Underestimated benefits from refuge habitats on benthic macroinvertebrate communities

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Study on pesticides and other agricultural stressors in Central Romania affecting benthic macroinvertebrates. Sampling sites selected to represent catchments with a wide range of agricultural intensity and little urban and industrial activities. We find no correlation between maximum TU and SPEARpesticides, but can show that upstream refuge habitats positively affect SPEARpesticides-values and mask effects from pesticides. Something about aquatic leaf decomposition here and on parasite prevalence in *Gammarids* and *Baetis*.

# 1 Introduction

Despite a turnover in the spectrum of compounds and changes in regulation, high pesticide exposure remains widespread in inland waters, especially in small water bodies such as streams of Strahler orders of four and below (Strahler, 1957; Szöcs et al., 2017). Studies in the last decades have repeatedly reported the exceedance of ecological and regulatory thresholds in small agricultural streams (Liess et al., 2021; Schäfer, 2019; Schulz, 2004). Freshwater macroinvertebrates have been identified as an organism group at risk from pesticides in freshwater systems (Malaj et al., 2014). In fact, several studies have linked pesticide exposure to a decrease in the fraction of vulnerable macroinvertebrates, using a metric developed to indicate pesticide stress (SPecies At Risk, also called SPEARpesticides) (Liess et al., 2021; Liess and Ohe, 2005; Schäfer et al., 2012b; Schäfer et al., 2007). Moreover, pesticide exposure can affect the fitness of exposed individuals and influence parasite prevalence in hosts, as shown for honey-bees (Di Prisco et al., 2013). Furthermore, tadpole susceptibility to trematode infections increased after herbicide exposure, whereas trematode density increased after insecticide exposure (Rumschlag et al., 2019). Parasites are important ecological players in ecosystems, and consequently, parasite abundance and diversity have been suggested as promising indicators for ecosystem health (Sures et al., 2017). Moreover, pesticide exposure has been associated with a decrease in leaf litter decomposition (LLD), a crucial ecosystem function in small streams (Schäfer et al., 2012a; Webster, 2007). Overall, pesticides present an important stressor for the freshwater macroinvertebrate communities and associated ecosystem functions in agricultural landscapes.

# 2 Materials and Methods

## 2.1 Study area, sampling site selection

The study area was located in Transylvania, north-west Romania around the city of Cluj-Napoca. The landscape in this rural study area is characterized by undulating terrain with hills reaching heights of several hundred meters (Fischer et al., 2012). At 19 small wadeable streams (3rd to 4th Strahler order, (Strahler, 1957)) a 100 m stream section was selected, respectively. The surrounding land use at all selected sampling sites was dominated by agriculture. No large urban settlements, industrial sites as well as municipal or industrial waste water treatment plants were located in the catchments upstream of the sampling sites to minimize effects of non-agricultural pesticide use and waste water.

## 2.2 Spatial analysis of catchments and land use types

The upstream catchments for the respective sampling sites and a stream network were derived using the free open-source software R, version 4.0.5, (R Core Team, 2021) and the R-package openSTARS (Kattwinkel and Szöcs, 2020) from the European digital elevation model with a cell size of 1000 m (EEA, 2004), cropped to the study area of north-west Romania. By intersecting the derived catchments with the Corine Land Cover data (Programme, 2020) land use types and respective proportions could be identified for each catchment. We aggregated relevant land use types into three groups named agriculture (representing “non-irrigated arable land”), pastures (representing “pastures” and “land principally occupied by agriculture”), and forest (representing “broad-leafed forest,” “coniferous forest” and “mixed forest”) and added up the respective proportions. Finally, catchments were checked for the presence of forested stream sections upstream of the sampling sites acting as a refuge habitats for sensitive macroinvertebrates. In order to be classified as an upstream refuge habitat, the forested stream sections had to be at least 500 m long, with the forest patch reaching a minimum width of 100 m at both sides of the stream, with a distance less than 10 km upstream of the respective sampling site (Bailey, 1966; Elliott, 1971; Knillmann et al., 2018; Orlinskiy et al., 2015). The upstream catchments of selected sampling sites differ between sizes from 7.5 km2 to 176.8 km2 with a mean size of 73.9 km2. Agricultural land use covered between 6.8 % and 61.4 % of the catchment areas (mean = 25.2 %) and was mostly located in plain areas along the main streams and its larger tributaries. Pastures covered mainly areas with a steeper slope or greater elevation compared to the agricultural areas, with cover reaching from 3.9 % to 70.1 % (mean = 34.9 %). In most catchments, forests were located along their boarders, making up between 2.9 % and 50.7 % of the catchment area (mean = 25.2 %), surrounding the sources of the main stream and its tributaries, which also marked landscapes with the greatest slopes and elevation (Figure 2.1) (Fischer et al., 2012). Land use classification in the CLC-2018 data gives no indication on the degree of mechanization of agricultural practices (e.g. human/animal labour or technical equipment). Furthermore, a previous study found no correlation between agricultural intensity (degree of mechanization and size of average field size) and maximum sumTUinvertebrates (Schreiner et al., 2021). Consequently and for reasons of simplicity, we considered only land use variables based on categories provided by the CLC-2018 data for our analysis.

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