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COMMUNITY ECOLOGY - ORIGINAL RESEARCH

Top predators affect the composition of naive protist communities, but only in their early-successional stage

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Abstract Introduced top predators have the potential to disrupt community dynamics when prey species are naive to predation. The impact of introduced predators may also vary depending on the stage of community development. Early-succession communities are likely to have small-bodied and fast-growing species, but are not necessarily good at defending against predators. In contrast, late-succession communities are typically composed of larger-bodied species that are more predator resistant relative to small-bodied species. Yet, these aspects are greatly neglected in invasion studies. We therefore tested the effect of top predator presence on early- and late-succession communities that were either naive or non-naive to top predators. We used the aquatic community held within the leaves of *Sarracenia purpurea*. In North America, communities have experienced the *S. purpurea* top predator and are therefore non-naive. In Europe, this predator is not present and its niche has not been filled, making these communities top-predator naive. We collected early- and late-succession communities from two non-naive and two naive sites, which are climatically similar. We then conducted a common-garden experiment, with and without the presence of the top predator, in which we recorded changes in community composition, body size spectra, bacterial density, and respiration. We found that the top predator had no statistical effect on global measures of community structure and functioning. However, it significantly altered protist composition, but only in naive, early-succession communities, highlighting that the state of community development is important for understanding the impact of invasion.

Keywords Aquatic top predators · Naive prey · Succession · Invasion · *Sarracenia purpurea*

Introduction

Top predators are known to have major impacts on community structure in both aquatic and terrestrial systems (Gurevitch and Padilla 2004; Salo et al. 2007; Estes et al. 2011) and are important for community dynamics (e.g., Hunter and Price 1992; Kneitel and Miller 2002) and ecosystem functioning (Hairston et al. 1960; Carpenter et al. 1985). Recently, with the increase in the movement of species around the world due to human transport, predators are being introduced to novel communities and are having a major effect (Gurevitch and Padilla 2004; Salo et al. 2007). This phenomenon has opened a new research path that tests whether novel predators have larger effects on invaded communities than on their native community. This prediction is based on the absence of shared evolutionary history between the predator and prey in the invaded communities [naive prey hypothesis (Elton 1958; Diamond and Case 1986; Cox and Lima 2006; Freeman and Byers 2006)] and

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support for this hypothesis has been demonstrated recently by Paolucci et al. (2013). In Paolucci et al.'s (2013) meta-analysis, alien predators had a 2.4 times stronger negative effect on prey compared to native predators, with similar effect sizes for herbivores and carnivores and for terrestrial and aquatic ecosystems (Paolucci et al. 2013; but see also Salo et al. 2007). The strong effects found by Paolucci et al. (2013) are illustrated by the well-known introduction of the brown tree snake (*Boiga irregularis*) to Guam (Savidge 1984, 1987; Wiles et al. 2003; Sih et al. 2010), where 90 % of the local bird species were exterminated. Of the six bird species that were least affected by the snake, four were introduced to Guam and had co-evolved with snake predators, and the other two occupy different habitats than the snake (Wiles et al. 2003).

The differential effect of introduced predators on naive vs. non-naive (i.e., co-evolved with a predator type similar to the introduced predator) communities has also been found in Australia, where introduced predators have a larger effect on communities than native predators (Salo et al. 2007). For island communities in general, the effects of novel predators are found to be particularly severe compared to on the mainland (Courchamp et al. 2003, but see Paolucci et al. 2013). This island concept can also apply to enclosed freshwater ecosystems, which are typically limited in size and isolated from other aquatic habitats. It is of little surprise then that examples exist where introduced aquatic predators strongly impact local communities [e.g., the Nile perch *Lates* spp. (Goldschmidt et al. 1993), the rainbow trout *Oncorhynchus mykiss* (Baxter et al. 2004), or the zander *Sander lucioperca* (Manchester and Bullock 2000)].

Despite the potentially large impact of novel predators on naive communities, few studies have addressed this question (Dickman 1996; Bruno et al. 2005; Cox and Lima 2006; Rodriguez 2006). Lowry et al. (2013) showed that less than 10 % of the research conducted on invasive species has focused on top predator invaders, and a majority of this research was conducted in terrestrial systems. Additionally, most of these studies used field observations and not experiments. Consequently, there is a major need to experimentally test the effect that top predators have on naive prey communities. This question is particularly relevant in freshwater systems (e.g., lakes, ponds), because barriers for the expansion of predators (e.g., waterfalls, terrestrial environment) occur more often in such systems and increase the likelihood that if novel predators are introduced, they will encounter naive prey communities (Moyle 1986; Cox and Lima 2006). Further, these systems are usually top-down controlled (e.g., Shurin et al. 2002), thus an invasion by a top predator is likely to have strong negative effects.

Aside from the naivety status, another aspect known to affect invasion success of a species is the successional stage of the host community (Connell and Slatyer 1977; Fabian et al. 2012; Oakley and Knox 2013). The organization of early-succession communities is difficult to predict due to idiosyncratic immigration events, but they are typically composed of small-bodied species with high dispersal and reproduction abilities (e.g., Odum 1969; del Moral and Wood 1993; Foster and Tilman 2000; Catford et al. 2012). Community structure changes through time because of the subsequent immigration of species with lower dispersal potential (Lortie et al. 2004), the addition of multiple trophic levels, and the biotic interactions within trophic levels (e.g., Odum 1969; Belyea and Lancaster 1999). This could lead to more predictable and stable communities, typical of late-succession (Clements 1916; but see Gleason 1926). Such communities are characterized by the presence of competitive large-bodied species (Sommer et al. 1986) that allocate more energy to exploit resources and for defense (Pianka 1970; Foster and Tilman 2000; Pomati et al. 2013). The transition between these states can be explained by the displacement of small-bodied species by larger and more competitive ones (e.g., Wootton 1993; Foster and Tilman 2000), and the preferential predation of abundant small-bodied species during early-succession (Sousa 1979; Wootton 1993; Hansson et al. 1998). Non-equilibrium dynamics are typically prevalent in early-succession compared to late-succession communities (Hutchinson 1961; Connell and Slatyer 1977; Rees et al. 2001) because of higher reproductive rates of early-succession species (Jiang et al. 2011) and stronger top-down control by predators (e.g., Schmitz et al. 2006). For predatory invaders, it is expected that they should benefit from the prevalence of small and fast-growing species that allocate more energy to reproductive output (offspring that are themselves easy prey) than to defense mechanisms (Pianka 1970; Walls et al. 1990). Thus, we hypothesize the impact of predation to be stronger in early- than in late-succession communities. In all, to understand the factors affecting the impact of an invasive predator, it is important to consider the possible combined effects between naivety status and successional stage of a community.

One reason for the lack of experiments addressing the effect of top predator invasion is that large-scale communities are complex, making the control of confounding factors challenging. Model systems of microorganisms can provide the tractability and high statistical power that is often difficult to obtain in larger-scale systems (Srivastava et al. 2004). The rainwater-filled leaves of *Sarracenia purpurea* is one such model system for aquatic communities (e.g., Miller and Kneitel 2005). This system shows the typical dynamics of larger aquatic food webs, but on small spatial and short time scales (e.g., Addicott 1974; Heard

1994; Kneitel and Miller 2002; Gotelli and Ellison 2006; Gray et al. 2006; Hoekman 2007). Whole communities can be easily sampled and used in experiments both in the field and in the laboratory. In *S. purpurea*'s native range in North America, insects fall and drown in the trapped rainwater. Bacteria and yeast colonize the system, decompose the insects, and liberate nutrients for the plant. A variety of protists and a rotifer species also colonize this community and consume the bacteria. These species come from a larger pool of species that are present in the local habitat (e.g., Bledzki and Ellison 2003). The highest trophic level is composed of the larvae of the endemic pitcher plant mosquito, *Wyeomyia smithii*, which feed on the protists and rotifers. Numerous studies using the *S. purpurea* model system have been conducted in the native range of North America (e.g., Addicott 1974; Bradshaw and Holzapfel 2001; Kneitel and Miller 2002; Gotelli and Ellison 2006; Miller and terHorst 2012).

S. purpurea has been introduced by seed into Europe and therefore lacks its native resident aquatic community. In Switzerland, it was planted in several locations in the Jura Mountains and Alpine regions in the late nineteenth century (Correvon 1947), and in several sites at lower elevations in the 1950s (Parisod et al. 2005). This introduction has allowed for the development of parallel aquatic communities in North America and Europe, but with different evolutionary histories. In Europe, where the inquiline communities have had a shorter time period to adapt to the environment of the *S. purpurea* leaves and develop shared evolutionary trajectories, evidence has shown that there may be a larger number of protist species present than in North America [51 protist morphospecies plus 17 species of coccal green algae inside *S. purpurea* leaves in a single site in Germany (Gebühr et al. 2006) versus 48 morphospecies found across North America by Buckley et al. (2010)]. The species composition of the inquiline *S. purpurea* community across Europe may also be more heterogeneous than in North America since we found little overlap in composition with Gebühr et al. (2006) in our study involving only first-year pitchers. Most importantly, the *W. smithii* larvae of the *S. purpurea* system have experienced the inquiline prey community in North America for at least 10,000 years. On the contrary, the communities that developed in Europe have never experienced *W. smithii* as a top predator, and very likely have never experienced any insect top predator in the leaf (Gebühr et al. 2006; Fragnière 2012), making them top-predator-naive communities. This island-like freshwater system is therefore ideal for addressing questions about the effects of novel predators on natural, naive aquatic communities.

Here, we conducted a full-factorial common-garden experiment where we manipulated the presence/absence of the *W. smithii* top predator in natural communities (protists

and bacteria) that did or did not share a habitat with the top predator and thus did or did not adapt avoidance or tolerance mechanisms against *W. smithii*. The non-naive communities come from two sites in Québec (Canada) and the naive ones come from two sites in Switzerland. Sites were chosen to match in climatic conditions and day length (similar latitude). Additionally, we tested the effect of the predator on early- and late-successional communities. First, we hypothesized that the predator-induced change in protist species composition should be stronger for naive communities because the species within *S. purpurea* leaves have not experienced *W. smithii* predation [naive prey hypothesis (Elton 1958; Diamond and Case 1986; Cox and Lima 2006; Freeman and Byers 2006)]. Second, early-succession communities, whose composition is typically dominated by small-bodied species allocating more energy to reproduction, should be more affected than late-succession communities that have been structured by predation and competition events (Clements 1916; Odum 1969; Kuno 1987; Louette et al. 2008; Kadowaki et al. 2012). Third, independent of community origin, the distribution of body size within a community should change due to preferential predation (Sommer et al. 1986; Wootton 1993; Hansson et al. 1998). Fourth, the effect of the top predator should cascade down to the bacterial trophic level because the bacteria will be released from the predation pressure of the protists (Kneitel and Miller 2002), an effect predicted to be strongest in early-succession and naive communities. Fifth, as a corollary, ecosystem functioning—measured as global respiration—should be highest in naive, early-succession communities because of a trophic cascade in the presence of a top predator [assuming that bacteria dominate in respiration (e.g., Simon et al. 1992; Gebühr et al. 2006)].

Materials and methods

We conducted a common-garden full-factorial experiment using communities from four sites, two successional stages per site, and two predator treatments (present = two third-instar mosquito larvae, or absent = no mosquito larvae). Each treatment was replicated four times for a total of 64 samples. We selected four climatically similar sites with regard to average July temperatures (averaged across 50 years, Worldclim data; www.worldclim.org). The sites also matched in day length due to their similar latitudes. Two of these sites were in the native range of *S. purpurea* in Québec (Lac des Joncs, 48°29′61.00″N, 68°77′15.55″W; Lac Rimouski, 48°18′32.71″N, 68°28′14.68″W), where the species were not naive to the top predator. The other two sites were in Switzerland (Les Embreux, 47°15′45.82″N, 7°6′57.79″E; Les Tenasses, 46°29′28.51″N, 6°55′16.04″E), where the species were naive to the top predator. At each

site, we marked randomly-selected leaves that were likely to open within several days. We returned to the sites 2 weeks later and marked a different set of leaves that were about to open. After 1 month since the first leaf markings, we returned to the field sites and collected water (the aquatic community) from all marked leaves. The aquatic communities that were collected from the 4-week-old leaves were designated as "late-succession communities" and the aquatic communities that were from 2-week-old leaves were designated as "early-succession communities". A 1-month duration is a reasonable interval for this community to reach late-succession because the community consists of bacteria and protists, which have fast generation times (Gray 2012). Note that early- and late-succession communities from Québec already experienced *W. smithii* predation at the time of sampling [*W. smithii* lay their eggs immediately after pitcher opening (Smith 1902)]. At each site, the communities of each successional time period were pooled and placed into sterilized containers. The samples were then cooled on ice packs and brought back to the laboratory, where they were sieved in order to remove large detritus pieces and, for Québec, invertebrate larvae in both early- and late-succession communities. Larvae were also removed by pipetting after careful visual inspection of each sample. Note that no Diptera larvae were found in the Swiss communities. All samples were chilled on ice to slow community dynamics until the start of the experiment (approximately 72 h later). The Swiss communities were transferred to North America under these constant conditions. Every visit to the four sites occurred on the same day, using a detailed protocol for marking, collecting, and storing samples.

On the same day that the experiment was started, fresh mosquito larvae were collected from *S. purpurea* water at the Lac de Joncs site. These larvae were brought back to the laboratory and placed into four replicate water baths for 30 min each. We measured bacterial cell density of each pooled community with a flow cytometer, standardized via dilution with sterilized deionized water. We then set up eight tubes for each successional stage at each site. We used 50-mL sterilized macrocentrifuge tubes containing 2-mL autoclaved glass beads to mimic the insect exoskeletons and detritus naturally found at the bottom of *S. purpurea* pitchers (Gray et al. 2014). Each tube was wrapped with opaque paper until the 25-mL mark to mimic the light availability present inside *S. purpurea* leaves. Using a sterilized pipette (one per treatment community), we transferred 20-mL of *S. purpurea* water containing standardized bacterial density into each macrocentrifuge tube. We then allocated the eight tubes for each site and successional stage into two predator treatments, allowing for four replicates in the no-top-predator and four in the top-predator treatment. In the top-predator treatment, we placed two third-instar

larvae of *W. smithii*, which corresponds approximately to average observed larval densities in mature leaves in northern latitudes (Nastase et al. 1995; Buckley et al. 2003; Hoekman 2007). One milliliter of sterilized fish food solution (5.4 g/L) was added to all samples as a feeding source for the community to standardize the nutrient content. All tubes were placed in a randomized block design for 7 days in an incubator (Sanyo MIR-154), which was programmed to the 50-year average July temperatures of the four sites (Worldclim Data), varying from 10 to 21 °C over 24 h (average temperature 15.5 °C), and natural light conditions. On every day of the experiment, we checked if the mosquito larvae had died or pupated. In such cases, we replaced the dead or pupating mosquito with a new third-instar larva that was collected at the same time and from the same site as the original mosquito. The spare larvae were stored under experimental conditions.

Measurements took place on days 0 and 6 (end). For day 0 (before the initiation of the experiment), we measured the initial protist composition and size spectra, and community respiration across all sites and successional stages. The same measurements, including changes in bacterial density, were taken on day 6. Bacterial density for each treatment was measured with a flow cytometer. Respiration of a 1-mL sample of each community was measured using the Micro-Resp TM system (James Hutton Institute, Scotland) according to the manufacturer's protocol (Carmen 2007). For the determination of protist community structure, an aliquot of 80 μL of each community was used. Observations of presence/absence and size spectra for all protist species were obtained using a compound microscope, with magnification ranging between × 100 and × 400. Furthermore, pictures and videos of the protists were taken to facilitate recognition and determination of the morphospecies and their classification into size spectra. For all morphospecies encountered, we used the reference of Lee et al. (2000) for identification. Cell sizes ranging from ca. 3–5 μm for the smallest species up to 150 μm for the largest species were measured using the pixel-counting feature of the program ImageJ (Rasband 2012). We assigned minimum and maximum cell size to each morphospecies according to Streble and Krauter (2002). Morphospecies were then classified into three size classes: small, <8 μm; medium, 8–40 μm; large, 40–150 μm. When dealing with colonies of protists, the size of a single cell was measured and the species were grouped into one of the three size classes accordingly. Note that we concentrated on species composition because it is already known that *W. smithii* has a strong effect on the abundance of protists (Addicott 1974; Hoekman 2007, 2010; Gray et al. 2014). Our choice was further motivated by the current interest in the impact of invaders on the diversity and species composition of resident communities (e.g., Hector et al. 2002; Fargione and Tilman 2005).

Statistical analyses

The difference in protist community composition when a top predator was present or absent (hypotheses 1 and 2) was determined with non-metric multidimensional scaling for early- and late-succession among each of the four sites on days 0 and 6 (Legendre and Legendre 1998); non-metric multidimensional scaling analyses were based on Jaccard distance matrices and performed using Primer 6.1 [version 6.1.6; Primer E 2006 (Clarke and Gorley 2006)]. We used two dimensions, which always resulted in stress values smaller than 0.08. We also conducted an analysis of similarity and a multivariate dispersion analysis for these treatments. Analysis of similarity was used to measure the compositional distance between replicate communities with and without predators; the output of the method is a global R-value, which indicates significantly different communities when $R > 0.5$. Multivariate dispersion provides an estimation of the dispersion of the replicates within each treatment; it computes an index of multivariate dispersion, a measure of the difference in dispersion between two treatments, where negative values indicate lower dispersion of communities with predators compared to communities without predators.

The effects of the top predator, successional stage, and naive/non-naive status of the community were evaluated with Gaussian generalized linear models on the following response variables: (1) the change between the start and the end of the experiment in protist species richness and body-size distribution of three body-size classes (hypothesis 3); (2) bacterial density (hypothesis 4); and (3) respiration (hypothesis 5) at the end of the experiment. All model residuals were checked for normality with quantile–quantile plots; no data transformation was necessary. For each analysis, we performed a model selection and we present the results for the model that yielded the lowest Akaike information criterion value. Due to small sample size, we further checked our results with the corrected Akaike information criterion; we found no difference in model selection based on both criteria. Analyses were performed in R (R Core Team 2013).

Results

Protist community composition

The presence of the top predator significantly affected protist community composition at the end of the experiment, but only when the community was naive to top predators and at an early stage of succession (Fig. 1; Table 1). Dispersion tests yielded significant values for these naive, early-succession communities (early Les Embreux and early Les Tenasses;
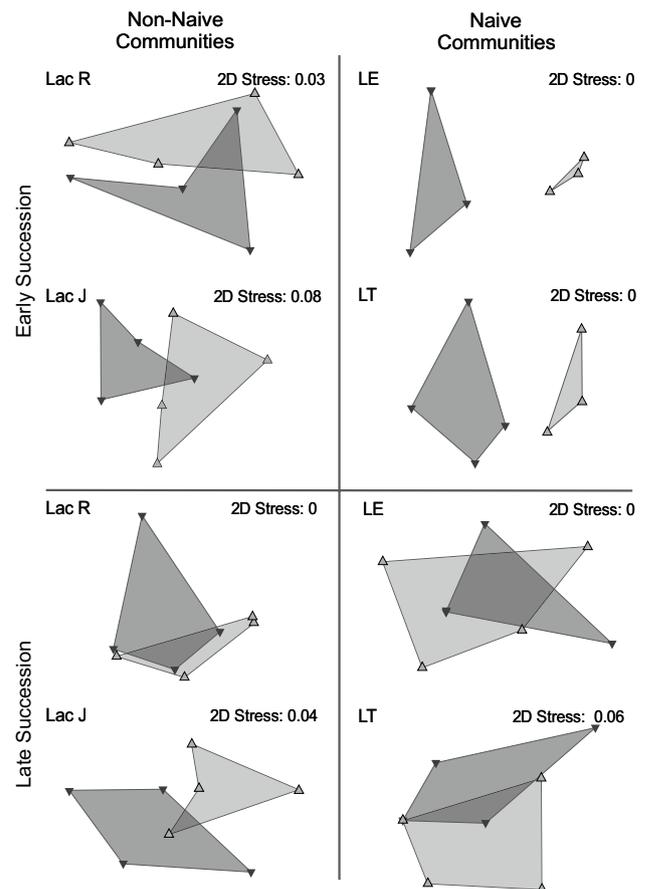
Fig. 1 Change in protist community composition. Each *triangle* represents one community in a two-dimensional (*2D*) non-metric multidimensional scaling plot [predator present (*gray triangle* and *light-gray shading* ($n = 8$ for each sampling site/$n = 32$ in total); predator absent (*black inverted triangle* and *dark-gray shading* ($n = 8$ for each sampling site/$n = 32$ in total)]. Nearby *triangles* have similar species composition [in the case of identical community composition, the symbols representing these communities completely *overlap*; see Les Embreux (*LE*) and Les Tenasses (*LT*) early-succession]. The *shaded envelopes* were added to help visualize: (1) the amount of community composition overlap that occurred between the predator/no predator treatments, and (2) how similar community composition was within a treatment (the *smaller* the *shaded area*, the more similar the communities are to each other). The presence of the top predator only significantly changed protist composition in naive, early communities (see Table 1). *LacR* Lac Rimouski, *LacJ* Lac des Joncs, *LE* Les Embreux, *LT* Les Tenasses

see Table 1). Interestingly, when the predator was present, the four replicated early-succession communities were very similar in composition within the two naive sites. It therefore appears that the presence of a top predator results in the composition of naive, early-succession communities converging in similarity (strongly negative values for index of multivariate dispersion in both groups; see Table 1). The composition of the non-naive communities and of all late-succession communities was not affected by the presence of a top predator.

Table 1 Results of analyses of similarity (ANOSIM) and multivariate dispersion (*MVDISP*) for the naive and non-naive communities, in early- and late-succession

Succession	Site	Global *R*	*p*-value	MVDISP with predator	MVDISP without predator	IMD value
Early	Non-naive (LacR)	−0.302	0.943	1.051	0.949	0.111
Early	Non-naive (LacJ)	−0.089	0.629	1.231	0.769	0.5
Early[a]	Naive (LT)[a]	**0.474**	**0.029**	**0.782**	**1.218**	**−0.472**
Early[a]	Naive (LE)[a]	**0.854**	**0.029**	**0.769**	**1.231**	**−0.5**
Late	Non-naive (LacR)	−0.036	0.686	0.923	1.077	−0.167
Late	Non-naive (LacJ)	0.177	0.171	0.859	1.141	−0.306
Late	Naive (LT)	−0.115	0.857	1.013	0.987	0.028
Late	Naive (LE)	−0.214	0.971	1.128	0.872	0.278

ANOSIM measures the compositional distance between communities with and without predators. A global *R*-value >0.5 indicates biologically significantly different communities. MVDISP provides an estimation of the dispersion of the replicates within each treatment; index of multivariate dispersion (*IMD*) is a measure of the difference in dispersion between the two predation treatments; *negative values* indicate lower dispersion of communities with predators compared to the communities without predator

Non-naive Québec sites [Lac Rimouski (*LacR*) and Lac de Joncs *(LacJ)*], *Naive* Swiss sites [Les Tenasses (*LT*) and Les Embreux (*LE*)]

[a] Early naive sites with boldface type indicating a global *R* value approximately equal or larger than 0.5

Change through time of protist community structure

The change in protist morphospecies richness from the beginning to the end of the experiment (Δ species richness) was independent of the presence of the top predator (Fig. S1a Supplemental Material). However, the change in species composition, measured as Jaccard distance over time, was affected by the presence of top predators. This change was particularly strong in early-succession (Fig. 2a) and in naive (Fig. 2b) communities, where communities containing a top predator became significantly different in protist composition compared to communities with no top predator. Globally, the top-predator-induced change in species composition was driven by nine out of 36 morphospecies, all of which belonged to the small- and medium-size classes. Four morphospecies were negatively affected, but interestingly this number was counterbalanced by five species that increased in occurrence in the treatments that contained top predators (see Fig. S2).

Species composition differed among sites and successional stage at the start of the experiment (Fig. S3). Although the compositions were different, there was generally an equal number of small- and medium-sized protists within early- and late-succession communities (naive and non-naive communities pooled); however, there were less large-sized protists in early-successional than late-successional communities (see legend of Fig. S3; Table S1 for details). At the end of experiment, the presence/absence of the predator had no statistically significant effect on the total number of morphospecies (Fig. S1a), or on the number of morphospecies in the three different size classes (all *p*-values >0.33). Only naivety status had a detectable influence for the medium-size class (Fig. S1b), in which
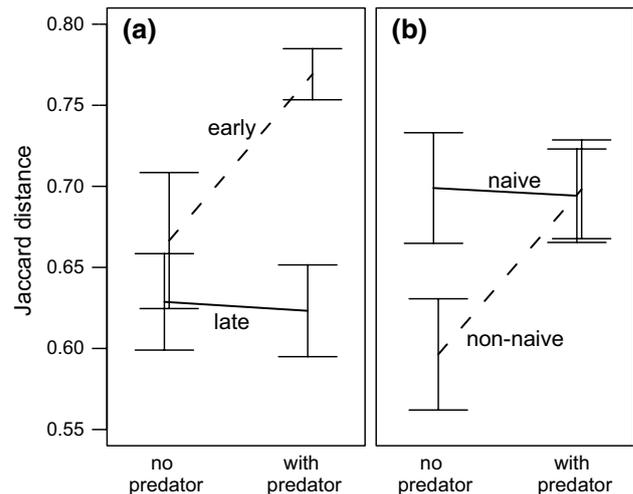
Fig. 2 Effect of succession, naivety status and top predator on protist community composition. Interaction plots for the distance in community composition (measured by Jaccard index comparing communities at the beginning and at the end of the experiment) for **a** early- (*dashed line*; n = 32) and late-succession (*solid line*; n = 32) communities, and **b** for naive (*dashed line*; n = 32) and non-naive (*solid line*; n = 32) communities, with and without the top predator. *Error bars* represent ±1 SE. Results of a Gaussian generalized linear model yield a significant effect for the presence of the top predator (parameter = 0.16, *p*-value = 0.003), and naivety status (parameter = 0.10, *p*-value = 0.017), but not of the successional stage (parameter = −0.04, *p*-value = 0.367)

there was a net loss of medium-sized species during the experiment for communities originating from Switzerland (naive), and a small gain in medium-sized species for communities originating from Québec (non-naive). There was also a marginally significant effect of succession and of

the interaction between origin and succession for medium-sized species.

Trophic regulation and ecosystem functioning

The effect of the top predator did not propagate to the bottom trophic level. There, the top-predator effect on bacterial density was statistically insignificant in both naive and non-naive communities (p-value $= 0.119$) and in early- and late-succession (p-value $= 0.547$; see Table S2 for details). This lack of a statistically significant effect on bacterial density was consistent with the results for bacterial respiration: we found that the top predator had no impact on respiration rate in naive and non-naive (p-value $= 0.32$) and in early and late communities (p-value $= 0.61$; see Table S4 for details). Instead, the only significant result was that non-naive communities respire less than naive ones ($p = 0.038$; Fig. S4a and see Table S3 for details). We also found a significant interaction between succession and naivety, in which respiration increases when communities transition from early- to late-succession in naive communities, but decreases in non-naive communities ($p = 0.041$; Fig. S4b and Table S3).

Discussion

We found that the composition of early-succession Swiss (naive) protist communities diverged significantly from communities with no top predator present. This result supports our first (naivety status) and second (successional stage) hypotheses, but only in the naive, early-succession treatment, where we had expected the strongest effect. Indeed, there was no evidence of a difference in protist composition induced by the predator between early- and late-succession for non-naive communities. This result can be explained simply by the fact that early-successional communities in Québec had already experienced the presence of *W. smithii*, and thus went through the biotic filter imposed by the predator. Indeed, *W. smithii* lay eggs in pitchers directly after they open (Smith 1902; Istock et al. 1975), so that protists experience predation early during community development. In the non-naive range, only species tolerant to *W. smithii* were likely to survive in the pitchers until sampling for the experiment took place.

Interestingly, we also found no evidence of a change for late-succession communities in the naive sites, suggesting that the filtering process of succession in the *S. purpurea* leaves selects species that are not only competitively superior, but also resistant to predation. We further found that the presence of a top predator did not change the size distribution of protist species, as was predicted.

Surprisingly, the effect on species composition did not propagate to the bottom trophic level, as found for freshwater systems (e.g., Shurin et al. 2002), nor did it affect ecosystem functioning. Overall, our results highlight the importance of considering the successional stage of communities, which is rarely discussed as having a large impact on invasion at the multi-trophic level. For example, in the extensive review on invasion research by Lowry et al. (2013), successional stage was not used as a criterion to classify the surveyed studies.

In Europe, where *S. purpurea* was recently introduced, the inquiline species had to make a transition from the bog habitat to that of leaves. Only a subset of species has made this transition (Gebühr et al. 2006; Fragnière 2012), and these species had neither co-evolved with the plant, nor had they experienced *W. smithii* predation. Every year when new leaves open, pioneer species randomly colonize and pass through the environmental filter imposed by the leaf habitat (e.g., air barrier to other aquatic habitats, high variability in temperature, pH and nutrients inside the leaf). Priority effects imposed by the already established inquiline species can then limit those arriving later (e.g., Kadowaki et al. 2012). These "neutral" factors are probably paramount for the organization of these early communities. Interestingly, in these naive, early-succession communities, in addition to the compositional change induced by the predator, we observed a strong convergence in morphospecies identity. This suggests the importance of predators in constraining the trajectories of community assembly in systems that are naive to predation.

We also hypothesized that the European late-succession communities should be affected by insect predation because they did not have time to develop strategies to cope with consumers, and thus vary in their response to predation. However, our results do not support this hypothesis, suggesting that protist species were equally tolerant to *W. smithii* in this successional stage. Since we did not observe a change in respiration and bacterial density between European and Canadian late-succession communities when a predator was present, naive communities appear to be as resistant to predation as non-naive ones. This result underlies the hypothesis that mechanisms of avoidance/tolerance against one predator are also effective against other similar types of filter-feeding predators (Anson and Dickman 2013).

Our results are consistent with other *S. purpurea* studies conducted in northern latitudes. From field experiments performed in Michigan (non-naive, native range, with a similar temperature range as our experiment), Hoekman (2007, 2010) found that, although protozoan biovolume greatly decreased in the presence of the mosquito top predator, bacterial density was unaffected. Further, Hoekman (2007) found that the species richness of non-naive prey

communities was affected, but only when 20 mosquito larvae were added to the system, which corresponds to high mosquito density in a natural setting (Nastase et al. 1995). It is likely the top predator would have affected the species richness of the non-naive communities in our study if more larvae had been added to the system (20 larvae vs. two larvae per community). Our results imply that the addition of only two mosquito larvae is sufficient to affect naive, early-succession communities. In this respect, the changes in the occurrence of species can be directly caused by predation, but also indirectly by a competition/predation trade-off favoring less-competitive species (Kneitel 2012).

We used a whole community (bacteria and protists) microcosm system to answer a question of general importance for conservation biology that is especially relevant at the ecosystem level. Simplified versions of larger-scale communities can provide tractability and high statistical power (Srivastava et al. 2004), allowing us to better understand what mechanisms may drive dynamics in more complex systems (Jessup et al. 2004). In this respect, we want to emphasize the importance of working with natural communities, where species have adapted to each other, so that they are more likely to epitomize larger-scale systems. Furthermore, our system is microbial, species of which are likely to evolve quite rapidly (Jessup et al. 2004). This characteristic of microbial systems makes it possible that the naive, early-successional communities in our study could have become more tolerant of the top predator if the experiment had been longer. The fact that the protist species were still vulnerable after 6 days suggests that larger-scale systems may take a long time to adjust to environmental changes. Our results also point to the importance of working with different successional stages. The observed effects of the top predator are subtle in our case, affecting only early-succession communities. Human activities such as deforestation and intensive agriculture reset many habitats worldwide to an early-successional stage. Since the effects of novel predators appear to be dependent on the successional stage (e.g., Estes et al. 2011), it is therefore necessary that conservation research consider information about the succession of the ecosystem.

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