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Microbial colonization and decomposition of invasive and native leaf litter in the littoral zone of lakes of different trophic state

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ABSTRACT

Riparian invasion by non-native trees may lead to changes in the quality of leaf litter inputs into freshwater ecosystems. Different plant species may affect the community of decomposers and the rate of litter decay in different ways. We studied the microbial colonization and decomposition of leaf litter of the invasive to Lithuania *Acer negundo* and native *Alnus glutinosa* during 64-day litterbag experiments in the littoral zones of mesotrophic and eutrophic lakes. The decomposition of *A. negundo* leaf litter proceeded faster than that of *A. glutinosa* irrespective of differences in the trophic conditions of the lakes. The amount of terrestrial and cellulose-degrading fungi (during the initial period) and bacterial numbers (during the experiment) were higher on *A. negundo* leaves than on *A. glutinosa* in both lakes. Differences in the assemblages of aquatic fungi colonizing the leaves of both types might be one of the reasons causing variation in their decay. The trophic conditions of the lakes did not significantly determine the extent of differences in decomposition rates between the two leaf species, but affected the microbial decomposers. The sporulation rate and diversity of aquatic fungi, especially on *A. glutinosa* leaves, was higher in the mesotrophic lake than in the eutrophic lake, while heterotrophic bacteria were more numerous on the leaves in the eutrophic lake. Generally, differences in the colonization dynamics of heterotrophs and the faster decay of *A. negundo* litter than of *A. glutinosa* suggest that the replacement of native riparian species such as the dominating *A. glutinosa* by invasive *A. negundo* may cause changes of organic matter processing in the littoral zones of lakes.

1. Introduction

Riparian forest strips play an important role in regulating anthropogenic nutrient transport into water bodies (Weissteiner et al., 2013). Although protecting against direct pollution, such buffer zones of riparian vegetation may have a negative impact due to the acceleration of littoral bioproductivity. Leaves of deciduous trees account for the major part of plant litter in forests (Gessner et al., 2010) and are known as an important nonpoint source of natural nutrient load (Camargo and Alonso, 2006). Thus, the decomposition of allochthonous natural organic matter entering a water body via fallen leaves and/or leaf litter leachates is an integral step in the cycling of nutrients (Meyer and Likens, 1979) and may be one of the reasons for eutrophication processes in the littoral zone of a water body.

Decomposition of leaf litter is largely mediated by fungi and bacteria that metabolize and convert leaf carbon into microbial biomass and make it more acceptable for invertebrates (Gessner et al., 1999). The activity of decomposers and the rate of leaf litter decomposition largely depend on litter quality (Lecerf and Chauvet, 2008) that

changes when native species are replaced by invasive ones (Casas et al., 2013). Different results have been obtained in aquatic studies on litter decomposition rates of native and invasive tree species. For example, the litter of the invasive *Ailanthus altissima* decomposed faster than that of six native riparian trees (Swan et al., 2008), while leaves of the invasive *Salix fragilis* decomposed slower than the native *Ochetophila trinervis* (Serra et al., 2013). No differences were observed in litter decomposition rates between the invasive *Acer platanoides* and the native *Populus trichocarpa* (Reinhart and VandeVoort, 2006). Shifts from native to exotic leaf litter inputs can alter, though not always, the microbial, fungal and invertebrate communities that colonize leaves (Bärlocher and Graça, 2002; Lecerf et al., 2007; Medina-Villar et al., 2015). Those effects depend on the leaf functional trait differences between the invasive and the native species (Hladysz et al., 2009; Claeson et al., 2014). The changes in leaf chemistry might selectively stimulate or inhibit fungal species resulting in a succession of species throughout leaf decomposition (Duarte et al., 2010), this highlighting the importance of specific studies in order to understand the effects of invasions on ecosystem functioning (Fargen et al., 2015).

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After its escape from cultivation during the 1930s, the invasive to Lithuania boxelder maple (*Acer negundo* L.) has become widely distributed and has been included on the list of invasive and annulled species since 2004 (Gudžinskas et al., 2014). *A. negundo* has a high invasiveness score and is the most widely distributed alien maple species in many Lithuanian habitats, including riparian zones of water bodies, particularly rivers, which are dominated by native black alder (*Alnus glutinosa* (L.) Gaertn.) (Gudžinskas et al., 2014; Janušauskaitė and Straigytė, 2011; Straigytė et al., 2015). The dynamic ecotones of a water ecosystem, which are characterized by water level fluctuations, create favorable conditions for colonization by this invasive species. Ecological changes in the shoreline could be especially deleterious for small shallow lakes. Changes in organic matter processing in the shoreline sediments may influence the exchange of nutrients, which in turn may affect the biological productivity in the water column. In Lithuania, there are more than 6000 lakes of natural origin, occupying an area equating to about 1.5% of the whole territory. Small lakes (< 50 ha) predominate, with only 14 lakes exceeding 1000 ha (Kilkus, 2005). Studies on various aspects of leaf litter decomposition have been carried out mostly in small forested streams or rivers (Gulis and Suberkropp, 2003a,b; Pascoal and Cássio, 2004; Artigas et al., 2008, 2011; Casas et al., 2013). Decomposition of plant material, mainly of macrophytes, has also been studied in lakes (Komínková et al., 2000; Carvalho et al., 2015). In this respect, investigation of the impact of *A. negundo* habitats expanding to lakeside ecotones becomes relevant in terms of changes in the quality of leaf litter entering the littoral zone of lakes.

Previously, we investigated the ecotoxicological effects of non-native *A. negundo* and native *A. glutinosa* leaf litter leachates obtained during 90-day decomposition of leaves under anaerobic (Krevš et al., 2013) and aerobic (Manusadžianas et al., 2014) conditions in laboratory experiments. Besides temporal and species-dependent differences in toxicity effects induced by these leachates on algae and invertebrates, it was found that boxelder maple leaves lost biomass more rapidly and released more bacteria into ambient water than leaves of black alder.

Taking into account current scientific reports and our own laboratory experiments, we put forward a hypothesis that under natural conditions *A. negundo* leaf litter would also show greater microbial colonization than native *A. glutinosa* leaf litter and, consequently, the decomposition rates of the invasive leaf litter would be higher than that of the native leaves. In addition, the extent of these differences could also depend on the trophic levels of the water body. To test our hypothesis, we examined in autumn the colonization dynamic of fungi and bacteria and the decomposition rates of fallen leaves of *A. negundo* and *A. glutinosa* by means of litterbag experiments in the littoral zones of the mesotrophic Lake Asveja and eutrophic Lake Riešė. The overall objectives of the study therefore were (1) to determinate the decomposition rates of native and invasive leaf species and to assess the influence of lake trophic condition on the extent of their differences between the two leaf species; (2) to compare the growth dynamics of fungi and bacteria associated with the leaves of the two types.

The present study, along with other similar studies that focus on the effects of alien riparian tree species on leaf litter decomposition rates and the communities of decomposers in the littoral zones of lakes, may be useful for riparian forest management strategies, predicting and minimizing the impact of invasive vegetation on aquatic ecosystems.

2. Materials and methods

2.1. Study sites and water analyses

The leaf litter decomposition experiment was performed in the littoral zones of Lake Asveja (55°02'35"N, 25°30'17"E) and Lake Riešė (54°46'59"N, 25°6'49"E) located in eastern Lithuania. Lake Asveja is the longest (21.9 km) lake in Lithuania with a mean depth of 14.7 m. Forest

cover in the catchment area is 56% and the trophic state of the lake is characterized as mesotrophic (Kalytė, 2010). The lake segment selected for the leaf litter colonization experiment was remote from urbanized areas, the bank was mostly steep and surrounded by riparian forest. Lake Riešė is situated in the suburbs of Vilnius city and undergoes anthropogenic impacts from the urbanized territories. It is a shallow lake (mean depth 2.5 m) and the surface area of Lake Riešė (0.85 km²) is approximately one tenth the extent of that of Lake Asveja (9.8 km²). The banks of the lake are low and some are surrounded by swampy areas. According to previous studies, Lake Riešė was assigned to the eutrophic type with frequent water blooming (Krevš and Kučinskienė, 2012). At the experimental sites of both lakes, the riparian zones are colonized by deciduous trees, mainly black alder. The littoral areas were overgrown with reeds and characterized by bottom sediments of grey sand with silt impurity and low water circulation.

On each sampling date, water temperature, pH and dissolved oxygen concentration were measured *in situ* using a portable universal MultiLine f/Set–3 m (WTW) and oxygen meter (Eutech Instruments). These parameters were measured on days 0, 4, 7, 14, 28, 42 and 64. Nutrient and biochemical oxygen demand (BOD₇) analyses of water samples were performed on days 0, 14, 42 and 64 in a certified analytical laboratory (JSC Water Investigations, Vilnius, Lithuania). Phosphate (P_{in}) was assessed via molybdate ascorbic acid method after digestion with sulphuric acid (ISO 6878). The total phosphorus (P_t) concentration was measured using persulphate-H₂SO₄ digestion and molybdate ascorbic acid methods. For inorganic nitrogen (N_{in}) analysis, nitrate was determined by Ion Chromatography (ISO 10304-1), ammonium by manual spectrometric method (ISO 7150-1). The total nitrogen (N_t) was determined using potassium persulfate-K₂S₂O₈ digestion following Cd-Cu reduction to NO₂ (ISO 11905-1), dissolved organic carbon (DOC) according to ISO 8245.

2.2. Experimental design

Recently fallen leaves of *Alnus glutinosa* and *Acer negundo* were collected after early autumnal frosts in November 2012. The method used for leaf litter collection allows to have as much as possible unbroken leaves that were fallen off at the same time. Exposed in the two lakes leaf litter was collected from the same trees and at the same site. The collecting site (Varėna district, Lithuania; 54°5'N, 24°40'E) was situated in a forest zone near the River Ūla, approximately 20 km away from main roads and industrial activity. In the laboratory, leaves were carefully cleaned of tree debris, dried at room temperature and stored in paper bags.

Decomposition experiments were carried out in autumn, starting the incubations on 13–14 September 2013 and continued for 64 days. Air-dried leaves from each tree species were incubated in the lakes using fine mesh bags of dimensions 17 × 32 cm with 1 mm mesh sizes (adapted from Artigas et al., 2011; Medina-Villar et al., 2015), which excluded access by larger invertebrates and prevented the loss of large fragments of material through the mesh. Each bag was carefully sewn across thus dividing it into two separate parts. Leaves of the same tree species were put in both separate parts. One part of the bag was filled with 2 g of air-dried unbroken single-species leaves for mass loss determination, while fifteen unbroken leaves of the same species were placed in other larger part of the bag for microbiological analyses. 21 bags containing *A. glutinosa* leaves and the same number of bags with *A. negundo* leaves were submerged onto the surface of bottom sediments in the littoral zones of the lakes at a water depth of 0.5 m. Each bags of the set with *A. glutinosa* leaves was attached to the outside of a separate cell of net mesh plastic boxes (50 × 30 cm, inside with stones) which were placed on the bottom of the lake in the littoral zone. The bags with *A. negundo* leaves were exhibited in the same way. Placement was done so that leaf bags horizontally touched the surface of the sediments.

Samples for laboratory analysis were taken at the beginning of the experiment (0-day), then after 4, 7, 14, 28, 42 and 64 days. At every

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