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Determining in situ periphyton community responses to nutrient and atrazine gradients via pigment analysis

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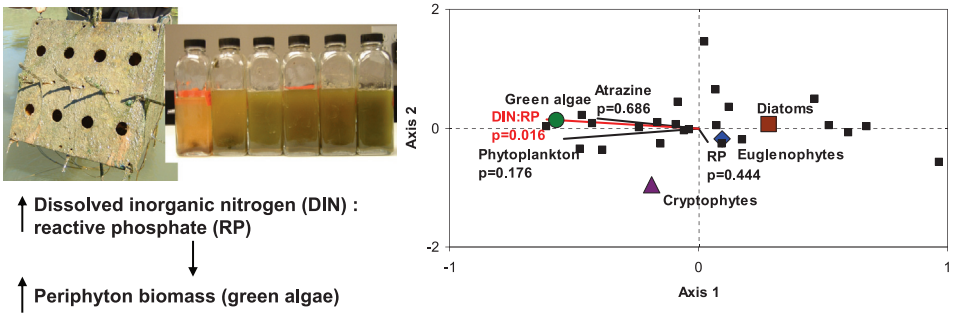
HIGHLIGHTS

- Agrochemicals (herbicides and fertilizers) may impair surface waters and periphyton
- In situ effects (experimental, existing gradients) assessed via pigment analyses
- Few effects of atrazine and nutrients were observed in periphytometer experiments
- Nitrate enrichment was related to increased biomass, particularly of green algae
- Effects of nitrogen enrichment superseded those of phosphorus and atrazine

GRAPHICAL ABSTRACT



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ABSTRACT

Agrochemicals, including fertilizers and herbicides, are significant contributors of non-point source pollution to surface waters and have the potential to negatively affect periphyton. We characterized periphyton communities using pigment markers to assess the effects of nutrient enrichment and the herbicide atrazine with in situ experimental manipulations and by examining changes in community structure along existing agrochemical gradients. In 2008, the addition of nutrients (20 mg/L nitrate and 1.25 mg/L reactive phosphate), atrazine (20 μg/L) and a combination of both nutrients and atrazine had no significant effect on periphyton biomass or community structure in a stream periphytometer experiment. In 2009, similar experiments with higher concentrations of atrazine (200 μg/L) at two stream sites led to some minor effects. In contrast, at the watershed scale (2010) periphyton biomass (mg/m^2 chlorophyll *a*) increased significantly along correlated gradients of nitrate and atrazine but no direct effects of reactive phosphate were observed. Across the watershed, the average periphyton community was composed of Bacillariophyceae (60.9%), Chlorophyceae (28.1%), Cryptophyceae (6.9%) and Euglenophyceae (4.1%), with the Bacillariophyceae associated with high turbidity and the Chlorophyceae with nitrate enrichment. Overall, effects of nitrate on periphyton biomass and community structure superseded effects of reactive phosphate and atrazine.

1. Introduction

In agricultural watersheds, aquatic primary producer communities, including periphyton, may be altered by exposure to agrochemicals such as herbicides and nutrients from fertilizers. Since periphyton is a

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dominant group of primary producers in mid-sized streams and rivers (Strahler stream order 4–6) (Vannote et al., 1980), agrochemicals have the potential to impair the environmental quality and ecological health of these systems. Nutrient enrichment can result in broad shifts in periphyton communities towards increased relative dominance of the Chlorophyceae (green algae), particularly filamentous taxa, in a variety of surface waters (e.g. Cattaneo, 1987; Carrick and Lowe, 1988; Dodds, 1991; Chételat et al., 1999; Rober et al., 2011; Ferragut and de Campos Bicudo, 2012). Changes may also occur at a finer taxonomic scale. For example, the Bacillariophyceae (diatoms) range in their sensitivity to eutrophication and organic pollution and are frequently used to assess water quality (reviewed in Kelly, 2013).

Direct effects of herbicides on periphyton are less clear because herbicides occur in surface waters at variable concentrations several orders of magnitude lower than nutrients with pulses occurring following rain events. Of the herbicides, atrazine is of particular interest due to its widespread usage on North American corn (maize) (*Zea mays* L.) crops, frequent detection in surface waters and concerns over its toxicity, mobility and persistence (Solomon et al., 1996; Gilliom et al., 2006; Stone et al., 2014). Laboratory and micro/mesocosm studies have shown that periphyton functional endpoints (e.g. primary production) may recover while community changes (e.g. species composition) persist following experimental exposure to herbicides in general (reviewed in Brock et al., 2000) and atrazine in particular (Hamala and Kollig, 1985). Early lentic enclosure studies noted a shift towards Bacillariophyceae dominated periphyton communities following exposure to atrazine (100 μg/L) (Herman et al., 1986; Hamilton et al., 1987).

Several studies have attributed atrazine exposure in the field to a shift towards atrazine tolerant Bacillariophyceae dominated periphyton communities but it is difficult to separate out effects of other stressors (e.g. Guasch et al., 1998; Dorigo et al., 2004). A number of laboratory and micro/mesocosm studies have aimed to tease apart the interaction between atrazine and correlated stressors such as nutrient enrichment on periphyton communities. The effects of atrazine and nutrients on periphyton are expected to be antagonistic but results have been mixed. For example, nutrients have been shown to have no effect on atrazine toxicity (Guasch et al., 2007). Others have found that both atrazine and nutrients can increase periphyton biomass through indirect effects of atrazine on phytoplankton (Rohr et al., 2008; Halstead et al., 2014). In contrast, Murdock et al. (2013) found conflicting trends between field and laboratory studies. A key challenge in risk assessment is finding the right balance between realistic field studies that may be variable and difficult to interpret and simplified experiments that may not be realistic of actual exposures and communities. In the present study, we aimed to find this balance by examining the effects of nutrients and atrazine on natural periphyton communities in situ using both experimental and field exposures.

The main objective of the present study was to assess the effects of high nutrients, exposure to the herbicide atrazine and their relative effects on periphyton community structure in situ using two approaches. First, natural communities of periphyton were exposed to experimental treatments of nutrients and atrazine at two stream/river sites using periphytometers. Second, periphyton communities were compared between paired sites surrounded by low or high agriculture and across existing gradients of nutrients and atrazine in a large agricultural watershed. It was hypothesized that nutrient enrichment would result in Chlorophyceae dominated communities and atrazine exposure would result in Bacillariophyceae dominated communities. It was predicted that nutrients and atrazine would have antagonistic effects and that the relative strength of each stressor would be concentration dependent. A secondary objective was to apply a chemotaxonomic pigment analysis method developed for classifying Southern Ocean phytoplankton communities to freshwater periphyton communities. Compared to microscopy, this approach reduces the time and taxonomic expertise required to determine changes in algal community structure.

2. Materials and methods

2.1. Study area

The South Nation River watershed, eastern Ontario, Canada (3915 km^2) is predominately agricultural with crops of corn and soybean (*Glycine max* L. (Merr.)) typically planted in tile-drained fields. The headwaters of this 177 km long river commence near the St. Lawrence River (44°40′41″N, 75°41′58″W) and the river flows north-easterly across a flat, poorly drained landscape until its confluence with the Ottawa River (45°34′24″N, 75°06′00″W) with a historical (1915–2011) average annual discharge of 44.3 m^3/s (Environment Canada, 2013). Twenty-four field sites located throughout the South Nation River watershed were selected for study (Fig. 1). Field sites were paired by matching 12 sites surrounded by low agriculture with 12 sites surrounded by high agriculture. Each pair was located along the same tributary with the low agriculture site located upstream of the high agriculture site (Fig. 1). The average distance between 10 of the 12 pairs was 8.8 ± 8.9 km (ranging from 1.5 to 33.7 km). Two pairs of sites were located along different tributaries due to a lack of accessible and suitable sites. Tributaries were generally hydrologically unconnected and varied in the intensity of surrounding agriculture. All sites (20 m stream length) were matched as closely as possible in terms of visible features such as steam width, bank slope and canopy cover.

2.2. Physical and chemical characteristics of field sites

Strahler stream order for all 24 field sites was determined from data provided by the South Nation River Conservation Authority and Ontario Ministry of Natural Resources (© Queen's Printer, 2013). Stream width was measured in triplicate at each site. Stream depth was measured in triplicate during base flow in August 2010. Maximum depth was measured mid-channel, while average depth was measured mid-channel as well as halfway between the mid-channel and each bank. Surface velocity was estimated by measuring the time for an orange wiffle ball to travel 1 m in triplicate in June and July 2010. Dissolved oxygen, pH, temperature and conductivity were measured with a HydroLab 4a Sonde (Hach Hydromet, Loveland, USA) once in May, June and July 2010 at all sites. Duplicate mid-channel, integrated water samples (1 L) were taken in polypropylene bottles in May, June and July 2010 to measure turbidity (LaMotte, Chestertown, USA) and planktonic chlorophyll *a*. Water samples (500 mL) for planktonic chlorophyll *a* analysis were filtered through 1.5 μm glass fiber filters (type 934-AH, Whatman, Mississauga, Canada) and frozen at −30 °C until extraction of algal pigments.

Agricultural intensity was calculated as the percentage of annual cropland in a 500 m radius surrounding each site (ArcMap v.10, ESRI, Canada Ltd, Toronto, Canada) from Landsat satellite imagery (30 m resolution) data provided by Agriculture and Agri-Food Canada (2008). Data used in Fig. 1 were from Natural Resources Canada (2009). Field sites were characterized by in-stream nitrogen (N) and phosphorus (P), with elevated concentrations representing enrichment from synthetic fertilizers and manure (Dubrovsky et al., 2010). Mid-channel, integrated water samples (300 mL) were collected in polyethylene terephthalate bottles for nutrient analysis in May, June and July 2010 with duplicate samples collected for approximately 10% of all samples. Time-weighted-average atrazine concentrations (56 days between 18 May and 22 July 2010) were determined by passive sampling with polar organic chemical integrative samplers (POCIS) deployed in triplicate as described in Dalton et al. (2014). In-stream and periphytometer reservoir nutrient and atrazine concentrations were also measured from duplicate samples on days 0, 7 and 14 of each periphytometer experiment. For atrazine analysis, subsamples (20 mL) were frozen and stored at −30 °C in pre-cleaned amber borosilicate vials until analysis. Nutrients, including reactive