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Short Communication

Allelopathic inhibition of primary producer growth and photosynthesis by aquatic fungi

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ABSTRACT

Autochthonous primary production is generally much reduced in forested headwater streams. Several hypotheses have been proposed for explaining this observation, among them, the low light intensity, or the strong constraints exerted by stream current. Allelopathic inhibition of competitors is a common ecological process in aquatic environments. Aquatic hyphomycetes are known to chemically inhibit bacteria and other fungi (including other aquatic hyphomycetes) but a possible allelopathic effect of aquatic hyphomycetes on primary producers has never been tested. The inhibitory effect of twelve aquatic hyphomycete species was tested on three diatom species. Nine aquatic hyphomycete species exhibited anti-diatom activity. Up to 100% diatom growth inhibition was observed. Our study reveals that such allelopathic interactions might be common in streams and probably involve an array of fungal compounds. We propose that the generally reduced primary production observed in forested headwater streams is, among other factors, due to the inhibition of primary producers by allelopathic compounds released by aquatic hyphomycetes.

1. Introduction

Forested headwater streams are generally characterized by low primary production (Fisher and Likens, 1973; Vannote et al., 1980). The low abundance of primary producers (PP) has most often been explained by shading from riparian trees (Vannote et al., 1980; Hill et al., 1995). However, in deciduous forests the incoming solar radiation intensity may be sufficient to sustain PP growth at least from autumn to spring, *i.e.* when there are no leaves on the trees. Alternative hypotheses for slow algal development, at least in some contexts, rely on stream water current (*e.g.* Peterson and Stevenson, 1989). Finally, PP may also be strongly nutrient-limited in these generally oligotrophic streams (Naiman et al., 2000). More recently, competitive exclusion between heterotrophic microbial decomposers and algae has also been proposed to explain the low abundance of PP in forested streams (Danger et al., 2013). Yet, if bacterial decomposers exhibit higher nutrient uptake rates than most PP due to their smaller size (Currie and Kalff, 1984; Danger et al., 2007), this discrepancy is less likely for fungal decomposers because their cells are larger. Indeed, aquatic hyphomycetes, which are among the most important microbial decomposers in headwater streams (Gessner et al., 2007), develop hyphae that are generally larger than most diatom cells occurring in such aquatic ecosystems.

In this study, we propose a supplementary hypothesis that has, to our knowledge, never been tested, for explaining the low amount of primary producers in headwater streams. We hypothesized that, in certain circumstances, primary production could be altered by chemical compounds released from aquatic hyphomycetes. This process, known as allelopathy, is well documented among aquatic microorganisms (Gross, 2003; Allen et al., 2016), but such interactions have never been investigated between aquatic hyphomycetes and PP.

2. Material & methods

The aquatic hyphomycete species used in this study were *Alatospora acuminata* (ALAC), *Anguillospora crassa* (ANCR),

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Anguillospora filiformis (ANFI), *Arbusculina moniliformis* (ARMO), *Articulospora tetracladia* (ARTE), *Clavariopsis aquatica* (CLAQ), *Flagellospora curvula* (FLCU), *Heliscus lugdunensis* (HULU), *Tetrachaetum elegans* (THEL), *Tetracladium marchalianum* (TEMA), *Tricladium chaetocladium* (TRCH) and *Tricladium splendens* (TRSP) (see Supplementary Material). All strains were isolated from forested headwater streams in south-western France. For Test 1, aquatic hyphomycetes were grown on 2% malt extract agar in Petri dishes incubated in the dark at 15 °C. For Tests 2 and 3, seven aquatic hyphomycete strains (ALAC, ANCR, ARMO, CLAQ, HULU, THEL and TRCH) were cultured in 50 mL of the mineral medium described in Arce Funck et al. (2015) with five alternative sources of organic carbon: glucose (5 g L^{-1}), cotton-strips, or twelve leaf litter discs (ø 12 mm) of alder (*Alnus glutinosa*), maple (*Acer pseudoplatanus*) or oak (*Quercus robur*). To reduce the release of leachates which could also interact with diatom growth, leaf discs and cotton-strips were autoclaved and rinsed in 40 mL of deionized water prior to being placed in sterilized culture medium. For each carbon source tested, a control was prepared, corresponding to sterile culture medium containing the corresponding carbon source but without fungi. Details of each test performed are given below.

The diatom strains used were *Fistulifera saprophila*, *Nitzschia palea*, and *Gomphonema parvulum*. All diatoms were cultured in axenic conditions with COMBO medium (Kilham et al., 1998) in a temperature-controlled chamber at 18 °C with a 16:8 light:dark cycle and 30 μmol m^{-2} s^{-1} light intensity. All experiments were carried out in these conditions (Fig. 1).

Test 1: To determine whether aquatic hyphomycetes produce allelopathic compounds, 6 mm-diameter cores were cut from the active margin of each aquatic hyphomycete solid culture. Cores were placed in 90 mm-diameter Petri dishes on solidified COMBO medium (1% agar) on which diatoms (4×10^6 cells for *F. saprophila* and *G. parvulum* or 2×10^6 cells for *N. palea*) had been homogeneously spread. Algal densities were based on cell size in order to have comparable biomass as described in Leflaive and Ten-Hage (2011). A sterile malt extract agar core, used as a control for culture medium effect, was placed on each Petri dish. The diatom photosynthetic efficiency of photosystem II (PSII yield), which is strongly linked to their physiological state (Baker, 2008), was measured with a Phyto-PAM fluorometer (Walz, Effeltrich, Germany) next to each core after 3 d for *F. saprophila* and *G. parvulum* or 5 d for *N. palea*.

Test 2: To determine whether inhibitory compounds from leaf litter could be released due to its decomposition by aquatic hyphomycetes, liquid cultures of aquatic hyphomycetes were filtered through sterile Polyethersulfone filters (0.22 μm pore size,

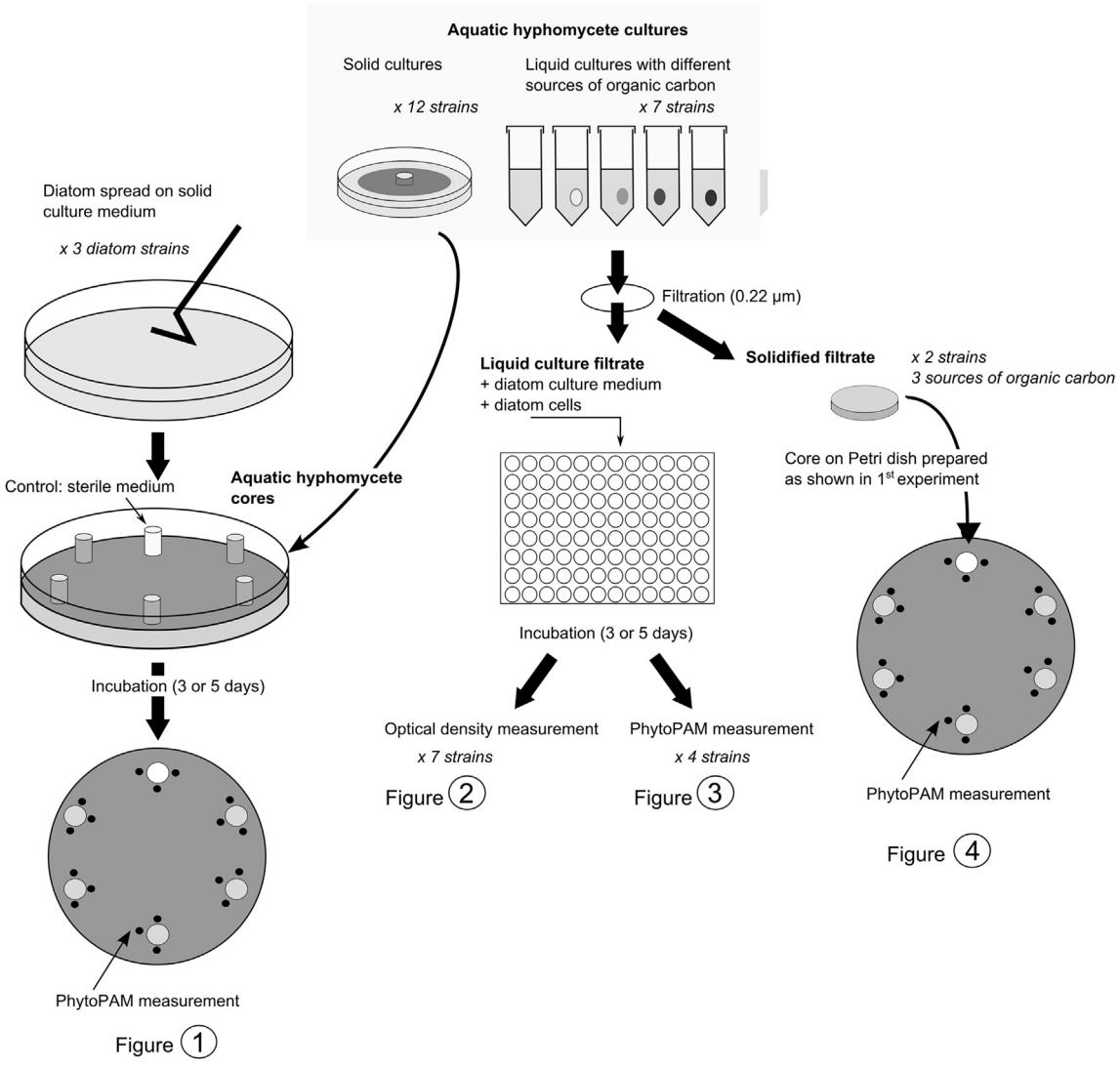

Fig. 1. Schematic representation of the experimental procedures in Test 1 (left panel), Test 2 (medium panel) and Test 3 (right panel).