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Assessing combined impacts of agrochemicals: Aquatic macroinvertebrate population responses in outdoor mesocosms

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HIGHLIGHTS

- Combined effects of agrochemicals were assessed in outdoor mesocosms inoculated with aquatic invertebrate assemblages.
- Environmentally realistic concentrations of binary mixtures showed additive species' responses.
- Tertiary mixtures affected species' responses indescribable from cumulative responses of the single exposures treatments.
- This indicates that in agricultural ditches, non-additive induced shifts in aquatic invertebrate assemblages might occur.

GRAPHICAL ABSTRACT

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ABSTRACT

Agricultural ditches host a diverse community of species. These species often are unwarrantedly exposed to fertilizers and a wide-array of pesticides (hereafter: agrochemicals). Standardized ecotoxicological research provides valuable information to predict whether these pesticides possibly pose a threat to the organisms living within these ditches, in particular macro-invertebrates. However, knowledge on how mixtures of these agrochemicals affect macro-invertebrates under realistic abiotic conditions and with population and community complexity is mostly lacking. Therefore we examined here, using a full factorial design, the population responses of macroinvertebrate species assemblages exposed to environmentally relevant concentrations of three commonly used agrochemicals (for 35 days) in an outdoor experiment. The agrochemicals selected were an insecticide (imidacloprid), herbicide (terbuthylazine) and nutrients (NPK), all having a widespread usage and often detected together in watersheds. Effects on species abundance and body length caused by binary mixture combinations could be described from single substance exposure. However, when agrochemicals were applied as tertiary mixtures, as they are commonly found in agricultural waters, species' abundance often deviated from expectations made based on the three single treatments. This indicates that pesticide-mixture induced toxicity to population relevant endpoints are difficult to extrapolate to field conditions. As in agricultural ditches often a multitude (approx. up to 7) of agrochemicals residues are detected, we call other scientist to verify the ecological complexity of non-additive induced shifts in natural aquatic invertebrate populations and aquatic species assemblages.

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1. Introduction

Agricultural ditches host many different organisms, and their water quality is important for the watersheds as well as the fringing terrestrial fields. Agricultural ditches collect a wide variety of pesticides from adjacent fields due to run off, direct drift and leaching (Nollet and Rathore, 2010) and they generally are the primary receivers of agrochemicals (Vijver et al., 2017). From the monitoring data that takes place in these water bodies (eg. Snoo et al., 2006), it is learned that in surface waters pesticides often co-occur in mixtures (Gilliom et al., 2006) with high collinearity (Vijver and van den Brink, 2014). It is well known that these pesticides can differentially impact various species within the aquatic community. Consequently, it is likely that pesticide exposure adversely impacts biodiversity and ecosystem processes such as primary production (eg. Relyea et al., 2005) and decomposition (eg. Schäfer et al., 2007; Hunting et al., 2016). Thus, obtaining reliable predictions on how these pesticides and their mixtures affect the environment and the organisms living therein is key for maintaining healthy ecosystems.

Following standardized protocols, the impact of single compounds and mixtures are mostly tested in the laboratory with easy-to-culture organisms (e.g. OECD, 2004; Barmentlo et al., 2015; Gessner and Tlili, 2016). These laboratory tests provide valuable information on whether chemicals impose a threat to organisms and are generally indicative for the toxicity of substances. However, within these laboratory approaches, abiotic (fluctuating water chemistry parameters such as changes induced by rain events and wind) and biotic (population and community interactions such as competition and predation) factors are often simplified or even overlooked. It is, however, well-known that abiotic factors such as pH, temperature and other chemistry parameters alter toxicity and bioavailability of chemicals (see examples in Holmstrup et al., 2010; Bundschuh et al., 2016; Barmentlo et al., 2017). Biotic conditions affecting ecological responses can also impact toxicity, for example through increasing predation pressure (Schulz and Dabrowski, 2001) or by modulating inter- and intraspecific competition (Liess, 2002; Kattwinkel and Liess, 2014). The variation in these abiotic and biotic variables is thus likely to alter toxicity under natural conditions compared to the standardized protocols. Exclusion of these variables may lead to uncertainties in the extrapolation of responses to field situations (Heugens et al., 2001; Clements et al., 2012; Halstead et al., 2014). These uncertainties are possibly even higher for mixtures of agrochemicals as combined effects may complicate the overall response (Côté et al., 2016; Gessner and Tlili, 2016).

To test for these uncertainties, this study aims to assess quantitatively the combined effects of multiple agrochemicals from single exposure under realistic conditions to individual macroinvertebrate species. We investigated the effects of single exposure as well as binary and tertiary mixtures of a commonly used insecticide, herbicide and nutrients to different endpoints of 9 functionally distinct aquatic macroinvertebrates species. In order to test these species under more (a)biotic context, we investigated them in assemblages for 35 days in an outdoor mesocosm experiment.

2. Material and methods

2.1. Species selection

The species assembly chosen (Table 1) consisted of aquatic macrofauna species that are often found in European aquatic ecosystems, particularly in semi-stagnant water bodies such as ditches (Verdonschot et al., 2011; Ieromina et al., 2015; see Appendix Table A1 for additional information on the species). The different test species and their abundancies (Table 1) reflected broadly the feeding mode trait distribution (eg. predator, grazer etc.; retrieved from www.freshwaterecology.info; Schmidt-Kloiber and Hering, 2012) as found in dune ditch systems in order to mimic a natural ditch food web (Ieromina et al., 2015).

Daphnia magna Straus were obtained from laboratory cultures of Leiden University (Leiden, The Netherlands). *Lymnaea stagnalis* Linnaeus were obtained from cultures from the Vrije Universiteit Amsterdam (Amsterdam, The Netherlands). Algae, fungi and microbial communities were collected from ditch water by filtering water over a 150 μm mesh. The sediment-dwelling species *Chironomus riparius* Meigen and *Tubifex* sp. Lamarck were purchased from VitalFish (Boskoop, The Netherlands). All other species were collected in March 2016 from water columns or sediments of ditches located in peaty nature reserves by sweeping nets. Organisms were kept at 4 °C for one day to acclimate prior experimental usage.

2.2. Experimental setup

In March – April 2016, a mesocosm experiment of 48 mesocosms was conducted in the botanical garden of Leiden (Leiden University, The Netherlands). In this setting, several abiotic variables were expected and observed to (co-)vary, including average air temperature (gradually increased from 5 °C to 15 °C), solar irradiance (481–2234 J/cm^2 per day), rain fall (0–10.1 mm/day), wind velocity (2.2–11.7 m/s), air-pressure (997–1030 hPa). Information on water quality parameters is provided in Section 3.3. Mesocosms consisted out of 65 L poly-ethylene tubs closed by 50% shadow cloth nets to prevent migration of the animals. A sediment layer of 8 cm depth was added to each mesocosm. The sediment was prepared from fine-grained, ignited quartz sand as mineral substrate (12.5 kg, grain size: 0.1–0.5 mm), ground dry hay (0.5 kg) which was pre-soaked and then mixed. The water column was prepared by 36 L of copper-free tap water and 4 L of filtered (planktonic net, mesh size 150 μm) ditch water in order to inoculate the mesocosms with natural micro communities (algae/bacteria/fungi).

The micro community was allowed to equilibrate for seven days prior to non-predacious macrofauna species (Table 1) were added. All animals were slowly cooled (1 °C/h, using an incubator) to the water temperature of the mesocosms while mixing in water from the mesocosm to avoid a temperature or medium shock. One day later, the top-predator *Notonecta glauca* Linnaeus was added. We observed all mesocosms to contain an additional copepod species *Cyclops* sp. at the end of the experiment. The nauplius larvae of *Cyclops* sp. Müller are 150–200 μm in size and therefore likely passed the sieve (mesh size 150 μm) when ditch water had been added to the mesocosms during microbial inoculation. *Cyclops* sp. is not expected to disrupt the simplified food web as it is common in most aquatic habitats that are susceptible to agricultural run-off (Kulkarni et al., 2013). To provide oxygen and to homogenize the water columns, mesocosms were gently aerated with air pumps throughout the duration of the experiment.

A full factorial design ($n = 6$) of imidacloprid (two levels; present and absent), terbuthylazine (two levels; present and absent) and nutrients (two levels: oligotrophic and eutrophic) was applied in a randomized fashion, resulting in eight different treatments (see below for all concentrations). The treatment in which pesticides were absent and nutrients were maintained at oligotrophic levels served as control treatment. In nutrient enriched mesocosms, we added 6.16 mL of liquid plant fertilizer (232 mg N: 133 mg P: 232 mg K – 7:4:7 combined with micro-elements) in order to approach nutrient concentrations that have been shown to stimulate fresh water algal growth (Ieromina et al., 2014). Imidacloprid and terbuthylazine were selected in this experiment as being representative for a large group, namely the neonicotinoids (neurotoxins) and triazines (photosynthetic inhibitors). Both pesticides commonly exceed the current water quality criteria for surface water concentrations in many European (Leiden University and Rijkswaterstaat-WVL, 2016; Vijver et al., 2017) and United States waters (USGS National Water-Quality Assessment (NAWQA) Program, 2017). The insecticide imidacloprid (99.7% purity, CAS Number: 138261-41-3) and herbicide terbuthylazine (99.4% purity, CAS Number: 5915-41-3) were purchased from Sigma-Aldrich (Zwijndrecht, The