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Specific responses to nitrogen and phosphorus enrichment in cyanobacteria: Factors influencing changes in species dominance along eutrophic gradients

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ABSTRACT

Anthropogenic eutrophication is a worldwide problem, causing proliferation of cyanobacterial masses, some of which may be toxic. However, little is known about whether the response to nutrient enrichment differs among cyanobacterial species. To address this issue, distinct patterns in growth and competitive response of benthic cyanobacteria under N and P nutrient regimes were studied. Nine cyanobacterial species, collected from Guadarrama river biofilms at several locations with different nutrient concentrations, were isolated and used for a series of N and P enrichment bioassays. In competition experiments with a mixture of all nine species, a great predominance of certain cyanobacteria over others was noted at high nutrient conditions, while under low nutrient conditions some others dominated. On the basis of these results four selected strains were subjected to a gradient of different concentrations of phosphate, nitrate and ammonium, in independent bioassays, both in monocultures and mixed cultures. Depending on the concentration of N and P, stimulation or inhibition of growth was observed. Some species grew better, dominating at high nutrient concentrations, while higher yields were recorded for others under low nutrient regimes, dominating in these conditions. Results from this study clarify previously published field observations, whereby a group of species occurred mostly in downstream nutrient-rich locations, while other was typical of upstream oligotrophic conditions. Our findings concerning differential growth in relation to nutrient concentrations may be useful for environmental management, because they help us predict which cyanobacteria may be expected to occur under certain conditions.

1. Introduction

Human disturbances give rise to a range of alterations in river systems, in which nitrogen and phosphorus inputs can have profound effects upon the quality of receiving waters, leading to eutrophication, which in turn causes proliferation of algal masses, some of which may be toxic (Dodds and Welch, 2000; Anderson et al., 2002). Other symptoms of eutrophication include deep water anoxia in lakes, taste and odour problems, and changes in the composition of aquatic communities

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(Lewis et al., 2011). Recognition of these problems has prompted a concerted worldwide effort to develop a rational predictive framework for preventing and managing freshwater eutrophication (European Communities, 2000; USEPA, 2000).

Cyanobacteria are among dominant primary producers in most freshwater environments, providing the principal energy base for many aquatic food webs (Scott and Marcarelli, 2012). They play important biogeochemical roles in terms of nutrient fixation and cycling within the ecosystem (Scott and Marcarelli, 2012). Nutrient enrichment has been closely linked to the stimulation of growth of masses of cyanobacteria, but it did not affect all species equally, although an increase of some harmful species has been reported (Anderson et al., 2002). Therefore, an improved understanding of the nature of taxon-specific responses to N and P influx may help to protect aquatic ecosystems against future pollution, optimise wastewater treatment procedures, and resolve the continuing debate about the respective roles of N and P in regulating human made eutrophication (Donald et al., 2013).

In previous reports, we described shifts in the structure and composition of benthic cyanobacterial communities in rivers with increasing eutrophication in the downstream direction (Perona et al., 1998, 1999; Douterelo et al., 2004; Perona and Mateo, 2006; Rodriguez et al., 2007). In the Guadarrama river (central Spain), molecular fingerprint analyses using temperature gradient gel electrophoresis (TGGE) were consistent with those obtained from microscopic observations of field-fixed samples, in which the cyanobacterial community composition at sampling locations differed between upstream and downstream locations, whereby some species predominated at downstream sites with high nutrient content, while others were scarce or absent (Loza et al., 2013a, b). However, assessing the causes of the presence or absence of species is difficult from field observations alone. The causal factors influencing cyanobacterial species composition are poorly understood. In trying to detect factors that may affect species distribution, statistical analyses have been carried out to detect significant correlations between relative abundances and water quality variables. However, many environmental factors are usually interrelated so species distributions may be correlated with a broad range of characteristics, although only one may reflect a causal relationship. Therefore, a better understanding of the factors controlling the relative dominance and/or coexistence phenomena in the environment is needed.

We hypothesized that specific characteristics of cyanobacterial species, such as differential physiological traits related to changes in N and P concentration, influence how species respond to changes in environmental conditions over space and time, leading to the observed shifts in the structure of the cyanobacterial communities. Such relationships have often been proposed, but rarely demonstrated experimentally. Empirical evidence of this nature would be helpful for interpreting species distributions in relation to environmental changes. Therefore, in this study, a series of bioassays were designed to test individually significant factors that may be responsible for the observed changes. The effects of different nutrient regimes (nitrogen and phosphorus) on the growth of isolated strains from the Guadarrama river were assayed in an attempt to identify causal relationships.

2. Materials and Methods

2.1. Cyanobacterial cultures

Cyanobacterial strains used for bioassays were isolated from epilithic biofilms from the riverbed of the Guadarrama river (central Spain, Madrid), as previously described (Loza et al., 2013b). Three culture media were used for the isolation in order to provide a wide range of nutrient concentrations, since we had previously found different patterns of cyanobacterial growth to be associated with the particular culture media used (Berrendero et al., 2008). CHU No. 10 modified (CHU10) medium (Chu, 1942; Gómez et al., 2009) was selected as a low nutrient content medium (0.99 mg P L^{-1}, 3.5 mg N L^{-1}), $BG11_0$ (Rippka et al., 1979) was selected as an intermediate nutrient level medium (5.5 mg P L^{-1}, 247 mg N L^{-1}), and Allen & Arnon medium (AAN) (Allen and Arnon, 1955) was chosen as a high nutrient concentration medium (61.9 mg P L^{-1}, 175 mg N L^{-1}). The nine isolated cyanobacteria used for the experiments were the coccoid and non-heterocystous filamentous species *Cyanobium* sp., *Aphanocapsa muscicola*, *Pleurocapsa minor*, *Pseudanabaena catenata*, *Leptolyngbya boryana*, *Leptolyngbya nostocorum*, *Phormidium* sp. and the two heterocyst-forming species *Nostoc carneum* and *Tolypothrix tenuis*. A detailed description of all isolates used in this study can be found in Loza et al. (2013b, c).

2.2. Experimental setup

2.2.1. Competition experiments with a mixture of all nine species

The differential growth of cyanobacterial strains was analyzed by measuring the percentage of the surface of 14-cm diameter agar plates colonized in the low nutrient CHU10 medium and the nutrient-rich AAN, described above, simulating the oligo-mesotrophic and hypertrophic conditions previously found in the river (Loza et al., 2013a,b). Isolates were inoculated on 2% agar-plates and were kept for 24 h in a 16:8 h light:dark regime at a temperature of 18 °C, with an irradiance of 20 μmol photon m^{-2} s^{-1}, without shaking, in order to fix inocula on the agar surface. After that, agar plates were covered by 5 mL of both liquid media (CHU10 and AAN medium) on each, and then put in the shaker under the same conditions. Media were replaced on day 10 of the experiment. Relative abundance was analyzed using a dissecting microscope (Leica; Leica Microsystems) and the percentage of the area colonized was calculated for each isolate. The experiment was replicated two times, yielding similar results. Values from a representative experiment are shown.

2.2.2. Nutrient gradient bioassays

On the basis of the results obtained from the competition experiments with all strains, a series of bioassays under a gradient of different concentrations of derived nutrients from nitrogen (nitrate (NO_3^-) and ammonium (NH_4^+) and phosphorus (PO_4^{3-}) were carried out. *T. tenuis*, *N. carneum*, *Phormidium* sp., and *L. boryana*. were selected for these bioassays. Independent experiments for each nutrient were performed over 20 days in CHU10 medium in which only the concentration of the ammonium, nitrate or phosphate was modified to final concentrations of 0.2, 3, 6, 10, 50 and 100 mg L^{-1} (ranges: 0.04–22.58 mg L^{-1} N– NO_3^-;