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# Understanding the combined influence of fine sediment and glyphosate herbicide on stream periphyton communities

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ABSTRACT

Pesticides and deposited fine sediment have independently been associated with changes in relative abundance and species richness in aquatic ecosystems, but the interplay between these two stressors in agricultural streams is poorly understood. A 28-day experiment in outdoor stream mesocosms examined the effects of four levels each of fine sediment coverage (0, 25, 75, 100%) and glyphosate-based herbicide concentration (0, 50, 200, 370 μg/L) on periphyton communities (algae and bacteria) in a fully factorial, repeated-measures design. Our aims were to determine whether (i) increased levels of sediment and glyphosate had individual and/or additive effects, (ii) increased sediment reduced the toxicity of glyphosate (antagonistic multiple stressor interaction), or (iii) sediment-adsorbed glyphosate prolonged the effects of exposure (synergistic interaction). We also assigned all algal taxa to three ecological guilds (low-profile, high-profile and motile growth forms) and separately determined their responses to the treatments. As individual stressors, sediment addition affected all algal community-level metrics, whereas glyphosate addition only affected algal community evenness. Bacterial taxon richness was unaffected by either stressor. In combination, however, significant overall sediment by glyphosate interactions were detected, demonstrating synergistic (algal evenness, high-profile and motile guilds) or antagonistic effects (low-profile guild). Our experiment underscores the importance of considering both structural and functional indicators, including algal guild representation, when assessing the mechanisms by which periphyton communities respond to multiple stressors.

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## 1. Introduction

Agricultural intensification worldwide has dramatically increased the use of pesticides (Konstantinou et al., 2006; Zhang et al., 2011) with widespread occurrence in neighbouring aquatic environments (Kreuger, 1998; Morin et al., 2010). Poor agricultural practices have also led to increased runoff of fine sediment that can alter the structure and functioning of aquatic communities (Townsend et al., 2008; Matthaei et al., 2010; Wagenhoff et al., 2011). However,

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the effects of pesticides in concert with fine sediment load in stream ecosystems remains poorly understood.

Glyphosate, the most commonly used agricultural herbicide in the world (Woodburn, 2000), has generally been regarded as environmentally safe because strong adsorption to soil reduces leaching and runoff into surface water (Giesy et al., 2000). In aquatic environments, moreover, this sediment-binding property coupled with microbial decomposition is reported to reduce glyphosate exposure to aquatic organisms (Giesy et al., 2000; Solomon and Thompson, 2003). Recent studies, however, have detected glyphosate in filtered stream water and indicate that glyphosate is more mobile or persistent than previously thought (Battaglin et al., 2005; Peruzzo et al., 2008).

Observational field studies (e.g. Sullivan et al., 1981; Pesce et al., 2008) cannot properly evaluate the impact of glyphosate contamination on stream algae because of simultaneous exposure to other stressors associated with intensified land use. Moreover, conventional, single-species, laboratory dose-response tests can be criticized for ignoring sublethal effects and the mediating effects of other abiotic stressors and species interactions (Relyea and Hoverman, 2006; Rohr et al., 2006). In contrast, well-designed community studies (microcosms and mesocosms) allow the individual impacts of co-occurring stressors to be tested in a reasonably realistic setting while controlling other relevant environmental variables (Culp et al., 2000; Larned, 2010). Mesocosm studies of this nature have been conducted with glyphosate but have so far produced conflicting results. For example, Austin et al. (1991) reported that glyphosate served as a source of phosphorus to benthic algae in oligotrophic experimental streams. On the other hand, in a pond mesocosm experiment Pérez et al. (2007) reported decreases in micro- and nanophytoplankton and increases in picocyanobacteria that were due to direct toxicological effects, rather than phosphorus enrichment. In another pond study, Vera et al. (2010) reported that periphyton communities were negatively affected by glyphosate, with diatoms being more susceptible than cyanobacteria, and concluded that a long-term (1-year) shift from clear to turbid water conditions may have been caused by the slow release of sediment-adsorbed glyphosate to the water.

Fine sediment (generally defined as inorganic particles less than 2 mm in diameter; Rabení et al., 2005) derived from adjacent land-use practices is well known to strongly influence periphyton communities (Allan, 2004; Schofield et al., 2004; Izagirre et al., 2009). Fine sediment increases turbidity, resulting in a decrease in primary production and food quality (Allan, 2004), impairs substrate suitability for periphyton and biofilm production (Allan, 2004; Graham, 1990), reduces organic content of periphyton cells (Graham, 1990), and smothers periphyton communities and eliminates sediment-intolerant taxa (Brookes, 1986). In addition, sediment may serve as a sink for contaminants that can subsequently be released (e.g. Cold and Forbes, 2004; Wu et al., 2005).

No previous mesocosm experiments have examined the interactive effects of glyphosate inputs and augmented fine sediment load on stream periphyton. Therefore, we investigate whether (i) increased levels of deposited fine sediment and glyphosate have individual and/or additive effects on stream periphyton (benthic algae and bacteria), (ii) increased sediment reduces the toxicity of glyphosate (antagonistic multiple stressor interaction), or (iii) adsorbed glyphosate prolongs the exposure period (synergistic interaction).

## 2. Material and methods

### 2.1. *Experimental design*

Our experiment was conducted from late austral spring to early summer (29 October to 10 December 2008) in 128 circular, flow-through stream mesocosms (25 cm external and 5 cm inner rim diameter, 9 cm high, nylon Microwave Ring Moulds; Interworld, Auckland, NZ) supplied by water and biota from the adjacent Kauru River in North Otago, New Zealand (170°44.6′ East, 45°6.5′ South, 98 m a.s.l.). This river is low in nutrients (nitrate: 44.1 ± 3.6 SE μg/L; ammonium: 24.87 ± 2.0 μg/L; dissolved reactive phosphorus: 4.0 ± 0.1 μg/L; Magbanua, 2012) and receives minimal glyphosate inputs (glyphosate in stream water: 0.005 ± 0.002 μg/L; Magbanua, 2012).

We added fine sediment (0, 25, 75, 100% surface cover) and applied four levels of glyphosate (0, 50, 200, 370 μg/L) to 96 mesocosms using a full factorial (4 glyphosate × 4 sediment levels × 2 replicates of each treatment combination), repeated-measures design with 32 mesocosms sampled for the principle biological response variables on five dates, with two dates before experimental manipulation and three after stressor imposition. To investigate glyphosate adsorption to deposited fine sediment, we used a further 32 mesocosms with 100% sediment cover (eight per glyphosate concentration).

Glyphosate treatments (commercial formulation *Glyphosate 360* with 360 mg/L active ingredient plus 10–20% polyethoxylated tallowamine (POEA) as the surfactant; Ravensdown Fertilizer Co-operative Ltd, Dunedin, New Zealand) were randomly distributed within two blocks of 4 × 16 mesocosms each (Fig. 1A). In practice, farmers apply the herbicide glyphosate together with POEA as surfactant; hence, we studied the effects of the commercial glyphosate formulation (active ingredient plus surfactant) rather than trying to separate the effects of its individual constituents. Sediment treatments (4 levels × 3 replicates + 4 extra replicates with 100% sediment cover) were randomly assigned within each set of 16 mesocosms. After a 14-day colonization period, sediment was added once at the start of the 28-day manipulative phase (day -7). Glyphosate was added continuously for seven days, starting one week later (days 0–7), after which the mesocosms were allowed to recover for two weeks (Fig. 1B). Stressor application was sequential to simulate the situation in real agricultural streams where particular sediment loadings are likely to be present before a pesticide runoff event (see Matthaei et al., 2006). The temporal design represents reasonably realistic environmental conditions where the highest concentrations of glyphosate occur shortly after application in the catchment area and following heavy rain, but dissipate thereafter with half-life values in water of 7–14 days (Giesy et al., 2000). Previous studies have used glyphosate exposures ranging from a few hours (e.g. Folmar et al., 1979) to many weeks (e.g. Austin et al., 1991), but with most lasting 2–14 days (e.g. Wong, 2000; Pesce et al., 2009; Vendrell et al., 2009).

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