Article

Functional Diversity and Primary Production Predict Future Patterns of Periphyton Productivity after Species Extinction

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Abstract: Understanding of the trait-based ecology of the periphytic algal community has increased in the last decade. However, the relationship between their functional diversity and ecosystem functions, such as primary production, has been speculated on, but yet not proven. Human impacts promote changes in biotic communities leading to a risk of extinction, with consequences for the functioning of aquatic ecosystems. In this study, we unraveled the associations between the taxonomic and functional components of periphytic algal diversity, stream eutrophication, and productivity patterns. Furthermore, we simulated future patterns of species extinction to predict how productivity may change when facing extinction. Primary production and taxonomic and functional diversity of the periphytic algal communities were estimated in five streams across a trophic gradient in the Ave River basin (northwest Portugal). Our results demonstrated that eutrophication led to a unimodal pattern of taxonomic diversity, while functional diversity tended to increase with increasing eutrophication. We found that only functional diversity had a positive association with primary production. The extinction estimations indicated that almost all species found in our study were at high extinction risk. When we spatially scaled our extinction simulations, we found poor-productive streams after the extirpation of a few species. However, at the regional scale, the ecosystem supports the extinction of at least 40% of species before turning into a poor-productive system. Intermediate levels of disturbance are probably beneficial for the diversity of periphytic algal communities, to a certain extent. Moreover, functionally diverse communities were more productive, and the alleged future extinction of species is likely to lead to poor-productive streams if regionally focused conservation initiatives are not implemented. We recommend that, using simulations of functional extinction, it is possible to infer how the loss of these microorganisms could alter ecosystem functioning, to better predict human impacts on aquatic ecosystems.

Keywords: benthic algae; extinction risk index; photosynthesis; periphytic algae; risk of extinction

1. Introduction

Species are becoming extinct at a rate of 1000 times faster than scientists expected during the Anthropocene [1,2]. This is due to human impacts on natural ecosystems, leading to habitat loss, climate change, pollution, over-exploitation, and biological invasions [1].

Habitat loss is evident in terrestrial ecosystems, but a subtle detrimental effect of habitat loss has also been identified in aquatic ecosystems, which depend on the surrounding terrestrial ecosystems [2,3]. Several studies have indicated that rare species (those with low local abundance and restricted geographical range) and specialist species (those with narrow habitat breadth) are at higher extinction risk, given their low population and their dependencies on resources and conditions that are negatively affected by human disturbances [4,5]. Human impacts promote changes in biotic communities with consequences for the functioning of aquatic ecosystems. For example, human-induced eutrophication is generally associated with shifts in the community structure of primary producers and changes in net primary production (NPP) that can fail to reach optimal NPP rates in aquatic systems [6,7]. Some studies have reported that intermediate levels of disturbance support high diversity of species, leading to increases in productivity [8].

Over the past two decades, the study of biodiversity has undergone a revolution [9], in which trait-based approaches have been used to better understand the assembly of communities and the consequences of species loss for ecosystem processes and services [10,11]. This revolution is because traits can enhance our ability to provide a mechanistic understanding of observed ecological patterns and may facilitate the formulation of generalizations of these patterns across species and ecosystems [10,12]. A functional trait can be any morphological, physiological, or phenological characteristic that indirectly affects species performance [13], and can determine where species live [14] and how they interact [15]. In this way, the use of trait-based approaches may help to predict how ecosystem processes change under environmental stress, identifying the group of species that regulates or controls that particular process and the traits that allow it to resist a given stressor [14].

The periphytic algal community (benthic algae) is composed of key primary producers in freshwater ecosystems. However, these algae are less studied than phytoplankton, and are sometimes overlooked. Due to their adhesion to substrates, they are important facilitators of plant litter decomposition [16] because algae exude upwards of 33% of the labile C available to heterotrophic microbes [17]. Taking into account that the best establishment of periphytic algal communities is related to their ability to adhere to substrates [18], the traits used in functional diversity evaluation are related to adherence to substrates, resistance to disturbance, and resource acquisition [19]. Considering the effect of their functional traits on ecosystem processes, algal species often present redundant functional traits (i.e., a trait shared by many species) that reflect their similar role in ecosystem functioning, such as primary production [20]. This high redundancy is an important functional aspect of algal communities, since it ensures ecosystem stability and happens mainly due to functional complementarity among algal species to support the same function in the ecosystem [21,22].

Some patterns of functional diversity of periphytic algae have been presented. In tropical floodplains, higher periphytic algal functional diversity has been related to higher water periods [23–26], with species traits associated with the limnological changes [27,28]. Considering biotic filters in establishing the functional diversity of periphytic algal communities, the herbivory by consumers of different trophic levels led to higher periphytic algal functional diversity in freshwater microcosms [28]. Studies in tropical streams and palm swamps have shown that most eutrophic environments have a reduced functional diversity and exhibit functional homogenization of periphytic algal communities [24]. From this perspective, periphytic diatoms are the most understudied groups [29–33], although diatom trait diversity may contribute more than 70% to primary production [34]. However, the relationship between functional diversity of periphytic algal communities and ecosystem processes, namely primary production, has not been shown.

The main goal of our study was to disentangle the relationships between eutrophication, functional diversity of periphytic algae, and primary production in temperate streams. Moreover, possible effects of future species extinction on functional diversity were assessed by analyzing extinction scenarios, considering that eutrophication has become increasingly intense and frequent. Even if this does not have an immediate effect on the community, it will lead to an "extinction debt" [35]; i.e., to the extinction of sensitive species. Specifically,

we aimed at answering the following questions: (I) How does eutrophication affect the taxonomic and functional diversity of periphytic algae? (II) How does taxonomic and functional diversity of periphytic algae affect primary production? (III) How do algal functional diversity and primary production respond to future extinctions of algal species? We hypothesized that (i) moderate levels of eutrophication provide higher taxonomic and functional diversity, and (ii) streams holding communities with higher functional diversity would have higher rates of primary production because the coexistence of more species with different traits would lead to higher functional diversity and to a better utilization of available resources. We also hypothesized that the loss of functional diversity would occur after the extinction of many species, due to high functional redundancy among algal species that maintain primary production.

2. Material and Methods

2.1. Study Area

The study was conducted in the Ave River basin (northwest Portugal) during spring 2013 (Figure 1). Five streams were chosen, presenting a trophic gradient as follows: Agra stream (oligotrophic stream, with the lowest nitrate and ammonium concentrations), Oliveira and Andorinhas streams (mesotrophic streams), Selho stream (eutrophic stream), and Couros stream (hypertrophic stream) [36]. The substrates present in the riverbed of streams were: stones and pebbles in the Agra and Oliveira streams, gravel and sand in the Andorinhas stream, and sand in the Selho and Couros streams. Further environmental and habitat characteristics of the sampling streams can be found in Dunck et al. [36,37]. Briefly, all five streams are similar in morphometric variables, such as size, current velocity, and abiotic conditions (e.g., temperature), and they mainly differ by the presence of riparian vegetation cover and by the influence of anthropogenic pressures that produced the trophic gradient [37].

Figure 1. Study area. Satellite images from Google Inc. Panel (**a**) represents Agra stream (oligotrophic), and Oliveira and Andorinhas streams (mesotrophic). Panel (**b**) represents Selho (eutrophic) and Couros (hypertrophic) streams.

2.2. Sampling Design

To provide substrates for algal colonization, four transparent polyethylene slides (7 cm × 2.5 cm, one side smooth and the other rough) were attached to mesh bags (5 mm mesh size, 30 cm × 23 cm), which were immersed in each stream (totaling twelve mesh bags and 48 slides per stream). We used artificial substrata to allow comparisons between streams, since they did not have the same dominant substrate [36]. The experiment started on 30 March 2013 and lasted for 28 days. The colonized slides were retrieved every seven days.

Three attached slides were randomly collected from each stream every seven days. Each slide was placed in dark flasks with distilled water. All samples were transported in cool boxes (4 °C) to the laboratory.

2.3. Physical and Chemical Water Parameters

The physical and chemical water parameters analyzed were dissolved oxygen, conductivity, pH, nutrient concentrations (nitrate, nitrite, ammonium, and phosphate) and solar radiations. Hydro-morphological parameters (maximum width, depth, and current velocity) were also assessed [36,37]

2.4. Laboratory Procedures

The periphytic algal material was removed from the slides (17.5 cm^2), considering both sides, with a toothbrush, scalpel blade, and jets of distilled water. This material was fixed and preserved in 0.5% acetic Lugol solution [38] to further assess algal density, algal biomass, and photosynthesis rate.

The algal density was quantified by applying the Utermöhl method [39] through an inverted microscope with 400× magnification. The counts were carried out in random fields until reaching at least 100 individuals (cells, colonies, and filaments) from the most abundant species in each sample and according to the species accumulation curve [40]. Species density was estimated according to Ros [41] and results were expressed as the number of individuals (unicellular, colonial, or filamentous) per unit area (ind/cm^2). Algal biomass was estimated based on chlorophyll-*a* concentrations in each sample, taking into account the scraped substrate area (each slide with 17.5 cm^2). To this end, the samples were filtered and the filters were macerated for further chlorophyll extraction, using 90% acetone, and quantification according to Golterman et al. [42]; the results were expressed as mg/cm^2.

We estimated the primary production rate of each stream by pulse amplitude modulation (PAM) fluorometry [43]. This method allows the analysis of the periphytic algal photosynthetic activity by chlorophyll fluorescence. We used a PAM-210 fluorometer (Heinz Walz GmbH, Germany) controlled via PAMWin software (for details, see [36]). Here, we only used the mean value of primary production values (ETR$_m$) of the two final steps of the colonization process (at 21 and 28 days), because 7 and 14 days were below the detection limit.

2.5. Algal Functional Traits

We selected the functional traits by considering traits that represent the ecological niche or that provide the most satisfactory establishment in their habitats, and that have been used in studies applying trait-based approaches to periphytic algae (e.g., [44]). The selected algal functional traits were: (i) life form (unicellular, filamentous, or colonial), (ii) intensity of adherence to substrate (loosely or firmly adhered; [45]), (iii) form of adherence (mobile, entangled, prostrated, pedunculated, with basal cell heterotrich; [18]), (iv) resistance to disturbance [18], and (v) size. Life form, adherence form and intensity of adherence, and size were directly analyzed in the individuals of each species; resistance to disturbance was analyzed using information from species identification specialized bibliographies [18,46,47].

2.6. Data Analysis

We tested spatial autocorrelation among the samples to ensure independence among them. For this, we used a matrix with the geographic coordinates of the sample sites and the raw data

of algal density, to which we applied Moran's I [48]. This analysis found no evidence of spatial autocorrelation among the study sampling points (I = 0.720, p = 0.493; [37]). Thus, we continued with data analyses considering that samples were independent.

A principal component analysis (PCA) was applied to ordinate the streams according to the physical and chemical parameters in the stream water, and to reduce the abiotic variation in the axes. Data were log (X + 1) transformed prior to this analysis. The first axis was used as a proxy for the eutrophication gradient for further analysis. We used the Shannon index to assess taxonomic diversity for each stream site (five sites) at each sampling time (four times).

To assess functional diversity, we used five traits to construct the functional matrix of species (life form, intensity of adherence to substrate, form of adherence, resistance to disturbance, and size). The functional matrix was standardized and transformed into a Gower's modified distance matrix according to Pavoine et al. [49]. These distance matrices were subsequently transformed into a functional dendrogram through a clustering method using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Functional diversity for each stream at each time was estimated considering the extent of trait differences between species through the mean pairwise distance index (MPD) [50]. To that end, we used the density of the algal species and the functional dendrogram, and the functional diversity representing the expected dissimilarity between two randomly selected species without replacement [51].

To evaluate the effects of eutrophication on taxonomic and functional diversity, we conducted polynomial and linear regression analyses using the functional diversity (MPD values) and taxonomic diversity (Shannon index) as response variables, and the first axis of the PCA (as a proxy for eutrophication gradient) as an explanatory variable [52,53]. To identify whether taxonomic and functional diversity can predict the patterns of primary production and biomass across the streams, we conducted polynomial and linear regressions using the taxonomic and functional diversity as explanatory variables of periphytic primary production (ETR_m values) and periphyton biomass accrual [52,53]. For each relationship, we built three statistical models, one simple linear regression (i.e., y ~ x), and two polynomial regressions that increased in degree from 2 to 3 (i.e., y ~ x + x^2 + x^3, in the highest degree considered in this study). Then, we compared the three models using partial F-tests and, whenever the models differed from each other, we selected the model that maximized the amount of variation of y explained by x (i.e., adjusted R^2) and minimized the residual standard error. Whenever the models did not differ, the simplest one was interpreted. We incremented our analysis with polynomial regressions due to several non-linear relationships between variables and maintained the linear regressions to capture any remaining linear associations among the variables.
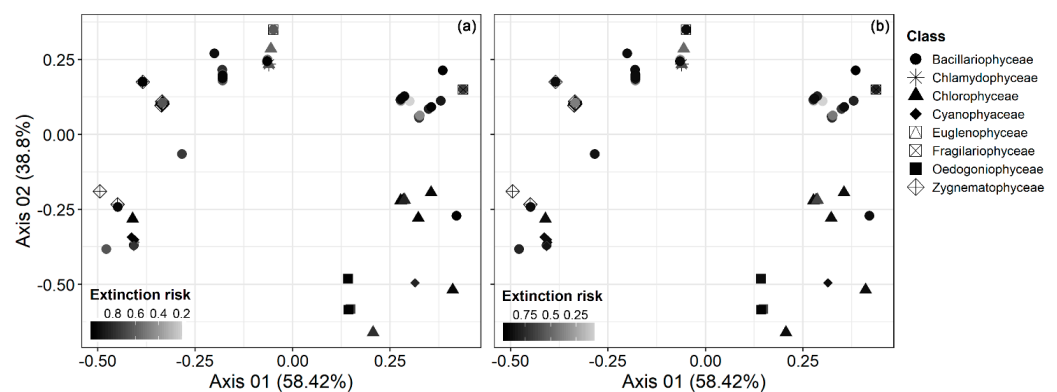
Because species with low abundance, restricted geographical distribution, and narrow habitat breadth are at higher risk of extinction [5,54–56], we calculated an extinction risk index based on species rarity and habitat breadth. To assess the rarity of species, we considered two spatial scales: local, at the stream level, and regional, considering all streams sampled in this study. At the local scale, we used the "scarcity" metric, while at the regional scale, we used the "restrictedness" index [5]. The scarcity metric considers species relative density at each stream, whereas restrictedness considers species occurrence across all streams relative to the total number of streams sampled [5]. Both metrics vary from 0 to 1, with values closer to 1 indicating locally scarce or geographically-restricted species. To assess species habitat breadth, we used the "tolerance" metric of the Outlying Mean Index analyses, which reflects the breadth of the set of environmental conditions in which the species are able to thrive relative to the habitat available (i.e., the whole set of environmental variables sampled) [57]. Since we needed an index that expresses how narrow the set of environmental variables is in which a species lives, instead of how broad it is, we inverted the tolerance (T) metric (i.e., by subtracting 1 from the tolerance of each species and then multiplying the result by −1). Finally, we summed species' rarity (r) at each scale with the inverse of the tolerance metric and divided it by two, which we

considered as our metric of species extinction risk (e_{risk}) (i.e., $e_{risk} = |(T - 1)| + r/2$). This final index of extinction risk (e_{risk}) varies from 0 to 1, with values closer to 1 indicating species with low abundance or restricted geographical distribution and narrow habitat breadth; thus, at higher risk of extinction. We used a Principal coordinate analysis (PCoa) to summarize the functional space occupied by species in relation to their extinction risk.

We conducted extinction simulations at both local and regional scales to investigate how functional diversity varies as algal species become extinct. Our extinction model extirpates species based on their extinction risk; thus, species at higher extinction risk are extinct first, leaving only species at lower extinction risk as extinction reaches an end. Moreover, we established ten extinction steps, from 0 to 90% of extinct species, and calculated the functional diversity of the community (MPD index) in each of these extinction steps. Furthermore, we developed a null model to compare with our scenario of vulnerable species extinction and inferred whether the found pattern differed from the random expectation. Our random scenario randomizes the order of extinction (i.e., the extinction risk index of a species) 1000 times while maintaining species traits and density. Then, the null model calculates the functional diversity of the community for each stream at each extinction step.
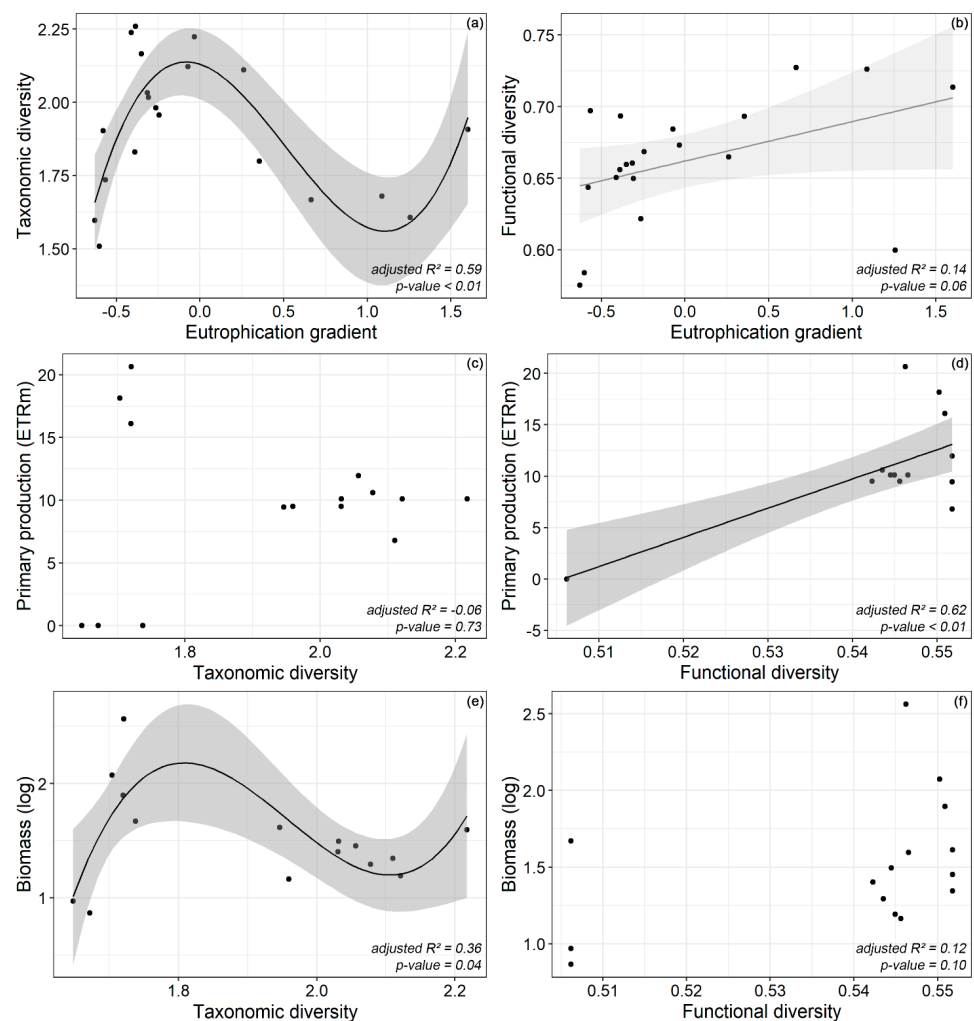
3. Results

We sampled 77 species of periphytic algae in five streams of the Ave River basin during the study period (Table A1). *Achnanthidium minutissimum*, *Eunotia sudetica*, and *Eunotia minor* were the dominant algal species in the Agra and Oliveira streams, while *Nitzschia palea* and *A. minutissimum* were dominant in the Selho River and Couros stream. The cyanophyceae *Chamaesiphon* sp.1 was the dominant algal species in the Andorinhas stream. The species with the highest extinction risk were *Sellaphora* sp.1 (0.993 in the extinction index we used) at local scale, and *Gomphonema turris* (0.967) at regional scale. Nevertheless, most species were considered at high extinction risk (Figure 2) (mean 0.843 $\pm$ 0.172 SD at local scale; and 0.803 $\pm$ 0.189 at regional scale). Conversely, *A. minutissimum* was the species with the lowest extinction risk at both local (0.195) and regional (0.067) spatial scales (Table A1).

Figure 2. Principal coordinates analysis summarizing the functional space occupied by the 77 periphytic algal species sampled in Agra, Oliveira, Andorinhas, Selho, and Couros streams. Shapes represent the taxonomic class to which each species belongs, and the grey scale in the bottom-left part represents the extinction risk of each species at (**a**) local scale and (**b**) regional scale, as calculated by their sensitivity to habitat alterations and taxonomic rarity.

Taxonomic diversity was higher in the middle of the eutrophication gradient, and lower at the extremes of the gradient (Figure 3a). The model that described this association (i.e., y ~ x + x^2 + x^3) was very unlikely to be observed at random ($p < 0.01$) and indicated that eutrophication explained ca. 59% of the variation in taxonomic diversity of the periphyton community (adjusted R^2 = 0.59). Functional diversity increased with increasing eutrophication (Figure 3b). Nevertheless, the model that described this relationship (i.e., y ~ x) indicated that eutrophication explained only 14% of the variation in functional diversity
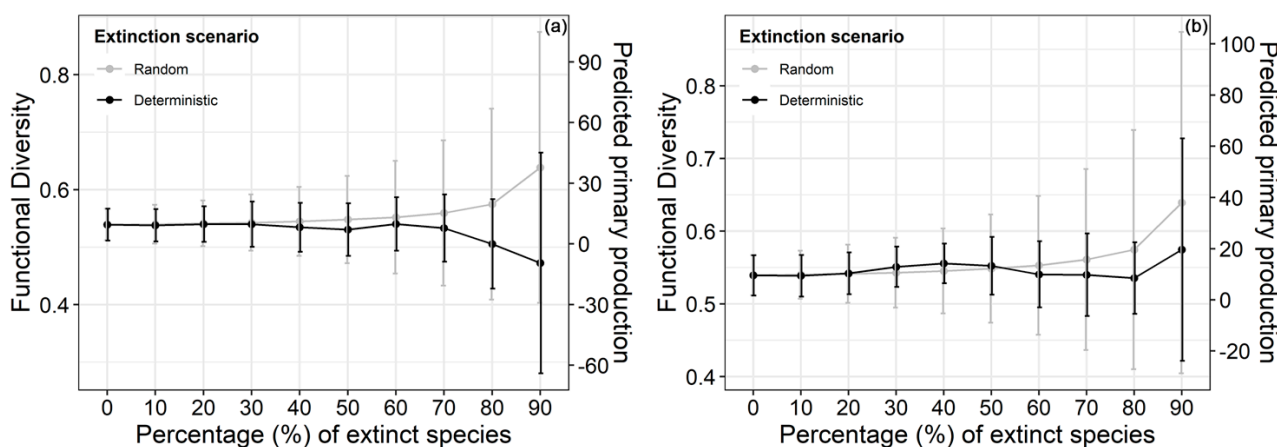
(adjusted $R^2 = 0.14$), and it is unlikely to observe this association at random ($p = 0.06$). Regarding primary production, there was no association between taxonomic diversity of periphytic algae and primary production (adjusted $R^2 = -0.06$; $p = 0.73$; Figure 3c), but there was a strong positive linear association between functional diversity and production (adjusted $R^2 = 0.62$; $p < 0.01$; Figure 3d). Finally, we found a non-linear association between taxonomic diversity and periphyton biomass (adjusted $R^2 = 0.36$; *p*-value = 0.04), in which the highest values of biomass were found at intermediate levels of taxonomic diversity (Figure 3e). Overall, functional diversity increased with increasing taxonomic diversity, but not in a linear way (see Appendix A for a detailed description of this association). There was no association between functional diversity and periphyton biomass (adjusted $R^2 = 0.12$; $p = 0.10$; Figure 3f).

Figure 3. Scatterplots representing the associations between eutrophication (based on the first axis of the PCA of the stream water variables) and taxonomic diversity (**a**) or functional diversity (**b**), between taxonomic diversity and primary production (**c**) or algal biomass accrual (**e**), and between functional diversity and primary production (**d**) or algal biomass accrual (**f**). Plots with a fit line represent associations between x and y that are very unlikely to be observed at random. Adjusted R^2 and *p*-values as returned by the model that best represented the variation of y explained by x. For the detailed results of model choice, see the Table A2.

The extinction simulations driven by species risk of extinction (i.e., a deterministic scenario) did not differ from the random expectations at both local and regional scales, as the functional diversity values were easily observed at random (Figure 4). Overall, uncertainty in values of functional diversity and estimated primary production (i.e., predicted by linear

regression, Figure 3d) increased with extinction (Figure 4). However, it is worth noting that, regardless of the extinction scenario, the predicted primary production would easily reach values closer to 0 as species were extinct at the local scale (Figure 4a). This is especially true after 70% extinction at the local scale, in which values of functional diversity start to decrease in the inverse direction of the random scenario (Figure 4a). Otherwise, at the regional scale, the species pool supports the extinction of at least 50% of species before estimated primary production values closer to 0 start to become increasingly common (Figure 4b). The estimation of primary production based on values of functional diversity predicts primary production values lower than 0 at some extinction steps, but negative values of primary production are very unlikely in the real world. Therefore, one may choose to interpret negative values of primary production in Figure 4 as poor-productive systems rather than negative production per se.

Figure 4. Graphical representation of average functional diversity and estimated primary production throughout species extinction scenarios at local (**a**) and regional (**b**) scales. Primary production was estimated for each value of functional diversity using the equation of the linear regression from Figure 3d. Mean values of functional diversity at each extinction step ($\pm$95% confidence intervals).

4. Discussion

In this study, extinction estimations indicated that almost all species were at high extinction risk. When we spatially scaled our extinction simulations, we could find poor-productive streams after the extirpation of a few species. However, at the regional scale, the ecosystem supports the extinction of at least 40% of species before turning into a poor-productive system. First, we observed that eutrophication led to a unimodal pattern of taxonomic diversity, a response already highlighted in several studies [58,59]. We also showed that functional diversity tended to increase with increasing eutrophication, but this relationship did not differ from that expected by chance. The mechanisms related to the unimodal response of species diversity can be explained by the subsidy-stress model proposed by Odum et al. [60]. An increase in taxonomic diversity occurred at moderate levels of nutrient enrichment due to a reduction in interspecific competition; therefore, more species are expected to coexist. However, a reduction in taxonomic diversity has been found at high nutrient levels due to the co-occurrence of other stressors that overlap with the subsidy effect of nutrients [61], and this allows the presence of few species that persist under extreme conditions.

According to previous studies, high levels of nutrients reduce functional beta diversity of periphytic algae [37]. However, in our study, in temperate streams this relationship was not distinct from random for the alpha component of functional diversity. Theoretical expectations indicate that plant species can be more functionally distinct at intermediate levels of resource gradients [62], and can more efficiently use resources. Conversely, the extremes of the gradient favored more specialized species with specific traits, as shown for aquatic plants [63] and planktonic organisms [64].

Considering our second hypothesis, we demonstrated that only functional diversity led to an increase in primary production. This positive association between periphytic algal functional diversity and primary production has been previously discussed, but not yet proven [24,28]. The evidence that biodiversity enhances ecosystem functions has emerged from both empirical and theoretical studies [12,65]. Most conclusions that biodiversity ecosystem functioning (BEF) relationships are positive have arrived from experimental manipulative studies, or field studies at local scales, and are conserved across several taxa and habitats [66,67]. Complementarity among species, due to niche differentiation or facilitative interactions, can explain the increase in ecosystem functioning when many species co-occur and interact [68]. Indeed, species with distinct traits within a community are expected to use resources in different ways, which would allow for better partitioning and more efficient utilization of available resources [69]. Since periphytic algal communities usually have many species interacting that contribute to primary production [70], and that can locally partition resource use over through time [71], the higher functional diversity and the higher diversity of traits in periphytic algae provided the greatest primary production, in accordance with our expectations. Our results corroborated the patterns for plants of higher coexistence of species with different traits related to higher functional diversity. However, observational field studies, like ours, can show variable relationships between functional diversity and primary production, particularly when increasing the spatial scales of observation [66].

Throughout this study, we also provided evidence that the functional diversity of periphytic algal communities was positively associated with primary production, but not with algal taxonomic diversity or algal biomass. Algal biomass has been used as a surrogate for primary production [8]; however, we showed here that this is a very distinct variable from primary production. Biomass accumulation (chlorophyll-a) is not necessarily related to higher photosynthetic rates, and configures distinct ecosystem processes. In this way, using algal biomass as a surrogate for primary production may lead to a misinterpretation of the relationship between periphytic algal production and ecosystem functioning.

The impact of extinction of periphytic algal species and their functional traits on primary production in streams with a distinct trophic gradient was analyzed. Taking into account our future extinction scenario, most species were considered at high extinction risk. This was particularly emphasized for the diatom *Eunotia bidens*, which had the highest extinction risk. Around the world, its distribution ranges from the Arctic [72] to the tropics, and mainly in oligotrophic and acidic waters with low conductivity [19]. In this way, eutrophication is a relevant factor that can lead to its extinction, as highlighted here. Extensive surveys of diatoms have been carried out in Europe, and Red Lists were proposed for Germany and Central Europe [73,74]. These lists have shown that organic pollution or artificial eutrophication in the last 15 years has led to a reduction in eutrophication-tolerant and -resistant species, and previously abundant species almost everywhere in Central Europe have been changed by other assemblages, dominated by taxa indicating mesosaprobic waters [74]. They also advocated that species diversity can be protected by conserving oligo to mesotrophic environments, since the diatom species present on the Red Lists are indicators of these environmental conditions. In this way, our results contribute to the understanding of the continued effects of eutrophication on microalgal extinctions, problems already pointed out two decades ago. On the other hand, *Achnanthidium minutissimum* was the species of least concern regarding extinction risk. This species is among the most commonly recorded periphytic algal species around the world [75,76]. The evidence for its distribution has been related to its tolerance to a wide range of environmental conditions, from acidic to alkaline waters, and from oligotrophic to eutrophic systems, a feature that remains intriguing [77], but that justifies its classification as an abundant species on the Red Lists [78].

Several studies have reported that planet Earth has reached the sixth mass extinction event, in which species are disappearing a thousand times faster than expected [1,79]. This high extinction rate is mainly due to human influence on natural environments, such as habitat degradation and climate change [1]. Therefore, the main targets of this extinction are rare and

sensitive species; i.e., those with low population densities and that only occur under specific environmental conditions [4,56]. The extinction of so many species will undoubtedly bring alterations to ecosystem functioning, especially if primary producers are extirpated from the environment [20]. In our case study, we found a high tolerance for the loss of functional diversity throughout extinction, especially at a local spatial scale, which may indicate that algal species are functionally redundant [21]. This high redundancy may ensure the maintenance of the functions provided by periphyton species (e.g., primary production), even after the extinction of some species (i.e., 40% at local scale and 20% at regional scale).

Here, we found that most algal species are at high extinction risk; i.e., have low population densities and are highly sensitive to environmental changes. However, the extinction of vulnerable algal species never differed from the random extinction scenario. Therefore, the pattern of functional diversity loss (and consequently, of productivity loss) that we found at local and regional scales could be easily observed at random. However, when considering that most algal species are at high risk of extinction, it is more likely that vulnerable species are extirpated if extinction randomly happens anyway. Nevertheless, it is worth noticing some differences in the pattern of functional diversity loss between local and regional spatial scales, and the consequences for primary production. On the one hand, at the local scale, one may observe poor-productive streams at any stage of extinction, although this chance progressively increases with species extinction. On the other hand, at the regional scale, the regional species pool supports at least 30% of species extinction before any indication of poor-productive streams in the region. These findings may be crucial for the conservation of temperate freshwater network systems, since they indicate that conservation initiatives focusing on the conservation of the whole region may prevent the loss of functionality and productivity during the sixth mass extinction event [1,54].

Some studies have been using functional diversity metrics as a measure of ecosystem functioning [55,80,81], as we did in this study. Indeed, we have evidenced that functional diversity is associated with primary production, an essential function provided by periphytic algal species in aquatic ecosystems. Apparently, intermediate levels of eutrophication benefit the number of periphyton species in streams, but this high taxonomic diversity does not translate into higher functional diversity or primary production. Our extinction simulation scenarios evidenced the high functional redundancy among species, which often had similar functional traits that reflected similar roles in the ecosystem. This high redundancy might ensure the resilience of the ecosystem when facing the sixth mass extinction crisis, at least for a short period. Nonetheless, functional losses will inevitably start to occur after some point in the extinction process, leading to decreases in primary production, consequently affecting all trophic levels presented in temperate streams. Therefore, conservation initiatives that aim to maximize primary production should focus on functionally diverse streams and should have regional coverage to mitigate the impacts of future species extinction.

5. Conclusions

We concluded that almost all the algal species found in our study were at high extinction risk. When we spatially scaled our extinction simulations, we found poor-productive streams after the extirpation of a few species. However, at the regional scale, the ecosystem supports the extinction of at least 40% of species before turning into a poor-productive system. The intermediate levels of disturbance should be beneficial for the number of periphytic species in a community, to a certain extent. Moreover, functionally diverse communities were more productive, and the alleged future extinction of species should lead to poor-productive streams if regionally-focused conservation initiatives are not implemented.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available in previews papers.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A

Table A1. Mean density of each of the 77 algae species sampled in each of the five streams (Agra, Andorinhas, Couros, Oliveira, and Selho), their respective taxonomical class and extinction risk index (which is based on species scarcity at the local scale, or restrictiveness at regional scale and sensitivity to habitat alterations).
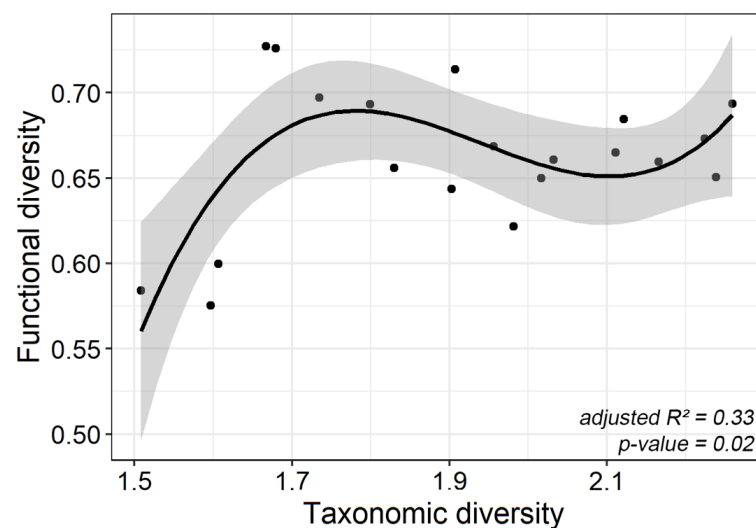
Taxon	Class	Streams					Extinction Risk	
		Agra	Andorinhas	Couros	Oliveira	Selho	Local	Regional
Achnanthidium minutissimum	Bacillariophyceae	2441.140	2295.273	1936.904	1306.662	1442.227	0.195	0.067
Actinotaenium cruciferum	Zygnematophyceae	0.000	36.009	0.000	100.243	0.000	0.935	0.900
Anabaena sp.1	Cyanophyaceae	0.000	66.641	0.000	0.000	0.000	0.962	0.900
Ankistrodesmus sp.1	Chlorophyceae	0.000	54.897	0.000	0.000	0.000	0.968	0.900
Aulacoseira alpigena	Bacillariophyceae	0.000	70.508	0.000	62.167	0.000	0.874	0.778
Aulacoseira granulata	Bacillariophyceae	0.000	0.000	0.000	85.667	0.000	0.941	0.900
Bulbochaete sp.1	Oedogoniophyceae	0.000	0.000	115.715	0.000	0.000	0.982	0.900
Chamaesiphon sp.1	Cyanophyaceae	62.188	85.858	0.000	1399.298	0.000	0.827	0.888
Characium sp.1	Chlorophyceae	0.000	0.000	978.561	0.000	0.000	0.794	0.900
Chlamydophyceae sp.1	Chlamydophyceae	0.000	0.000	248.278	0.000	0.000	0.934	0.900
Chlorococcales sp.1	Chlorophyceae	0.000	0.000	1510.967	0.000	502.838	0.650	0.604
Oscillatoriales sp.1	Cyanophyaceae	0.000	0.000	6596.910	0.000	0.000	0.570	0.900
Cocconeis placentula	Bacillariophyceae	0.000	51.586	0.000	56.886	54.219	0.941	0.780
Cosmarium bioculatum	Zygnematophyceae	0.000	0.000	0.000	78.553	0.000	0.958	0.900
Cosmarium reniforme	Zygnematophyceae	0.000	15.538	0.000	103.720	0.000	0.890	0.816
Cosmarium sp.1	Zygnematophyceae	0.000	57.231	0.000	0.000	84.640	0.952	0.900
Cosmarium sp.2	Zygnematophyceae	0.000	59.793	0.000	0.000	0.000	0.956	0.933
Cosmarium sp.3	Zygnematophyceae	0.000	50.764	0.000	0.000	0.000	0.971	0.917
Cosmarium undulatum	Zygnematophyceae	0.000	14.983	0.000	35.611	0.000	0.987	0.900
Cymbella sp.1	Bacillariophyceae	0.000	59.886	0.000	0.000	0.000	0.962	0.900
Desmidiaceae sp.1	Zygnematophyceae	0.000	77.631	0.000	13.333	59.219	0.935	0.867
Desmodesmus sp.1	Chlorophyceae	0.000	0.000	0.000	0.000	67.778	0.982	0.900
Bacillariophyceae sp.1	Bacillariophyceae	10.528	0.000	0.000	761.147	0.000	0.737	0.867
Encyonema minutum	Bacillariophyceae	168.251	28.445	35.417	25.667	145.883	0.673	0.372
Epibolium sp.1	Chlorophyceae	0.000	0.000	0.000	113.439	0.000	0.946	0.900
Euastrum sp.1	Zygnematophyceae	0.000	0.000	0.000	18.524	0.000	0.986	0.900
Euglena sp.1	Euglenophyceae	0.000	0.000	62.688	0.000	0.000	0.979	0.900
Eunotia bidens	Bacillariophyceae	0.000	25.542	0.000	0.000	0.000	0.987	0.900
Eunotia bilunaris	Bacillariophyceae	4.615	215.655	0.000	185.384	35.038	0.834	0.694
Eunotia minor	Bacillariophyceae	1215.610	0.000	0.000	0.000	0.000	0.656	0.900
Eunotia sp.3	Bacillariophyceae	0.000	146.669	0.000	691.584	512.852	0.741	0.755
Eunotia sp.1	Bacillariophyceae	0.000	4058.203	1475.347	0.000	0.000	0.573	0.767
Eunotia sp.2	Bacillariophyceae	0.000	1233.523	0.000	0.000	0.000	0.602	0.933
Eunotia cf. *sudetica*	Bacillariophyceae	0.000	1395.014	0.000	25.382	0.000	0.717	0.788
Eunotia sudetica	Bacillariophyceae	1688.213	3579.021	0.000	888.084	1141.484	0.462	0.424
Fragilaria capucina	Bacillariophyceae	0.000	48.357	22.229	0.000	0.000	0.968	0.889
Frustulia saxonica	Bacillariophyceae	0.000	113.544	0.000	0.000	669.451	0.795	0.900
Gomphonema augur	Bacillariophyceae	0.000	71.737	0.000	0.000	0.000	0.965	0.900
Gomphonema gracile	Bacillariophyceae	0.000	54.321	55.237	14.111	58.108	0.940	0.780
Gomphonema parvulum	Bacillariophyceae	374.761	884.445	2057.510	394.970	1089.236	0.344	0.092
Gomphonema sp.1	Bacillariophyceae	671.534	0.000	23.899	30.417	56.719	0.856	0.761
Gomphonema turris	Bacillariophyceae	0.000	13.667	0.000	0.000	0.000	0.981	0.967
Hanszchia sp.1	Bacillariophyceae	0.000	13.056	0.000	3399.648	55.053	0.622	0.859
Hipodonta sp.1	Bacillariophyceae	0.000	0.000	0.000	20.834	0.000	0.986	0.900
Leiblenia sp.1	Bacillariophyceae	0.000	0.000	0.000	1482.785	955.783	0.642	0.790

Table A1. *Cont.*

Taxon	Class	Streams					Extinction Risk	
		Agra	Andorinhas	Couros	Oliveira	Selho	Local	Regional
Luticola sp.1	Bacillariophyceae	0.000	0.000	0.000	0.000	108.439	0.948	0.900
Mesotaenium sp.1	Zygnematophyceae	0.000	45.334	0.000	167.146	0.000	0.824	0.690
Monoraphidium arcuatum	Chlorophyceae	0.000	77.423	0.000	0.000	74.334	0.948	0.865
Monoraphidium contortum	Chlorophyceae	0.000	46.742	0.000	0.000	0.000	0.982	0.900
Monoraphidium sp.1	Chlorophyceae	0.000	0.000	0.000	68.443	0.000	0.959	0.900
Monoraphidium griffithi	Chlorophyceae	0.000	210.047	0.000	66.810	18.629	0.825	0.650
Monoraphidium longisculum	Chlorophyceae	51.084	0.000	0.000	0.000	0.000	0.952	0.900
Mougeotia sp.1	Zygnematophyceae	0.000	0.000	0.000	81.667	0.000	0.944	0.900
Navicula cryptocephala	Bacillariophyceae	199.714	1186.379	743.822	623.319	3032.617	0.571	0.312
Navicula sp.1	Bacillariophyceae	0.000	0.000	0.000	0.000	1626.798	0.618	0.900
Niszchia palea	Bacillariophyceae	56.188	745.046	788.123	295.459	1441.840	0.624	0.354
Oedogonium sp.1	Oedogoniophyceae	5.264	0.000	229.596	0.000	67.890	0.749	0.626
Oedogonium sp.2	Oedogoniophyceae	0.000	0.000	175.406	0.000	0.000	0.973	0.933
Pennales sp.1	Bacillariophyceae	0.000	0.000	0.000	96.491	0.000	0.954	0.900
Phormidium sp.1	Cyanophyaceae	0.000	0.000	0.000	51.429	0.000	0.964	0.900
Pinnularia acrosphera	Bacillariophyceae	0.000	0.000	0.000	0.000	6037.365	0.629	0.900
Pinnularia rostrada	Bacillariophyceae	0.000	0.000	0.000	0.000	18.133	0.983	0.900
Pinnularia sp.1	Bacillariophyceae	0.000	51.586	0.000	29.276	0.000	0.948	0.828
Pinnularia sp.2	Bacillariophyceae	0.000	104.779	0.000	0.000	0.000	0.942	0.900
Planothidium sp.1	Bacillariophyceae	0.000	0.000	0.000	0.000	93.556	0.922	0.900
Pseudoanabaena catenata	Cyanophyaceae	33.945	91.538	132.625	13.667	75.696	0.811	0.662
Pseudoanabaena skuja	Cyanophyaceae	202.780	0.000	0.000	0.000	1721.926	0.757	0.854
Scenedesmus sp.1	Bacillariophyceae	0.000	0.000	44.001	0.000	0.000	0.970	0.900
Scenedesmus sp.2	Bacillariophyceae	0.000	0.000	25.167	0.000	0.000	0.982	0.900
Sellaphora sp.1	Bacillariophyceae	0.000	48.948	0.000	0.000	0.000	0.993	0.900
Staurastrum sp.1	Zygnematophyceae	16.528	0.000	0.000	0.000	5.375	0.985	0.900
Stauroneis sp.1	Bacillariophyceae	0.000	104.779	0.000	0.000	0.000	0.942	0.900
Stigeoclonium sp.1	Chlorophyceae	34.556	0.000	827.506	0.000	110.105	0.586	0.496
Surirella angusta	Bacillariophyceae	397.420	67.106	0.000	54.593	46.620	0.637	0.397
Surirella linearis	Bacillariophyceae	0.000	42.282	0.000	0.000	0.000	0.975	0.900
Tabellaria fenestrata	Fragilariophyceae	0.000	0.000	0.000	90.991	0.000	0.956	0.900
Ulnaria ulna	Bacillariophyceae	0.000	0.000	32.750	95.472	73.557	0.868	0.797

Results of the associations between taxonomic diversity (Shannon index) and Functional diversity of the periphytic algae sampled in the five temperate streams considered in this study.

Functional diversity increased with taxonomic diversity until medium diversity sites, and then decreased at sites with high taxonomic diversity, and finally it increased again at sites with the highest taxonomic diversity (Figure 2). The model that best described the variation in functional diversity explained by taxonomic diversity was a polynomial of degree three (i.e., $y \sim x + x^2 + x^3$), which explained about 33% of the association (adjusted $R^2 = 0.33$; *p*-value = 0.02; Figure A1).

Figure A1. A scatterplot representing the association among taxonomic diversity (Shannon index) and functional diversity (mean pairwise distance index, MPD). Adjusted R^2 and *p*-values as returned by the model that best represented the variation of y explained by x.

Table A2. Model selection results. Bold row represents the models that were selected based on the criteria described in the methods section of this study.

Associations	Partial F-Tests						Models		
	Residual DF	RSS	DF	Sum of Squares	F	p	Adjusted R^2	Residual SE	p
Functional diversity ~ Taxonomic diversity									
$y \sim x$	18	0.032					0.016	0.042	0.269
$y \sim x + x^2$	17	0.028	1	0.004	3.281	0.089	0.065	0.225	0.219
$y \sim x + x^2 + x^3$	**16**	**0.019**	**1**	**0.009**	**7.363**	**0.015**	**0.335**	**0.035**	**0.023**
Taxonomic diversity ~ Eutrophication gradient									
$y \sim x$	18	0.966					0.005	0.232	0.309
$y \sim x + x^2$	17	0.857	1	0.109	5.016	0.040	0.065	0.225	0.219
$y \sim x + x^2 + x^3$	**16**	**0.348**	**1**	**0.509**	**23.416**	**0.000**	**0.597**	**0.147**	**0.000**
Functional diversity ~ Eutrophication gradient									
$y \sim x$	**18**	**0.028**					**0.136**	**0.040**	**0.061**
$y \sim x + x^2$	17	0.025	1	0.004	2.416	0.140	0.200	0.038	0.058
$y \sim x + x^2 + x^3$	16	0.023	1	0.001	0.906	0.355	0.195	0.038	0.093
Taxonomic diversity ~ Primary production									
$y \sim x$	**13**	**524.020**					**−0.067**	**6.349**	**0.730**
$y \sim x + x^2$	12	504.710	1	19.305	0.503	0.493	−0.113	6.485	0.754
$y \sim x + x^2 + x^3$	11	422.060	1	82.656	2.154	0.170	−0.015	6.194	0.459
Functional diversity ~ Primary production									
$y \sim x$	**13**	**63.760**					**0.620**	**3.791**	**0.000**
$y \sim x + x^2$	12	63.619	1	0.141	0.025	0.878	0.595	3.911	0.002
$y \sim x + x^2 + x^3$	11	62.986	1	0.632	0.110	0.746	0.654	3.614	0.002
Taxonomic diversity ~ Biomass									
$y \sim x$	13	2.497					−0.022	0.438	0.417
$y \sim x + x^2$	12	2.465	1	0.031	0.262	0.619	−0.093	0.453	0.676
$y \sim x + x^2 + x^3$	**11**	**1.321**	**1**	**1.145**	**9.537**	**0.010**	**0.361**	**0.347**	**0.048**
Functional diversity ~ Biomass									
$y \sim x$	**13**	**2.140**					**0.124**	**0.406**	**0.108**
$y \sim x + x^2$	12	2.110	1	0.030	0.186	0.675	0.065	0.419	0.265
$y \sim x + x^2 + x^3$	11	1.797	1	0.313	1.916	0.194	0.131	0.317	0.224

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