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Plastic substrate deviated to a great extent from the stone and reed substrata with regard to the parameters measured, whereas the biofilms developing on reed and stone substrata were quite similar. We conclude that for water quality monitoring purposes, sampling from green reed during spring time is not recommended, since this is the colonization time of periphyton on the newly growing reed, but it may be appropriate from the second half of the vegetation period. Stone and artificially placed old reed substrata may be appropriate for biomonitoring of shallow soda lakes in both (spring and autumn) periods inasmuch as they showed highly similar results regarding all measured features.

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Comparative algological and bacteriological examinations on biofilms developed on different substrata in a shallow soda lake

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Abstract Benthic diatoms of lakes are a tool for ecological status assessment according to the European Water Framework Directives. In this study, we followed an integrative sample analysis approach in order to find an appropriate substratum for the water qualification oriented biomonitoring of a shallow soda lake, Lake Velencei. Six types of substrata (five artificial and one natural), i.e. andesite, granite, polycarbonate, old reed stems, plexi discs and green reed, were sampled in May and in November. Total alga and diatom composition, chlorophyll-*a* content of the periphyton, surface tension and roughness of the substrata and carbon source utilisation of microbial communities were analysed. Water quality index was calculated based on diatom composition. Moreover, a novel statistical tool, a self organising map was used to relate algal composition to substratum types. Biofilms on plastic substrates deviated to a great extent from the stone and reed substrata with regard to the parameters measured, whereas the biofilms developing on reed and stone substrata were quite similar. We conclude that for water quality monitoring purposes, sampling from green reed during spring time is not recommended, since this is the colonization time of periphyton on the newly growing reed, but it may be appropriate from the second half of the vegetation period. Stone and artificially placed old reed substrata may be appropriate for biomonitoring of shallow soda lakes in both spring and autumn since they showed in both seasons similar results regarding all measured features.

Keywords Biomonitoring · Natural and artificial substrata · Water qualification

Introduction

Surfaces exposed to adequate moisture, mainly solid-liquid phase interfaces, provide a niche for microbial colonisation, where the majority of microorganisms are thought to exist naturally as biofilms (SUTHERLAND, 2001). Biofilms provide a both spatially and temporally heterogeneous milieu, comprising an organised, 3-dimensional structure of microbial communities embedded in an exopolysaccharide matrix (EPS), with a network of water-filled channels (JENKINSON and LAPPIN-SCOTT, 2001). Bacteria are observed to be the primary colonisers, followed by the adhesion of other bacteria and different microorganisms, among others algae, with each cell potentially serving as a new surface for the wide variety of further colonising cells (ÁCS et al. 2000). The composition and the architecture of the biofilm are subject to intrinsic as well as extrinsic factors, the latter including several physico-chemical attributes of the surrounding environment and the nature of the substratum.

Periphytic algal studies in lentic environments lagged behind phytoplankton investigations. Nowadays, in several countries of the EU, in the investigations connected to EU Water Framework Directive (WFD, European Parliament, 2000, directive 2000/60/EC), benthic algae are considered key-organisms for the assessment of the ecological quality of watercourses. Palaeolimnology has been using transfer functions based on weighted averaging for a long time, which are developed on the basis of the composition of the recent flora and fauna and the belonging measurable chemical parameters and on the strength of the model, knowing the composition of the fossil flora and fauna, the environmental parameters of that time are inferred (e.g. CHRISTIE and SMOL 1993, SCHNITCHEN et al. 2006). It is clearly visible from these results that the epilithic algal community of the littoral zone shows good correlation with the water quality parameters and can be used well in the assessment of trophic condition of lakes in biomonitoring.

In Germany, water qualification investigations based on the benthos of lakes have a long history. HOFMANN (1994) described the bases of the use of benthic algae in lakes as the indicator species of trophic and saprobic condition, in which she wrote that the difference in species composition on stone and plant did not cause significant change to the indices. Later, she (HOFMANN 1999) developed a new index (trophic index: TI) for the more precise assessments, which was used subsequently by others also in the country (e.g. SEELE et al. 2000). Later it was built into the national monitoring program too and developed further (SCHAUMBURG et al. 2005), a lake diatom index was worked out (DI_{Seen}), one element (module) of which was the TI, as the other module a reference index (reference species ratio, RAQ) was calculated, then by means of the two modules, the lake diatom index (DI_{Seen}) was computed. They suggested stone and the surface of the sediment as substrate, where the water level was at least 30 cm, far away from the macrophyton.

In the work of BLANCO et al. (2004), the indicator role of diatoms was investigated in six Spanish lakes of different water qualities for the determination of the trophic and saprobic condition of the lakes. The indices calculated by the OMNIDIA software were used in the qualification. Their results showed that the investigation of epiphytic algae and the diatom indices calculated by OMNIDIA software could be used well in the biological monitoring of lake ecosystems too for the estimation of the ecological condition, although the indices were basically developed for flowing waters.

POULICKOVA et al. 2004 investigated the indicator role of the diatoms of the littoral zone in Austrian lakes near the Alps, samples were collected from the surface of alive reed, stone and sediment too. The results showed that the species composition of the various substrates was significantly different that influenced the values of the indices, hereby the qualification. According to his opinion, the most suitable substrate in the lake is the alive reed, because both dead reed, both stone and sediment accumulates the dead diatoms from the previous years. Alive reed grows newly in every year however, and the community developed on it reflects the actual condition. During the investigations of GRIMES et al. (1980), it was unambiguously shown that there was not significant difference between the diatom composition of the periphyton of dead and alive reed.

By comparing the element content of the periphyton of alive and dead reed in Lake Balaton and Lake Velence with the element content of the reed, LAKATOS (1983) found significant positive correlation in the case of the alive reed, while in the case of dead reed there was not any significant correlation. This is an important statement also because it indicates that dead reed rather serves only as substrate for the developing periphyton on it, so until the beginning of a considerable decomposition, probably the algal composition of the periphyton developing on it reflects more to the chemical components of the water than the periphyton on alive reed, which is in considerable metabolistic connection with the reed.

The introduction of artificial substratum use, through which more standardised and reproducible results were gained, has circumvented this problem to a certain extent, and artificial substrata have been extensively used for the study of freshwater periphyton (reviewed e.g. in KRALJ et al. 2006).

A proposal was formulated by KING et al. (2006) concerning the sampling of benthos of lakes for the assessment of the ecological condition. For the proposal, she surveyed numerous literatures and summarized the most important statements regarding the topic. According to his opinion however, the type of the substrate should be selected in a way that it is the most characteristic substrate of the littoral region of the lake. In the question of the substrate, the available data in the literature is not sufficient yet and is very contradictory too.

The community level BIOLOG (Hayward, California, USA) assay is a rapid and cultivation-independent method to distinguish between microbial communities based on temporal, spatial and physicochemical parameters (GARLAND, 1997). Community level physiological profiling (CLPP) of bacterial biofilms has proved an effective measure of biofilm condition changes due to copper-induced toxicity (MASSIEUX et al. 2004).

Shallow soda lakes are characteristic for the Hungarian plane but also occur elsewhere in Europe (e.g. Spain). Up to now, there are no existing guidelines as to the periphyton based water quality analysis of this lake type. The aim of our study was to find an appropriate substratum for the biomonitoring of shallow, sodic lakes, and to compare the biofilm development in an early and late phase of the vegetational period. Based on our previous studies, the Lake Velencei was chosen as a model system to gain new experience on the subject of substratum preference. A multidisciplinary approach was followed using analytical chemistry, community-level physiological profiling and microscopic alga investigations along with novel statistical tools.

Materials and methods

Study site and sample collection

Lake Velencei is the third largest lake of Hungary (24.5 km^2) with a 615 km^2 water catchment region. The lake is situated at the foot of the Velencei Mountains (which consists mainly of granite), 45 km SW from the capital, so it is an important recreation centre. It is a shallow soda lake, rich in dissolved salts and generally mesotrophic. The water level of the lake is regulated by two reservoirs situated in its catchment area and generally fluctuates between 140 and 160 cm measured at Agárd near to our sampling point (N 47°11'21", E 018°34'41"). On the SW part of the lake, a large reed-belt can be found and the water colour is dark brown. This part belongs to a nature conservation area. The middle and NE part of the lake is the "open water" area with grey water colour caused by mud stirred up by the wind. This part belongs to the holiday area. Since the larger, open water area of the lake is used for bathing, field experiments were conducted here.

Four different artificial substrata (granite [G], andesite [A], plexi [P] discs (made by Satunaplex Ltd. Balatonfűzfő, Hungary), and polycarbonate [PC] slides (made by Arla-plast Ltd. Sweden)) and two natural substrata (old reed [OR] stems and living green reed [GR] stems) were used for biofilm development. The common reed (*Phragmites australis* (Cav.) Trin ex Steudel) stems located near the sample holder served as green reed substratum. The additional substrata (with the exception of plexi in May, when it was not included) in ten replicates were placed into the same plexi holder (Fig. 1a), which was fixed to a rack equipped with a float (Fig. 1b) to keep the samples approximately 30 centimetres below water surface. Biofilms developed on different substrata were studied in spring and autumn 2002. Surface sterilized granite, andesite and polycarbonate slides (12 x 3 x 0.7 cm) and plexi discs with a diameter of 3 cm as well as old reed stems (all in ten replicates) placed in the same distance from one another and in vertical position were dipped into the lake water on 15 April and 23 September. Following six weeks' colonization they were collected on 27 May and 4 November, as well.

At the end of the colonisation periods (in May and November), green reed samples from the same depth were also collected. Samples were transported to the laboratory in a dark cooling box within 2 hours of sampling. The biofilm from different substrata was washed into separate flasks filled with known volume of sterilized physiological saline solution (0.89 g NaCl l^{-1}, pH 8.0) with the help of a sterile brush. Composite samples – collected from the same type of substrata – were divided into subsamples for different further analyses.

Surface tension and roughness of substrates

In order to determine the surface tension of the substrata, advancing and receding contact angles of water and formaldehyde droplets on the different substrata were measured by a contact goniometer. Based on the measured values, the polar and dispersion components of the surface tension were calculated according to Fowkes geometrical mean equation. Polarity (P) can be calculated based on the following equation $P = \frac{\gamma_S^{pol}}{\gamma_S} \cdot 100$, where γ_s is the surface tension and γ_s^{pol} is its polar component of the solid material.

Determination of roughness was carried out using AFM (Atomic Force Microscopy) at a resolution of 10 x 10 µm. Section analysis provides the roughness information on an arbitrary cross section of the surface. Typical roughness parameter given to characterize the the surface roughness is Ra, which represents the mean roughness, which is the mean value of a surface cross section relative to the center line.

Algal abundance and identification

A part of the biofilm samples was preserved in Lugol's iodine solution. The quantitative determination and the identification of algae were completed by UTERMÖHL'S (1958) method, according to the statistic instructions of LUND et al. (1958). For identification of diatoms, samples were treated with H_2O_2 and mounted in Naphrax.

Chlorophyll-a analysis

Another part was prepared on the day of sampling for chlorophyll-*a* measurement. Following the method of GOODWIN (1976) the chlorophyll-*a* content was extracted by cc methanol and measured by a photometer at 653, 666 and 750 nm. The chlorophyll-*a* concentration was calculated by the following equations: $C_a = 17.12 * E_{666} - 8.68 * E_{653}$, and the chlorophyll-*a* concentration ($\mu g\ cm^{-2}$) = $m * C_a * 1000 * M^{-1} * t^{-1}$, where m: the volume of methanol used for the extraction (ml), M: the volume of the sample filtered onto the glass fiber filter (ml).

Carbon source utilisation of bacterial communities

From the third part of the biofilm samples, a sole-carbon-source-utilisation test of bacterial communities was carried out. In order to inoculate BIOLOG GN2 microplates, an initial 10^{-1} biofilm dilution was prepared by suspending wet biofilm – equivalent to 10 g of dried matter – in 100 ml of sterile saline solution. Serial dilutions were prepared up to a dilution factor of 10^{-3}. A 20 ml aliquot of each dilution was shaken for 10 min and left settling for 10 min, in order to avoid interference in the assay by co-extracted biofilm components, causing unspecific turbidity and absorbance. From the diluted biofilm samples, 150-150 µl volumes were inoculated into the wells of BIOLOG GN2 microplates, providing 95 different carbon sources and a redox indicator (tetrazolium violet). The majority of carbon sources on BIOLOG GN2 plates are carbohydrates (30), carboxylic acids (24) and amino acids (20). Polymers (5), amines/amides (6) and miscellaneous compounds (10) are represented in lower numbers (PRESTON-MAFHAM et al., 2002). The detection of the substrate utilisation - based on the reduction of the tetrazolium dye and therefore the optical density values - were measured at 595 nm with an ELISA Reader (Labsystems Multiscan PLUS), after incubation at 25°C from 24 to 96 hours. The type and number of the carbon sources utilised by the bacterial communities were evaluated according to GARLAND and MILLS (1991).

Statistical analysis

Diatom diversity and evenness values were calculated using the Shannon-Weaver index. Self Organizing Map (SOM) method introduced by KOHONEN (2001) was used for grouping the samples and evaluates the structure of diatoms and the epiphyton. SOM can make a projection of the database into a two dimensional hexagonal map. Closely related communities are placed into neighbouring hexagons by their similarities, while samples with different communities are in distant hexagons. The SOM can display the groupings of samples and species together; therefore, each species can be evaluated by its importance. The number of output neurons is an important factor in the analysis, and the suggestion of VESENATO (2000) was used, which determines the output number as: $Noutput = 5x\sqrt{Nsamples}$. The rough tuning phase includes 2000, while the fine tuning phase lasted contains 8000 iterations.

The Structuring Index (SI) was originally developed to define the species that show the strongest influence and the organization of the SOM map (PARK et al. 2005). TISON et al. (2004) used the SI to evaluate relevant diatom species in the classification of diatom communities. The set of species showing high SI can be considered as the indicator species. Taxa showing strong gradient display high SI values, whereas species showing weak gradient present low SI values. Thus, the higher the value of SI, the more relevant the variable is to the structure of the map. For clustering the SOM we used the K-means clustering technique. It is an algorithm to classify or to group objects based on attributes (in this case species composition) into K number of group. The grouping is done by minimizing the sum of squares of distances between data and the corresponding cluster centroid as the square error of each data point is calculated and clusters reformed such that the sum of square errors is made to be minimum. The SOM Toolbox (http://www.cis.hut../projects/ somtoolbox) was used to implement the SOM under a MATLABs environment.

The diatom index (IBD) was calculated by using OMNIDIA V4. software.

Comparison of the substrate oxidation results originating from the different samples was accomplished by statistical analysis (principal component analysis [PCA]), using the SYNTAX 2000 software package (PODANI, 2000).

Results

Among the tested substratum types, granite had the highest surface roughness, polycarbonate and plexi substrata were by two orders of magnitude smoother (granite: 510 nm, andesite: 420 nm, polycarbonate: 9 nm, plexi: 4 nm) (Fig. 2). The polarity values were 76.2 for the granite, 68.4 for the andesite, 60.4 for the plexi, 39.2 for the polycarbonate and 22.9 for the old reed substrata. The old reed substratum had the highest dispersion and lowest polar component of the surface tension while granite had the highest polar component and the lowest dispersion (Fig. 3).

Studying the periphyton communities developed on different substrata in Lake Velencei, more than 120 algal species were found and most of them belonged to diatoms. Comparing the species number, Shannon diversity and evenness values of the different substrata, the smallest values were found on the plastic substrata in almost all cases (Fig. 4). Certain species such as *Navicula cryptocephala* Kütz. and *Oedogonium capitellatum* Wittrock were more dominant in the spring, while others such as e.g. *Nitzschia palea* (Kutzing) W.Smith and *Ctenophora (Fragilaria) pulchella* (Ralfs ex Kutz.) Williams et Round dominated in the autumn (Fig. 5). The communities grown on plexi and polycarbonate substrata were strongly dominated by *Achnanthidium minutissimum* (Kütz.) Czarnecki. Other characteristic species were *Microcystis* *aeruginosa* (Kütz.) Kütz. for granite, *Oedogonium capitellatum* for andezite, *Gomphonema olivaceum* var. *olivaceum* (Hornemann) Brébisson for both granite and andezite, while for green and old reed no significant differences were detected in the dominance of algal species.

According to the SOM analysis based on the algological data (species composition and their abundance), a strong seasonality can be observed (Fig. 6). Substratum types formed five distinct groups. In May granite, andesite and old reed formed a group, while polycarbonate and green reed formed a group, which clearly separated from the ones originated from November. In autumn plexi and polycarbonate as well as granite, green reed and old reed comprised two distinct groups, while andesite was loosely connected to the latter (Fig. 6). IBD indices displayed the most analogous values on the granite, andesite and old reed substrata both in May and November, respectively (Fig. 7).

The chlorophyll-*a* content was highest on the granite (326 μg cm^{-2}) and andesite (328 μg cm^{-2}) in May, and on the old reed in November (51 μg cm^{-2}), and it was the lowest on green reed (24 and 0.5 μg cm^{-2}) on both occasions.

Microbial BIOLOG substrate utilization patterns of biofilm communities developed on different substrata were compared on the average well color development value (AWCD; average of absorbance values from 95 wells). With the exception of green reed substratum, the AWCD values were higher in May than in November (Fig. 8). Out of green reed substratum, the higher substrate utilization values were measured in case of granite and old reed substrata on both occasions. According to the bacteriological investigations, carbohydrates (sucrose, α-D-glucose, maltose, D-mannitol and D-trehalose) were the most preferred among the carbon sources. The number of carbon sources characteristic for certain microbial communities was the largest in the case of granite while this number was lower in the case of the other substrata types. Among the carbon sources the utilization of D-fructose and D-gluconic-acid was characteristic in May, while glycogen, tween 80, D-mannose and D-sorbitol in November. Community level physiological profiles (CLPP) based on the colour development on BIOLOG plates were examined by principal component analysis (PCA), as well. In PCA, microbial communities from different substrata served as objects and the 96 hour absorbance values of carbon source utilization as variables. According to the ordination, the first two PC axes accounted for 69 and 63% of the variance in the data in May and November samples, respectively (Fig. 9). In May, samples from andesite, granite and old reed substrata grouped tightly together along PC1, while polycarbonate and green reed clearly separated from them. As Fig. 9 indicates, in November the differences among the substratum types increased as the objects scores diverged along the PC1. Apart from this, in autumn CLPP of bacterial communities from granite and old reed substrata were the most similar to each other with their highest object scores for Axis 1.

Discussion

The potential of epilithic microalgae to derive nutrients from rock substrata has not been extensively examined so far. However, it can be assumed that it depends on the rock`s chemical composition, porosity, crystal size and other features. ROSEMARIN and GELIN (1978) related the trend observed in substratum specificity to the roughness of the surface: among the different artificial substrata, red granite and lightly sandblasted plexiglass were better than smooth glass. Similarly, KRÖPFL et al. (2006) have found that surface properties of substrata greatly influence developing biofilms. Nevertheless, ROSOWSKI et al. (1986) showed that the organic layer developing on the substrata may mask the substratum effect already after two weeks of colonization.

Differences in the polar and dispersal components of the surface tension of the different substrata used in our study seemed to have an influence on the composition of the mature periphyton, as well. Comparing species number, Shannon diversity and evenness values of algae and AWCD values of bacterial communities detected on the different substrata, smaller values were found on the smooth surfaced plastic substrata (plexi and polycarbonate), than on the rougher surfaced ones (andesite, granite and reeds). As it can also be seen from the diatom composition, this may have been primarily caused by the dominance of *A. minutissimum* on the plastic substrata.

Based on the results of the SOM analysis, it can be stated that the separation of plastic substrata - both in May and November - is in good correspondence with the dominance of certain species. In May, the two rocks (granite, andesite) and old reed showed the greatest similarity to each other and it was reflected in the chlorophyll-*a* contents, diatom indices and the carbon source utilisation test of bacterial communities, as well. In November, the most similar periphyton communities were also detected on the granite and old reed substrata, while the plexi was greatly deviant both in algological and in bacteriological respects. The analysis of BIOLOG carbon source utilization patterns revealed a more expressed heterogeneity among the same type of substrata (e.g. rocks, plastics or reeds) compared to the different type of substrata, especially granite and old reed. In both (May and November) sample series, granite and old reed had the most similar and the most diverse bacterial communities according to their BIOLOG carbon source utilization, respectively.

Comparing the metabolic fingerprints as well as algological data of biofilm samples, the positions of object scores and the results of SOM analysis of natural substrata indicated that bacterial and algal communities developed on green and old reed substrata were more dissimilar in May than in November. In an earlier study performed on green and old reed stem surfaces of Lake Velencei in summer (ÁCS et al., 2003) no significant differences were found in the species composition algae and metabolic activities of bacterial communities in mature biofilms. These results suggest that the differences between them on green and old reed start to diminish already in summer.

The obvious separation of the green reed substratum in this study was probably caused by the different colonization phase of the periphyton (early colonization phase communities differ from mature communities). There are three different hypotheses concerning the interaction between epiphytes and their host plants: a) some studies showed a positive nutrient interaction (e.g. WETZEL 1983), when the substratum is a second nutrient source for the algae; b) others indicate a negative interaction, when the macrophyte releases allelopathic substances that inhibit epiphyte growth (e.g. ANTHONI et al.1980); c) a third hypothesis states that macrophytes are only a neutral site for attachment (e.g. CATTANEO and KALFF 1979). Our results based on the aligned algological and bacteriological studies on biofilms indicated that in May mainly dissimilar or opposite plant-microbe interactions could be dominant on green and old reed substrata whereas in November these interactions became more complex and related to each other. As BURKHOLDER (1996) also pointed out, each of these hypothesized interactions might be true, depending on the season, water quality (with respect to nutrient content) and plant substratum conditions. Significantly different epiphyte communities have been found on natural as opposed to plastic plants in mesotrophic and oligotrophic lakes (CATTANEO and KALFF 1978), while in eutrophic lakes, they did not exhibit any substratum preference (EMINSON and MOSS 1980).

Summarising, both the algological and bacteriological investigations showed that periphyton developing on stone and reed were not significantly different. Especially in the case of old reed, even the diatom indices showed similar values. However, it has to be noted, that in this experiment cut, sterilized and artificially placed reed was used as old reed rather than naturally growing, rooted reed from the lake. Using rooted reed might have resulted in greater differences of the community composition between reed and stone periphyton by November, since active microbiological degradation processes would have influenced algal composition to a greater extent.

Conclusions

For water quality monitoring purposes, sampling from green reed during spring time is not recommended, since this is the colonization time of periphyton on the newly growing reed, consequently, algal and bacterial composition cannot unambiguously be in consonance with water quality but it may be appropriate from the second half of the vegetation period. Stone and artificially placed old reed substrata may be appropriate for biomonitoring of shallow soda lakes in both (spring and autumn) periods inasmuch as they showed highly similar results regarding all measured features.

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Figures captions

Fig. 1 Frames for the artificial substrata (above) and their floating holder used in Lake Velencei.

Fig. 2 Atomic force micrographs of the surface of the artificial substrata.

Fig. 3 Polar and dispersal components of the surface tension of the different substrata (G=granite, A= andesite, P= plexi, PC= polycarbonate, OR= old reed).

Fig. 4 Diversity and evenness of diatom communities on different substrata (Div=diversity, M=May, N=November, Ev=Evenness, G=granite, A= andesite, P= plexi, PC= polycarbonate, OR= old reed, GR= green reed).

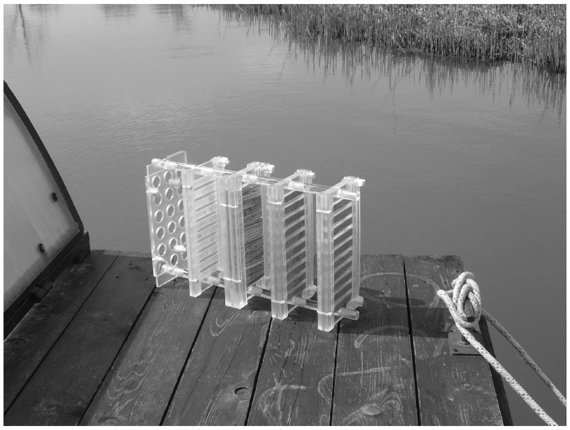
Fig. 5 Dominant alga species in the samples. (MAER= *Microcystis aeruginosa* (Kütz.) Kütz., AMIN= *Achnanthidium minutissimum* (Kütz.) Czarnecki, CPLA= *Cocconeis placentula* Ehrenberg var. *lineata* (Ehr.)Van Heurck, CAFF= *Cymbella affinis* Kutz, CCIS= *Cymbella cistula* (Ehrenberg) Kirchner, CLAC= *Encyonema (Cymbella) lacustre* (Agardh) F.W.Mills, DTEN= *Diatoma tenuis* Agardh, FPUL= *Ctenophora* (*Fragilaria) pulchella* (Ralfs ex Kutz.) Williams et Round, GOLI= *Gomphonema olivaceum* var. *olivaceum* (Hornemann) Brébisson, NCRY= *Navicula cryptocephala* Kutz., NPAL= *Nitzschia palea* (Kutzing) W.Smith, RABB= *Rhoicosphaenia abbreviata* (C.Agardh) Lange-Bertalot, MGRI= *Monoraphidium griffithii* (Berk.) Kom.-Legn., OECA= *Oedogonium capitellatum* Wittrock. See more abbreviation on Fig. 4).

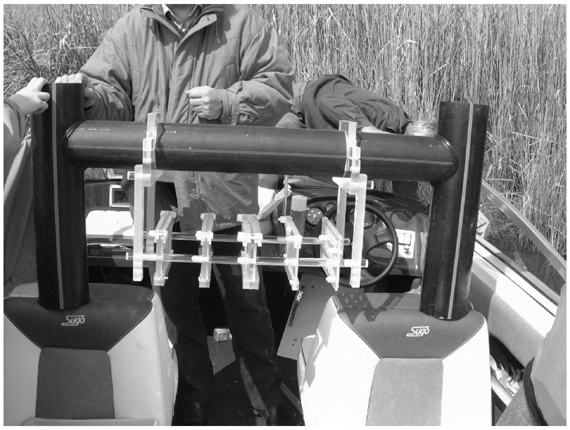
Fig. 6 Classification of the samples through SOM, the SOM virtual units were classified into 4 clusters by K-means clustering algorithm (see abbreviation on Fig. 4).

Fig. 7 Values of the indices IBD (Indice Biologique Diatomées) in May and November, based on the benthic diatom composition of the different substrata (see abbreviation on Fig. 4).

Fig. 8 Differences of the average well color development (AWCD) values of microbial communities detected on BIOLOG GN2 microplates originating from different substrata placed in Lake Velencei (see abbreviation on fig. 4).

Fig. 9 Scattergram of substrata based on bacterial data (the colour development on BIOLOG plates. A= in May, B= in November. M=May, N=November, see more abbreviations on Fig. 4).

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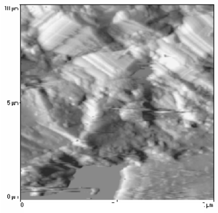

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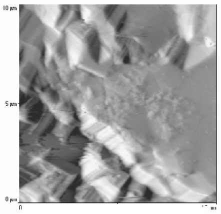

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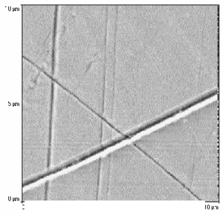

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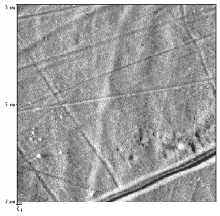
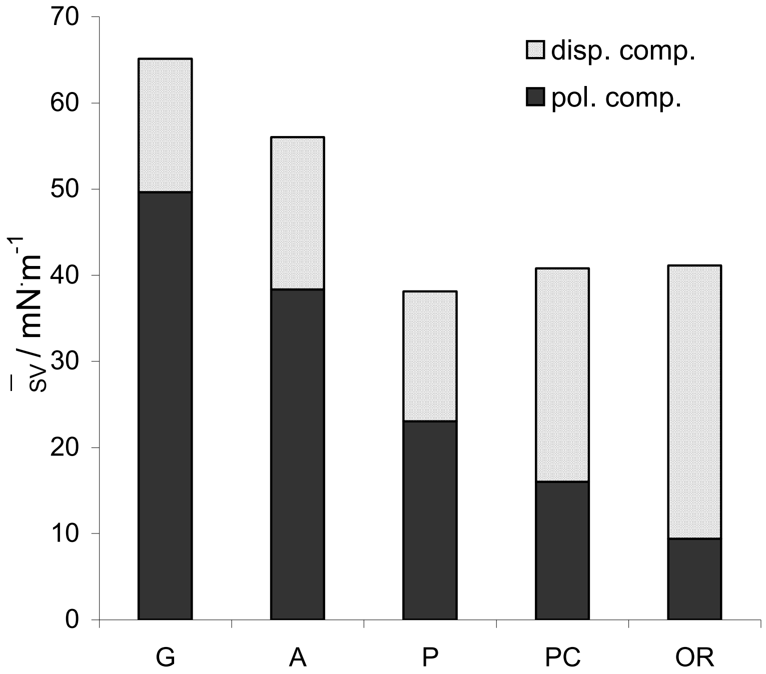


Polycarbonate


70
60
50
40
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0
disp. comp.
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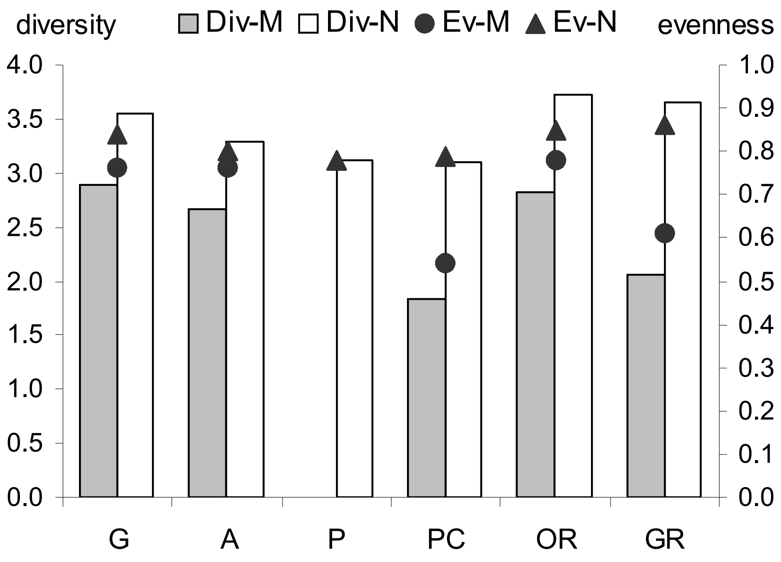
OR


diversity
▨ Div-M
☐ Div-N
● Ev-M
▲ Ev-N
evenness
4.0
3.5
3.0
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1.0
0.5
0.0
1.0
0.9
0.8
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line figure

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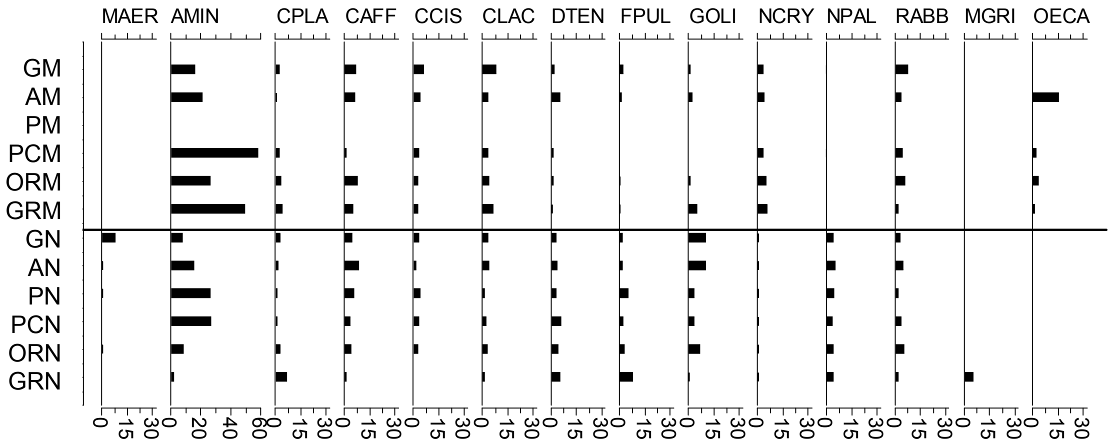

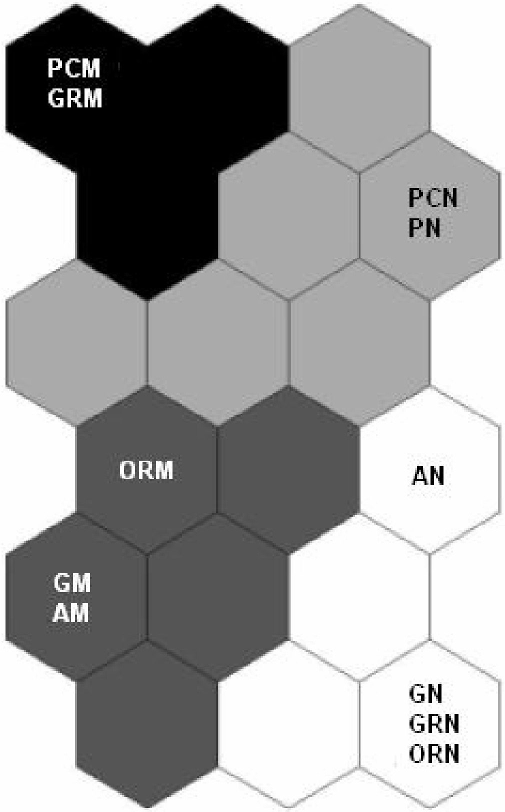
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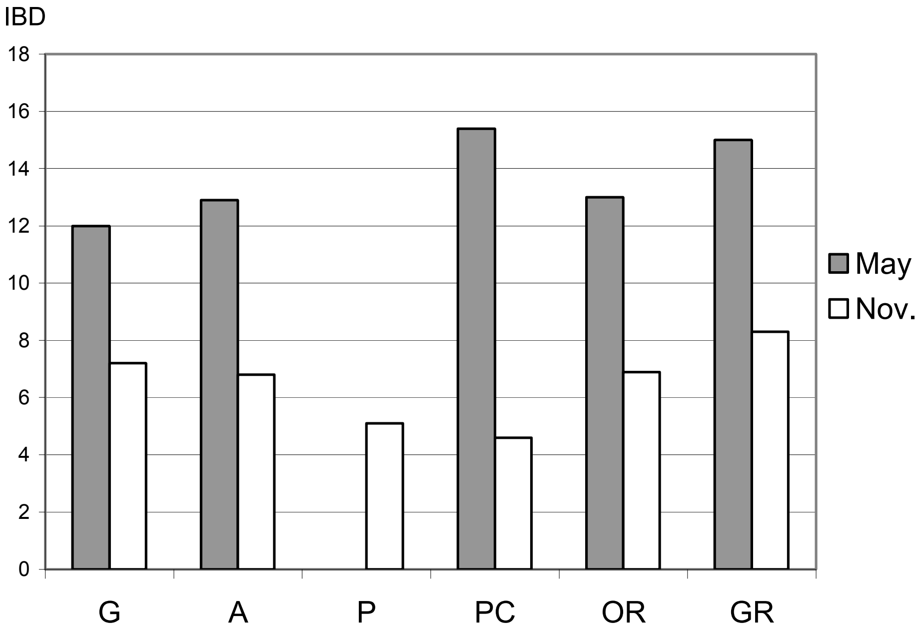
IBD
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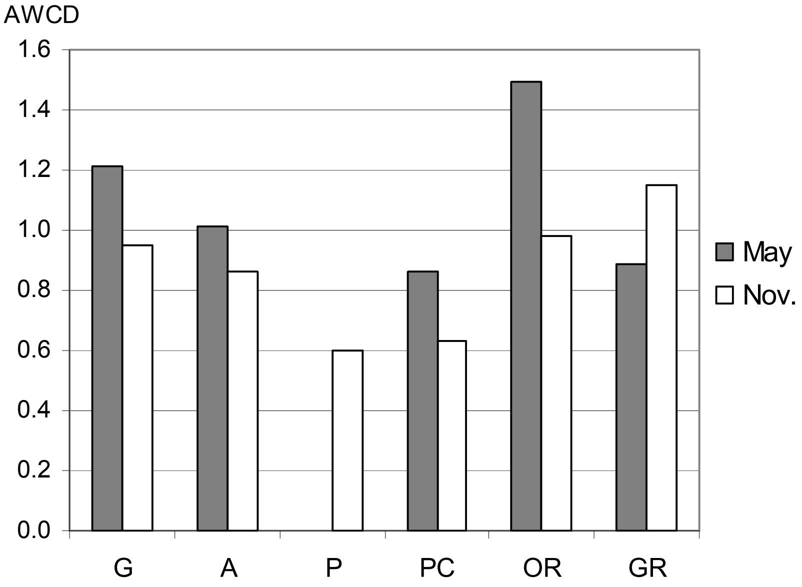
AWCD
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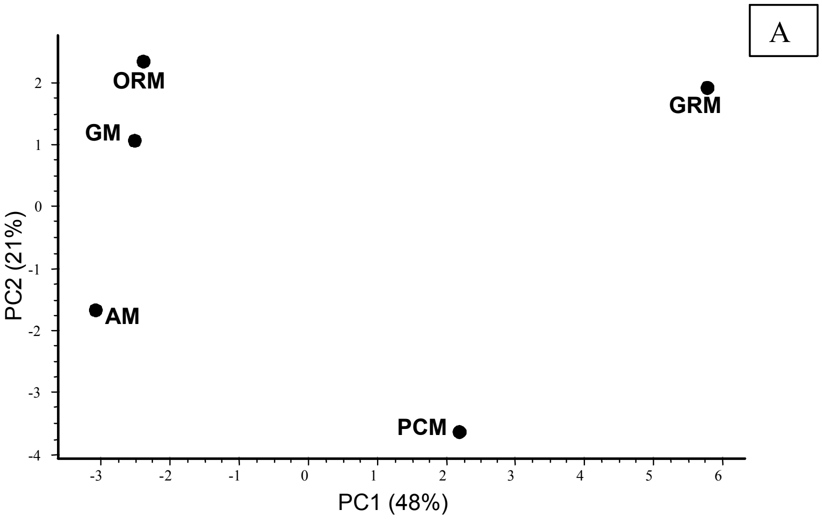
■ May
☐ Nov.


A
ORM
GRM
GM
AM
PCM
PC2 (21%)
PC1 (48%)
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-2
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-2
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5
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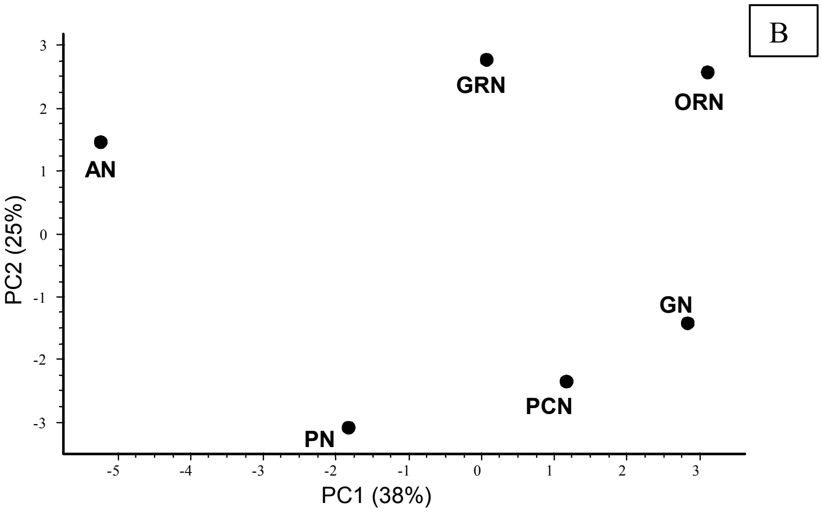
B
GRN
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PCN
PN
PC2 (25%)
PC1 (38%)
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