DIVERSITY-ECOSYSTEM FUNCTIONING RELATIONSHIP IN BENTHIC DIATOM ASSEMBLAGES IN RIVERS

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Abstract

Biodiversity-Ecosystem Functioning (BEF) relationships are important indicators of ecosystem health and species interactions. Despite their extensive study in terrestrial plants, the BEF relationship in benthic diatoms in rivers has been considerably understudied. In the present study, benthic diatom samples from nine rivers across Greece were obtained and species richness and abundance, diatoms functional traits and biomass production (as chlorophyll-a concentration) were measured. The observed relationship was not universal and differed among rivers, presenting also a strong response on environmental factors like flow and geology. This is the first extensive study of BEF in benthic river diatoms and gives important insights on possible mechanisms that require further research.

Keywords: biomass production, chlorophyll-a, functional diversity, Greece.

1. Introduction

Ecosystem functioning comprises of ecosystem processes that account for ecosystem health and sustain ecosystem services. Diversity plays a pivotal role in driving ecosystem functioning and the form of the Biodiversity-Ecosystem Functioning (BEF) relationship is indicative of species interactions and their contribution to ecosystems. BEF studies, especially early ones, mainly focus on positive relationships (i.e., increase of ecosystem functions with increased diversity). More recent research and meta-analysis, suggest different relationships (e.g., negative or hump-shaped relationships), depending on the type and duration of the study (i.e., observational field or experiment), the ecosystem type and the taxa studied (Daam *et al*., 2019). Most research occurred in terrestrial plants, whereas aquatic environments and especially freshwater remain understudied (Daam *et al*., 2019). River benthic biofilm studies are nearly missing (but see Smeti *et al*., 2019), making the importance of field observations in these systems imperative for our understanding of BEF relationships in phytobenthos.

A major component of phytobenthos in rivers are diatoms, unicellular algae with cell wall of silica, responsible for 50% of O_2 production and important indicators of water quality. Their growth depends on nutrient concentrations, and they contribute immensely to biofilm primary productivity, an important ecosystem function. A surrogate of primary productivity in algae is the concentration of chlorophyll-a (a measure of biomass accumulation) which can also be associated to the total volume of the cells in the community (i.e., biovolume). Thus, the important contribution of benthic diatoms in a fundamental ecosystem function together with the fact that they are the most diverse protists, constitute them an ideal group of organisms to study the BEF relationship.

The use of community biovolume as a measure of ecosystem function highlights the importance of cell size as functional trait. Other important traits are related to adherence to substrates and life forms and are increasingly used in describing benthic diatom assemblages. Growing evidence suggests that functional richness could be more important in driving ecosystem functions than taxonomic richness (Abonyi *et al.,* 2018). Despite their importance, functional diversity metrics are not widely used to BEF studies, denoting another gap in the research of the BEF shape.

The aim of this study was to identify the shape of the BEF relationship in benthic diatom assemblages in rivers using both taxonomic and functional diversity metrics. We further investigate possible environmental explanatory factors that underpin it. This is the first extensive field survey in rivers towards this aim and it provides useful insights in the function and diversity of these overlooked systems.

2. Material and Methods

2.1 Field sampling

Nine rivers across Greece (Nestos, Lisos, Fonias, Spercheios, Mornos, Alfeios, Arkadikos, Neda, Evrotas) were sampled in summer 2020. These rivers were selected based on available access and sampling substrate (stones) as well as due to their differences in terms of size, geology and environmental conditions. Pollution levels slightly differed even between sites of the same river, based on a biological quality diatom index, but quality classes didn't seem to play an important role in the BEF relationship (results not shown). In each river, five sampling sites were sampled across their linear flow from upstream to downstream, apart from Arkadikos and Lisos, where only four samples were taken. To ensure replication, in each site three spots were sampled, comprising of three stones each. From each stone, two surfaces of defined area were scraped, one to be used for chlorophyll analysis (immediately put in a dark bag and freezed) and the other for species identification and counting (preserved with 70% ethanol). This ensured the direct comparison between species diversity and productivity. At each site, physico-chemical parameters (Temperature, DO, pH, Conductivity, Turbidity) were also measured in-situ using a Portable multiparameter Aquaprobe and water was collected for the determination of main nutrients (NO2, NO3, NH4, TN, PO4, TP, SiO4).

2.2 Analysis of samples

In the laboratory, after filtration through 0.45 μm pore size membrane filters, nutrients were determined by a Skalar San++ Continuous Flow Analyzer (APHA, 1980). For the determination of chlorophyll, the trichromatic equations were applied (Jeffrey and Humphrey 1975), where all three main chlorophylls were measured and corrected for (Chl-a, Chl-b, Chl-c). Chl-a is a measure of the whole phytobenthos biomass production, whereas Chl-c is more indicative of the biomass produced by benthic diatoms.

Diatom species samples were treated with hot hydrogen peroxide to remove organic matter and obtain clean frustules, used for diatom species identification (Battarbee, 1986). Clean frustules were mounted with Naphrax, identified to species level with a light microscope at 1000X magnification and counted until no more new species were detected in each sample. For the taxonomy, the work of Cantonati *et al.* (2017) was mainly used. Functional traits used were linked to cell size (L/W ratio, biovolume), substrate adherence (high profile, low profile, motile and planktonic guilds), life forms (colonial, singular) and nitrogen fixation (Rimet & Bouchez, 2012).

2.3 Data analysis

Taxonomic diversity (Species Richness (S) and Evenness (J)) and functional diversity (Functional Richness based on functional traits assigned to species) indices were defined. The form of the relationship between the different diversity metrics and Chl-a and Chl-c was determined, both within each river and with the aggregated dataset. For the aggregated dataset, to define more clearly linear trends, Chl-a concentrations were log-transformed. Data analyses and illustrations were performed in R (v.4.0.3), using packages vegan v.2.5-7 (Oksanen *et al.*, 2020), BAT v.2.7.1 (Cardoso *et al.*, 2021) and ggplot2 (Wickham, 2016).

3. Results

Overall, the sampled rivers presented different environmental conditions, as depicted in the physico-chemical parameters and nutrient concentrations measured (Fig. 1). This had also an effect on diversity indices and biomass production (Fig. 1).

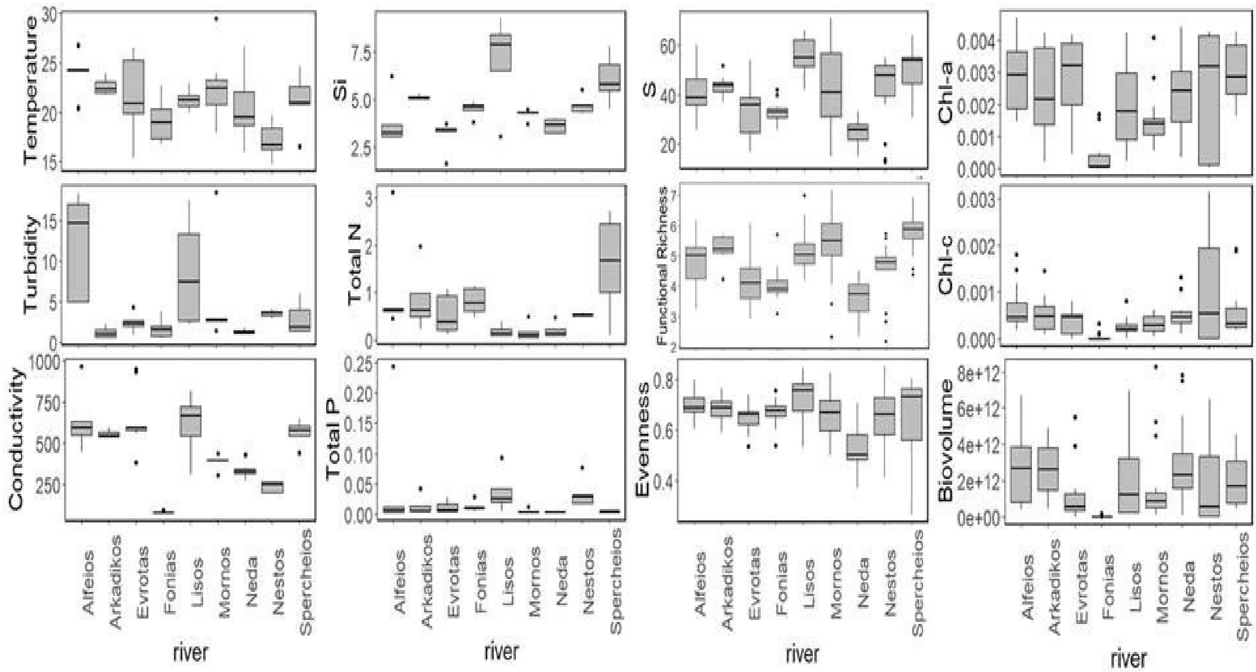

Fig. 1: Physico-chemical (Temperature, Turbidity, Conductivity), Nutrient concentration (Si, TN, TP), Diversity (Species richness –S, Functional Richness, Evenness) and Biomass (Chl-a, Chla-c, Total biovolume) variation between the nine rivers of the study.

Alfeios and Lisos presented the highest turbidity, Spercheios presented the highest TN concentration, Lisos and Nestos presented the highest TP concentration, Lisos and Spercheios presented the highest Si concentration. Lisos, Nestos and Spercheios were the rivers with the highest species richness. Functional richness presented the same trend with species richness, with the exception of Mornos that presented an increased functional richness. Fonias was the river with the lowest biomass production. All the above presented results were significant following Tukey post-hoc test. Chl-c and Total biovolume - biomass production metrics only based on benthic diatoms- presented the same trend as Chl-a – the metric of total biomass production- thus ensuring its use interchangeably.

The relationship between species richness and ecosystem functioning (i.e., biomass production measured as Chl-a concentration) is variable between the different rivers sampled (Fig. 2a). Indeed, Chl-a was best explained when the interaction between species richness and river was also considered (adjusted R^2=0.55, $p<0.001$). This variation is also apparent when considering other diversity metrics (Evenness J and Functional Richness, Fig. 2b,c). However, the overall relationship (when all samples were pooled together) is positive, albeit rather weak (adjusted R^2 =0.047, $p<0.01$, Fig. 2d). When considering the flow intensity as an additional explanatory variable, the relationship stays positive, whereas sites with faster flow present increased biomass production (adjusted R^2=0.15, $p<0.001$, Fig. 2e). A strong interaction effect is apparent when testing for the substrate geology. When testing for siliceous substrate, the BEF relationship is positive, whereas in non-siliceous substrate, there is no relationship (Fig.2f). Results are similar when testing for relationships between species richness and Chl-c (not presented).

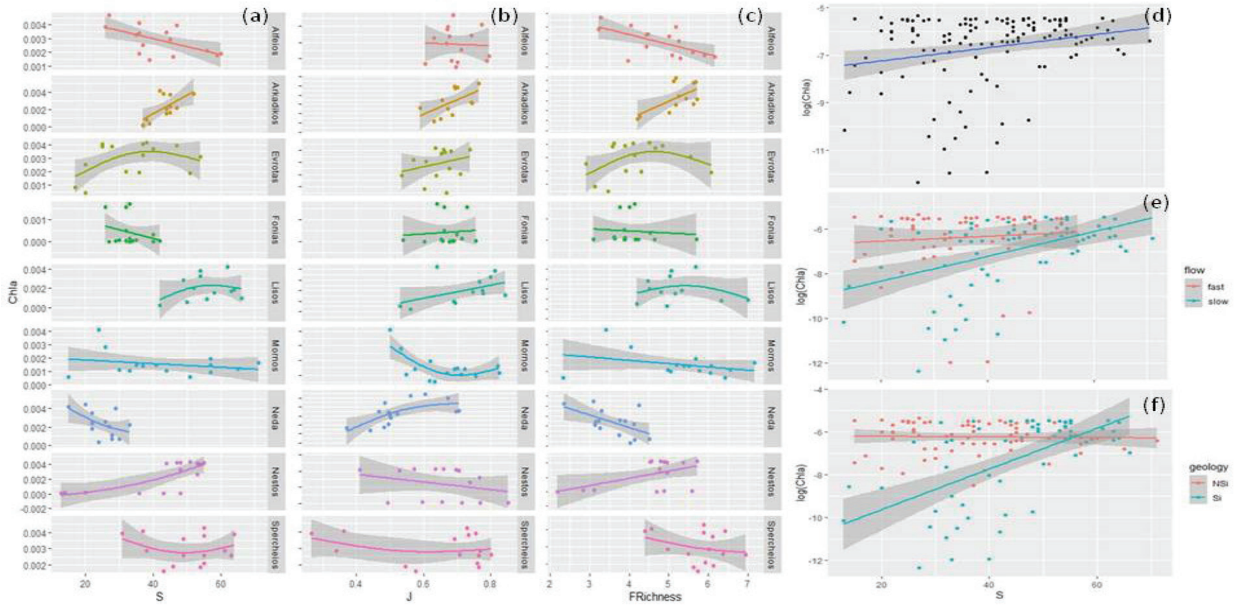

Fig. 2: Diversity (presented as species richness –S on x-axis in panels a,d,e,f) and ecosystem functioning (presented as Chl-a on y-axis) relationships in each river (a-S, b-Evenness J, c-Functional Richness), (d) the whole dataset, (e) at different flow levels (fast vs slow) and (f) in siliceous (Si) or non-siliceous (NSi) substrate. In panels d,e,f the y axis is log-transformed, to better express the linear trends.

4. Discussion/Conclusion

The shape of BEF relationship in benthic river diatoms is not universal. Different species interactions occur at different rivers, indicating a strong environmental effect on the form of the BEF relationship. Indeed, the response of biomass production to species richness depended on multiple environmental factors (i.e., geology, flow). The most pronounced effect is the dependence of the positive BEF relationship on river geology: the increase of species number results in an increase in biomass production only in rivers with siliceous substrate. Geology is linked to phosphorus concentrations, as calcareous soils can retain phosphorous, contrary to siliceous soils (Skoulikids, 2018), thus, changing nutrient stoichiometry. Therefore, it is possible that the effect of geology indicates an effect of differing nutrient limitation and species competition on the BEF relationship (i.e., on species richness and biomass production). Competition for available nutrients shapes microalgae communities and affects biomass production (Tilman, 1982), whereas the shape of the BEF relationship depends on species life-history traits (i.e., growth rate and competitive ability for nutrients) (Smeti *et al.*, 2018).

Functional traits important for river ecosystems and life-history traits of diatoms could provide a mechanistic understanding of the shape of the BEF relationship and consequently give us important insights on ecosystem health and protection. These aspects could not be controlled in this first extensive field survey, as in all field surveys. However, we gained important insights on the complexity of the drivers and factors emerged in this study (i.e., geology, nutrient concentrations, flow), that will be used for further exploration using experimental set-ups or numerical modeling.

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