Environmental Pollution 202 (2015) 32–40

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Responses of primary production, leaf litter decomposition and associated communities to stream eutrophication

Bárbara Dunck [a, *, 1], Eva Lima-Fernandes [b, 1], Fernanda Cássio [b], Ana Cunha [c], Liliana Rodrigues [a, d], Cláudia Pascoal [b]

[a] Graduate Program in Ecology of Continental Aquatic Environments, University of Maringá, Maringá, Paraná, Brazil
[b] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Braga, Portugal
[c] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB, University of Minho Pole), Department of Biology, University of Minho, Braga, Portugal
[d] Department of General Biology and Center of Research in Limnology, Ichthyology and Aquaculture, University of Maringá, Brazil

ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form
12 March 2015
Accepted 14 March 2015
Available online

Keywords:
Fungi
Invertebrates
Periphyton
Streams
Subsidy-stress model

ABSTRACT

We assessed the eutrophication effects on leaf litter decomposition and primary production, and on periphytic algae, fungi and invertebrates. According to the subsidy-stress model, we expected that when algae and decomposers were nutrient limited, their activity and diversity would increase at moderate levels of nutrient enrichment, but decrease at high levels of nutrients, because eutrophication would lead to the presence of other stressors and overwhelm the subsidy effect. Chestnut leaves (*Castanea sativa* Mill) were enclosed in mesh bags and immersed in five streams of the Ave River basin (northwest Portugal) to assess leaf decomposition and colonization by invertebrates and fungi. In parallel, polyethylene slides were attached to the mesh bags to allow colonization by algae and to assess primary production. Communities of periphytic algae and decomposers discriminated the streams according to the trophic state. Primary production decomposition and biodiversity were lower in streams at both ends of the trophic gradient.

1. Introduction

Human impacts promote changes in biotic communities with consequences to the functioning of aquatic ecosystems (Goudie, 1999; Pascoal et al., 2003; Loreau and de Mazancourt, 2013). Excess nitrogen and phosphorus in freshwaters (Liang et al., 2014; Smith et al., 1999) mainly from urbanization (Agostinho et al., 2005), deforestation (Allan, 2004) and increased use of agricultural fertilizers has led to eutrophication, which is one of the leading causes of water pollution worldwide (Vörösmarty et al., 2010).

Primary production and decomposition are two key complementary ecosystem processes that ensure organic matter turnover, nutrient cycling and the provisioning of many ecosystem services (Hooper et al., 2012). Leaf litter decomposition and primary production have been extensively studied in freshwaters, but researchers have rarely examined both processes *in tandem* (Danger et al., 2013). Nitrogen and phosphorus are proven regulators of aquatic primary production, although the response of primary producers may be altered by other factors, such as light limitation, hydrology and herbivory (Smith et al., 2006). Nutrients, at moderate levels, can stimulate primary production and, consequently, the production of organisms at higher trophic levels, such as invertebrates (Niyogi et al., 2007). This higher ecosystem production might be linked to higher diversity of producers and consumers (Rosenzweig, 1995; Thompson and Townsend, 2005). However, high levels of eutrophication can lead to algal blooms that are stressful to several organisms due to low dissolved oxygen and poor habitat quality (Niyogi et al., 2007).

Leaf litter decomposition responds to the increase in nutrient availability in the stream water through effects on microbial and invertebrate communities that drive this process (Pascoal et al., 2003; Duarte et al., 2009). Moderate nutrient concentrations in the stream water are reported to stimulate fungal diversity, biomass and activity (Pascoal and Cássio, 2004; Ferreira et al., 2006; Duarte et al., 2009; Fernandes et al., 2014). Similarly, invertebrate

* Corresponding author. Periphytic Algae Laboratory, Av. Colombo, 5790, Sala 08 Bloco G-90, University of Maringá (UEM), CEP 87020-900 Maringá, PR, Brazil.
E-mail address: dunck.barbara@gmail.com (B. Dunck).
[1] Both authors gave equal contribution.

http://dx.doi.org/10.1016/j.envpol.2015.03.014
0269-7491/

diversity, biomass and density seem to be enhanced by moderate nutrient levels (Greenwood et al., 2007; Chung and Suberkropp, 2008; Rosemond et al., 2010; but see Ferreira et al., 2006). Fungi are able to uptake nutrients from the stream water (Gessner et al., 2007) thereby stimulating their biomass production, diversity and activity (Ferreira and Chauvet, 2011; Fernandes et al., 2014). Under these conditions, invertebrates may benefit from the increased fungal biomass on leaf litter and enhance their biomass and activity. However, in highly eutrophic streams, a reduction in fungal biomass and diversity (Baldy et al., 2007; Duarte et al., 2009) as well as in invertebrate biomass, diversity and density is often observed (Pascoal et al., 2005a; Lecerf et al., 2006; Baldy et al., 2007). Inorganic nitrogenous compounds, such as ammonia (Lecerf et al., 2006; Duarte et al., 2009), and the hypoxic conditions commonly associated with eutrophic streams (Pascoal and Cássio, 2004; Pascoal et al., 2005a) can negatively affect the biota in detritus-based foodwebs.

Here, we used an integrative approach to assess effects of eutrophication in streams by examining leaf litter decomposition, primary production and associated periphytic algae, fungi and invertebrates. According to the subsidy–stress model (Odum et al., 1979), we expected a unimodal response of leaf litter decomposition and productivity to a trophic gradient. We hypothesized that, when nutrients were limited, biomass and activity of primary producers and decomposers would exhibit a subsidy response (increase) to moderate levels of nutrient enrichment, but a stress response (decrease) at high levels of nutrients, because eutrophication would lead to the presence of other stressors, that could overwhelm the subsidy effect of nutrients (Rosenzweig, 1995; Mittelbach et al., 2001). We also expected that at moderate nutrient levels, more species would coexist because competitively dominant species would not monopolize all resources, creating opportunities for less competitive species (Odum et al., 1979). Finally, we expected that general response patterns to eutrophication would be similar across communities, but the stress response thresholds might vary among periphytic algae, fungi and invertebrates. To test these hypotheses, mesh bags containing chestnut leaves (*Castanea sativa* Mill) were immersed in five streams of the Ave River basin (northwest Portugal) with different eutrophication levels to allow colonization by fungi and invertebrates, and to follow leaf decomposition. In parallel, periphytic algae and primary production were examined on polyethylene slides that were attached to the leaf bags.

2. Material and methods

2.1. Study area

The experiment was carried out in five streams of the Ave river basin (northwest Portugal) during spring 2013. Agra Stream flows through a mountain area with little human influence. Oliveira and Andorinhas streams flow through areas influenced by minor agricultural activities and suffer from diffuse nutrient inputs. Selho River flows near the city of Guimarães while Couros Stream crosses the city. Study sites in Selho River and Couros Stream were downstream the city and surrounded by agricultural fields, therefore influenced by diffuse nutrient inputs. Agra, Oliveira and Andorinhas streams were bordered by riparian vegetation mainly composed of alder (*Alnus glutinosa* Gaertn.), oak (*Quercus* sp.) and chestnut (*C. sativa*). The riverbed in Agra and Oliveira streams was composed of stones and pebbles, while in Andorinhas Stream was composed of gravel and sand. The Selho River and the Couros Stream were bordered by a narrow corridor of riparian vegetation composed of alder and poplar (*Populus* sp.), and sand was the dominant substrate.

2.2. Physical and chemical parameters of stream water

Dissolved oxygen, conductivity and pH were measured *in situ* with field probes (Multiline F/set 3 no. 400327, WTW). Water samples were collected in plastic bottles, transported in cool boxes (4 °C) and analysed on the same day. Nutrient concentrations in the stream water were measured by spectrophotometry (DR2000 spectrophotometer, Hach company, Loveland, CO, USA), according to manufacturer specifications, as follows: nitrate by the cadmium reduction method, nitrite by the diazotization method, ammonium by the salycilate method, and phosphate by the ascorbic acid method.

Hydro-morphological measures (maximum width, depth, and current velocity) were taken according to Wetzel and Likens (1991) (Table 1). Maximum and minimum solar radiations (μmol m^{-2} s^{-1}) were estimated through radiometer model LI-250 (Li-COR, Inc.) connected to a quantum sensor model Li-190SA (Table 1).

2.3. Experimental setup

Chestnut leaves were collected before abscission in autumn 2009 and stored air-dried. Twelve coarse mesh bags (5 mm mesh size; 30 × 23 cm) were filled with 3 g (±0.001 g) of air-dried leaves. Four transparent polyethylene slides were attached to each mesh bag (7 cm × 2.5 cm) and used as substrate to allow colonization by periphytic algal community.

On 30 March 2013, mesh bags were immersed in each river for 28 days. Three coarse-mesh bags and attached slides were randomly collected from each stream every seven days. Each mesh bag was individually placed in plastic bags and each slide was placed in dark flasks with distilled water. All samples were transported in cool boxes (4 °C) to the laboratory.

The periphytic material was removed from the slides (17.5 cm^2) with a toothbrush and jets of distilled water, fixed and preserved in 0.5% acetic Lugol solution (Bicudo and Menezes, 2006). Three slides were used to assess community attributes, namely algal density, algal biomass, and photosynthesis rate.

From each bag, leaves were washed under tap water through sieves (250 and 800 μm mesh) to retain the invertebrates. Leaves were cut into 12 mm diameter disks, and used to assess fungal biomass and sporulation. The remaining leaf material was freeze-dried (48 h ± 24 h) to constant mass, weighed to the nearest 0.0001 g, and then ignited (500 °C, 4 h) and reweighted to calculate the ash-free dry mass. Leaf disks used for fungal biomass and sporulation were also freeze-dried and weighted to the nearest 0.0001 g.

2.4. Periphytic algae

The algae were quantified by applying the Utermöhl method (1958) through inverted microscope with 400× magnification. A minimum of 100 individuals (cells, colonies and filaments) were identified and counted from random fields taking into account the most abundant species according to the species accumulation curve (Ferragut and Bicudo, 2012). Species density was estimated according to Ros (1979) and results expressed as number of individuals per unit area (ind/cm^2). The algal richness was estimated from this analysis.

Algal biomass was estimated based on chlorophyll-*a* concentration in each sample, adapted to substrate area scraped. Chlorophyll-*a* analysis was done using 90% acetone extraction according to Golterman et al. (1978) and results were expressed as mg/cm^2.

The algal photosynthetic activity in each stream was estimated by chlorophyll fluorescence analysis by pulse amplitude modulation (PAM) fluorometry (Schreiber et al., 1986). A PAM-210