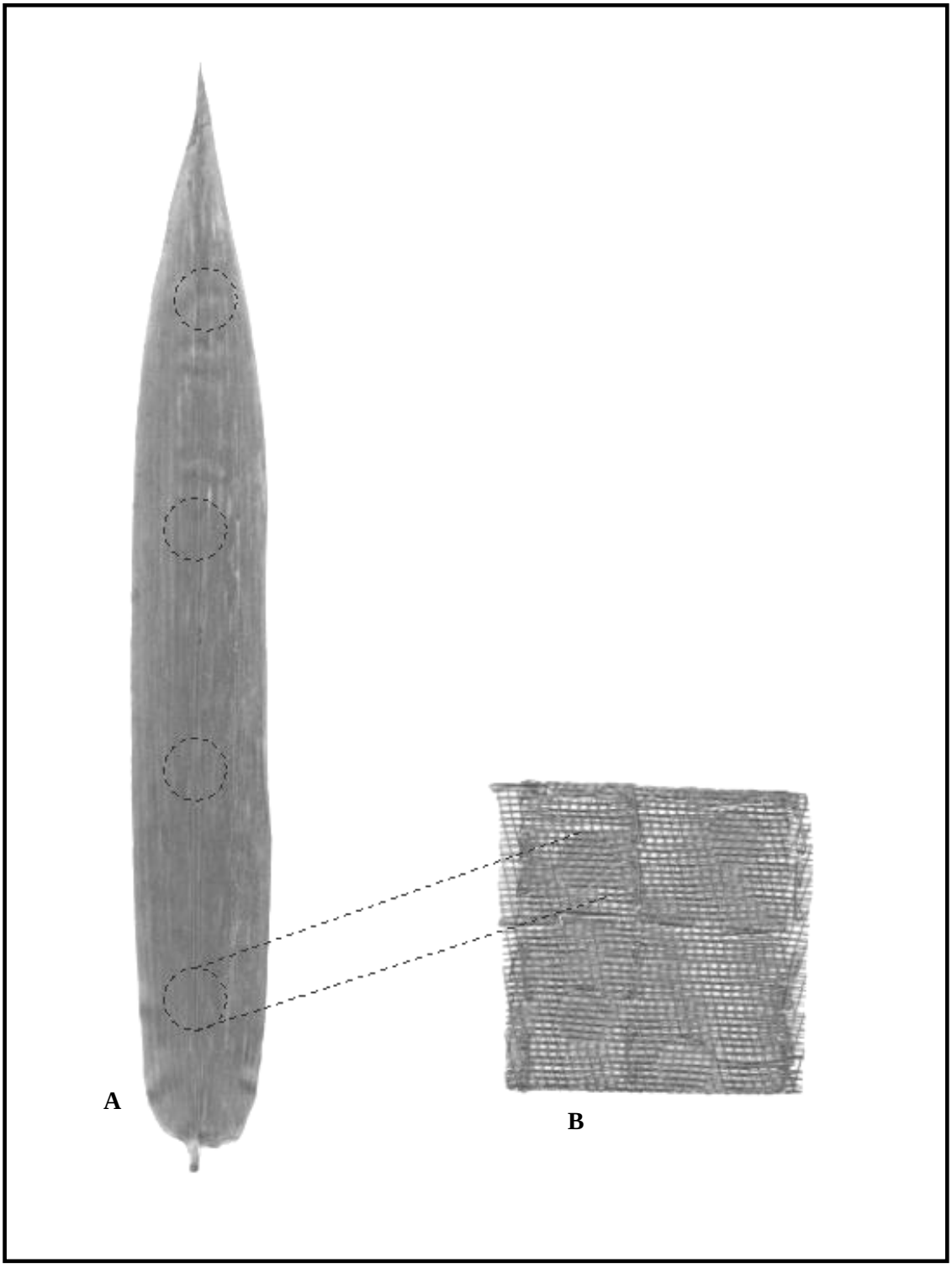

Figure 2. (A) Leaf zones used for selecting the 10-mm diameter discs with the cork borer, and (B) example of the 6 cm x 6 cm plastic mesh bags for the leaf discs.

(Suberkropp and Klug, 1976). Each litter bag was placed inside a sterile bag and transported to the laboratory for further analysis (Benfield, 1996).

In the laboratory, the bags were cleaned with sterile distilled water and one disc of each bag was mounted on a slide and stained with lactophenol cotton blue. In addition, one disc of each bag was aerated for ten days in sterile tubes with 30 ml of sterile water from the creek and a combination of streptomycin sulfate and penicillin G-potassium (0.5 ml for each 100 ml) (Fig. 3E and Fig. 3F). Antibiotics were incorporated to inhibit the growth of bacteria on the surface of the leaf discs, thus permitting the growth of aquatic hyphomycetes (Millie-Lindblom and Tranvik, 2003). Ten discs recovered from each cage were aerated at room temperature using an air pump (Santos and Betancourt, 1997) to produce turbulence to oxygenate the water, promote the flow of nutrients, and induce spore formation (Sridhar and Bärlocher, 2000). After the incubation period, the discs were mounted on slides and stained with lactophenol cotton blue. The slides were observed under a compound microscope (Nikon Opticphot-2) and the conidia were identified and counted at total magnifications of 150X and 600X.

Sampling with bamboo litter bags

Five whole bamboo leaves were cleaned, as previous described, and placed inside each of fifteen bags made with plastic mesh of 2 mm^2 (18 cm wide x 17 cm length), which were cleaned as described before (Suberkropp, 1995) (Fig. 3C). Fifteen bags of leaves were placed in a metallic mesh cage (15 cm high x 57 cm wide x 93 cm length). The field study started in October 2005 by introducing three cages (one in each study

site) and ended in December 2005. The cages were submerged in the same stations where the previous field experiment was conducted. Every 14 days, two bags were collected from each site, washed with water from the creek, and transported in sterile bags. In the laboratory, three discs were cut from each leaf using a sterilized 10-mm diameter core borer (Fig.3D). One disc (from each leaf) was mounted and stained with lactophenol cotton blue for observation and the others were aerated in tubes with sterile distilled water at room temperature (Fig. 3E). One tube had the same combination of antibiotics described above to reduce the bacterial growth and the other tube had sterile water. After 10 days of incubation, discs were placed on slides and stained with lactophenol cotton blue. The leaf discs were examined under a compound microscope to identify conidia as in the previous section.

Scanning electron microcopy (SEM) to observe bamboo discs

Three samples of bamboo leaves were used from bamboo leaves bags collected from each site from October to December 2005. The samples were fixed overnight with 2% glutaraldehyde in phosphate buffer, and refrigerated.. The samples were then rinsed three times with phosphate buffer for a period of 15 minutes per rinse to eliminate the excess fixing solution. An ascending dehydration procedure was used to replace the water and the buffer solutions without distorting the sample. Fixed material was dehydrated with alcohol at 10 to 95% (in 10% increments) and culminated with three rinses in absolute alcohol (Lu et al., 1998; Hyde and Wong, 1999) for 20 minutes (Muñoz and Jordán, 1997). The alcohol was substituted with liquid carbon dioxide for the critical point method (Electron Microscopy EMS 850). The critical point method dries the

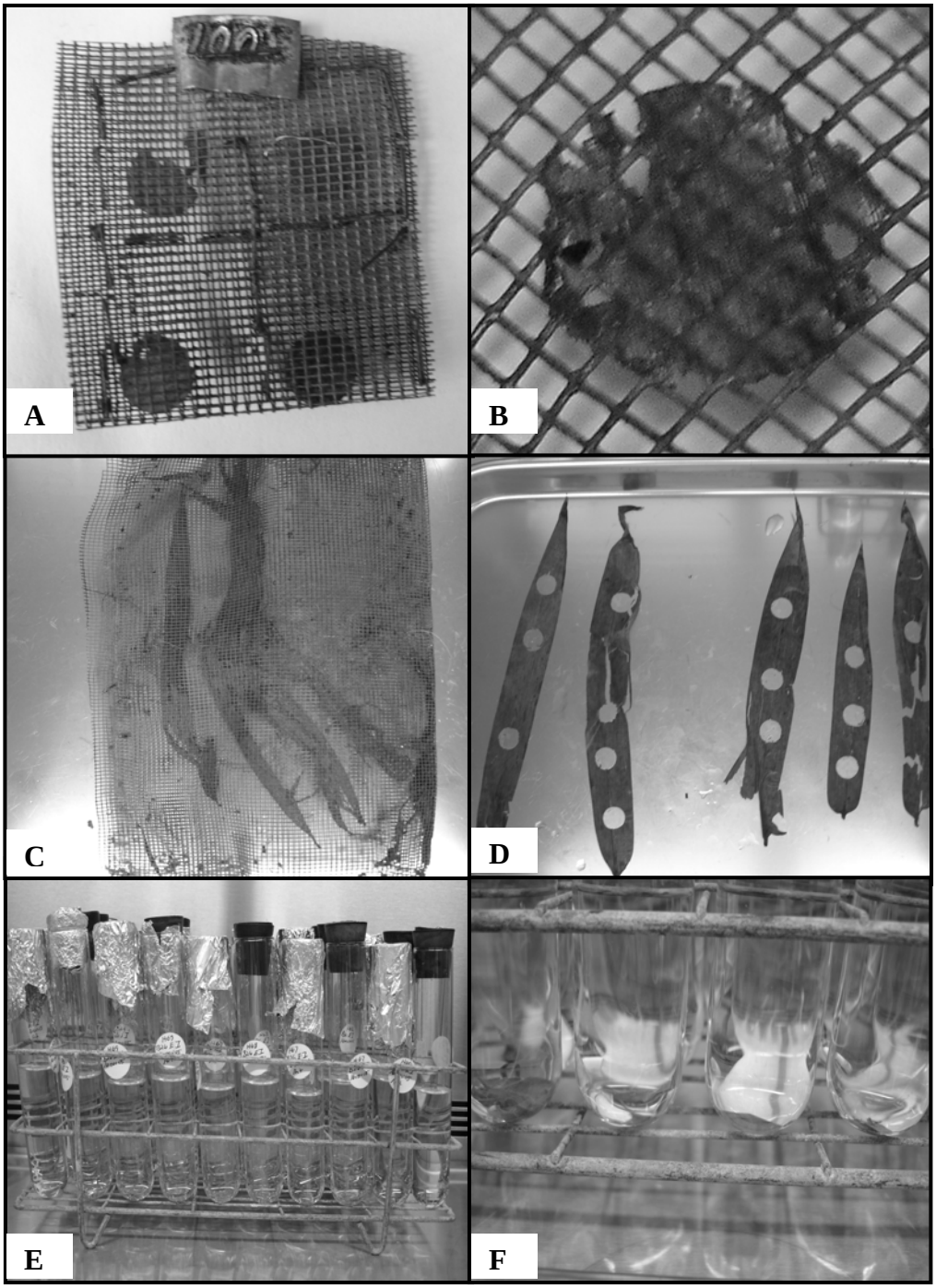

Figure 3. Procedure used to study aquatic hyphomycetes associated with the decomposition process of the bamboo leaves: (A) leaf discs in mesh bags, (B) bamboo leaf disc, (C) bamboo litter bag, (D) bamboo leaves cut into discs, (E) tubes used to aerate the leaf discs, and (F) enlargement of the bottom of the tubes with bamboo leaf discs inside.

samples and prevents the collapse of the cells (Bozzola and Russell, 1999). Dried samples were mounted on aluminum SEM specimen mount stubs using spectro-grade carbon adhesive tape. The samples were coated with gold by the sputter coating procedure (Electron Microscopy Sciences 550X). Samples were stored in plastic boxes for storing specimen stubs and put in a desiccator to prevent rehydration, until examination with a JEOL, model JSM-5410LV, scanning electron microscope at 10kV.

Naturally occurring submerged leaves samples

Bi-weekly, from November 2004 to January 2005, submerged leaves of the riparian vegetation were collected randomly from each site and stored within sterile plastic bags (Whirl-pak ®). Various discs of 10 mm of diameter were cut from each of three leaves (per site), rinsed with sterile distilled water. Each disc was mounted on a slide, stained with lactophenol cotton blue and observed.

Submerged leaves from each site were also collected as previously stated for the period between October and December 2005. In this case, discs were aerated for 10 days at room temperature in 200 ml of autoclaved distilled water in a 300 ml flask (Sridhar and Bärlocher, 2000; Garnett et al., 2000). The entire volume from the flask was filtered through a 0.8 μm or 5 μm pore size membrane filter (Millipore®). The filters were stained with lactophenol cotton blue and heated for 30 minutes at 60°C. The conidia trapped in the filters were counted and identified.

Water samples for the conidial pool determination

Conidial concentrations in the water were estimated by the filtration technique (Webster and Descals, 1981). From each site, a water sample was taken in 500-ml sterile jars (I-CHEM®) and filtered through a 0.45 to 5.0-µm pore size membrane filters (Millipore®) with a hand-operated pump water. The filters were stained with lactophenol cotton blue and heated at 60^{o}C for 30 minutes. A water sample of 100 ml was filtered from each site between November 2004 and January 2005. Then quantity of filtered water sample was changed to 500 ml, for the sampling carried out between October and December, 2005. The conidia trapped on the filter were stained and fixed with lactophenol cotton blue, identified, and counted.

Precipitation

Precipitation data were obtained of the Atmospheric Caribbean Research Center. The meteorological station is located at the Mayagüez Airport. Precipitation was calculated by adding the precipitation registered the days before each sampling date, using a period between the last day of sampling and the day before sampling. For the first sampling, the data obtained since the day the cages were submerged on the stream until the day before the sampling were used to calculate the precipitation that could have affected the monitoring. For the other samplings, the data of precipitation since the last sampling date until the day before the sampling event were used.

Fungal species identification

Aquatic hyphomycetes reported in this study were identified according to the taxonomic keys of Ingold (1975), Gulis et al. (2005), Nawawi (1985), Nilsson (1964), and Santos and Betancourt (1997).

Statistical analyses

The two periods of sampling were treated separately because of the differences between the sampling protocols. The main comparisons in this study were among types of samples (bamboo discs, leaf litter, and water samples) within each period of sampling. Spearman correlations were made to examine the relation among precipitation, temperature, dissolved oxygen, pH, nitrate and phosphate, and the number of species found in bamboo leaves. A Kruskal Wallis analysis was used to analyze the physico-chemical parameters comparing the temperature, D.O. concentration, and the pH among sites, and among dates of sampling. Also, this test was used to compare the physico-chemical parameters previously mentioned between sampling dates. In addition, the Kruskal Wallis test was used to evaluate the number of conidia obtained in the various treatments of the bamboo leaves (aerated, aerated with antibiotics and non-aerated). A Wilcoxon test was used to analyze the differences in the number of conidia documented from the discs aerated in the presence of antibiotics and those aerated without antibiotics.

Spearman correlations, Kruskal Wallis analyses, and Wilcoxon tests were used to analyze most of the information because the data did not have a normal distribution and could not be transformed. These tests were nonparametric statistics; Spearman correlation

is the nonparametric analog of Pearson's correlation, Kruskal Wallis is an analog of one-way analysis of variance, and Wilcoxon test is a *t*-test.

An ANOVA was conducted to compare the number of conidia counted on non-aerated bamboo discs with those counted on aerated bamboo discs. Data transformations using Log10 and/or Log (X+1) were made for some of the analyses, like in the t-test. A cluster analysis for the three study sites based on the percentage of relative abundance of species found on bamboo leaves during the second period of study was made. Distance was measured with Jaccard (1-S) and the linkage method was averaged (UPGMA) using the mean of relative abundance of the species. Jaccard is a similarity coefficient that varies from a minimum of 0 when the compared units are different, to 1 when they are identical. All statistical analyses were done with InfoStat Version 2006e.1 (InfoStat, Argentina).

The percentage frequency of occurrence was calculated for each fungal species using the following equation (Gulis, 2001; Zhou and Hyde, 2002);

Percentage frequency of occurrence on a substrate =

$$\frac{\text{Number of samples on which a given species occurred}}{\text{Number of samples examined}} \times 100$$

The percentage of abundance for each fungal species was calculated using the following equation:

Percentage of relative abundance =

$$\frac{\text{Number of conidia on a specific species}}{\text{Total number of conidia counted}} \times 100$$

RESULTS

Physico-chemical factors

From February to May 2004, water temperature was generally higher and, there was a reduction of rainfall during this period. During the months from September 2004 to January 2005, a decrease in water temperature was observed. These behaviors were similar to changes in the air temperature. However, the dissolved oxygen in the stream did not have drastic changes.

The physical and chemical parameters of the three study sites were summarized in table 2 and 3. During the first sampling period, from November 2004 to January 2005 (Fig.4), temperature ranged from 22.4 to 23.8^{o}C (mean: 23.1 ^{o}C) upstream (site 1), 22.9 to 24.0^{o}C (mean: 23.5 ^{o}C) in the midstream section (site 2), and 22.7 to 24.9^{o}C (mean: 24.1 ^{o}C) downstream (site 3). For the second sampling period, from October to December 2005, the water temperature was from 23.3 to 25.4^{o}C for site 1 (mean: 24.6 ^{o}C), 22.9 to 25.4 ^{o}C (mean: 21.6 ^{o}C) for site 2, and 23.0 to 26.0 (mean: 24.5 ^{o}C) for site 3.

During the first sampling period the dissolved oxygen (D.O.) concentration ranged from 4.41 to 6.18 mg/L (mean: 5.48 mg/L) for site 1, 4.40 to 6.32 mg/L (mean: 5.28 mg/L) for site 2, and 4.96 to 7.51 mg/L (mean: 6.40 mg/L) for site 3, with a significant variation among study sites (H: 15.56, p: 0.0004) (Appendix 1). The pH ranged from 6.9 to 7.6 (mean: 7.3) for site 1, 6.9 to 7.8 (mean: 7.5) for site 2, and 7.2 to 7.9 (mean: 7.6) for site 3. The mean nitrate and phosphate concentrations were, respectively, 1.87 mg/L and 0.33 mg/L for the upstream station (site 1), 2.66 mg/L and 0.26 mg/L for the midstream station (site 2), and 2.89 mg/L and 0.27 mg/L downstream (site 3).

During the second sampling period, D.O. ranged from 5.86 to 6.90 mg/L for site 1 (mean: 6.35 mg/L), 6.24 to 7.74 mg/L (mean: 7.11 mg/L) for site 2, and 6.79 to 8.30 mg/L (mean: 7.47 mg/L) for site 3. The pH fluctuated from 7.5 to 7.9 (mean: 7.86) for site 1, 7.02 to 8.12 (mean: 7.93) for site 2, and from 7.87 to 8.21 (mean: 8.05) for site 3. Mean nitrate and phosphate concentrations recorded for each site were, respectively; 1.28 mg/L and 0.20 mg/L for site 1, 2.89 mg/L and 0.31 mg/L for site 2, and 2.24 mg/L and 0.23 mg/L for site 3 (Fig. 5).

During the first sampling period, a moderate relation between D.O. and pH was obtained (Spearman's coefficient = 0.64, $p = 2.3x\ 10^{-6}$), as well as for temperature and D.O. (Spearman's coefficient = 0.45, $p = 7.5\ x\ 10^{-4}$). A weak relation was observed between pH and temperature (Spearman's coefficient = 0.30, p = 0.02; see Appendix 2). For the second sampling period, pH and the D.O. had a moderate relation (Spearman's correlation = 0.53, $p = 1.5x10^{-4}$) (Appendix 4).

During the twelve months of the study (combination of both periods and other data), there was a weak correlation between temperature and D.O. (Spearman's correlation = 0.14; p = 0.04), but a moderate correlation between pH and D.O (Spearman's correlation = 0.53, p = 0.00) (Appendix 6). When comparing the mean physico-chemical parameters among sampling dates within same sampling sites, the Kruskal Wallis test (H-value) suggested that there were significant differences in temperature and pH (Appendix 7). Temperature ($H = 184.6$, $p < 0.0001$) and pH ($H = 160.36$, $p < 0.0001$) fluctuated among dates of collection, but the D.O. concentration ($H = 9.31$, $p < 0.0001$) showed little *intra-site* variation among dates during the whole study. Using all data, the Kruskal Wallis test indicated that pH ($H = 29.84$, $p < 0.0001$) and D.O.

(H = 78.33, p < 0.0001) had a significant variation among sites in contrast with the temperature (H = 0.24, p = 0.8885), (Appendix 8). In overall, nitrate and phosphate concentrations were not strongly correlated (Spearman's coefficient of 0.13, p = 0.08; see Appendix 9).

Aquatic hyphomycetes associated with bamboo leaves

During the first period of colonization, bamboo discs began to disintegrate by the third sampling date (December 27, 2004). During the last date of monitoring of the first period (January 14, 2005) four bags without discs were collected from the cages. During the aeration process, some leaf discs broke down and could not be mounted, thus reducing the number of samples that provided information about colonization of the bamboo leaves. During the second season of sampling, some leaves were broken down and mesh bags with fragments of leaves could be observed during the third sampling. Bamboo leaves during the second sampling of this period (November 2nd, 2005) were partially fragmented and by the last sampling some bags were empty.

Aquatic and aero-aquatic hyphomycetes found in Quebrada de Oro

Thirty-seven species of aquatic and aero-aquatic hyphomycetes were identified in Quebrada de Oro during this study. Of these, twenty-four were observed in water samples, twenty-three on the bamboo leaves, and ten on leaf litter. Those species observed on bamboo leaves belong to the genera *Anguillospora*, *Campylospora*, *Helicomyces*, *Phalangispora*, *Pyramidospora*, *Tetraploa*, and *Tripospermum*. Conidia belonging to the genera *Beltrania*, *Brachiospora*, *Clavariopsis*, *Clavatospora*, *Diplocladiella*, *Lunulospora*, and *Trinacrium* were only observed from water samples. Conidia of *Triscelophorus* were observed only on naturally occurring leaf litter samples.

In general, twenty-seven species were found during the first period of monitoring (November, 2004 to January, 2005), from which thirteen were found in water samples, eighteen were identified on bamboo leaves, and only four species were observed on the leaf litter.

During the second sampling period, a total of fifteen species were observed on the bamboo leaves, nineteen in the water samples, and eight species on the leaf litter. *Campylospora filicladia* was the numerically dominant species during this period of study, observed in each type of sample from the three sites (Table 5), followed by *Helicomyces* sp., *Campylospora chaetocladia*, and *Pyramidospora casuarinae*. However, the most frequently found species in bamboo leaves was *Helicomyces* sp. (17.5% occurrence), followed by *C. filicladia* (12.5%).

First sampling period: samples using leaves discs in mesh bags

A total of 7 fungal genera were found on bamboo leaf discs, yielding 16 species, during the first period. For site 1, the most common (higher occurrence) genera on bamboo leaves were *Anguillospora* (with 2.5%) and *Helicomyces* (with 3.75%). The most common genera in bamboo leaves for site 3 were *Campylospora*, *Helicomyces*, and *Pyramidospora*, occurring in 5%, 5%, and 11.25% of the samples, respectively. The dominant species on bamboo leaves were members of *Helicomyces* (3.75%) for site 1 and *Pyramidospora casuarinae* (3.8%) and *Pyramidospora* sp. (3.8%) for site 3.

Table 2. Physico-chemical factors recorded at the three sampling stations.

	Sampling Date	Site 1			Site 2			Site 3		
		Temp. (°C)	D.O. (mg/L)	pH	Temp. (°C)	D.O. (mg/L)	pH	Temp. (°C)	D.O. (mg/L)	pH
1	Feb. 24, 2004	23.6 (0.0)	6.14 (0.07)	7.6 (0.02)	24.1 (0.0)	7.71 (0.14)	8.0 (0.01)	24.5 (0.0)	8.31 (0.04)	8.0 (0.01)
2	March 6, 2004	24.1 (0.0)	6.22 (0.17)	6.9 (0.01)	24.1 (0.02)	6.16 (0.17)	7.0 (0.01)	24.0 (0.0)	7.29 (0.11)	7.0 (0.0)
3	March 22, 2004	24.2 (0.03)	5.95 (0.09)	6.6 (0.03)	24.6 (0.02)	6.46 (0.28)	6.9 (0.03)	23.6 (0.04)	7.54 (0.06)	7.1 (0.12)
4	April 7, 2004	26.4 (0.03)	6.01 (0.14)	7.6 (0.01)	25.3 (0.03)	6.50 (0.05)	7.6 (0.01)	24.6 (0.03)	7.19 (0.03)	7.5 (0.01)
5	April 20, 2004	25.3 (0.0)	6.51 (0.06)	7.2 (0.03)	24.8 (0.0)	6.78 (0.29)	7.6 (0.05)	25.0 (0.0)	7.83 (0.16)	7.7 (0.02)
6	May 4, 2004	25.7 (0.02)	5.62 (0.14)	7.3 (0.05)	25.7 (0.0)	6.90 (0.07)	7.4 (0.03)	24.9 (0.0)	7.42 (0.16)	7.4 (0.06)
7	Sept. 27, 2004	25.6 (0.0)	4.98 (0.05)	5.0 (0.07)	25.9 (0.0)	6.55 (0.03)	7.3 (0.10)	26.0 (0.0)	6.81 (0.07)	7.6 (0.04)
8	Oct. 15, 2004	25.4 (0.0)	5.53 (0.14)	7.2 (0.05)	25.4 (0.08)	3.07 (0.39)	7.1 (0.18)	25.2 (0.02)	7.16 (0.04)	7.6 (0.03)
9	Nov. 5, 2004	24.1 (0.0)	5.68 (0.11)	7.4 (0.03)	24.7 (0.05)	5.73 (0.27)	7.7 (0.01)	24.5 (0.0)	7.37 (0.10)	7.7 (0.02)
10	Nov. 28, 2004	23.1 (0.0)	6.08 (0.03)	7.4 (0.02)	24.0 (0.03)	5.72 (0.23)	7.6 (0.0)	24.3 (0.0)	7.39 (0.10)	7.7 (0.01)
11	Dec. 14, 2004	23.8 (0.03)	5.95 (0.16)	7.6 (0.01)	--	--	--	24.9 (0.02)	7.13 (0.09)	7.8 (0.01)
12	Dec. 27, 2004	22.9 (0.03)	5.02 (0.23)	7.6 (0.03)	23.6 (0.0)	6.10 (0.12)	7.8 (0.0)	22.8 (0.03)	6.10 (0.23)	7.8 (0.02)
13	Jan. 14, 20005	23.6 (0.03)	5.29 (0.14)	7.1 (0.05)	23.7 (0.0)	4.74 (0.21)	7.4 (0.04)	24.5 (0.03)	5.46 (0.17)	7.5 (0.02)
14	Jan. 28, 2005	22.8 (0.0)	5.09 (0.19)	6.9 (0.02)	22.9 (0.0)	4.55 (0.09)	7.1 (0.03)	23.9 (0.03)	5.91 (0.35)	7.3 (0.05)
15	Oct. 19, 2005	25.3 (0.0)	6.24 (0.15)	7.7 (0.0)	25.3 (0.02)	6.81 (0.11)	8.1 (0.02)	26.0 (0.03)	6.98 (0.11)	8.0 (0.01)
16	Nov. 2, 2005	25.3 (0.05)	6.48 (0.03)	7.7 (0.01)	25.0 (0.0)	7.40 (0.12)	7.9 (0.0)	24.6 (0.0)	7.44 (0.11)	8.1 (0.01)
17	Nov. 16, 2005	24.6 (0.02)	6.36 (0.17)	7.9 (0.0)	24.5 (0.0)	7.41 (0.10)	8.0 (0.0)	24.5 (0.05)	7.78 (0.06)	8.2 (0.01)
18	Nov. 30, 2005	23.2 (0.0)	6.34 (0.22)	7.6 (0.01)	23.1 (0.03)	7.13 (0.22)	7.5 (0.20)	23.1 (0.06)	7.69 (0.27)	7.9 (0.01)
19	Dec. 13, 2005	--	--	--	22.9 (0.0)	6.79 (0.19)	8.0 (0.0)	--	--	--

-- No data.
Data represent means of four measurements at each site.
Standard error is included within parenthesis.
Gray lines and yellow lines represent the results of the first and second periods, respectively, for the bamboo leaves colonization experiment.
*No data on nitrite were measured or only one value was available to calculate nitrate-N.

Table 3. Measures of nitrate and phosphate in the water column at each sampling site.

	Date	Site 1		Site 2		Site 3	
		Nitrate (mg/L)	Phosphate (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
1	Feb. 24, 2004	5.375 (0.34)	1.313 (0.01)	1.530 (0.11)	0.300 (0.02)	1.98 (0.21)	0.870 (0.21)
2	March 6, 2004	3.417 (0.30)	0.643 (0.17)	4.038 (0.46)	0.280 (0.0)	3.240 (0.27)	0.258(0.01)
3	March 22, 2004	1.936 (0.45)	0.335 (0.14)	3.295 (0.15)	0.260 (0.05)	2.677 (0.04)	0.248 (0.01)
4	April 7, 2004	0.946* (0.19)	0.323 (0.02)	2.882* (0.43)	0.315 (0.02)	1.231* (0.71)	0.278 (0.01)
5	Sept. 27, 2004	1.890* (0.10)	0.051 (0.03)	6.292* (0.41)	0.168 (0.03)	4.191* (0.36)	0.353 (0.10)
6	Oct. 15, 2004	0.392 (0.32)	0.138 (0.03)	1.494 (0.12)	0.030 (0.03)	0.960 (0.65)	0.200 (0.03)
7	Nov. 5, 2004	1.716 (0.10)	0.853 (0.21)	4.059 (0.15)	0.500 (0.12)	1.910 (0.27)	0.298 (0.03)
8	Nov. 28, 2004	2.172 (0.22)	0.345 (0.10)	3.084 (0.44)	0.305 (0.02)	2.532 (0.09)	0.258 (0.02)
9	Dec. 14, 2004	2.162 (0.28)	0.265 (0.11)	--	--	3.094 (0.27)	0.233 (0.02)
10	Dec. 27, 2004	2.462 (0.22)	0.235 (0.06)	2.067 (0.23)	0.255 (0.01)	3.100 (0.18)	0.250 (0.03)
11	Jan. 14, 20005	1.442 (0.37)	0.503 (0.15)	2.594 (0.69)	0.303 (0.0)	2.720 (0.07)	0.273 (0.01)
12	Jan. 28, 2005	1.116 (0.34)	0.300 (0.10)	2.896 (0.52)	0.163 (0.09)	2.987 (0.14)	0.348 (0.04)
13	Oct. 19, 2005	1.595 (0.12)	0.180 (0.05)	3.710 (0.20)	0.203 (0.0)	2.297 (0.23)	0.200 (0.01)
14	Nov. 2, 2005	0.660 (0.12)	0.200 (0.02)	3.226 (0.19)	0.280 (0.03)	3.045 (0.39)	0.240 (0.05)
15	Nov. 16, 2005	2.116 (0.78)	0.193 (0.01)	1.843 (0.47)	0.290 (0.01)	1.613 (0.48)	0.210 (0.02)
16	Nov. 30, 2005	0.746 (0.33)	0.243 (0.03)	3.106 (0.10)	0.295 (0.02)	2.016 (0.42)	0.258 (0.03)
17	Dec. 13, 2005	--	--	2.586 (0.90)	0.500 (0.08)	--	--

-- No data
Standard error in parenthesis.
* No data of nitrite were measured or only one measurement of nitrite was used to calculate nitrate-N.
The nutrients concentrations are means of four measurements for each site, based on a single sample.
Gray lines and yellow lines represent the results of the first and second periods, respectively, of the bamboo leaves colonization

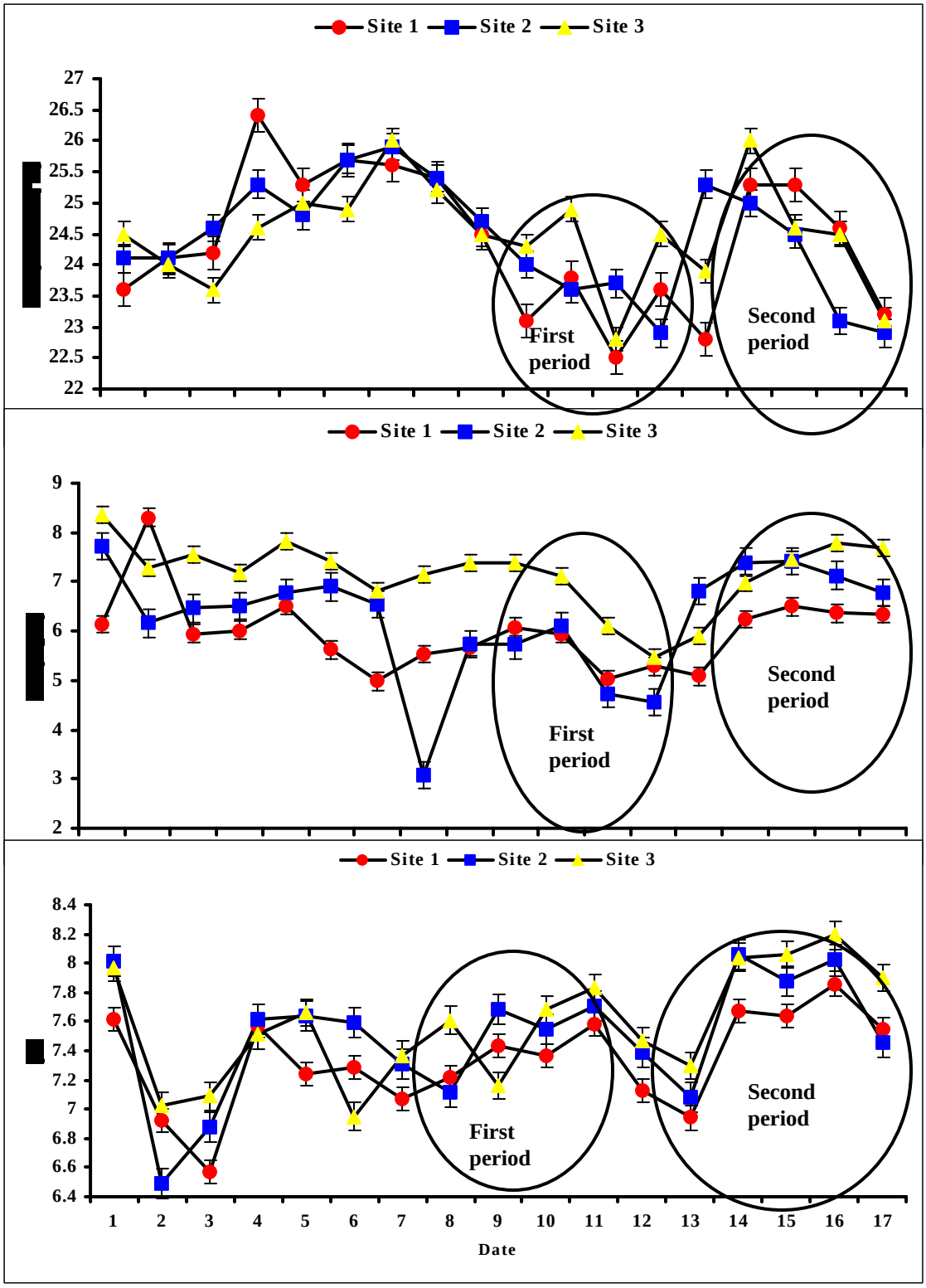

Figure 4. Patterns of physical and chemical parameters measured during the study. Circles represent the two periods of the bamboo leaves colonization study. Vertical bars indicate ± SE. Refer to Table 3 for abbreviation of dates.

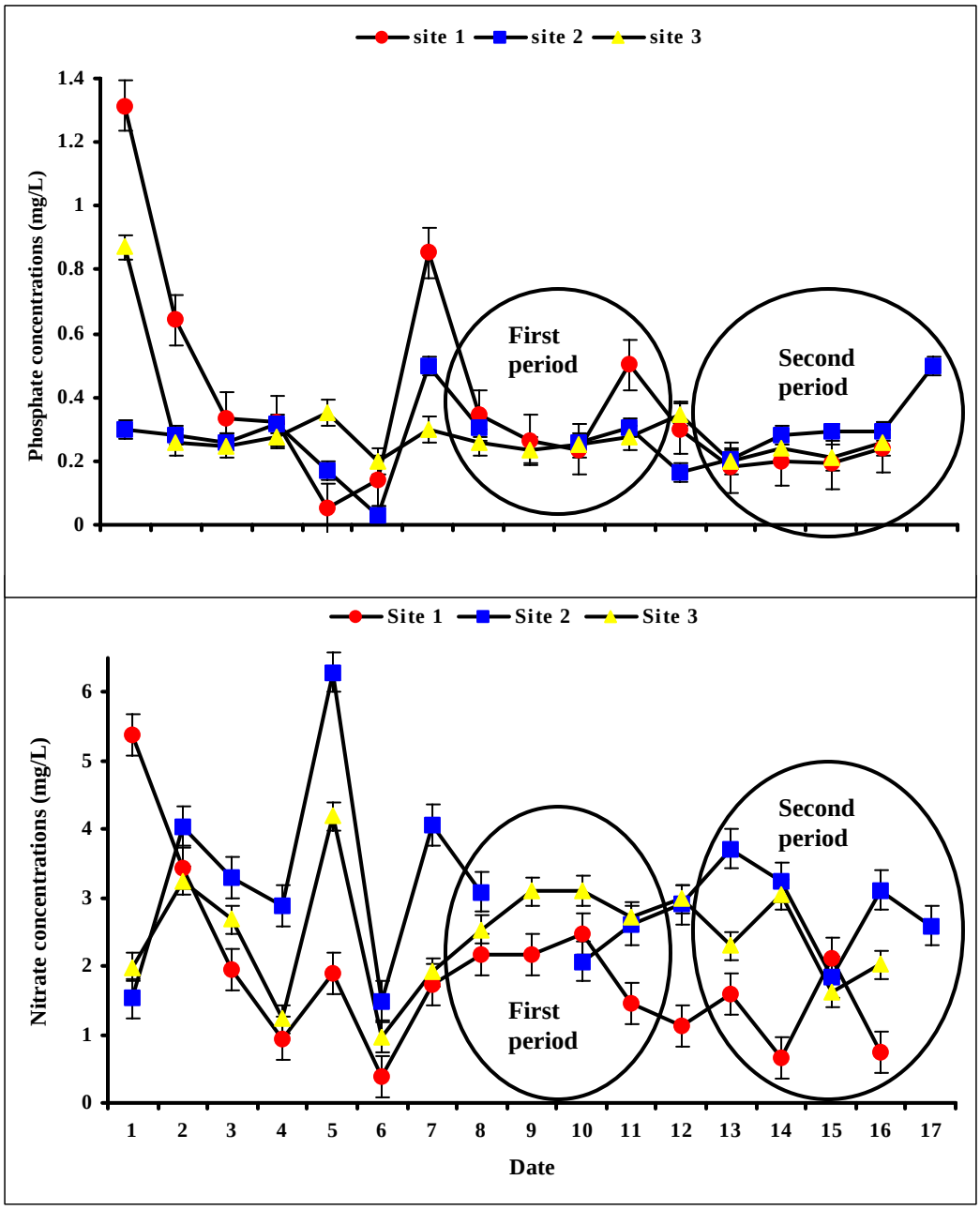

Figure 5. Mean concentration of phosphate and nitrate at the three sampling sites. Circles represent the two periods for the bamboo leaves colonization study. Vertical bars indicate ± SE. Refer to Table 4 for the abbreviation of dates.

During the first sampling period, the number of species associated with the bamboo leaf discs were six for site 1 and thirteen for site 3. The most abundant species was *Campylospora* sp. for site 1 (8.2 % relative abundance) and *Anguillospora* sp. for site 3 (6.2% relative abundance). At both sites, higher numbers of species were observed when the study started, but numbers decreased with time. The number of conidia on bamboo samples on the second sampling date (December 14, 2004) was higher than on the other sampling dates. Only species from *Campylospora* and *Helicomyces* for site 1 and *Helicomyces* for site 3 were found associated with the bamboo leaf disc during this sampling date.

Second sampling period: sampling using whole bamboo leaves in litter bags

In general, 28 taxa were found from bamboo litter bags in Quebrada de Oro between October and December 2005. The number of species observed on bamboo leaves for site 1 was high at the beginning of the study, with 7 species, and decreased later. *Helicomyces* sp. (11.7% occurrence) and *Helicomyces colligatus* (5% occurrence) were the predominant species for site 1, and *C. filicladia* (10% occurrence) and *Campylospora* sp. (5.8% occurrence) were so for site 3. Also, *Helicomyces* sp. was the most abundant for site 1 (17.1%) and *C. filicladia* was so for site 3 (32.1%). The number of conidia observed was high in the first sampling date, but decreased by the second sampling date; then increased again, and eventually decreased for both study sites. Site 2 was the area with the lowest number of recorded species and conidia compared with the other two sites. The number of species on bamboo leaves was generally low, fluctuating

from 1 to 4 species. Also, the number of conidia found on bamboo leaves was low with a peak in the last sampling date.

Bamboo leaves at site 3 harbored more species than those from sites 1 and 2. The species composition and abundance were more similar between sites 1 and 3, than compared to site 2. A cluster between site 1 and site 3 was observed by a cluster analysis. Sites 1 and 3 had a Jaccard (1-S) similarity coefficient of 0.87, indicating that both sites had a were similar in their percentages of relative abundance of species found on bamboo leaves. These sites had six species in common.

Relation between temperature, pH, D.O., nitrate, phosphate, precipitation and aquatic and aero-aquatic hyphomycetes on bamboo leaves

During the first period, there were no relation between the physico-chemical parameters and the fungal species found associated to bamboo leaves. Dissolved oxygen (D.O.) was the parameter most related to the number of fungal species found on bamboo leaves during the first period (November 2004 to January 2005), but no significantly. For example, *Campylospora chaetocladia*, *C. parvula*, *P. casuarinae*, *P. fluminea*, *P. ramificata*, *Pyramidospora* sp., *Tetraploa* sp., and *T. porosporiferum* showed a higher correlation with the concentration of D.O. (Spearman's correlation coefficient = 0.67, p = 0.08) (Appendix 10).

Few species were highly correlated with the physico-chemical parameters during the second period of study. Only nine species had a high relation with temperature, seven species with phosphate and one species with D.O (Appendix 11).

About 4.1 cm of pluvial precipitation were registered during the first period and 37.7 cm for the second period. Precipitation promoted erosion and evident sedimentation

in the cage areas. Also, organic matter, like leaves and branches, blocked the cages and prevented the full contact of the bamboo leaves with the water. During each sampling date, the cage was opened and cleaned of leaves, small branches, and sediments that accumulated inside and around the cage. The high water levels moved the cage out of the stream (December 27, 2004). To prevent this incident, each cage was attached to the bottom of the stream. These incidents may have precluded the interaction of the aquatic mycoflora with the bamboo leaves, deteriorated the leaves, and covered them with sediments.

Precipitation could have affected the colonization of bamboo leaves by aquatic hyphomycetes. During the first period of study, more species were found associated with bamboo leaves for sites 1 and 3 during the days when the precipitation was high. However, there was no significant correlation between precipitation and the number of species found on the bamboo leaves during both periods (Appendix 12 to 16).

Scanning electron microscopy to observe bamboo discs

Using SEM, *Campylospora* and *Helicomyces* were found associated with bamboo leaves. The presence of abundant mycelia and reproductive stages of the genus *Helicomyces* suggests that this taxon is involved in the decomposition of bamboo leaves (Fig. 6).

The same predominant species were found using light microscopy and SEM. SEM confirmed the germination of *Campylospora filicladia* on bamboo leaves (Fig. 7). Other microorganisms, such as bacteria, algae, and protozoans, were observed associated with the bamboo leaves under study (Fig. 8).

Aerated bamboo discs versus non aerated bamboo disc

The number of conidia found on aerated discs was higher than that on normal discs (not aerated) in this study. In the first phase of the study, the aeration technique promoted sporulation on disc samples from site 1, but this effect was not statistically significant [Appendix 16; T test independent, T= 0.33, p = 0.7703] (Fig. 9). For site 3, more conidia were observed on aerated discs than on the non-aerated discs with antibiotic; although, the numbers of produced conidia were not significantly different (Appendix 17; T test independent, T= 1.04, p = 0.4058). Leaf discs aerated for a few days became covered with mycelia and a small number of conidia (not identified). Conidiophores and conidia of *Helicomyces* and *Campylospora* were observed on the surface of aerated discs. Also, conidia of *Anguillospora* were observed on this type of disc.

Aerated and non-aerated bamboo-discs studied during the first period of study (from sites 1 and 3) did not have a significant variation in their number of conidia (Appendix 18; ANOVA, F = 2.29, p = 0.2272). The same pattern was observed in the second phase of the study, where some discs were aerated with and without antibiotic. Fewer conidia were found on aerated discs leaf. Results showed no differences between discs aerated with antibiotics and discs aerated without antibiotics (Fig. 10). When comparing results from site 1 and site 3, there were no significant variations in the number of conidia between aerated (with and without antibiotics) and non-aerated discs (Appendix 19; site 1: H = 0.62, p = 0.7434, site 3: H = 0.47, p = 0.8061).

Table 4. Fungal species associated with filtered water samples, leaf litter discs, and bamboo leaves, from November 2004 to January 2005 in Quebrada de Oro.

Fungal Species	**Site 1**			**Site 3**		
	Water (# samples)	**Leaf litter (# samples)**	**Bamboo (leaves)**	**Water (# samples)**	**Leaf litter (# samples)**	**Bamboo (leaves)**
Anguillospora crassa	-	-	1.03 (1.25)	-	- -	-
Anguillospora longissima	3.6 (25)	-	-	-	- -	-
Anguillospora pseudolongisima	-	-	-	3.6 (25)	- -	-
Anguillospora sp.	3.6 (25)	-	1.03 (1.25)	-	- -	6.2 (1.25)
Campylospora chaetocladia	3.6 (25)	-	-	3.6 (25)	- -	2.06 (1.25)
Campylospora parvula	-	-	-	-	- -	1.03 (1.25)
*Campylospora sp.**	-	-	8.2 (1.25)	3.6 (25)	- -	2.06 (2.5)
Clavariopsis azlanii	10.7 (25)	-	-	-	- -	-
Diplocladiella scalaroides	3.6 (25)	-	-	-	- -	-
Flabellocladia sp.	3.6 (25)	-	-	-	- -	-
Helicomyces colligatus	3.6 (25)	-	-	3.6 (25)	- -	-
Helicomyces sp. 1	10.7 (25)	-	-	-	- -	-
Helicomyces sp. 2	3.6 (25)	-	-	-	- -	4.64 (1.25)
Helicomyces sp. 3	-	-	-	-	- -	2.06 (1.25)
Helicomyces spp.	32.1 (50)	4.8 (25)	6 (3.75)	-	- -	4.12 (2.5)
Phalangispora nawawii	3.6 (25)	-	-	-	- -	-
Pyramidospora casuarinae	-	-	-	-	- -	4.12 (3.75)
Pyramidospora fluminea	-	-	-	-	- -	4.12 (2.5)
Pyramidospora ramificata	-	-	-	-	- -	2.06 (1.25)
Pyramidospora sp.	-	-	-	-	- -	5.15 (3.75)
Tetraploa aristata	-	-	1.03 (1.25)	-	- -	-
Tetraploa sp.	-	-	-	-	- -	1.03 (1.25)
Trinacrium cf. subtile	-	-	-	3.6 (25)	- -	-
Tripospermum porosporiferum	-	-	-	-	- -	1.03 (1.25)
Tripospermum sp.	-	4.8 (25)	1.03 (1.25)	-	- -	-
Triscelophorus acuminatus	-	52.4 (25)	-	-	- -	-
Triscelophorus sp.	-	38.1 (25)	-	-	- -	-
Total of species	**11**	**4**	**6**	**5**	**0**	**13**
Total of conidia	**23**	**42**	**18**	**5**	**0**	**79**

Data indicate percentage of relative abundance per site; parentheses indicate percentage of occurrence per site.

* *Campylospora* sp. was designated as the species described by Santos (1996).

- = not found

Table 5. Fungal species associated with filtered water samples, leaf litter discs, and bamboo leaf-discs, from October to December, 2005 in Quebrada de Oro.

Fungal Species	Site 1						Site 2						Site 3					
	Water (# samples)		Leaf litter (# samples)		Bamboo (leaves)		Water (# samples)		Leaf litter (# samples)		Bamboo (leaves)		Water (# samples)		Leaf litter (# samples)		Bamboo (leaves)	
Anguillospora filiformis	.37	(25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anguillospora longissima	2.21	(25)	-	-	-	-	-	-	-	-	-	-	6.62	(25)	-	-	.09	(.83)
Anguillospora sp.	.74	(25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beltrania rhombica	1.84	(25)	6.17	(25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brachiosphaera sp.	.74	(25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Campylospora chaetocladia	-	-	-	-	.09	(.83)	.37	(25)	-	-	-	-	7.35	(50)	.41	(25)	14	(4.2)
Campylospora filicladia	3.7	(50)	.8	(25)	.17	(.83)	.37	(25)	4.12	(25)	.6	(1.7)	-	-	.8	(50)	32.1	(10)
Campylospora parvula	3	(75)	-	-	-	-	-	-	-	-	-	-	3.7	(50)	-	-	-	-
*Campylospora sp.**	-	-	-	-	-	-	-	-	2.5	(25)	-	-	1.10	(25)	-	-	1.8	(4.2
Campylospora spp.	.74	(50)	-	-	-	-	1.47	(50)	-	-	-	-	-	-	-	-	15.1	(5.8)
Clavariopsis azlanii	7.7	(75)	-	-	-	-	1.10	(25)	-	-	-	-	7	(100)	-	-	-	-
Clavatospora tentacula	2.6	(50)	-	-	-	-	1.47	(25)	.8	(50)	-	-	36.8	(100)	-	-	-	-
Diplocadiella scalaroides	.37	(25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Helicomyces colligatus	-	-	-	-	4.2	(5)	-	-	-	-	1.4	(.83)	-	-	-	-	.43	(1.7)
Helicomyces roseus	-	-	-	-	-	-	-	-	-	-	.52	(.83)	-	-	-	-	-	-
Helicomyces sp. 2	-	-	-	-	5.6	(2.5)	-	-	-	-	-	-	-	-	-	-	-	-
Helicomyces sp. 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.09	(.83)
Helicomyces sp. 4	-	-	-	-	.17	(.83)	-	-	-	-	1.6	(.83)	-	-	-	-	-	-
Helicomyces spp.	3	(50)	.8	(20)	17.1	(11.7)	-	-	-	-	1.4	(3.3)	.37	(25)	-	-	1.6	(2.5)
Helicomyces torquatus	-	-	-	-	.17	(.83)	-	-	-	-	-	-	-	-	-	-	.09	(.83)
Lunulospora curvula	.37	(25)	-	-	-	-	-	-	-	-	-	-	3.7	(50)	-	-	-	-
Phalangispora nawawi	-	-	-	-	-	-	-	-	-	-	-	-	.37	(25)	-	-	.09	(.83)
Pyramidospora casuarinae	-	-	18.11	(25)	.09	(.83)	.37	(25)	63.8	(25)	-	-	-	-	-	-	.09	(.83)
Pyramidospora ramificata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	(.83)
Pyramidospora sp.	-	-	-	-	-	-	-	-	-	-	-	-	.37	(25)	-	-	.17	(.83)
Tetraploa sp.	-	-	.8	(50)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trinacrium sp.	-	-	-	-	-	-	-	-	-	-	-	-	.37	(25)	-	-	-	-
Tripospermum sp.	-	-	-	-	-	-	-	-	.8	(25)	-	-	-	-	-	-	-	-
Total of species:	**13**		**5**		**8**		**6**		**5**		**5**		**11**		**2**		**13**	
Total of conidia:	**74**		**65**		**319**		**14**		**175**		**63**		**184**		**3**		**774**	

Data show percentage of relative abundance per site; parentheses show percentage of occurrence per site.

* *Campylospora* sp. was designated as the species described by Santos (1996).

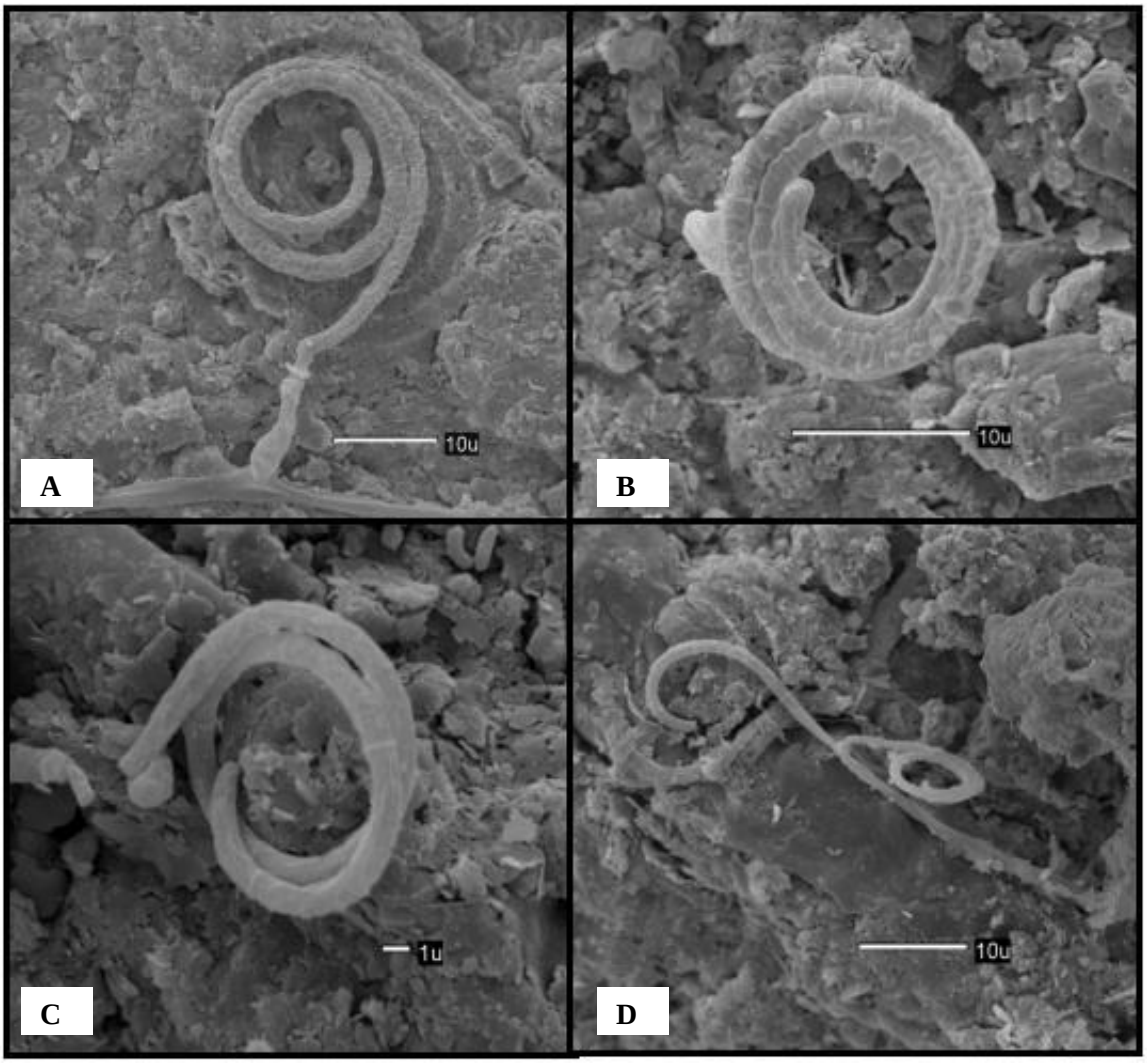

Figure 6. *Helicomyces* spp. conidia observed by SEM.

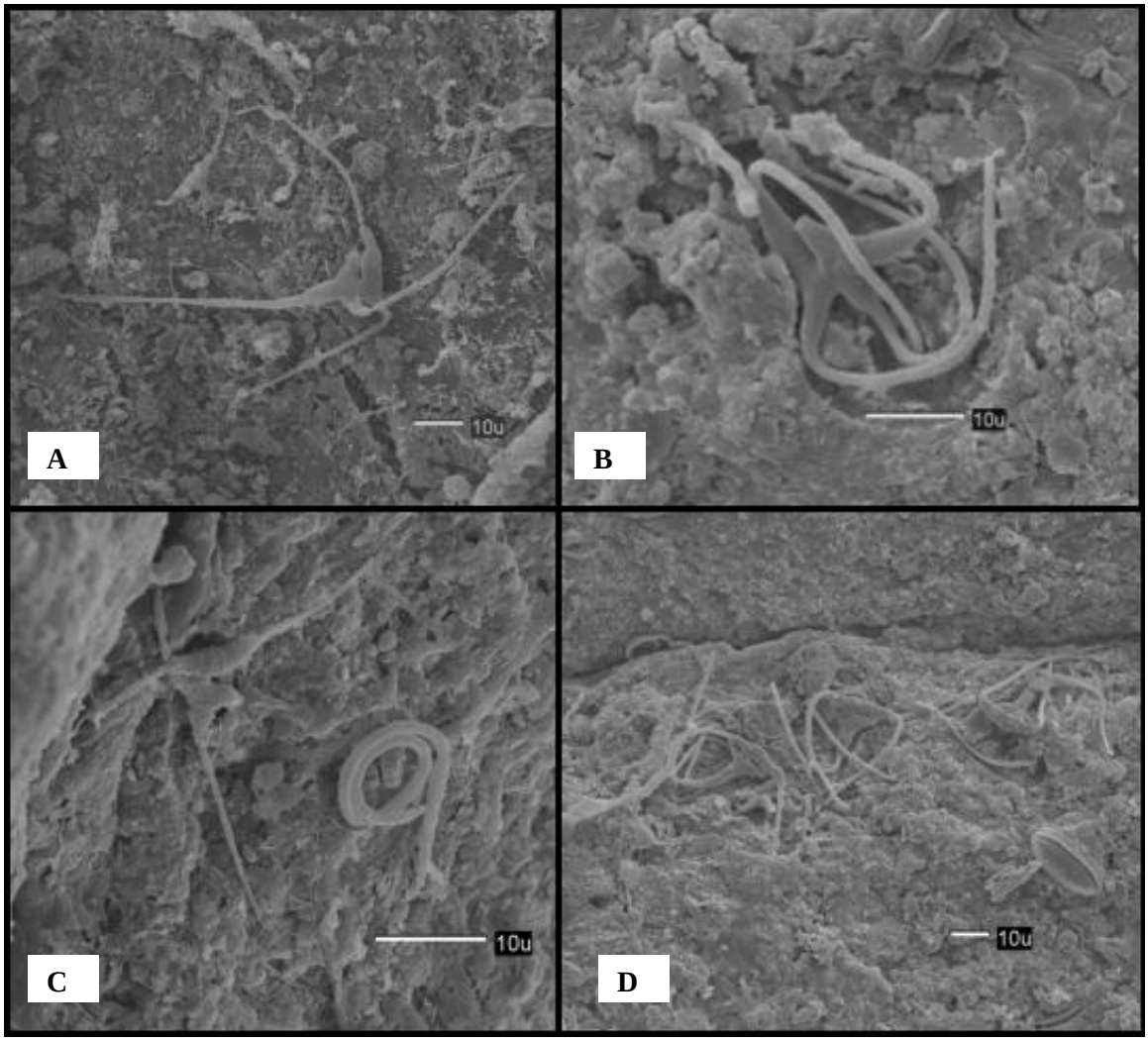

Figure 7. Diverse species of *Campylospora* observed on bamboo leaves by SEM: (A) *Campylospora* sp., (B) *C. chaetocladia*, (C) *C. filicladia*, and (D) *Campylospora* cf. *chaetocladia*.

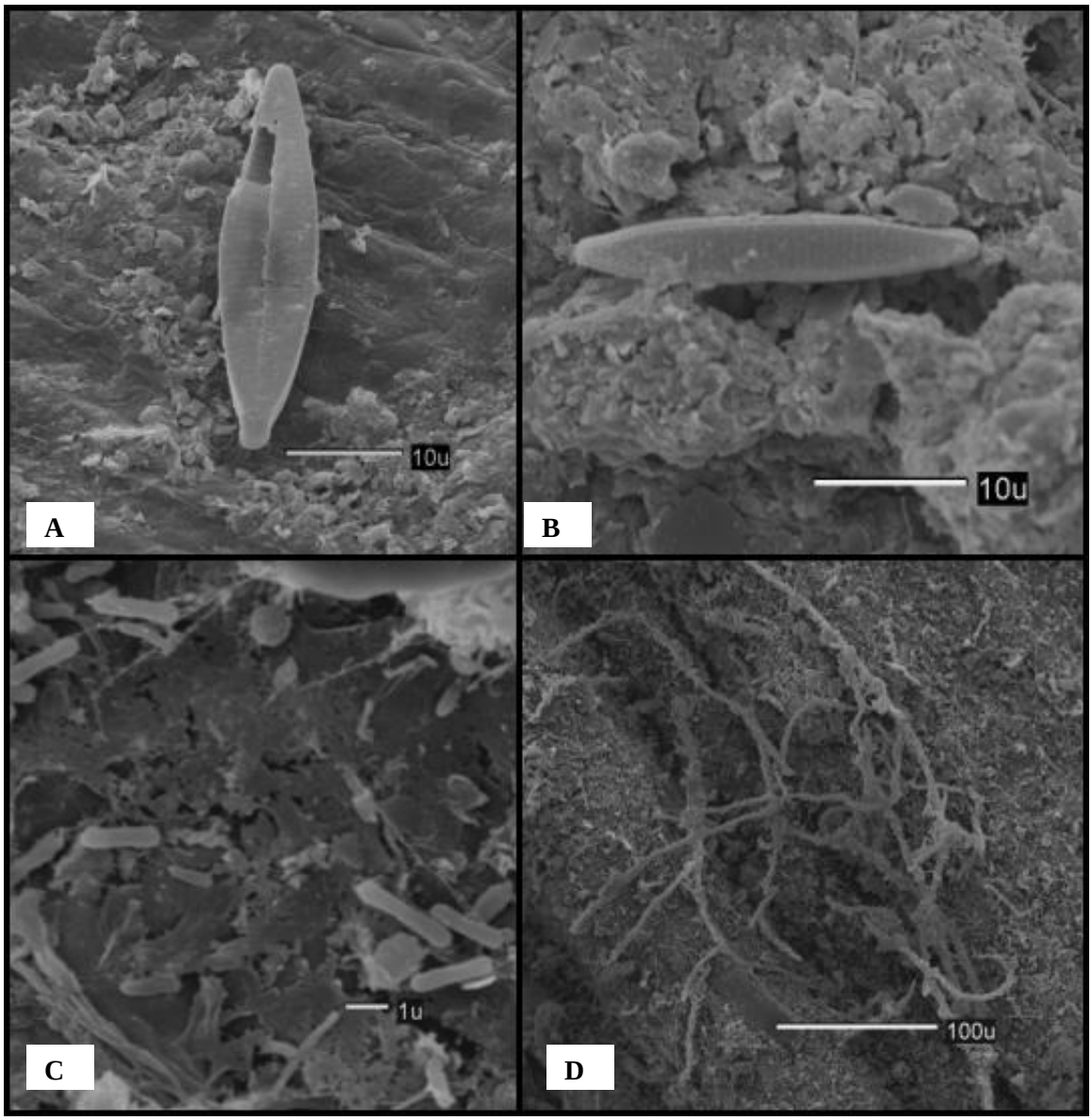

Figure 8. Other microorganisms found on the surface of bamboo leaves: (A and B) diatoms, (C) bacteria, and (D) mycelia.

The addition of antibiotic to the water for aeration apparently did not stimulate more sporulation, as supported by Wilcoxon test (Appendix 20).

Water and naturally-occurring leaf litter samples collected in Quebrada de Oro

First period of sampling:

A total of thirteen species were found from the water samples and only four species were observed on the naturally-occurring leaf litter between November 2004 and January 2005. Ten species were observed from water samples of site 1 and five species from site 3 (Table 4). For site 1, the most abundant fungus in the water samples was *Helicomyces* sp., with 50% of occurrence and 32.1% of abundance. All five species found in the water column for site 3 were observed in the same frequency and abundance.

Only four species were observed from naturally-occurring leaf litter samples obtained from site 1 and no species were detected from samples of leaf litter from site 3 during this first phase of the study. *Triscelophorus acuminatus* was the most abundant species in the leaf litter samples from site 1.

Second period of sampling:

Thirteen species were found in water samples collected in Quebrada de Oro between October and December 2005. *Clavariopsis azlanii* and *Clavatospora tentacula* were found at the three sites but only from the water samples (Table 5). The percentages of occurrence for these two species were: 75% and 50%, respectively, for site 1; 25% both for site 2, and 100% both for site 3.

A total of 13 species were found from water samples from site 1. Here, the number of species in the water was high in the first sampling date, declined, and increased again in the last sampling date. *Clavariopsis azlanii* had a 7.7 % of abundance, followed by *C. filicladia* (3.7%) and *Helicomyces* sp. (3%). *Pyramidospora casuarinae* was the most abundant species on leaf litter samples.

For site 2, water samples and leaf litter samples contained low number of species, but there were two abundant species in the water column: *Campylospora* sp. and *Clavatospora tentacula*. *C. tentacula* was also abundant in water samples from site 3. For site 2, about 63.8% of the conidia counted in leaf litter samples belonged to *P. casuarinae*.

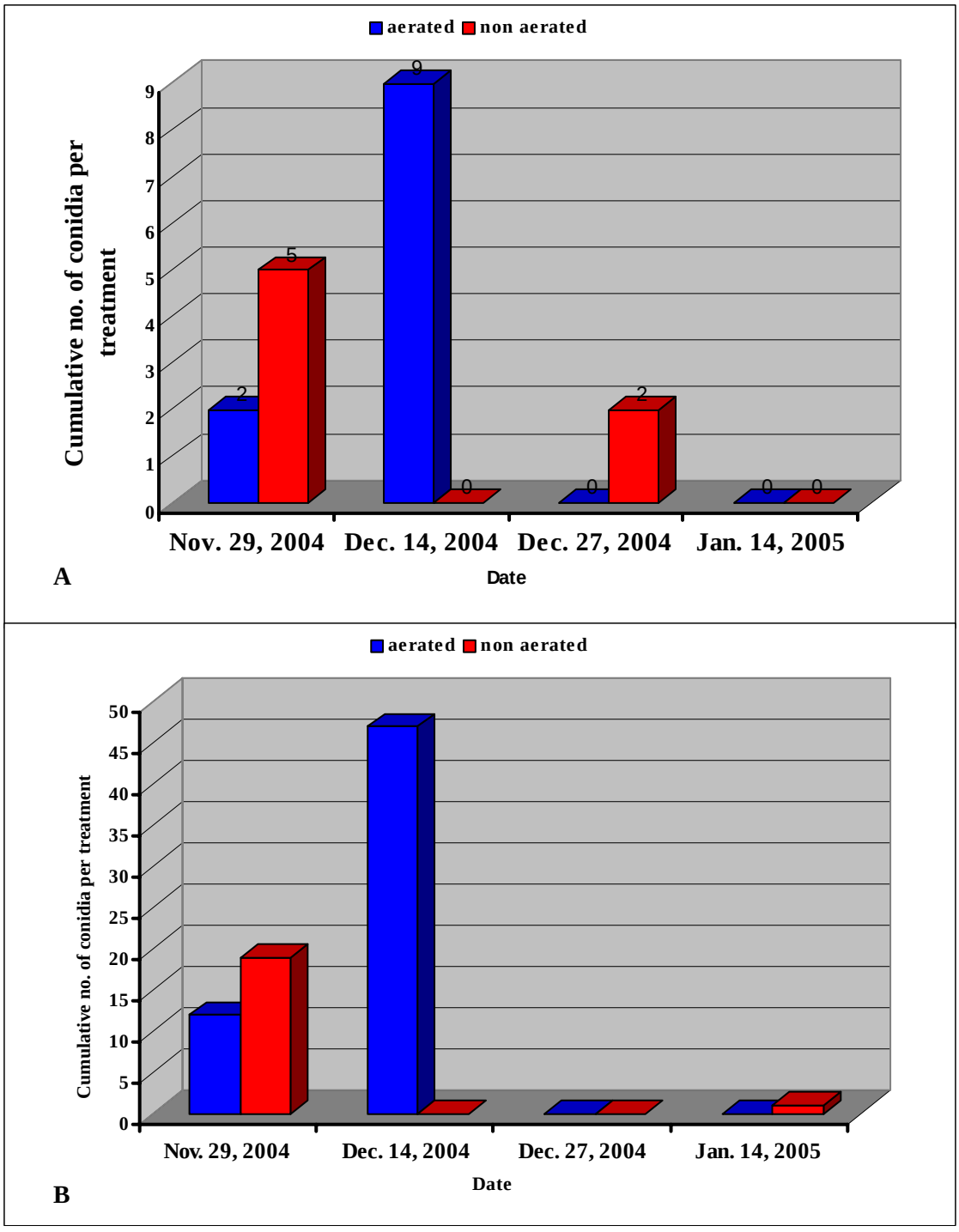

Figure 9. Number of conidia observed in the two different treatments on leaf discs (non- aerated discs and aerated discs with antibiotic) applied to leaf discs during the first phase of study: (A) Site 1, and (B) Site 3.

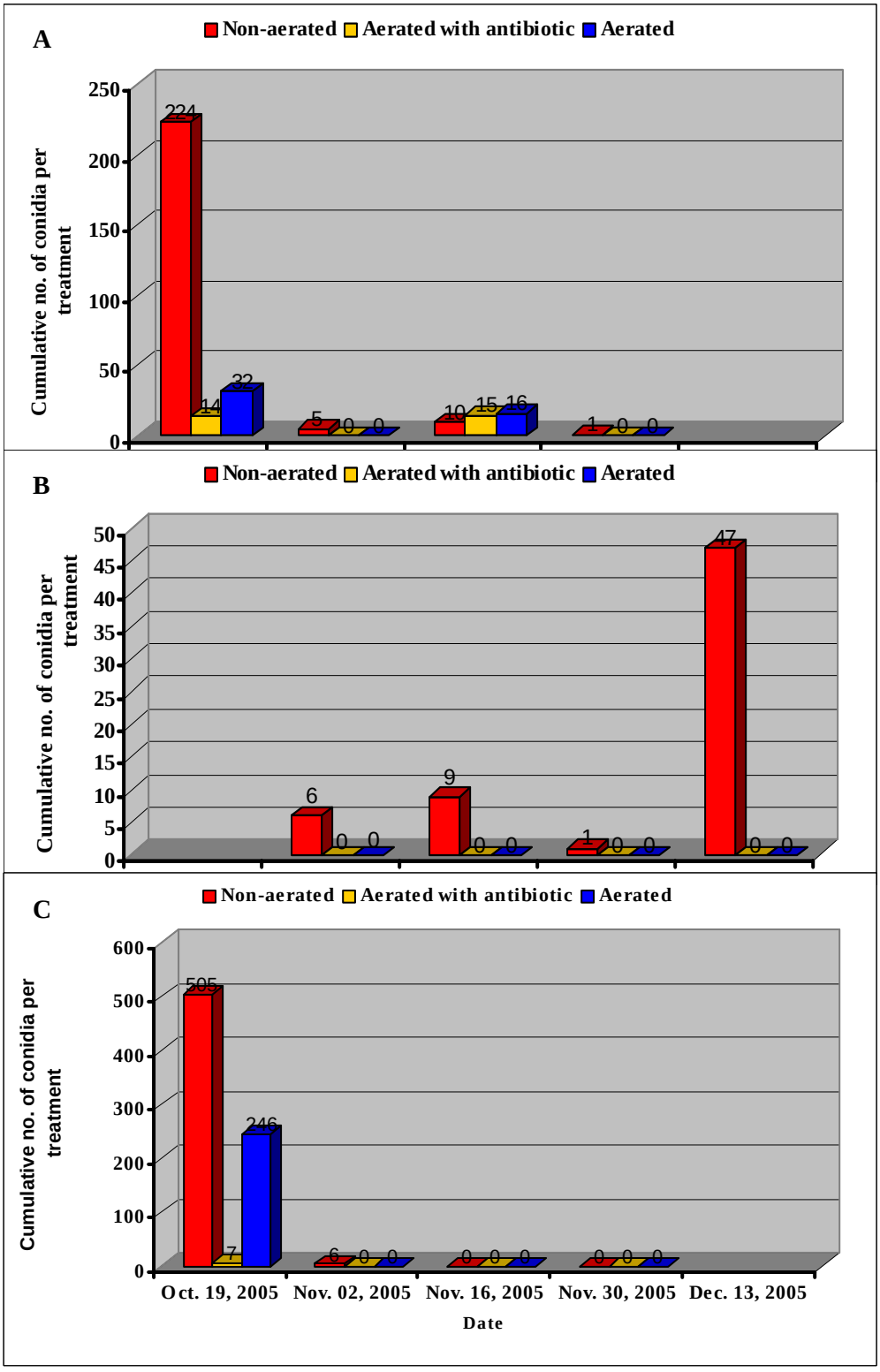

Figure 10. Number of conidia observed on the three different treatments on leaf discs (non-aerated, aerated without antibiotic, and aerated with antibiotic) applied to leaf discs during the second period of study: (A) Site 1, (B) Site 2, and (C) Site 3.

DISCUSSION

Physical-Chemical Factors

Quebrada de Oro is a surface water system, a type of resource designed as a source for drinking water, an area for preservation of aquatic species, and recreation by the Environmental Quality Board (E.Q.B.) (2003). This stream had a variety of pollution inputs, but the temperature, D.O., and pH registered during this study did not reflect impairment in the water quality. The water temperature in the study sites of Quebrada de Oro was below the standard implemented by the E.Q.B., which stipulates a temperature not higher than 32.2^{o}C for surface waters (Environmental Quality Board, 2003).

The mean D.O. was 6.42 mg/L, which is above the 5.0 mg/L limit implemented by the E.Q.B as a critical value for surface waters in Puerto Rico. The mean pH value for the stream was within standard values (6-9) and individual pH measures taken during most of the sampling dates were within this range. A direct effect of the temperature in the solubility of D.O. as was proposed by diverse authors (Hauer and Hill, 1996; Allan, 1995) was not observed. There was a positive correlation between the D.O. and pH, which could be influenced by the heterotrophic respiration in the system.

When contrasting the two sampling periods, the temperature, pH, and D.O. registered between November 2004 and January 2005 (first period) were slightly lower, but nitrate and phosphate concentrations were slightly higher. D.O. for site 3 was higher than in the other two sites during both periods. Shallower waters and faster flow at site 3 might have promoted higher diffusion of the oxygen. Within sites 1 and 2 there was more deposition of organic matter. The input of organic compounds into the stream should have significant impacts on the concentration of D.O. in the stream. The pH values

during both study periods were circum-neutral to slightly basic, but somewhat higher during the second period.

Nitrogen and phosphorus have different properties and are involved in diverse processes. Both are introduced to the aquatic ecosystem by rain, runoff, ground water and drainage basins, industrial, and residential waste. In some areas of Puerto Rico, the high total phosphorus concentrations are a problem and are primarily related to organic sources (Sotomayor-Ramírez et al., 2001).

The concentration of nitrate was lower for site 1 than in the other two sites during both periods. Site 1 is located in a secondary forest that could function as a buffer zone for the stream (Lowrance et al., 1984; Carpenter et al., 1998). In contrast, concentrations of nitrate in the study sites at Quebrada de Oro were much higher than those obtained in Mameyes River, a larger stream whose higher recorded concentration was approximately 0.25 mg/L (Ortiz-Zayas et al., 2005).

The main reservoir of nitrogen is the air and nitrogen can exist in diverse forms (Lowrance et al., 1984). Nitrogen can be retained through sediment deposition and adsorption of dissolved nutrients by the riparian ecosystem (Lowrance et al., 1984). Precipitation supplies nitrogen to the terrestrial ecosystem, but riparian vegetation helps in the retention of most of it, and only a small amount of nitrogen is drained into the water. Site 3 was an example of a riparian zone without a buffering forest and it evidently had a high impact of anthropogenic activity. This site was the area of study with the highest concentration of nitrate, followed by site 2.

Decomposition of the bamboo leaves

As imported organic matter, submerged leaves pass through different stages during their decomposition process. Within the first days after entering to the stream, soluble nutrients leach into the water (Webster and Benfield, 1986), and then microorganisms colonize the leaves. These colonizers begin the process known as conditioning, where the constituents of the leaf change by the effect of the enzymes, allowing the establishment of other aquatic microorganisms and the use of the substrates as food by microorganisms and macroconsumers (Benfield, 1996). Conditioning also leads to the fragmentation of the leaves and the reduction of the organic matter (Suberkropp and Klug, 1980). Apparently, this process occurred more rapidly on bamboo leaves than expected. Nutrients like N and P are known to rapidly leach from bamboo leaves (O'Connor et al., 2000). Mathuriau and Chauvet (2002) found in a study made in a stream of Colombia that *Croton* and *Clidemia*, two plant genera found also in Puerto Rico, had a rapid mass loss with only 5% of mass and 46%, respectively, of mass remaining after 29 days of immersion. In our study, fragmentation of bamboo leaves occurred as decay advanced, observing a complete loss of samples in 42 days. However, unlike in the present work, a study made in Puerto Rico by O'Connor et al. (2000) found that the decay of bamboo leaves was slower than leaves of *Syzygium jambos* and *Guarea guidonia*, observing only 40% of initial mass loss, after 42 days.

O'Connor et al. (2000) indicated that the composition of bamboo leaves could have an effect in the losses of leaf mass by mechanical processing. The chemical composition and texture of the leaves is known to affect breakdown, for example, the leaf concentration of nitrogen, carbon, tannins, cellulose and lignin (Mathuriau and Chauvet

2002). The anatomy of bamboo leaves should have an effect in the leaf breakdown. This leaf type has groups of bulliform cells in the upper epidermis that can occupy half leaf thickness, air spaces, lower mesophyll and lower epidermis (Rao, 1987). The vascular bundle arrangement is similar to those of other grasses, located parallel in the entire blade. To determine how the bamboo leaf composition affects interactions of aquatic hyphomycetes with the foliar tissues, it is necessary to use a different technique to monitor leaf decomposition. Leaf mapping was effective for Abdel-Raheem (2004) in the study of the structure of the aquatic hyphomycetes communities and the fungal diversity on *Eucalyptus rostrata* leaves, and might prove to be useful in the study of bamboo leaves.

Rainfall detached a variety of particles and transported them into the stream. The current moved sediments through the cage, allowing the interaction of many particle types with the surface of bamboo leaves, fragments of dematiaceous mycelia, dead insects, spores of terrestrial fungi, and sediments on the samples were observed. Water flow is an important factor that removes potential substrates (i.e. leaves) before fungi could colonize them. At the same time, physical factors, like currents and abrasion, could have effects on the bamboo leaves. The fragmentation of bamboo leaves could be the combination of microbial activity, invertebrates, and physical factors. Runoff and high flows have been shown to increase fragmentation and accelerated the breakdown of leaves softened by microbial activity (Paul et al., 2006). Mathuriau and Chauvet (2002) noticed that turbulent water flow provoked a release of fine particulate from leaves of *Croton* organic matter. According to our observations, bamboo leaves were fragmented rapidly in Quebrada de Oro, but bamboo is a predominant plant in some zones of the

stream, and has a leaf fall rate that exceeds that of other species during some seasons of the year (O'Connor, 2000), thus, maintaining an input of leaf litter to the stream and giving the impression that its leaf litter decays slowly.

The number of fungal species on bamboo leaves was similar to that in the water samples, but the species compositions were different. Some species found on bamboo leaves were not found in the other two types of samples (water and leaf litter of other plants). There were about eleven species during the first period and seven during the second period that were found only on the bamboo leaves.

Effect of physical-chemical factors on the hyphomycetes associated with the bamboo leaves

Nutrients have an effect on leaf breakdown and microbial communities in stream, but this effect is variable (Suberkropp and Chauvet, 1995). Fungi can assimilate inorganic nitrogen from the environment and use it for the production of intracellular compounds.

In this study, a weak to moderate relation between the concentration of nitrate and phosphate in the water and the diversity of fungi associated with bamboo leaves was observed. Nevertheless, Gulis and Suberkropp (2004) found that conidia concentration increased with an increase on the concentration of nitrogen and phosphate in the stream. Another study found that conidia production appeared to be stimulated by low concentration of nitrate, but high levels could suppress the activity, and phosphate did not stimulate significantly the production of conidia (Sridhar and Bärlocher, 1997). Total spore production also increased significantly with an increase nitrogen and phosphorus in a study by Sridhar and Bärlocher (2000).

However, the concentration of nitrate and phosphate in Quebrada de Oro was not as high as in the studies by Suberkropp and Chauvet (1995), Sridhar and Bärlocher (1997), and Gulis and Suberkropp (2004). The highest concentrations registered in this study were 3.7 mg/L of nitrate and 0.5 mg/L of phosphate, compared with 70 mg/L of nitrate and 1 mg/L of phosphate as observed by Sridhar and Bärlocher (1997).

The temperature registered in this study had a moderate to high correlation with the number of conidia found on the bamboo leaves that were experimentally submerged. Temperature influences the enzymatic activity and the amount of growth (Moore-Landecker, 1996), and also affects the sporulation of some fungi. Chauvet and Suberkropp (1998) observed that high temperatures (over 25^{o}C) were unfavorable for growth and sporulation of some aquatic fungi (i.e. *Tetracladium marchalianum*).

Chamier (1992) suggests that the effect of pH on the different fungi could be indirect; for example, the pH influences the concentrations of inorganic aluminum and calcium ion available in the water lowering the buffering capacity of the water. At the same time, the pH has a direct relation with the capability of the fungi to degrade the organic matter by influencing the enzymes activity (Suberkropp and Klug, 1980). Plant cell-wall polymerases have an optimum pH, but they vary considerably and show activity over a range of pH values. But in our present study, the pH was not significantly correlated with the species found on bamboo leaves.

Most of the studies about the effect of nutrients and physico-chemical factors were conducted in temperate zone where these streams are different from their tropical counterparts, making it difficult to compare their results with those in the present study. The physico-chemical parameters that are usually monitored influence synergistically the

behavior of other components in the water. Therefore, the measured physico-chemical parameters in the current study are not necessarily the factors directly influencing the fungi.

Fungi on bamboo leaves, leaf litter, and stream water from Quebrada de Oro

During the first period of sampling, there were more species associated with bamboo leaves than in the water column. Four more species were found in the water column for the second period rather than associated with bamboo leaves. The quantity of filtered water sample was changed from 100 mL during the first period to 500 mL during the second period. However, the difference between the numbers of species counted on bamboo leaves versus observed in water samples was lower. Santos-Flores (1996) observed sixty-five species in the water column of the same stream. Like in the present study, Abdel-Raheem (2004) observed much lower numbers of species using filtration methods than employing other methodologies. Also, the species found on bamboo leaves differ from the mycoflora observed during the study, suggesting that the substratum (bamboo leaves) might stimulate or inhibit the colonization by fungi in the stream. From site 1, two of the four species associated with the leaf litter during the first period were observed on bamboo leaves, and during the second period of study, three of the five species observed on leaf litter were found on bamboo leaves. One of the five species associated with the leaf litter was found on the bamboo leaves from site 2, and two of them were recorded in samples from site 3. The lower diversity of aquatic hyphomycetes was found in the leaf litter samples. In a tropical stream, in India, the highest diversity of species was found using this technique, i.e. the examination of naturally occurring

submerged litter (Sridhar and Kaveriappa, 1984). However, they observed higher occurrences of fungi over the months of July to December, similar to what was found in the present study.

The number of species associated with bamboo leaves for site 1 was lower than for site 3 during the first period of study. The same results were observed during the second period. During the first period, ten of the thirteen species found on the bamboo leaves for site 3 were also found associated with samples collected for site 1. Also, bamboo leaves situated for site 3 were colonized by species that also occurred in the stations upstream. These species were observed on the samples of sites 1 and 2 during the second period.

The fungal community composition could also be related with the type of riparian vegetation (Chan et al., 2000). The riparian zone on site 1 consisted of mixed wood forest, shrubs, and palms. The littoral stream on site 2 had more bamboo species, shrubs, and grasses, than trees. Upstream morphology of Quebrada de Oro should have allowed the retention of organic matter as well as the development of more species (*sensu* Bärlocher, 1982). However, more species were found associated with bamboo leaves located downstream (site 3). The riparian area of site 3 had lower vegetation cover, dominated by grasses, bamboo and pines. Site 3 is located in a straight zone, where leaves were rapidly flushed away, and the only retention of leaves was within the cage. Therefore, the low input of substrate most have been washed downstream.

Garnett et al. (2000) proposed that the fungal species richness on submerged substrates is initially low, rises to a peak, and then decreases or maintains stable. But the species found on bamboo leaves did not show that common pattern. In both study

periods, most of the species increased at the beginning and then showed a rapid decrease as was also observed by Mathuriau and Chauvet (2002) with *Croton* and *Clidemia* leaves in a stream in Colombia. Some species were observed sporadically during the sampling season and did not have a clear pattern of colonization on bamboo leaves. During the first period, conidium *Tripospermum* and *Tetraploa aristata* were observed only on bamboo leaf samples for site I, and *Campylospora parvula*, *Tripospermum porosporiferum* and *Tetraploa* sp. was observed on bamboo leaf samples for site 3. During the second period, *Pyramidospora casuarinae* was observed once on bamboo samples for site 1 and 3.

In the Ave River in Portugal, the aquatic hyphomycetes had a peak of sporulation between the first and the third week of immersion (Pascoal et al., 2003). Maharning and Bärlocher (1996) observed an increment in conidia production after four weeks, with a subsequent decline. In the current study, bamboo leaves were submerged for over three weeks in Quebrada de Oro, observing a peak of species and conidia production by the third week that were equivalent to those measured on the first sampling day.

Bamboo leaf discs: aerated versus non aerated

In the present study a closed system was used which maintained all the metabolites and components of the decomposition in the surrounding solution. Sridhar and Bärlocher (1997) found that lecheates of sugar maple leaves suppressed the sporulation when added to a microcosm. On the aerated discs of the present study, mycelia were observed growing on the leaf discs, suggesting that compounds liberated during the decomposition of bamboo discs not necessarily had negative effect on fungal growth. Also, the use of leaf discs could increase the access of the fungi to the tissue,

promoting in the colonization of the fungi, and diminish the effects of other factors in the microcosms. Wright and Covich (2005) proposed that cutting the leaf provides access of bacteria to the tissue.

Bacterial growth on the leaf promotes competition for habitat (Sridhar and Bärlocher, 1997). When comparing the discs aerated in the presence of antibiotics with those aerated without antibiotics, no significant difference was found in the number of fungal species ($F = 2.29$, $p = 0.2272$). Nevertheless, Wright and Covich (2005) found higher biomass on fungi on leaves (treated with antibiotic to minimize bacteria) than on controls (no antibiotic) in a study made with leaves of cecropia (*Cecropia scherberiana*) and tabonuco (*Dacryodes excelsa*). The objective of using antibiotic in the present study was also to reduce bacteria associated with the bamboo leaf discs. However, it possible that the bamboo leaf samples do not had a high density of bacteria associated and the effectively of the antibiotic was not noticed. Perhaps, the concentration of nutrients available on the tubes (closed system) could have inhibited the sporulation. Fungi do not waste energy in the production of conidia if there are nutrients available for vegetative growth (Bärlocher, 1992). Many fungi sporulate when deprived from nutrients, but avoid sporulation when in a surplus of nutrients is available (Carlile et al., 2001). Fungi produce conidia for dispersal to a new habitat or on survival, and sometimes sporulation is stimulated by interplay of different environmental factors.

Comparison with others studies made in Puerto Rico

The number of fungal species on bamboo leaves was higher than the number of species associated with other exotic plant species reported in previous studies in Puerto

Rico, such as *Erythrina peoppigiana*. Santos-Flores (1996) found that four aquatic species (*A. crassa*, *C. chaetocladia*, *Clavariopsis azlanii* and *P. casuarinae*) colonized the leaves of *E. peoppigiana* in the same stream. The limited number of species found on the leaves of *E. peoppigiana* contrast with sixty-five species reported from water and foam samples in the same study. The low number of species is remarkable considering the high number of samples observed by Santos-Flores (1996). Santos-Flores (1996) observed about 54 leaves discs per sampling station for every sampling day compared with about 30 leaves discs in this research. In contrast with the pattern of colonization by Santos-Flores (1996), where an initially low number of fungi increased with time, in the bamboo leaves the opposite was observed, a high number species initially colonized the samples and then decreased with time. Santos-Flores (1996) suggested that the competition for substrate with *Fusarium* sp., the dominant species in that study, accounted for the low number of aquatic hyphomycetes on leaf litter of *E. peoppigiana*. The frequencies of occurrence of aquatic hyphomycetes and their colonization vary among leaf species due to the composition of the substratum (Abdel-Raheem, 1997). Therefore, the differences between aquatic hyphomycetes found in bamboo leaves and *E. peoppigiana* leaves could be related to the type of leaf.

Another study conducted in Puerto Rico with an exotic plant, *Syzigium jambos*, reported twelve species associated with the leaf litter of this plant (Justiniano and Betancourt, 1989a), fewer than on bamboo leaves. Four species identified from bamboo leaves in Quebrada de Oro overlapped with those found in *S. jambos* in Maricao. The dominant species on *S. jambos* were: *A. crassa*, *A. longissima*, *Campylospora* sp., *Flagellospora penicilloides*, and *Lunulospora curvula*. *Campylospora filicladia*,

Helicomyces colligatus, and *Helicomyces* sp. were the dominant species on bamboo leaves. Justiniano and Betancourt (1989a) observed about 399 discs of *S. jambos* (15 mm diam.) during their study. However, in the present study we observed about 520 discs of bamboo leaves, which mean about 120 discs more than Justiniano and Betancourt.

In natural stages, bamboo decomposition was more slowly than other riparian vegetation, providing habitat for aquatic species (O'Connor et al., 2000). Also, the nutrient dynamics for *S. jambos* differed from that of bamboo, which immobilizes low concentration of nutrients. In contrast with the results observed in the Luquillo Experimental Forest (O'Connor et al., 2000), the breakdown of bamboo leaves was fast in Quebrada de Oro. Thirty days after submersion in the stream, empty leaf bags were found in some traps. Mechanical process could have helped in the decomposition of this type of leaf in Quebrada de Oro.

A study made in Puerto Rico with native plants (*Buchenavia capitata, Cordia borinquensis*, *Dacryodes excelsa*, *Manilkara bidentada*, and *Sloanea berteriana*) in a small stream at the Caribbean National Forest, showed a limited number of aquatic hyphomycetes. About six to eight hyphomycetes species associated with leaf discs of these plants were reported (Padgett, 1976). Six species were found on leaf discs of *B. capitata*, *C. borinquensis,* and *D. excelsa*; seven on *M. bidentada*, and eight on *S. berteriana*. From those, four aquatic hyphomycetes were observed on the bamboo leaves in the present study: *Anguillospora longissima*, *Campylospora chaetocladia*, *Pyramidospora casuarinae* and *Tripospermum* sp.

According to Padgett (1976), *C. borinquensis* was the leaf-type with the lowest occurrence of fungi suggesting that its composition inhibits the colonization by aquatic

hyphomycetes. Also, a lower number of species was reported on the leaf discs than the number represented in foam samples (17 species). A possible substrate specificity of some aquatic hyphomycetes was suggested in that study. The diversity of riparian plants promotes substrate variety for the decomposers, like freshwater fungi (Sridhar et al., 2000), and at the same time maintains a diversity of species on the aquatic habitat.

If the current study is compared with other studies made in the same stream, we documented a lower diversity (with sixteen genera of aquatic and aero-aquatic hyphomycetes). Betancourt and Justiniano (1989) identified thirty-five species in the same stream, which belonged to twenty-eight different genera. Santos-Flores (1996) recorded sixty-five species and forty-nine genera on foam and water samples from Quebrada de Oro, obtaining a high number of conidia and diversity in foam samples. Both studies had higher diversity of species than the current report. These studies included foam samples, which had more suspended conidia, and are proven effective traps for conidia (Chan et al., 2000). Conidia of aquatic and aero-aquatic hyphomycetes concentrate in the foam and are observed in a high number and variety (Gessner at al., 2003). Foam sample were not collected in the present study because the area designed for the research did not promote the accumulation of foam. The morphology of the stream in the three sites does not provide downstream rapids and accumulation of branches that promote the natural production of foam.

Leaf samples

The number of species on the leaf litter was higher during the second period. If we compare the techniques used in the first and second periods to monitor the leaf litter,

the second one was more efficient on the recovery of species. The morphology and anatomy of the leaves of some species of plants found in Quebrada de Oro, like *Castilla elastica,* do not allow passage of light through the samples and affected visibility under the microscope. Some leaves were too opaque to permit observation of fungal structures on the surface.

Aeration of leaves in distilled water and the further collection of spores produced by the filtration technique have been suggested as indicators of the ecological contribution of fungi in a particular stream (Garnett et al., 2000). The technique of aerated leaf litter, with subsequent filtration to observe conidia allowed the recovery of conidia from the samples. However, there was no information on how long the naturally-occurring leaf litter had been submerged in the stream. On the current study this technique did not provide the same results obtained in other studies. Aerated leaf discs allowed observing more conidia of some species, but the diversity and the abundance of the spores obtained by this technique was lower than for water column samples and by observation of the whole bamboo leaves.

The number of species found in this study suggests that Quebrada de Oro still provides favorable conditions for aquatic and aero-aquatic hyphomycetes. However, the number of species, when compared with previous studies from the same stream, was lower in the current work, suggesting a significant reduction in fungal diversity in this stream in the past twenty years. This study suggests that aquatic and aero-aquatic hyphomycetes might play a role in the decomposition process of bamboo leaves in this subtropical stream.

CONCLUSIONS

- The temperature, D.O., pH, nitrate and phosphate values registered during this study did not change drastically overtime. These factors probably did not influence significantly the colonization of bamboo leaves in Quebrada de Oro.

- The genera *Helicomyces*, *Campylospora*, and *Pyramidospora* were the dominant aquatic fungi in the colonization of bamboo leaves in Quebrada de Oro.

- Aquatic and aero-aquatic hyphomycetes such as *Campylospora* and *Helicomyces* used bamboo leaves as substrate for their growth and reproduction. At least, twenty-two species of aquatic and aero-aquatic hyphomycetes were found associated with bamboo leaves during this study.

- Fungi found associated with bamboo leaves were part of the aquatic fungal community (i.e. present in the water column or on other substrate) in Quebrada de Oro. About ten species of the twenty-four species found on the water column were observed on bamboo leaves. There was higher abundance of conidia in the water column than on bamboo leaves.

- The same number of species and the same predominant genera were documented using two different techniques to monitor the colonization of the bamboo leaves in three areas of study at Quebrada de Oro: leaf discs in mesh bags and bamboo leaves bags.

- When compared with other research made with native vegetation and with other exotic plants in Puerto Rico, bamboo leaves provided a good habitat for some aquatic hyphomycetes.

- The number of species observed in natural occurring leaves in the study sites of Quebrada de Oro was lower than the number reported on bamboo leaves. Contrary to other studies, randomly collected leaves provided little information about the aquatic hyphomycetes community of the stream.

- The aquatic hyphomycetes diversity in Quebrada de Oro has decreased with time when compared with other studies made in the same creek, which reported higher number of conidia and higher diversity of species.

RECOMMENDATIONS

To provide more information about the decomposition process of the bamboo leaves and the fungi associated with this activity, it is recommended to:

- extend the period of monitoring and reduce the time between collections during the first days, after the bamboo leaves are submerged.
- use molecular techniques to determine the biomass of the fungal community associated with the substrate.
- modify the aeration system by changing the water used to aerate the leaf discs during the process, reduce the input of air to avoid fragmentation of the discs, measure the temperature and pH, and avoid the incorporation of antibiotics to the system to maintain a system more similar to the natural condition.
- use the water of the aeration system (including water changes) to observe conidia by a filtration technique.

To observe the structure of aquatic hyphomycetes communities in Quebrada de Oro, it is suggested to:

- increase the number of collected samples of water and leaf litter through the creek.
- measure total nitrogen, total phosphate, and the water flow in the stream.

To compare the interaction of aquatic organisms during the decomposition process of the bamboo leaves and the use of this substrate in the food web in tropical streams, it is recommended to:

- design similar research in other streams of Puerto Rico where bamboo is part of the riparian vegetation.
- study aquatic organisms that use bamboo leaves as habitat in other streams of Puerto Rico.

LITERATURE CITED

Abdel-Raheem, A.M. 2004. Study of the effect of different techniques on diversity of freshwater hyphomycetes in the River Nile (upper Egypt). Mycopathologia. 157:59-72.

Abdel-Raheem, A.M. 1997. Colonization pattern of aquatic hyphomycetes on leaf peaks in subtropical stream. Mycopathologia. 138:163-171.

Allan, J.D. 1995. Physical factors of importante to the biota. In: Stream Ecology: structure and function of running waters, pp. 45-82. Kluwer Academy Publishers, Dordrecht.

Baldy, V., and M.O. Gessner. 1997. Towards a budget of leaf litter decomposition in a first-order woodland stream. Ecology. 320:747-758.

Bärlocher, F. 1992. Ecology of aquatic hyphomycetes. Springer-Verlag, Germany. 225pp.

Bärlocher, F. 1982. On the ecology of Ingoldian fungi. Bioscience. 32(7):581-586.

Bärlocher, F., and M.A.S. Graça. 2002. Exotic riparian vegetation lowers fungal diversity but no leaf decomposition in Portuguese streams. Freshwater Biology. 47:1123-1135.

Bärlocher, F. and J.J. Oertli. 1978. Colonization of conifer needles by aquatic hyphomycetes. Canadian Journal of Botany. 56(1):57-62.

Bärlocher, F., J.J. Oertli and R. Guggenheim. 1979. Accelerated loss of antifungal inhibitors from *Pinus leucodermis* needles. Transactions of the British Mycology Society 72(2):277-281.

Benfield, E.F. 1996. Leaf breakdown in stream ecosystems. In:Hauer and Lamberti (eds). Methods in stream ecology, pp. 579-589. Academic Press, San Diego.

Betancourt, C., and M. Caballero. 1983. Aquatic hyphomycetes (Deuteromicotina) from Los Chorros, Utuado, Puerto Rico. Caribbean Journal of Science. 19(3-4):41-42.

Betancourt, C., and J. Justiniano. 1989. Hongos ingoldianos presentes en el arroyo de Oro, Mayagüez, Puerto Rico. International Journal of Mycology and Lichenology. 4(1-2):1-8.

Betancourt, C., J. Cruz, and J. García. 1987. Los hifomicetos acuáticos de la Quebrada Doña Juana en el Bosque Estatal de Toro Negro, Villalba, Puerto Rico. Caribbean Journal of Science. 23(2):278-284.

Betancourt, C., J. Cruz, J. García, and L. Galarza. 1986. Estudio preliminar de los hifomicetos acuáticos (Deuteromicotina) de la República Dominicana. Caribbean Journal of Science. 22(1-2):49-51.

Boulton A.J. and P.I. Boon. 1991. A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf?. Australian Journal of Marine and Freshwater Research. 42:1-43.

Bozzola, J.J. and L.D. Russell. 1999. Electron Microscopy: Principles and techniques for biologist. 2nd ed. Jones and Bartlett Publishers, Sudbury. 670pp.

Burgos J. and C.A. Shearer. 1983. Hifomicetos acuáticos de Magallanes (Chile). Boletín Micológico. 1:143-146.

Cardona, N., and R. Rivera. 1986. Hifomicetos acuáticos del Río Cañas, barrio Río Cañas, Mayagüez, Puerto Rico. Unpublished.

Carlile, M.J., S.C. Watkinson, and G.W. Gooday. 2001. Spores, dormancy and dispersal. In: The Fungi, pp. 185-242. 2nd ed. Academic Press, London.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications. 8(3):559-568.

Chamier, A.C. 1992. Water chemistry. In:F. Bärlocher (ed.). Ecology of aquatic Hyphomycetes, 153-171. Springer-Verlag, Germany.

Chan, S.Y., T.K. Goh, and K.D. Hyde. 2000. Ingoldian fungi in Lam Tsuen River and Tai Po Kau forest stream, Hong Kong. Fungal Diversity. 5:109-118.

Chauvet, E., and K. Suberkropp. 1998. Temperature and sporulation of aquatic hyphomycetes. Applied Environmental Microbiology. 64(4):1522-1525.

Chan, S.Y., T.K. Goh, and K.D. Hyde. 2000. Ingoldian fungi in Hong Kong. Fungal Diversity. 5:89-107.

Environmental Quality Board. 2003. Puerto Rico Water Quality Standards Regulation. Commonwealth of Puerto Rico, Governor's Office.

Ewel J.J. and J.L. Whitmore. 1973. The ecological zones of Puerto Rico and the U.S. Virgin Islands. Forest Service Research Paper ITF-18. Institute of Tropical Forestry and Forest Service, U.S. Department of Agriculture, 71 pp.

Farrelly, D. 1984. The book of the bamboo. Sierra Club Books, San Francisco, 332 pp.

Francis, J.K. 2000. *Bambusa vulgaris* Schrad ex Wedl. In:J.K. Francis and C.A. Lowe (

eds.). Silvics of native and exotic trees of Puerto Rico and the Caribbean Islands, pp. 73-78. U.S. Department of Agriculture and Forest Service, Río Piedras. General Technical Report ITS-15.

Findlay, S.E.G. and T.l. Arsufii. 1989. Microbial growth and detritus transformations during decomposition of leaf litter in a stream. Freshwater Biology. 21:261-269.

Fidlay, S.E.G, J. Tank, S. Dye, H.M. Valett, P.J. Mulholland, W.H. McDowell, S.L. Johnson, S.K. Hamilton, J. Edmonds, W.K. Dodds, and W.B. Bowden. 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microbiol Ecology. 43:55-56.

Garnett, H., F. Bärlocher and D. Giberson. 2000. Aquatic hyphomycetes in Catamaran Brook:colonization dynamics, seasonal patterns, and logging effects. Mycologia. 92(1):29-41.

Gessner, M.O., and E. Chauvet. 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75(6):1807-1817.

Gessner, M.O., F. Bärlocher, and E. Chauvet. 2003. Qualitative and quantitative analyses of aquatic hyphomycetes in streams. In:Freshwater mycology, Tsui and Hyde (eds.). Fungal Diversity Press, Hong Kong. Pp.127-157.

Gessner, M.O., M. Thomas, A.M. Jean-Louis and E. Chauvet. 1993. Stable succesional patterns of aquatic hyphomycetes on leaves decaying in a summer cool stream. Mycological Research. 97(2):163-172.

Gulis, V. 2001. Are there any substrate preferences in aquatic hyphomycetes? Mycological Research. 105(9):1088-1093.

Gulis, V., and K. Suberkropp. 2004. Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycetes conidia in transport. Mycologia. 96(1):57-65.

Gulis, V., L. Maranova and E. Descals. 2005. An illustrated key to the common temperature species of aquatic hyphomycetes. In: Graça, Barlocher and Gessner (eds.). Methods to study litter decomposition: A practical guide, pp. 153-167. Springer, The Netherlands.

Hackney, C.T., D.E. Padgett, and M.H. Posey. 2000. Fungal and bacterial contributions to the decomposition of *Cladium* and *Typha* leaves in nutrient enriched and nutrient poor areas of the Everglades, with a note on ergosterol concentrations in Everglades soils. Mycological Research. 104(6):666-670.

Hamilton, S.L. 1973. Aquatic hyphomycetes flora of two rain forest streams in Northeastern Puerto Rico. Thesis M.S. Ohio State University, Columbus, 44 pp.

Hauer, F.R., and W.R. Hill. 1996. Temperature, light and oxygen. In: Hauer and Lamberti (eds.), Methods in stream ecology, pp. 93-106. Academic Press, San Diego.

Hershey, A.E., and G.A. Lamberti. 2001. Stream macroinvertebrate communities. In: R.J. Naiman & R.E. Bilby (eds.). River ecology and management, pp. 169-199. Springer, New York.

Hyde, K.D. 2001. Where are the missing fungi? Does Hong Kong have any answers? Mycological Research. 105(12):1515-1518.

Hyde, K.D., and S.W. Wong. 1999. *Didymella aptrootii* sp. nov. from bamboo Submerged in freshwater. Australasian Mycologist. 18(3):54-59.

Hyde, K.D., D.Q. Zhou, and T. Dalisay. 2002. Bambusicolous fungi: A review. Fungal Diversity. 9:1-14.

Hyde, K.D., W.H. Wo, E.H.C. McKenzie, and T. Dalisay. 2001. Saprobic fungi on Bamboo culms. Fungal Diversity. 7:35-48.

Ingold, C.T. 1974. Foam spora from Britian. Transactions of the British Mycology Society. 63(3):487-497.

Ingold, C.T. 1975. An illustrated guide to aquatic and water-borne hyphomycetes (Fungi Imperfect) with notes on their biology. Freshwater Biological Association. 30:97.

Jiménez, Y.M. 1986. Hongos ingoldianos presentes en espuma de la Quebrada El Guamá, San Sebastián, Puerto Rico. Unpublished.

Judziewicz, E.J., R.J. Soreng, G.Davidse, P.M. Peterson, T.S. Filqueiras and, F.O. Zuloaga. 2000. Catalogue of new world grasses (Poaceae):I. Subfamilieas Anomochlooideae, Bambusoideae, Ehrhartoideae, and Pharoideae. Contributions from the United States National Herbarium. 39:1-128.

Justiniano, J., and C. Betancourt. 1989a. Colonización de hojas de *Syzygium jambos* L. por hongos ingoldianos. Caribbean Journal of Science. 25(3-4):101-110.

Justiniano, J., and C. Betancourt. 1989b. Factores físicos y químicos del Río Maricao y su efecto sobre los hongos ingoldianos. International Journal of Mycology and Lichenology. 4(1-2):307-318.

Kendrick, B. 2003. Analysis of morphogenesis in hyphomycetes:new characters Derived from considering some conidiophores and conidia as condensed hyphal systems. Canadian Journal of Botany. 81:75-100.

Lamberti, G.A., and S.V. Gregory. 1996. Transport and retention of CPOM. In: F.R. Hauer and G.A. Lamberti (eds.). Methods in stream ecology, pp. 217-229. Academic Press, California.

Lei Cai, Keqin Zhang, E.H.C. McKenzie and K.D. Hyde. 2003. Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. Fungal Diversity. 13:1-12.

Liese, W. The structure of bamboo in relation to its properties and utilization. In: Bamboo and its use. International Symposium on Industrial use on Bamboo, Ed. Intern. Trop. Timber Organizationa, Chinese Academy of Foresrty. China, 1992.

Lowrance, R., R. Todd, J. Fail, O. Hendrickson, R. Leonard, and L. Asmussen. 1984. Riparian forests as nutrient filters in agricultural watersheds. BioScience. 34(6):374-377.

Lu, B.S., K.D. Hyde, and W.H. Ho. 1998. Spirodecospora gen. nov. (Xylariaceae, ascomycotina), from bamboo in Hong Kong. Fungal diversity. 1:169-177.

Maharning, A.R., and F. Bärlocher. 1996. Growth and reproduction in a aquatic hyphomycetes. Mycologia 88(1):80-88.

Mans, C., T. Denward and L.J. Tanvik. 1998. Effects of solar radiation on aquatic macrophyte litter decomposition. Oikos. 82:51-58.

Mathuriau, C., and E. Chauvet. 2002. Breakdown of leaf litter in a neotropical stream. Journal of the North American Benthological Society. 21(3):384-396.

McClure, FA. 1967. The bamboos: a fresh perspective. Harvard University Press, Massachusetts, pp. 343.

Mille-Lindblom C. and L.J. Tranvik. 2003. Antagonism between bacteria and fungi on decomposing aquatic plant litter. Microbial Ecology. 45:173-182.

Moore-Landecker, E. 1996. Growth. In: Fundamentals of the fungi, 279-310 pp. 4th ed. Prentice Hall, New Jersey.

Muñoz, C.A. and F. Jordán. 1997. Manual de microtécnica para tejidos vegetales. Unpublished Manual.

Murphy, R.J., O. Sulaiman, and K.L. Tranvik. 1997. Fungal deterioration of bamboos cell walls. In:G.P. Capman (ed.), The bamboos, pp. 323-332. Academic Press, London.

Nawawi, A. 1985. Aquatic hyphomycetes and other water-borne fungi from Malaysia. Malayan Nature Journal 39:75-134.

Newel S.Y. & R.D. Fallon. 1988. Litterbags, leaftags, and decay of nondoscided intertidal leaves. Can. J. Bot. 67:2324-2327.

Nilsson, S. 1964. Freshwater hyphomycetes:taxonomy, morphology and ecology. Symbolae Botanicae Upsalienses. 2:5-124.

O'Connor, P.J., A.P. Covich, F.N. Scatena and L.L. Loope. 2000. Non-indigenous bamboo along headwater streams of the Luquillo Mountains, Puerto Rico: leaf fall, aquatic leaf decay and patterns of invasion. Journal of Tropical Ecology. 16:499-516.

Ortiz-Zayas, J.R., W.M. Lewis Jr., J.F. Saunders III,J.H. McCutchan Jr., and F.N. Scatena. 2005. Metabolism of a tropical rainforest stream. Journal of North American Benthological Society. 24(4):769-783.

Padgett, D.E. 1976. Leaf decomposition by fungi in a tropical rainforest stream. Biotropica. 8(3):166-178.

Pascoal, C., M. Pinho, F. Casio, and P. Gomes. 2003. Assessing structural and functional Ecosystem condition using leaf breakdown studies on a polluted river. Freshwater Biology. 48:2033-2044.

Paul, M.J., J.L. Meyer and C.A. Couch. 2006. Leaf breakdown in streams differing in catchment land use. Freshwater Biology. 51:1684-1695.

Rajashekhar, M. and K.M. Kaveriappa. 2000. Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. Hydrobiologia. 444:149-153.

Rao, A.N. 1987. Anatomical studies on certain bamboos growing in Singapore. In:A.N. Rao, G. Dhanarajan and C.B. Sastry (eds.). Recent research on bamboos, pp. 209-226. The Chinese Academy of Forestry, People's republic of China and International Development Research Centre, Canada.

Raviraja, N.S., K.R. Sridhar and F. Bärlocher. 1998. Breakdown of *Ficus* and *Eucalyptus* leaves in an organically polluted river in India: fungal diversity and ecological functions. Freshwater Biology. 39:537-548.

Read, S.J., S.T. Moss and E.B.G. Jones. 1992a. Germination and development of attachment by conidia of aquatic Hyphomycetes: light microscope studies. Canadian Journal of Botany. 70:831-837.

Read, S.J., S.T. Moss and E.B.G. Jones. 1992b. Attachment and germination of conidia. In: Bärlocher (ed.).The ecology of aquatic hyphomycetes, pp. 135-151. Springer-Verlag, Berlin.

Rosset, J. and F. Bärlocher. 1985. Aquatic hyphomycetes; influence of pH, Ca^{2+} and HCO_3^- on growth in vitro. Transactions of the British Mycology Society. 84(1):137-145.

Rosset, J., F. Bärlocher and J.J. Oertli. 1982. Decomposition of conifer needles and deciduous leaves in two Black forest and two Swiss Jura streams. Internationale Revue der Gesamten Hydrobiologie. 67(5):695-711.

Santos-Flores, C. 1996. A contribution to the knowledge of the aquatic and water-borne hyphomycetes of Puerto Rico, with a study on the colonization pattern on leaves of *Erythrina peoppigiana* (Walp.) O.F. Cook in Quebrada de Oro at Mayagüez. M.S. Thesis. University of Puerto Rico, Mayagüez, P.R., 334 pp.

Santos-Flores C., and C. Betancourt. 1994. Aquatic hyphomycetes (Deuteromycotina) from Río Loco at Susúa State Forest, Puerto Rico. Caribbean Journal of Science. 30(3-4):262-267.

Santos, C.J. and C. Betancourt. 1997. Aquatic and water-borne Hyphomycetes (Deuteromicotina) in streams of Puerto Rico (including records from other Neotropical locations). Caribbean Journal of Science. Special Publication (2):1-116.

Santos-Flores C., A.M. Nieves-Rivera and C. Betancourt. 1995. Hyphomycetes with multiradiate conidia in stream foam from Puerto Rico. Caribbean Journal of Science. 31(1-2):49-56.

Sati, S.C. and M. Bewal. 2005. Aquatic hyphomycetes as endophytes of riparian plant roots. Mycologia. 97(1):45-49.

Schlickeisen E., T.E. Tietjen, T.L. Arsuffi and A.W. Groeger. 2003. Detritus processing and microbial dynamics of an aquatic macrophytes and terrestrial leaf in a thermally constant. Microbial Ecology. 45:411-418.

Schoenlein-Crusius, I.H., and R.A. Piccolo Grandi. 2003. The diversity of aquatic hyphomycetes in South America. Brazilian Journal of Microbiology. 34:183-193.

Shanmughavel, P. and K Francis. 1997. Balance and turnover of nutrients in bamboo Plantation (*Bambusa bambos*) of different ages. Biology and Fertility of Soils. 25:69-74.

Soderstrom, T.R. and C.E.Calderón. 1979a. A commentary on the bamboos (Poaceae:Bambusoideae). Biotropica. 11(3):161-172.

Soderstrom, Th.R., and C.E. Calderón. 1979b. Distribution and environment of the

Bambusoideae. In:Makoto Numata (ed.). Ecology of grasslands and bamboolands in the world, pp. 223-236. Gustav Fischeer, Verla Jena.

Sotomayor-Raímerz, D., G. Martínez, and L.J. Olivieri. 2001. Phosphorus status of stream waters in Puerto Rico:1989-1997. The Journal of Agriculture of the University of Puerto Rico. 85(1-2):1-15.

Sridhar, K.R. and F. Bärlocher. 2000. Initial colonization, nutrient supply and fungal activity on leaves decaying in streams. Applied and Environmental Microbiology. 66(3):1114-1119.

Sridhar, K.R. and F. Bärlocher. 1997. Water chemistry and sporulation by aquatic hyphomycetes. Mycological Research. 101:591-596.

Sridhar, K.R., and F. Bärlocher. 1994. Viability of aquatic hyphomycetes conidia in foam. Canadian Journal of Botany. 72:106-110.

Sridhar, K.R., and F. Bärlocher. 1992. Aquatic Hyphomycetes in spruce roots. Mycologia. 84:580-584.

Sridhar, K.R., and K.M. Kaveriappa. 1989. Colonization of leaves by water-borne hyphomycetes in a tropical stream. Mycological Research. 92(4):392-396.

Sridhar, K.R., and K.M. Kaveriappa. 1984. Seasonal occurrence of water-borne fungi in Konaje stream (Manglore), India. Hydrobiologia. 119:101-105.

Sridhar, K.R., G. Krauss, F. Bärlocher, R. Wennrich, and G.-J. Krauss. 2000. Fungal diversity in heavy metal polluted waters in central Germany. Fungal Diversity. 5:119-129.

Suberkropp, K. 2001. Microorganisms and organic matter decomposition. In: R.J. Naiman & R.E. Bilby (eds.). River ecology and management, pp. 120-143. Springer, New York.

Suberkropp, K. 1998. Effect of dissolved nutrient on two aquatic hyphomycetes growing on leaf litter. Mycological Research. 102(8):998-1002.

Suberkropp, K. 1995a. The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. Canadian Journal of Botany. 73(1):S1361-S1369.

Suberkropp, K. 1991. Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. Mycological Research. 95(7):843-850.

Suberkropp, K.,and E. Chauvet. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology. 76(5):1433-1445.

Suberkropp, K., and M.J. Klug. 1980. The maceration of deciduous leaf litter by aquatic hyphomycetes. Canadian Journal of Botany. 58:1025-1031.

Suberkropp, K. and M.J. Klug. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology. 57:707-719.

Suberkropp, K., and S.H. Weyers. 1996. Application of fungal and bacterial production methodologies to decomposing leaves in streams. Applied and Environmental Microbiology. 62(5):1610-1615.

Suberkropp, K., T.L. Arsuffi and J.P. Anderson. 1983. Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. Applied and Environmental Microbiology. 46(1):237-244.

Tan, T.K. and L.L. Koh. 1995. Variations if foam spora in a lowland tropical forest stream. Nova Hedwigia. 60(3-4):519-526.

Tanaka, K. and Y. Harada. 2004. Bambusicolous fungi in Japan (1):four *Phaeosphaeria* species. Mycoscience. 45:377-382.

Tsui, C.K.M., and Hyde K.D. 2003. Introduction. In:Tsui and Hyde (eds.). Freshwater Mycology, pp.1-9. Fungal Diversity Press, Hong Kong.

Tubaki, K. 1957. Studies of Japanese Hyphomycetes (III). Aquatic group. Bulletin of National Science Museum of Tokyo. 41:249-268.

Vale, Y. 1995. Hifomicetos acuáticos en el Río Chuco Ramos, Aguada, Puerto Rico. Unpublished.

United States Geological Survey of Washigton D.C. 20242, and Department of Public Works of Puerto Rico. 1964. Mayagüez Quadrangle.

United States Department of Agriculture and University of Puerto Rico. 1969. Soil survey of Mayagüez area of western Puerto Rico. U.S. Department of Agriculture-Soil Conservation Service, Washington D.C.

Wai Hong, H., K.D. Hyde and I.J. Hodgkiss. 2004. *Cataractispora receptaculorum*, a new freshwater ascomycete from Hong Kong. Mycologia. 96(2):411-417.

Webster J.R., and E.F. Benfield. 1986. Vascular plant breakdown in freshwater Ecosystem. Annual Review of Ecology and Systematics. 17:567-594.

Webster J. and E. Descals. 1981. Morphology, distribution, and ecology of conidial fungi in freshwater habitats. In:Kole and Kendrick(eds.). Biology of conidial fungi, pp. 295-351. Vol. 1. Academic Press, New York.

Wright, M.S., and A.P. Covich. 2005. Relative importance of bacteria and fungi in a tropical headwater stream:leaf` decomposition and invertebrate feeding preference. Microbial Ecology. 49:536-546.

Wong, M.K.M., T. Goh, I.J. Hodgkiss, K.D. Hyde, V. Mala Ranghoo, C.K.M. Tsui, W. Ho, W.S.W. Wong, and T. Yuen. 1998. Role of fungi in freshwater ecosystems. Biodiversity and Conservation. 7:1187-1206.

Zhou, D.Q. and K.D. Hyde. 2002. Fungal succession on bamboo in Hong Kong. Fungal Diversity. 10:213-227.

Zhou, D. and K.D. Hyde. 2000. Submersisphaeria bambusicola sp. nov. from bamboo in Hong Kong. Fungal Diversity. 4:181-186.

Appendices

Appendix 1. Kruskal Wallis test made to the physical-chemical parameters using study site as criterion of classification monitored during the first period (November, 2004 to January, 2005)

Variable	Site	N	Mean	S.D..	Median	H	p
Temperature	1	20	23.14	0.48	23.10	18.28	0.0001
Temperature	2	16	23.54	0.41	23.65		
Temperature	3	20	24.06	0.74	24.30		
Variable	Site	N	Mean	S.D.	Median	H	p
D.O.	1	20	5.48	0.54	5.46	15.56	0.0004
D.O.	2	16	5.28	0.74	5.24		
D.O.	3	20	6.40	0.84	6.43		
Variable	Site	N	Mean	S.D.	Median	H	p
pH	1	20	7.32	0.25	7.38	11.28	0.0035
pH	2	16	7.46	0.27	7.53		
pH	3	20	7.62	0.22	7.68		

Appendix 2. Correlations between temperature, pH and D.O. measure during the first period (November, 2004 to January, 2005). The test was made using the statistics program Infostat.

Spearman's Correlations: coefficient/probability

	Temperature	D.O.	pH
Temperature	1.00	7.5E-04	0.02
D.O.	0.45	1.00	2.3E-06
pH	0.30	0.64	1.00

Appendix 3. Correlations between phosphate and nitrate measure during the first period. The test was made using the statistics program Infostat.

Spearman's Correlations: coefficient/probability

	Phosphate	Nitrate
Phosphate	1.00	0.44
Nitrate	-0.10	1.00

Appendix 4. Correlation between temperature, pH and D.O. measured during the second period of study. The test was made using the statistics program Infostat.

Spearman's Correlations: coefficient/probability

	Temperature	D.O.	pH
Temperature	1.00	0.09	0.55
D.O.	-0.24	1.00	1.5E-04
pH	0.08	0.53	1.00

Appendix 5. Correlation between nutrients measure during the second period of study. The test was made using the statistics program Infostat.

Spearman's Correlations: coefficient/probability

	Phosphate	Nitrate
Phosphate	1.00	0.44
Nitrate	0.11	1.00

Appendix 6. Correlations between physical-chemical parameters measure during all the study. The test was made using the statistics program Infostat.

Spearman's Correlations: coefficient/probability

	Temperature	D.O.	pH
Temperature	1.00	0.04	0.17
D.O.	0.14	1.00	0.00
pH	0.09	0.53	1.00

Appendix 7. Kruskall Wallis test made to physical-chemical parameters registered during all the study using sampling date as criteria of classification

Variable	Date	N	Mean	S.D.	Median	C	H	p
Temperature	April 20, 2004	12	25.03	0.21	25.00	1.00	184.60	<0.0001
Temperature	April 7, 2004	12	25.38	0.78	25.25			
Temperature	Dec. 13, 2005	4	22.90	0.00	22.90			
Temperature	Dec. 14, 2004	8	24.31	0.60	24.30			
Temperature	Dec. 27, 2004	12	22.95	0.50	22.80			
Temperature	Feb. 24, 2004	10	24.11	0.39	24.10			
Temperature	Jan. 14, 2005	12	23.91	0.43	23.70			
Temperature	Jan. 28, 2005	12	23.18	0.50	22.90			
Temperature	March 22, 2004	12	24.15	0.44	24.20			
Temperature	March 6, 2004	12	24.08	0.06	24.10			
Temperature	May 4, 2004	12	25.43	0.39	25.70			
Temperature	Nov. 2, 2005	12	24.95	0.28	25.00			
Temperature	Nov. 28, 2004	12	23.79	0.53	24.00			
Temperature	Nov. 30, 2005	12	23.12	0.09	23.15			
Temperature	Nov., 16, 2005	12	24.55	0.08	24.55			
Temperature	Nov.5, 2004	12	24.43	0.26	24.50			
Temperature	Oct. 15, 2004	12	25.33	0.14	25.40			
Temperature	Oct. 19, 2005	12	25.53	0.32	25.30			
Temperature	Sept. 27, 2004	12	25.83	0.18	25.90			

Variable	Date	N	Mean	S.D.	Median	C	H	p
D.O.	April 20, 2004	12	7.04	0.69	6.78	1.008	9.31	<0.0001
D.O.	April 7, 2004	12	6.57	0.53	6.50			
D.O.	Dec. 13, 2005	4	6.79	0.38	6.90			
D.O.	Dec. 14, 2004	8	6.54	0.68	6.53			
D.O.	Dec. 27, 2004	12	5.74	0.64	5.93			
D.O.	Feb. 24, 2004	10	7.48	0.97	7.81			
D.O.	Jan. 14, 2005	12	5.16	0.45	5.23			

D.O.	Jan. 28, 2005	12	5.18	0.72	4.92			
D.O.	March 22, 2004	12	6.65	0.76	6.46			
D.O.	March 6, 2004	12	6.56	0.61	6.43			
D.O.	May 4, 2004	12	6.65	0.82	6.84			
D.O.	Nov. 2, 2005	12	7.10	0.49	7.26			
D.O.	Nov. 28, 2004	12	6.40	0.80	6.11			
D.O.	Nov. 30, 2005	12	7.05	0.72	7.10			
D.O.	Nov., 16, 2005	12	7.18	0.66	7.37			
D.O.	Nov.5, 2004	12	6.26	0.88	5.86			
D.O.	Oct. 15, 2004	12	5.25	1.81	5.51			
D.O.	Oct. 19, 2005	12	6.68	0.41	6.74			
D.O.	Sept. 27, 2004	12	6.11	0.85	6.53			
Variable	Date	N	Mean	S.D.	Median	C	H	p
pH	April 20, 2004	12	7.51	0.21	7.62	1.00	160.36	<0.0001
pH	April 7, 2004	12	7.57	0.05	7.57			
pH	Dec. 13, 2005	4	8.04	0.01	8.04			
pH	Dec. 14, 2004	8	7.70	0.15	7.70			
pH	Dec. 27, 2004	12	7.73	0.13	7.80			
pH	Feb. 24, 2004	10	7.87	0.18	7.96			
pH	Jan. 14, 2005	12	7.34	0.16	7.39			
pH	Jan. 28, 2005	12	7.11	0.17	7.10			
pH	March 22, 2004	12	6.90	0.31	6.87			
pH	March 6, 2004	12	6.98	0.05	6.99			
pH	May 4, 2004	12	7.43	0.15	7.45			
pH	Nov. 2, 2005	12	7.86	0.18	7.89			
pH	Nov. 28, 2004	12	7.54	0.13	7.56			
pH	Nov. 30, 2005	12	7.69	0.26	7.83			
pH	Nov., 16, 2005	12	8.03	0.14	8.03			
pH	Nov.5, 2004	12	7.59	0.12	7.66			
pH	Oct. 15, 2004	12	7.35	0.28	7.30			
pH	Oct. 19, 2005	12	7.92	0.19	8.03			
pH	Sept. 27, 2004	12	7.33	0.25	7.42			

Appendix 8. Kruskal Wallis test made to the temperature, D.O. and pH measured during all the study and the site was used as criteria of classification

Variable	Site	N	Mean	S.D.	Median	H	p
Temperature	1	71	24.37	1.11	24.20	0.24	0.8885
Temperature	2	71	24.42	0.93	24.60		
Temperature	3	72	24.48	0.82	24.50		
Variable	Site	N	Mean	S.D.	Median	H	p
D.O.	1	71	5.86	0.55	5.92	78.33	<0.0001
D.O.	2	71	6.23	1.18	6.53		
D.O.	3	72	7.16	0.74	7.25		
Variable	Site	N	Mean	S.D.	Median	H	p
pH	1	71	7.34	0.32	7.40	29.84	<0.0001
pH	2	71	7.57	0.38	7.64		
pH	3	72	7.66	0.31	7.66		

Appendix 9. Correlations between phosphate and nitrate measure during all the study. The test was made using the statistics program Infostat.

Spearman's Correlations: coefficient/probability

	Phosphate	Nitrate
Phosphate	1.00	0.08
Nitrate	0.13	1.00

Appendix 10. Spearman's correlation made to physical-chemical factors and species found on the bamboo leaves during the first period of bamboo colonization study (November 2004 to January 2005)

Spearman's correlation: Coefficient/probability

	Tempt.	pH	D.O	Nitrate	Phosphate	*Campylospora* sp	*C. parvula*	*Helicomyces* sp.	*P. Fluminea*	*T. porosporiferum*
Temperature	1.00	0.61	0.23	0.57	0.86	0.31	0.21	0.95	0.21	0.21
pH	0.19	1.00	0.10	0.04	0.02	0.46	0.21	0.53	0.21	0.21
D.O.	0.45	0.62	1.00	0.17	0.40	0.24	0.08	0.71	0.08	0.08
Nitrate	0.21	0.79	0.52	1.00	0.06	0.96	0.31	0.57	0.31	0.31
Phosphate	-0.07	-0.91	-0.32	-0.71	1.00	0.46	0.44	0.40	0.44	0.44
Campylospora sp.	0.39	0.28	0.45	-0.02	0.28	1.00	0.04	0.17	0.04	0.04
C. parvula	0.48	0.48	0.67	0.38	0.29	0.78	1.00	0.10	0.01	0.01
Helicomyces sp.	-0.02	-0.24	0.14	-0.21	0.32	0.52	0.62	1.00	0.10	0.10
P. fluminea	0.48	0.48	0.67	0.38	0.29	0.78	1.00	0.62	1.00	0.01
T. porosporiferum	0.48	0.48	0.67	0.38	0.29	0.78	1.00	0.62	1.00	1.00

	Temp	pH	D.O.	Nitrate	Phosphate	*Tetraploa* sp.	*C. chaetocladia*	*P. ramificata*	*A. Crassa*	*Tripospermum* sp.
Temperature	1.00	0.61	0.23	0.57	0.86	0.21	0.21	0.21	0.61	0.61
pH	0.19	1.00	0.10	0.04	0.02	0.21	0.21	0.21	0.80	0.80
D.O.	0.45	0.62	1.00	0.17	0.40	0.08	0.08	0.08	0.31	0.31
Nitrate	0.21	0.79	0.52	1.00	0.06	0.31	0.31	0.31	0.61	0.61
Phosphate	-0.07	-0.91	-0.32	-0.71	1.00	0.44	0.44	0.44	0.13	0.13
Tetraploa sp.	0.48	0.48	0.67	0.38	0.29	1.00	0.01	0.01	0.10	0.10

C. chaetocladia	0.48	0.48	0.67	0.38	0.29	1.00	1.00	0.01	0.10	0.10
P. ramificata	0.48	0.48	0.67	0.38	0.29	1.00	1.00	1.00	0.10	0.10
A. crassa	0.19	0.10	0.38	0.19	0.58	0.62	0.62	0.62	1.00	0.01
Tripospermum sp.	0.19	0.10	0.38	0.19	0.58	0.62	0.62	0.62	1.00	1.00

	Tempt.	pH	D.O	NO3	PO4	*Helicomyces* sp. 2	*Helicomyces* sp. 3	*T. aristata*	*Anguillospora* sp	*P. casuarinae*	*Pyramidospora* sp.
Temperature	1.00	0.61	0.23	0.57	0.86	0.08	0.08	1.00	0.52	0.21	0.21
pH	0.19	1.00	0.10	0.04	0.02	0.08	0.08	0.31	0.67	0.21	0.21
D.O.	0.45	0.62	1.00	0.17	0.40	0.13	0.13	1.00	0.10	0.08	0.08
Nitrate	0.21	0.79	0.52	1.00	0.06	0.13	0.13	0.45	0.72	0.31	0.31
Phosphate	-0.07	-0.91	-0.32	-0.71	1.00	0.99	0.99	0.79	0.33	0.44	0.44
Helicomyces sp. 2	0.67	0.67	0.57	0.57	0.01	1.00	0.01	0.10	0.24	0.10	0.10
Helicomyces sp. 3	0.67	0.67	0.57	0.57	0.01	1.00	1.00	0.10	0.24	0.10	0.10
T. aristata	0.00	0.38	0.00	0.29	0.10	0.62	0.62	1.00	0.24	0.10	0.10
Anguillospora sp.	0.24	0.16	0.63	0.14	0.37	0.45	0.45	0.45	1.00	0.02	0.02
P. casuarinae	0.48	0.48	0.67	0.38	0.29	0.62	0.62	0.62	0.88	1.00	0.01
Pyramidospora sp	0.48	0.48	0.67	0.38	0.29	0.62	0.62	0.62	0.88	1.00	1.00

Appendix 11. Spearman's correlation made to physical-chemical factors and species found on the bamboo leaves during the second period of bamboo colonization study (October to December 2005)

Spearman's correlation: Coefficient/probability

	Temperature	pH	D.O	Nitrate	Phosphate	*Campylospora* spp.	*C. filicladia*	*C. chaetocladia*	*Campylospora* sp.
Temperature	1.00	0.95	0.34	0.68	0.84	0.06	0.02	0.02	0.04
pH	0.02	1.00	0.03	0.26	0.34	0.05	0.23	0.27	0.09
D.O. (mg/L	-0.29	0.67	1.00	0.05	0.75	0.18	0.65	0.86	0.23
Nitrate (mg/L)	0.13	0.34	0.59	1.00	0.71	0.13	0.06	0.38	0.10
Phosphate	0.06	0.28	0.10	0.11	1.00	0.13	0.14	0.02	0.04
Campylospora spp.	0.56	0.60	0.40	0.46	0.45	1.00	0.01	0.01	2.7E-03
C. filicladia	0.68	0.36	0.14	0.56	0.44	0.75	1.00	0.01	0.02
C. chaetocladia	0.71	0.33	0.05	0.27	0.72	0.79	0.75	1.00	2.7E-03
Campylospora sp.	0.62	0.51	0.36	0.49	0.62	0.90	0.72	0.90	1.00

	Temperature	pH	D.O.	Nitrate	Phosphate	*P. ramificata*	*A. longissima*	*Pyramidospora* sp.	*H. colligatus*
Temperature	1.00	0.95	0.34	0.68	0.84	0.04	0.04	0.04	0.10
pH	0.02	1.00	0.03	0.26	0.34	0.09	0.09	0.09	0.86
D.O.	-0.29	0.67	1.00	0.05	0.75	0.23	0.23	0.23	0.08
Nitrate	0.13	0.34	0.59	1.00	0.71	0.10	0.10	0.10	0.08
Phosphate	0.06	0.28	0.10	0.11	1.00	0.04	0.04	0.04	0.51
P. ramificata	0.62	0.51	0.36	0.49	0.62	1.00	9.1E-04	9.1E-04	0.07
A. longissima	0.62	0.51	0.36	0.49	0.62	1.00	1.00	9.1E-04	0.07
Pyramidospora sp.	0.62	0.51	0.36	0.49	0.62	1.00	1.00	1.00	0.07
H. colligatus	0.50	-0.05	-0.53	-0.53	0.20	0.55	0.55	0.55	1.00

	Temperature	pH	D.O.	Nitrate	Phosphate	*Helicomyces* sp.	*P. nawawi*	*H. torquatus*	*P. casuarinae*
Temperature	1.00	0.95	0.34	0.68	0.84	0.21	0.04	0.02	0.03
pH	0.02	1.00	0.03	0.26	0.34	0.48	0.09	0.33	0.37
D.O.	-0.29	0.67	1.00	0.05	0.75	0.02	0.23	0.95	0.59
Nitrate	0.13	0.34	0.59	1.00	0.71	0.13	0.10	0.45	0.34
Phosphate	0.06	0.28	0.10	0.11	1.00	0.16	0.04	0.02	0.32
Helicomyces sp.	0.38	-0.21	-0.69	-0.46	0.42	1.00	0.07	0.02	0.11
P. nawawi	0.62	0.51	0.36	0.49	0.62	0.55	1.00	4.3E-03	3.3E-03
H. torquatus	0.70	0.29	0.02	0.23	0.71	0.69	0.86	1.00	0.02
P. casuarinae	0.67	0.27	0.16	0.29	0.30	0.48	0.88	0.73	1.00

	Temperature	pH	D.O.	Nitrate	Phosphate	Helicomyces sp. 2	Helicomyces sp. 4	Helicomyces sp. 3	Helicomyces roseus
Temperature	1.00	0.95	0.34	0.68	0.84	0.06	0.48	0.18	0.61
pH	0.02	1.00	0.03	0.26	0.34	0.35	0.27	0.06	0.09
D.O.	-0.29	0.67	1.00	0.05	0.75	0.61	0.98	0.08	0.29
Nitrate	0.13	0.34	0.59	1.00	0.71	0.43	0.87	0.14	0.52
Phosphate	0.06	0.28	0.10	0.11	1.00	0.05	0.09	0.34	0.15
Helicomyces sp. 2	0.57	0.28	0.15	0.24	0.58	1.00	4.3E-03	0.01	0.01
Helicomyces sp. 4	0.21	0.33	0.01	-0.05	0.52	0.86	1.00	0.04	2.7E-03
Helicomyces sp. 3	0.41	0.58	0.53	0.45	0.29	0.75	0.63	1.00	0.01
Helicomyces roseus	0.15	0.51	0.32	0.20	0.44	0.75	0.90	0.75	1.00

Appendix 12. Analysis of correlation for the precipitation registered and the number species found associated with the bamboo leaves for site 1 during the first period of study (November, 2004 to January 2005)

Date	Precipitation (mm)	# Species
Nov. 28, 2004	29.75	4
Dec. 14, 2004	3.25	2
Dec. 27, 2004	5.00	1
Jan. 14, 2005	3.00	0

Shapiro-Wilks (modified)

Variable	n	Mean	S.D.	W*	p (one tail)	
Precipitation (mm)	4	10.25		13.03	0.69	0.0073
# species	4	1.75		1.71	0.97	0.8212

Spearman's Correlation: coefficient/probability

	Precipitation (mm)	# species
Precipitation (mm)	1.00	0.17
# species	0.80	1.00

Appendix 13. Analysis of correlation for the precipitation registered and the number species found associated with the bamboo leaves for site 3 during the first period of study (November, 2004 to January 2005)

Date	Precipitation (mm)	# Species
Nov. 28, 2004	29.75	11
Dec. 14, 2004	3.25	2
Dec. 27, 2004	5.00	0
Jan. 14, 2005	3.00	1

Shapiro-Wilks (modified)

Variable	n	Mean	D.E.	W*	p (one tail)	
Precipitation (mm)	4	10.25		13.03	0.69	0.0073
# species	4	3.50		5.07	0.78	0.0788

Spearman's Correlation: Coefficient/probability

	Precipitation (mm)	# species
Precipitation (mm)	1.00	0.49
# species	0.40	1.00

Appendix 14. Analysis of correlation for the precipitation registered and the number species found associated with the bamboo leaves for site 1 during the second period of study (November to December 2005)

Date	Precipitation (mm)	# Species
Oct. 19, 2005	142.494	8
Nov. 2, 2005	146.300	3
Nov. 16, 2005	63.500	2
Nov. 30, 2005	24.638	1

Shapiro-Wilks (modify)

Variable	n	Mean	S.D.	W*	p (one way)	
Precipitation (mm)	4	94.23	60.08	0.85	0.2289	
# species	4	3.50	3.11	0.86	0.2686	

Pearson's Correlation: coefficient\probability

	Precipitation (mm)	# species
Precipitation (mm)	1.00	0.27
# species	0.73	1.00

Appendix 15. Analysis of correlation for the precipitation registered and the number species found associated with the bamboo leaves for site 3 during the second period of study (October to December 2005)

Date	Precipitation (mm)	# Species
Oct. 19, 2005	142.494	12
Nov. 2, 2005	146.300	3
Nov. 16, 2005	63.500	0
Nov. 30, 2005	24.638	0

Shapiro-Wilks (modify)

Variable	n	Mean	S.D.	W*	p (one tail)	
Precipitation (mm)	4	94.22	60.06	0.85	0.2284	
# species	4	3.75	5.68	0.79	0.0889	

Pearson's Correlation: coefficient\probability

	Precipitation (mm)	# species
Precipitation (mm)	1.00	0.28
# species	0.72	1.00

Appendix 16. T Test of independent samples used to compared the number of conidia found on aerated disc and non- aerated discs of bamboo for site 1 during the first period

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	Varianza(1)	Varianza(2)	p(Var.Hom.)	T	gl	p	prueba
Type of disc	LOG10_# conidia	{aerated}	{non-aerated}	2	2	0.63	0.50	0.21	0.08	0.6967	0.33	2	0.7703	Bilateral

Appendix 17. T Test of independent samples used to compared the number of conidia found on aerated disc and non- aerated discs of bamboo for site 3 during the first period

Clasific	Variable	Grupo(1)	Grupo(2)	n(1)	n(2)	media(1)	media(2)	Varianza(1)	Varianza(2)	p(Var.Hom.)	T	gl	p	prueba
Type disc	LOG10_# conidia	{aerated}	{non-aerated}	2	2	1.38	0.64	0.18	0.82	0.5528	1.04	2	0.4058	Bilateral

Appendix 18. Analysis of variance of the bamboo discs aerated and non-aerated During the first period of study

Analysis of variance

Variable	N	R²	R² Aj	CV
LOG10_# conidia	5	0.43	0.24	70.10

Analysis of Variance (SS type III)

F.V.	SS	df	MS	F	p-value
Model	0.94	1	0.94	2.29	0.2272
Type of disc	0.94	1	0.94	2.29	0.2272
Error	1.23	3	0.41		
Total	2.18	4			

Test: LSD Fisher Alfa:=0.05 SMD:=1.84480

Error: 0.4115 df: 3

Type of disc	Medias	n	
non-aerated	0.56	3	A
aerated	1.45	2	A

Different letters indicate significant differences (p<= 0.05)

Appendix 19. Non-parametric analysis of variance made to compare the number of conidia on non-aerated discs, aerated discs with antibiotic and aerated discs without antibiotic observed from the three sites of study during the second period.

Site 1: Kruskal Wallis Test

Variable	Type of disc	N	Mean	S.D.	Median	H	p
# conidia	aerated	4	12.00	15.32	8.00	0.62	0.7434
# conidia	aerated w ant.	4	7.25	8.38	7.00		
# conidia	non-aerated	4	60.00	109.40	7.50		

Site 2: Kruskal Wallis Test

Variable	Type of disc	N	Mean	S. D.	Median	H	p
# conidia	aerated	4	0.00	0.00	0.00	7.38	0.0061
# conidia	aerated w ant.	4	0.00	0.00	0.00		
# conidia	non-aerated	4	15.75	21.09	7.50		

Site 3: Kruskal Wallis Test

Variable	Type of disc	N	Mean	S.D.	Median	H	p
# conidia	aerated	4	61.50	123.00	0.00	0.47	0.8061
# conidia	aerated w ant.	4	1.75	3.50	0.00		
# conidia	non-aerated	4	127.75	251.52	3.00		

Appendix 20. The different treatments made to the bamboo leaves from the three sites of study were compared using a non-parametric analogues T test for independent samples

Site 1: Wilcoxon rank sum test
Exact calculation of probability

Clasific	Variable	Group 1	Group 2	n(1)	n(2)	Mean(1)	Mean(2)	DS(1)	DS(2)	W	p(2 tails)
Type of disc	# conidia	aerated	aerated w ant.	4	4	12.00	7.25	15.32	8.38	20.00	0.6571

Site 2: Wilcoxon rank sum test
Exact calculation of probability

Clasific	Variable	Group 1	Group 2	n(1)	n(2)	Mean(1)	Mean(2)	DS(1)	DS(2)	W	p(2 ails)
Type of disc	# conidia	aerated	aerated w ant.	4	4	0.00	0.00	0.00	0.00	0.00	sd

Site 3: Wilcoxon rank sum test
Exact calculation of probability

Clasific	Variable	Group 1	Group 2	n(1)	n(2)	Mean(1)	Mean(2)	DS(1)	DS(2)	W	p(2 tails)
Type of disc	# conidia	aerated	aerated w ant.	4	4	61.50	1.75	123.00	3.50	18.50	>0.9999