The role of environmental variables in the colonization pattern of aquatic hyphomycetes in a semi-tropical canal water habitat

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Abstract

The canal water hyphomycete distribution was studied using three techniques of study during the years 2006 and 2007. The maximum number of conidial species was observed by the membrane filtration technique and compared to randomly collected submerged leaves and bait leaves. Among the most fluctuating and influential factors in the canal, temperature and organic debris deposition appeared to be significant.

Three distinct colonization patterns could be detected for the different species of aquatic hyphomycetes well regulated by the environmental variables. The dominant species of aquatic hyphomycetes occurring throughout the year in canal water and favored by high temperature included Articulospora proliferata, Flagellospora penicillioides, Flagellospora sp. B., Lunulospora curvula, Tetracadium marchalianum and Triscelophorus monosporus. Five species namely, Anguilliospora sp. C., Heliscus tentaculus, Mycofalcella iqbalii, Pyramidospora casuarinae and Scorpiosporium sp. I. made their appearance in the rainy season and also strongly responded to the increase in amount of leaf debris. Another six species namely, Alatospora acuminata, Anguilliospora longissima, Articulospora tetracladia, Lemonniera spp. and Tetrachaetum elegans formed the typical winter assemblage. The Principal Component Analysis indicated ordination trends of canal water hyphomycetes to be comparable by different techniques with respect to temperature and organic biomass deposition.

Key words: Aquatic hyphomycetes, Distribution patterns, Temporal communities

1. Introduction

The colonization patterns of aquatic hyphomycetes have been mostly studied in the temperate regions of the world like America (Triska, 1970; Suberkropp and Klug, 1976; Suberkropp, 1984), Australia (Thomas et al., 1989, Canada (Bärlocher and Kendrick, 1974; Bärlocher, 1989), England (Shearer and Webster, 1985a, b), France (Chauvet, 1991; Fabre, 1998a, b), Hungary (Gönczöl, 1989; Gönczöl and Révay, 1999; Gönczöl et al., 1999) and Pakistan (Iqbal et al., 1979, 1980; Iqbal et al., 1990; Iqbal, 1992). In such streams the drastic seasonal variation gives a corresponding clear temporal distribution pattern of these fungi, so that they can be distinctly called summer and winter species. Fewer ecological studies have been done in the semi-tropical or tropical habitats like Egypt (Abdel-Raheem, 1997) Texas, North America (Akeridge and Koehn, 1987) Western Ghats, India (Sridhar and Kaveriappa, 1984) Morocco (Chergui, 1990) and South Africa (van der Merwe and Jooste, 1988). Temperature in shallow, temperate mountain streams often fluctuates between 4°C and 16°C in Pakistan (Iqbal et al., 1979; 1980) and between 17°C and 30°C in streams of tropical origin in Western Ghats (Sridhar et al., 1992). In freshwater streams located in the temperate areas, a lower temperature regime mostly between 0°C and 20°C is observed with larger diurnal and seasonal fluctuation, thus the temperature regime in the studied site (between 10°C and 26°C) overlaps the higher temperature regime of the temperate streams (Firdaus-e-Bareen and Iqbal, 1994).

The canals represent a new habitat for the freshwater fungi. They are part of the irrigation network in the plains of Punjab. They can be considered semi-tropical water bodies on leveled and lower altitude in comparison with temperate freshwater streams present at higher altitude (Firdaus-e-Bareen and Iqbal, 1994; Arshad and Firdaus-e-Bareen, 2009). An attempt was made to study the aquatic hyphomycete spora by all techniques because according to Firdaus-e-Bareen and Iqbal (1997), data generated by all possible techniques depicts the exact reflection of the actual

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spora. This study was carried out to observe how the colonization patterns of aquatic hyphomycetes differ under the influence of environmental variables in this semi-tropical habitat.

2. Materials and methods

These studies were carried out in the Lahore Branch of the BRB Canal, at the University of the Punjab, Lahore, Pakistan, during the years 2006 and 2007. No sampling could be done during the month of January due to annual closure of canal water for removal of silt and organic debris.

2.1. Description of the sampling site

The detail of the experimental site has been described in Firdaus-e-Bareen and Iqbal (1994, 2003). The climatic situation of the area renders it a semi-tropical habitat. The riparian vegetation consists of trees such as Bombax ceiba L., Dalbergia sissoo Roxb., Eucalyptus spp, Mangifera indica L., Populus euramerica (Dade) Guinier, Salix babylonica L. and S. terasperma Roxb., shrubs such as Callistemon lanceolatus DC., Cassia angustifolia Valil., Hibiscus rosa-sinensis L. with occasional occurrence of native greases and herbs all along. The leaves of Populus euramerica and Salix babylonica are the main contributors, due to their abundance along the banks.

The canal is annually closed in January for the removal of silt and accumulated organic debris when the bed is leveled in order to provide full space for the uniform flow of water. Water is allowed into it again in the last week of January. So the latest part of winter (February and March) the canal lacks organic debris accumulations, while in early winter it reaches the maximum of deposition of debris.

The canal represents a massive body of water with uniform, slow and steady flow rate of water in comparison to mountain streams which are shallow bodies of water coming across rapid altitudinal changes so that many falls and pools are formed in their course. Thus their flow rate is variable and often very fast. The canal water is quite turbid especially during the rainy season due to erosion from the banks and addition of runoff water. The temperate, freshwater streams are not turbid and in these streams autumn is distinct and earlier than winter. In the semitropical canals, the leaf fall overlaps winter during the lowest temperature regime. So, the winter in the canal is quite comparable to the autumn of the temperate streams.

2.2. Determination of physico-chemical variables in the canal

The physico-chemical properties of the canal water were monitored at daily intervals. The diurnal fluctuation of temperature in the canal was recorded daily using a Maximum and Minimum thermometer. The average temperature was calculated for each day. The pH of canal water was determined on a portable pH meter. The conductivity and Total Dissolved Solids (TDS) were determined with the help of an auto ranging portable water proof microprocessor EC/ TDS/ NaCl °C meter (Model HI 9835). The amount of Total Solids (TS) was determined by both filtration and evaporation methods. The mean monthly values were calculated for each variable.

The flow rate of water in the canal was observed to be constant. An average flow rate in the center and along both banks was calculated and found to be 20m s⁻¹. It was observed that the maximum organic matter traveled with water in the upper 20 cm layer before deposition. A wooden sampler (1 m broad and 20 cm deep) with a loose nylon net fitted in it was suspended in the water for 15 minutes so that all the organic debris was collected in the net as the water traveled through it. Three samples at the center and three near each bank were collected and taken into the laboratory. The organic matter was spread on a blotting paper and allowed to dry in an oven at 60°C for 24 hours. The mean value of dry biomass in grams was calculated for each day and expressed as dry weight in grams per meter square area of water.

2.3. Study of canal water hyphomycetes

The distribution pattern of aquatic hyphomycetes in the canal was studied canal using three techniques, membrane filtration, direct observation of benthic leaves and leaf baiting. For the membrane filtration technique, at least one liter of water was filtered through the membrane filters of 8 μm pore size. At least 6 filters were prepared daily during the period from January 2006 to December, 2007. When water was too turbid, more than 6 filter membranes were necessary. However, on days, turbidity was so high that it did not allow more than 100 ml of water to pass through and it was impossible to observe the filters for conidia. The filter membranes were stained in Trypan Blue and processed in an oven for half an hour at 60°C. They were left overnight. The next day, three 1cm² pieces were randomly cut from each filter membrane. Total number of conidia of each species was recorded. The percentage frequency of occurrence of each species was calculated from the total number of conidia observed. Total number of conidia obtained on all filters was pooled to calculate the number of conidia per liter of water for each day. Later, the data for each month was pooled.
For the second technique, submerged leaves were randomly sampled from the canal daily during the years 2006 and 2007. The leaves were washed with tap water. Ten leaves were randomly selected and three 1 cm square discs were cut from each leaf so that at least one side of the disc was the margin of lamina. The discs from each leaf were incubated in a separate glass Petri dish of 9 cm diameter in shallow distilled water. The Petri dish was incubated for 24 hours at canal water temperature. The next day each disc was observed directly in the water and also mounted in Trypan Blue stain under a cover slip. The developing and released conidia were observed. The percent frequency of occurrence of each species was calculated on the basis of its presence on the number of leaves studied. The average monthly frequencies for each species were calculated.

Leaf baiting was carried out in the canal using of four common tree species Dalbergia sissoo Roxb., Eucalyptus camaldulensis Dehn., Populus euramerica (Dade) Guinier and Salix babylonica Linn. The leaves were collected from the canal banks during the December, 2005 and baiting was started for the years 2006 and 2007. For each experiment, the leaves were submerged for a period of one month. The leaves were retrieved for study at daily intervals. A new experiment was set up every 15 days. Thus, 22 such baiting experiments were set during the year from February to December, 2006 and 22 for the year 2007. No experiment could be set up during January because of canal closure. Leaves were selected and sewn individually in small nylon bags (5 cm²) of 2 mm mesh size. Hundreds of such bags were prepared and tied by a thin metallic wire to a metallic rod of 0.8 cm diameter. This rod with all the small nylon bags was submerged horizontally about 20 cm under the canal water by drilling between the bricks on one side of the canal bank. The purpose was to expose the leaves against the flow of water. Twenty small nylon bags, 5 of each tree species, were sampled daily and brought to the laboratory. One disc of 1 cm² was randomly cut and incubated in glass Petri dishes of 3 cm diameter in shallow distilled water at canal water temperature on each sampling date. Each disc was observed 24 hours later directly in water as well as in Trypan Blue stain under a cover slip. The developing and detached conidia were observed on each disc, and the frequency of occurrence for each species was calculated as explained before. The average monthly frequencies for each species were calculated.

2.4. Multivariate analyses

For multivariate analysis, Community Analysis Package (CAP) version 4 (Pisces Conservation Ltd., 2008) was used. The Principal Component Analysis (PCA) plots of correlation were prepared between physico-chemical variables and seasons. The PCA correlation with individual environmental variables if present was also studied. A multivariate PCA was carried out on square root transformed data of frequency of aquatic hyphomycetes to study the relationships between the aquatic hyphomycete communities and the technique used for study.

3. Results

The fluctuation in the important physicochemical parameters of the canal water during the years 2006 and 2007 is shown in Figure 1(a-f).

Among the most important physical parameters, the greatest fluctuation was shown in the temperature and the amount of dry biomass of fallen debris. Keeping in view the environmental variables, the year could be divided into four distinct periods.

1. The late winter regime: The months of February and March represented a low temperature regime, without inorganic and organic debris representing a microcosm (microenvironment) control environment because the water in the canal was discharged after removal of substratum from the canal. The organic matter is added at a slow pace all the year round. One small peak was formed during May and June when the leaves of many trees are partly shed due to drought. The other prominent peak was formed during the months of November and December. After December this peak abruptly comes to zero point in January because of canal closure and removal of silt and debris.

2. The summer regime: The months of April to June represent the summer period of high temperature (above 15°C) with an adequate amount of organic matter as a substratum for aquatic hyphomycetes.

3. The summer regime with seasonal rainfall: The summer months of July and August have a high temperature accompanied by heavy rains under the influence of the Monsoon winds and their influence continues till September. Organic matter content is similar to the summer regime.

4. The early winter regime: The period between October and December represents another period of low temperature accompanied by the maximum amount of organic matter. Therefore, in considering the distribution patterns of aquatic hyphomycetes as well as the physical characteristics, these four distinct periods were taken into consideration.

Only two factors indicated correlations with seasonal changes in PCA vs. variable graphs, temperature and dry biomass of debris. Temperature was minimum in the early winter regime and maximum in August and September (Figure 2a). Similarly biomass was minimum in February and maximum in December (Figure 2b).
Figure 1(a-f). The annual fluctuation of physico-chemical variables in the canal (mean values during 2006 and 2007).
Figure 2a. Temperature factor showing correlation with the Principal Axis 1. February and December indicate the lowest regime while August and September indicate the highest regime.

Figure 2b. Dry Biomass of organic debris showing correlation with the Principal Axis 1. In the canal February represents the time period with minimum biomass deposition while December represents the month of maximum biomass availability.

The species of canal water hyphomycetes showing uniform distribution throughout the year obtained by all techniques and on all types of leaves included *Articulospora proliferata*, *Dimorphospora folicola*, *Flagellospora penicillioides*, *Flagellospora* sp. B., *Lunulospora curvula*, *Tetracladium marchalianum* and *Triscelophorus monosporus* (Figure 3). However, only *Tetracladium marchalianum* showed a curve similar to a winter species with much higher frequency of occurrence values during both the early and late low temperature regimes. Another difference was the increasing trend in the frequency of occurrence of these uniform species on random leaves with increasing deposition of organic matter during the year (Figure 3b).

The species of canal water hyphomycetes in which frequency of occurrence was governed by either rain or both rainfall and deposition of organic debris included *Anguillospora* sp. C., *Heliscus tentaculus*, *Mycofalcella iqbalii*, *Pyramidospora casuarinae* and *Scorpiosporium* sp. I. (Figure 4).

The group of canal water hyphomycetes whose frequency values increased during the low temperature regime, no matter organic matter was present (early winter) or absent (late winter, after canal closure) included *Alatospora acuminata*, *Anguillospora longissima*, *Articulospora tetracladia*, *Lemonniera* spp. and *Tetrachaetum elegans* (Figure 4).
5). However, some fluctuations were shown in the curve for *Alatospora accuminata* on bait leaves of *Eucalyptus camaldulensis* and *Salix babylonica* (Figure 5 d,f). This group of species comprised of only a few well known winter species and showed the lowest frequency values.

![Graphs showing frequency of occurrence for various species](image)

Figure 3. The species of hyphomycetes showing uniform occurrence throughout the year by membrane filtration, random leaf sampling and leaf baiting of *Dalbergia sissoo, Populus euramericana, Eucalyptus camaldulensis* and *Salix babylonica*. (♦ = *A. proliferata*, □ = *D. foliicola*, ■ = *F. penicillioides*, ▲ = *Flagellospora* sp. B, x = *L. curvula*, * = *T. marchalianum*, ● = *T. monosporus*).
Figure 4. The species of hyphomycetes governed by rainfall and deposition of leaf debris observed by membrane filtration, random leaf sampling and leaf baiting of Dalbergia sissoo, Populus euramericana, Eucalyptus camaldulensis and Salix babylonica. (♦= Anguillospora sp. C, ■= H. tentaculus, ▲= M. iqbalii, x= P. casuarinae, □ Scorpiosporium sp. I).
Figure 5. The species of hyphomycetes appearing in winter observed by membrane filtration, random leaf sampling and leaf baiting of *Dalbergia sissoo*, *Populus euramericana*, *Eucalyptus camaldulensis* and *Salix babylonica* (♦ = *A. acuminata*, ■ = *A. longissima*, ▲ = *A. tetracladia*, × = *L. aquatica*, * = *L. centrosphaera*, ● = *T. elegans*).
The multivariate ordination using the Principal Component Analysis (PCA) performed on yearly data for average monthly frequencies of canal water hyphomycetes during the year by different techniques also showed similar trends with respect to technique used. The data of aquatic hyphomycetes obtained through the membrane filtration technique indicated a diverse trend with specific species showing higher values during different periods of the year (Figure 6). The occurrence of species on random leaves were strongly regulated by biomass deposition as the frequency values showed greater values towards the end of the year i.e., the early winter regime (Figure 7).

![PCA Plot - Covariance - Aquatic hyphomycetes in water vs. seasonal distribution](image)

Figure 6. PCA plot of the first (54.69%) and second axis (17.22%) carried out with the data on aquatic hyphomycetes present in the water. The dominance species are indicated by the length of vector while their seasonal affinities are indicated by vector orientations.

![PCA Plot - Covariance - Aquatic hyphomycetes on random leaves vs. seasonal distribution](image)

Figure 7. PCA plot of the first (44.49%) and second axis (33.20%) carried out with data on aquatic hyphomycetes found on randomly collected leaves. The dominance species are indicated by the length of vector while their seasonal affinities are indicated by vector orientations.

Among the bait leaves, similar trends in ordination of species were observed in all leaves except *Eucalyptus camaldulensis* (Figure 8-11). *Tetracladium marchalianum* showed greatest values in the winter regime for all bait leaves. *Clavariopsis aquatica* showed greater values towards the late winter and summer regimes. *Heliscus tentaculus*, *Lumulospora curvula* and *Tricelophorus monosporus* showed greater values in the low temperature regime with greater biomass deposition.
Figure 8. PCA plot of the first (47.59%) and second axis (25.95%) carried out with data on aquatic hyphomycetes found on bait leaves of *Dalbergia sissoo*. The dominance species are indicated by the length of vector while their seasonal affinities are indicted by vector orientations.

Figure 9. PCA plot of the first (45.80%) and second axis (30.52%) carried out with data on aquatic hyphomycetes found on the bait leaves of *Eucalyptus camaldulensis*. The dominance species are indicated by the length of vector while their seasonal affinities are indicted by vector orientations.
4. Discussion

The geographical distribution of aquatic hyphomycetes extends from the arctic to the equator although certain species are found to be dominant in temperate and others in the tropical latitudes (Bärlocher, 1992). According to Gönczöl and Revay (1999) it is difficult to separate the effects of temperature and available substrate and one cannot determine which factor has a greater influence on the temporal distribution of individual species. Rajashekhar and Kaveriappa (2003) have shown the role of physico-chemical variables in bringing about the differences in fungal communities. They found the riparian vegetation to be the strongest factor in showing correlation with aquatic hyphomycete species. In a later study Gönczöl et al., (2003) showed that abiotic factors play a greater role in structuring the hyphomycete communities than the type of leaf litter and riparian vegetation. In this study similar colonization patterns have been observed by all techniques and the community structure seems to be most influenced by abiotic factors than by the technique used. Among the physico-chemical variables, the most influential factors regulating community structure were the availability of leaf debris and temperature of water. Nikolcheva and Bärlocher (2005)
using both the traditional and molecular techniques of studying aquatic hyphomycetes have shown that the substrate did not significantly affect the conidial communities and season seemed to play a more significant role in structuring communities. In structuring hyphomycete communities, temporal dynamics play a greater role than spatial dynamics (Fabre, 1998b) and time of the year and its related factors such as water chemistry or quality and quantity of available substrata show a greater effect on the occurrence and abundance of species than the temperature only (Chauvet, 1991).

In the canal, time as compared to temperature only appeared to be more important. It is because the early winter community (October to December) differed significantly from the late winter community (February and March) both with reference to species assemblage as well as the total number of species. It is not merely because of temperature which is similar in both communities but because of a time related factor which is the availability of leaf debris, the greatest amount being available in early winter and the smallest in late winter. Therefore, in the canal, the clearing of the substratum in January reshapes the late winter community in February, whereby the inoculum in the canal is brought in the form of colonized substrata and spores are brought with the incoming water itself. The canals have their origin from the large rivers in the plains of Punjab which form by the pooling water of mountain streams in the northern regions of Pakistan where lots of submerged substrata are present. The importance of deposition of leaf debris is also shown by the fact that some species made their appearance not in response to temperature but to accumulation in leaf biomass as well as stimulation for sporulation through rainfall. There are certain freshwater hyphomycetes responding to rainfall and making their appearance only after a heavy rainfall occurs showing the probable role of rainfall in stimulating conidiogenesis (Iqbal et al., 1980). Thomas et al., (1989) have also shown that the species composition in water is most affected by temperature and rainfall. In the canal, these included *Anguillospora* sp. C, *Heliscus tentaculus*, *Mycofalcella ighali*, *Pyramidospora casuarinae* and a species of *Scorpiosporium*. All of these can also be considered summer species, occurring in the high temperature regime with uniform circumneutral pH. Among these species, Chauvet (1991) has associated *H. tentaculus* to high pH and temperature conditions.

Species of aquatic hyphomycetes are known to exhibit temperature preferences. Species known as typical ‘summer species’ include *Clavatospora tenuicola*, *Lunulospora curvula*, *Flagellospora penicillioides*, *Lunulospora curvula*, *Tetracladium marchalianum*, *Triscelophorus monosporus* (Gönczöl, 1975; Suberkropp, 1984; Chauvet, 1991; Gessner et al., 1993; Gönczöl and Révay, 1999). Species known as typical ‘winter species’ include *Articulospora tetracladia*, *Clavariopsis aquatic*, *Flagellospora curvula*, *Leomniera aquatic*, *L. centrosphaera*, *Taenospora gracilis* and *Tetracladium elegans* (Suberkropp, 1984; Chauvet, 1991; Gessner et al., 1993; Chauvet and Suberkropp, 1998; Gönczöl and Révay, 1999). Most of the top ranking species in the semi-tropical canal water hyphomycetes belong to the summer assemblage of aquatic hyphomycetes (Firdaus-e-Bareen and Iqbal, 2003). All the species of aquatic hyphomycetes occurring uniformly in the canal included in this group were *Articulospora proliferata*, *Dimorphospora folicola*, *Flagellospora penicillioides*, *Flagellospora* sp. B., *Lunulospora curvula*, *Tetracladium marchalianum* and *Triscelophorus monosporus*. This is a well known summer assemblage of temperature streams found only when the temperature is above 10°C. Temperature between 15-25°C represents upper ranges for many streams in temperate zones of the world (Chauvet and Suberkropp, 1998) and temperature in the canal exactly conforms to this situation (Firdaus-e-Bareen and Iqbal, 1994). *Articulospora proliferata* and *D. folicola* are new to the list of summer species. Czeczuga and Orlowska (1996) have reported *Articulospora proliferata* in autumn in Poland. Gönczöl and Révay (1999) have also reported the uniform occurrence of *Anguillospora crassa*, *D. folicola* and *Tetracladium splendens* in the Morgo stream in Hungary with an irregular temporal distribution. These are similar findings especially in the case of *D. folicola*. However, *L. curvula* showed a clear response to increase in available leaf debris while *F. penicillioides* formed a peak in response to highest temperature in summer. Thus *F. penicillioides* should be referred to as a typical summer species.

*Tetracladium marchalianum* showed a pattern characteristic of a winter species. *Tetracladium marchalianum* is known to have a greater affinity for warmer temperature (Gönczöl, 1975; Suberkropp, 1984, 1991; Chauvet, 1991; Gessner et al., 1993). In the canal, although it showed a curve similar to the winter species, it never disappeared even in the high temperature regime, confirming earlier observations. But the greater frequency of occurrence during the low temperature regime shows that low temperature favors sporulation in *T. marchalianum*. Moreover, the low temperature regime in the canal water is quite comparable to the summer of temperature streams. This is in line with van der Merwe and Jooste (1988) who were of the opinion that *T. marchalianum* is less favored by elevated temperature. According to Suberkropp and Klug (1981) *T. marchalianum* and *T. setigerum* behave like low temperature species. In the present study, data obtained by all the techniques indicates that it has a uniform occurrence in the canal, although the pattern of distribution was similar to the low temperature species. Other scientists have also found similar consistent and uniform patterns for aquatic hyphomycetes e.g. in central Himalayan streams *Alatospora acuminata*, *Anguillospora longissima*, *Tetracladium elegans*, *Tetracladium marchalianum* and *T. setigerus* were uniformly reported both below and above 16 °C (Belwal et al., 2008). In another study Rajashekhar and Kaveriappa (2003) have observed *Anguillospora longissima*, *Lunulospora curvula* and *Triscelophorus monosporus* to be most abundant uniformly in all samples in the semi-tropical Western Ghat region of India. The major colonizers in a semitropical habitat in Egypt, *A. acuminata*, *Lunulospora curvula*, *Tetracladium marchalianum* and *Triscelophorus monosporus* have been found to be the major colonizers on all types of leaves (Abdel Raheem, 1977). The results in the canal are similar with respect to uniform species.

The winter assemblage in the canal was much more restricted consisting of those species which appeared periodically when the temperature was low. They consisted of the well known winter species like *Alatospora*
acuminata, Anguillospora longissima, Articulospora tetracladia, Lemonniera aquatica and L. centrosphaera and Tetrachaetum elegans. Almost all of them are known winter species (Suberkropp and Klug, 1981; Chauvet and Suberkropp, 1998). In the canal many well known winter species like Clavariopsis aquatica and Flagellospora curvula exhibited a different behavior. Both were earlier known for their affinity to low temperature. Therefore they may represent new ecological variants of the existing species that have adapted themselves to live in a different set of ecological conditions with a higher temperature regime.

5. Conclusions

1. The semitropical canal water habitat shows prevalence of summer assemblage of aquatic hyphomycetes with periodic occurrence of winter species during the low temperature regime.
2. The technique used for study had little impact on the overall colonization patterns of aquatic hyphomycetes observed in the canal.
3. All the environmental variables had an overall impact on the colonization pattern of aquatic hyphomycetes, the temperature and biomass deposition being the most influential factors regulating the ordination of communities.

References


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