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COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Disentangling the roles of diversity resistance and priority effects in community assembly

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Abstract The assembly of many biological communities is constrained by the resistance exerted by resident species to immigration (biotic resistance). Two important mechanisms contribute to the generation of biotic resistance: diversity resistance and priority effects. These mechanisms have been explored through theoretical models and laboratory experiments, but the importance of their interplay in the assembly of natural communities remains untested. We used a mesocosm experiment with communities of aquatic plants and zooplankton assembled from natural propagule banks to test whether and how diversity resistance, mediated by the diversity of the resident community, and priority effects, mediated by the timing of immigrants' arrival, affect the establishment of immigrant species and community diversity. In plant communities, immigration success decreased with increasing resident-species richness (diversity resistance) and arrival time (priority effects). Further, diversity resistance was stronger in communities colonized later in the season, indicating that these mechanisms interacted to reinforce biotic resistance. This interaction ultimately determined species richness and beta-diversity in plant communities. For zooplankton, in contrast, neither the diversity of resident communities nor the time of arrival affected the establishment of immigrant species. In these communities, beta-diversity was explained by species sorting, namely biotic effects mediated by plant assemblages. Our results show that the progressive buildup of communities generates an interaction between diversity resistance and priority effects that eventually determines community diversity, unless species sorting mediated by environmental filtering supersedes the effect of biotic resistance. Therefore, disentangling the mechanisms underlying biotic resistance contributes to understand how species diversity is ultimately determined.

Keywords Biotic resistance · Immigration · Species diversity · Macrophytes · Zooplankton

Introduction

Community structure and dynamics are driven by the balance between competitive (deterministic) and stochastic exclusion of species (Tilman 2004; Gravel et al. 2006). However, disentangling deterministic and stochastic community outcomes has been a major challenge in modern ecology (Chase and Myers 2011; Vellend et al. 2014), as assembly processes are difficult to quantify and the history of colonization during early assembly stages is difficult to assess at later (stable) stages where other assembly processes, such as environmental filtering, might supersede chance colonization patterns (Fukami and Morin 2003; Pu and Jiang 2015). Further, both chance colonization and ecological drift introduce stochasticity in community composition, and their relative importance in explaining compositional variation is difficult to disentangle (e.g. Chase

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2007). Therefore, the study of historical processes is an important research avenue in community ecology (Chase 2003; Fukami and Morin 2003; Chase and Myers 2011).

The history of colonization is particularly determinant when the species that arrive first in the habitat patch are maintained, and not excluded by later immigrants, across time until a possible equilibrium state in species composition is reached. The maintenance of initial compositional states may result from biotic resistance, in which the pioneer, resident community prevents immigrant species from colonizing the habitat patch. Resistance to immigration has been proposed to be stronger (1) in resident communities with higher diversity, a process known as "diversity resistance" (Elton 1958), and/or (2) at later stages of community assembly, due to competitive exclusion by already-established populations/species, a process known as "priority effects" (sensu Drake 1991).

Species are not likely to arrive simultaneously in a given habitat patch; thus the sequence and history of arrival maintained by priority effects have been demonstrated to affect species establishment and community composition (Drake 1991; Chase 2003; Mergeay et al. 2011). The dominance of early colonizers (priority) is manifested through demographic effects, since the reproduction and growth of already-established species may limit the availability of resources and/or generate inadequate conditions to potential immigrants. If the competitive advantages of later immigrants are ineffectual against well-established founders, biotic resistance prevails over niche requisites to determine community composition (e.g. Berlow 1997). Therefore, early-arriving species (founders) might be maintained by priority effects, so that the history of colonization determines community structure at each habitat patch (Chase 2003; Allen et al. 2011). Given that dispersal from the regional pool of species is often stochastic, priority effects may result in high compositional variation among local communities (i.e. high beta-diversity), particularly if alternative stable community states characterized by the dominance of different pioneer species assemblages in otherwise similar habitats can be produced (Shurin et al. 2004; Jiang et al. 2011).

As the number of species increases during community assembly, diversity resistance might also contribute to maintain initial compositional states. Diversity resistance has been studied in the context of invasion biology, mainly in plants (e.g. Kennedy et al. 2002), but also in the aquatic environment such as in zooplankton communities (Beisner et al. 2006). The resistance of communities to invasion by exotic species or immigration by native species is expected to decrease with increasing resident-species diversity (Levine and D'Antonio 1999; Levine et al. 2004; Davis 2005). Although this mechanism relies on a clear negative relationship between resident-species richness and the number of successful colonizers, positive and null relationships have also been reported, especially when effects are analyzed over different spatial scales (Fridley et al. 2007). Depending on the strength of resistance (i.e. on the number of resident species) and the carrying capacity of the habitat patch, the establishment of immigrants might result in either increased species richness if their niche is complementary to that of residents, or stable and even decreased species richness if immigrants outcompete and replace residents (Case 1990, 1991).

Diversity resistance and priority effects might act simultaneously during community assembly, since the number of species in a given community is expected to increase as species arrive. Hence, the magnitude of the priority effect might depend on the number of already-established species (Jiang et al. 2011). The interplay between priority effects and diversity resistance in determining the assembly and diversity of naturally assembled communities remains poorly studied. Seasonal aquatic communities provide excellent model systems for this purpose, since communities are assembled by organisms that emerge from propagule banks and by immigrants arriving from the regional pool of species throughout the growing season—a process easy to reproduce under mesocosm conditions.

We used a mesocosm experiment that assembled communities of aquatic plants and zooplankton from propagule banks collected in natural aquatic ecosystems to investigate whether two mechanisms of biotic resistance, priority effects and diversity resistance, hamper the establishment of immigrant species and ultimately determine community diversity. First, we assessed the independent and interactive effects of diversity resistance and priority effects on community invasibility by varying the timing of the colonization attempts (different inoculation times along the growing season) and the diversity of the resident community. We expected increasing biotic resistance via diversity resistance and priority effects to, independently or interactively, decrease the number of immigrant species. Second, we investigated whether different levels of biotic resistance lead to different diversity patterns. We hypothesized that biotic resistance (1) affects the species richness (i.e. alpha-diversity) of developed communities if immigrants are added to the community or cause the extinction of resident species, rather than simply replacing resident species (Case 1990, 1991) and (2) affects community compositional variation (i.e. beta-diversity), as less resistant communities are more permeable to homogenization promoted by immigration from the regional pool of species. Third, we assessed whether the abiotic and biotic environment could affect community composition due to species sorting, possibly obscuring biotic resistance processes.

Materials and methods

Experimental approach

This experiment builds on the opportunities provided by a restoration project that, based on an adaptive approach, recreated in 2005 an experimental array of ponds of identical size and shape in an area of marshland reclaimed for agriculture in the late 1960s (Santamaría et al. 2005). The area was first flooded by natural rainfall in the winter of 2005–2006 and natural colonization took place over the following years.

In October 2007, we selected seven ponds, out of 24 monitored in the previous years, of varying aquatic plant species richness: from 0 to 7 species, spanning the whole range of species richness at the 24 ponds after 2 years of colonization. We then collected samples of the upper layer of sediment (8 samples of 5 cm depth and 1 m^2 surface) at the pond's centre and edges to build our "resident" mesocosm communities. For each pond, all sediment samples were thoroughly mixed, subdivided in four fractions and used as sediment and propagule source in each of the four separate mesocosms, making up a total of 28 tanks (4 × 7 ponds; see experimental design in Fig. 1). Thus, resident communities in the different mesocosms emerged from the source sediment. Mesocosms were set in 680 dm^3 tanks (110 × 95 × 65 cm) filled up with approximately 550 L of propagule-free freshwater and covered with a mesh screen (1 × 1.5 mm mesh size) to reduce colonization by wind- or bird-transported propagules. For an additional set of four tanks, we used commercial potting clay as sediment to estimate invasion rates under fully vacant conditions and control for undesired colonizations (e.g. propagules transported by wind) in the treatment without subsequent inocula ("noSnoI", see below). Each of the four tanks, set for each of the seven types of sediment, was assigned to a different treatment, corresponding to the timing of a single inoculation event (three treatments): inoculation at the moment of tank filling, "I0", 2 months later, "I2", 4 months later, "I4", plus a control (no inoculation, "noI"). The inoculum representing the set of potential colonizers from regional natural wetlands was created by sampling and thoroughly mixing sediment samples from three neighbouring, mature wetlands known to host different plant and zooplankton species. Equal-volume aliquots were prepared at the moment of tank filling and preserved dry until the moment of inoculation. The mixture of dry sediment inoculated at each mesocosm included the seeds and resting eggs of plants and zooplankton present in the propagule bank and acted as inoculation agents. We chose these neighbouring wetlands as propagule sources because they represent the most likely source of colonizers for the restored wetlands used to establish the resident communities.

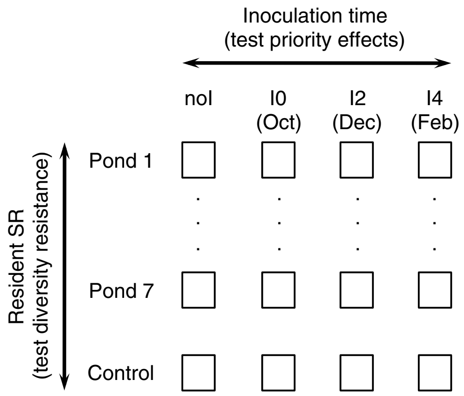

Fig. 1 Diagram representing the experimental design. *Squares* represent mesocosm communities. Each *pond* (from 1 to 7) represents the set of four mesocosm communities built from the propagules contained in the sediment collected in different source natural ponds

At the time of each inoculation, we measured the vegetation cover using a grid of 48 points equally spaced to cover the complete surface of the mesocosm and collected samples of zooplankton by filtering through a 90 µm mesh, and 6 L of water was collected across the whole water column. A final assessment of the plant and zooplankton communities took place 2 months after the last inoculation (in April 2008), 6 months after the onset of the experiment, using the same procedures. In addition, we identified the different charophyte species over the whole surface of the mesocosms based on laboratory inspection of sampled specimens, which was not possible in previous surveys (see species lists in Online Resource 1, Tables S1, S2). The species composition and diversity of mesocosm communities matched those of source pond communities (personal observation for aquatic plants; see Badosa et al. 2010 and Frisch et al. 2012 for zooplankton). The low species richness in source ponds, and in turn in the mesocosms, can be explained to some extent by dispersal limitation, as many species from the regional pool might not have had sufficient time to colonize the restored ponds (Frisch et al. 2012). Hence, fast colonizers might be overrepresented in our resident mesocosm communities. Immigrant species established at each mesocosm were identified as those found in the post-inoculum surveys that were absent from the resident community throughout the entire season, i.e. absent from the noI control tanks in all surveys and from the I0, I2 or I4 tanks before their inoculation. We show the detailed data matrix of resident and immigrant species per mesocosm in Online Resource 2. The composition dynamics of resident species was assessed, and controlled for, in each control tank ("noI"; for the seven ponds) by assessing whether the number of species became saturated along the growing season. The number of species saturated before the last survey in every tank, except for plants in one tank, meaning that the probability of identifying immigrants as residents is negligible. A few zooplankton species appeared in the noSnoI tank (i.e. propagule-free sediment and no subsequent inoculum), probably as "accidental immigrants". Although mesocosms were not close to the source ponds of resident (>3.5 km) and immigrant (>5.5 km) propagules, there were ephemeral ponds and wetlands in the immediate neighbourhood (<500 m). This probably explains the origin of the accidental invasions, probably through transportation by wind, as all but one were copepods and rotifers (see Online Resource 1, Table S2). We excluded these species from all analyses, to make sure that we truly dealt with resident and inoculated zooplankton species. However, to inspect possible biases derived from this exclusion, we also present the results for the analyses that included these accidental invaders (Online Resource 3). To control for possible variations in the abiotic environment that could affect our results, we also measured in each tank conductivity, pH and dissolved oxygen with a Hach-Lange HQ40d portable digital instrument, turbidity with a nephelometer (Hanna HI93703) and chlorophyll *a* by filtering a known volume of water through a 0.7 μm pore-size glass microfiber filter, extracting chlorophyll in 90 % acetone and measuring its concentration by fluorometry (Welschmeyer 1994).

Data analysis

Effect of biotic resistance on immigration success

Biotic resistance was assessed by testing the effect of the two possibly interacting mechanisms: diversity resistance, tested by varying the resident-species richness, and priority effects, tested by varying the time of inoculation (Fig. 1). We were only able to perform this analysis for the I2 and I4 treatments, since at the time of inoculation of the I0 mesocosms (the onset of the experiment), species from the resident community had not yet emerged. For aquatic plants, we used the data from the last survey (end of the experiment) because plants do not show short-term temporal dynamics in species composition, but rather a continuous buildup along the growing season (see dynamics in Online Resource 1, Fig. S1). Moreover, using the last survey allowed us to distinguish charophyte species (after collecting all the biomass in each tank), increasing the resolution of our analysis. For zooplankton, we used the survey immediately after each inoculation (i.e. 2 months after), because these organisms showed temporal dynamics along the growing season (see dynamics in Online Resource 1, Fig. S1) and, thus, by using the last survey, we would have risked excluding early-senescing species from the number of established immigrants. For this analysis, we also considered already-established "accidental immigrants" as resident species, as they could exert biotic resistance over immigrant species.

To determine the effects of resident-species richness and inoculation time on the number of immigrant species that became established, we fitted generalized linear models (GLM) with Poisson error distributions and log link functions. The GLMs included the number of immigrant species as the dependent variable, and the number of resident species present at the time of inoculation, inoculation time and their interaction as independent variables. In addition, we confronted the effect of community density, measured as the summed abundance of all emerged resident species, against diversity resistance to investigate their relative importance in explaining immigration success. For this purpose, we compared the explanatory power of the different models via the second-order Akaike information criterion (AICc) and assessed statistical significance of model terms by performing likelihood ratio tests (LRT). To discard possible leverage effects due to low sample sizes, we performed a leave-one-out cross-validation procedure. The potential (confounding) effects of abiotic factors were tested separately by fitting single-predictor GLMs to the number of immigrant species established. Although multiple regression was not possible due to sample size problems, we did not find evidence of collinearity (all correlation coefficients among environmental variables were lower than 0.31). All models described above were performed using the R base package (R Development Core Team 2014).

Effect of biotic resistance on community diversity

Total species richness at the end of the experiment was the result of the number of resident species that emerged throughout the growing season, including those that could still emerge after the inoculation of immigrants and the number of immigrant species that became established. To assess whether biotic resistance affected total species richness by regulating the number of immigrant species, we proceeded in two steps. First, we tested whether different levels of biotic resistance led to variation in total species richness, using a linear model with two predictors, the time of inoculation and the number of resident species that had emerged at the time of inoculation. This allowed us to test whether biotic resistance resulted in differences in total richness, but not in the relative contributions of the immigrant and resident fractions, generated by the addition of immigrant species and the maintenance or reduction of resident species. Second, we estimated the relative contribution of resident and immigrant fractions by regressing the total species richness on each richness fraction. In addition, we used linear mixed models (LMM) with inoculation time as a fixed effect and pond (origin of the sediment) as a random effect to compare total species richness differences between all inoculation time levels (I0, I2, I4 and noI) while controlling for differences in initial resident-species richness and composition. Statistical significance was assessed by performing likelihood ratio tests (LRT). The same type of model was used to test for possible abiotic effects (one model for each abiotic predictor). These statistical analyses were performed using the R base package (R Development Core Team 2014).

Effects of biotic resistance and environment on community composition

Most measures of compositional variation (beta-diversity) are not independent of the variation in alpha-diversity (i.e. local species richness), since beta-diversity tends to increase with increasing alpha-diversity due to purely stochastic effects (Chase et al. 2011). To account for the effect of alpha-diversity, we used the Raup–Crick index (Chase et al. 2011), which measures species turnover based on a randomization procedure in which species are assigned randomly to each site (here, each "mesocosm") up to the observed species richness. The estimated turnover index measures the degree to which species composition is more or less similar to that expected by random chance. The effect of biotic resistance on compositional variation was assessed by performing non-metric multidimensional scaling (NMDS) and comparing the degree of clumpiness between the sets of mesocosms inoculated at different times. To test whether the abiotic and, in the case of zooplankton, biotic environment could explain species compositional variation, we used distance-based redundancy analysis (dbRDA; Legendre and Anderson 1999). Because dbRDA uses positive distances, we standardized the Raup–Crick index, which ranges from −1 to +1, by subtracting the minimum value in the distance matrix to each value and dividing each of the resulting values by their maximum (causing the distances to vary between 0 and 1; see Stegen et al. 2013). The abiotic matrix consisted of a reduced set of environmental variables selected by means of a forward selection procedure, using the adjusted coefficient of determination (R^2) and the alpha-level (=0.05) as stopping criteria (according to Blanchet et al. 2008). Biotic effects in zooplankton communities were estimated using co-correspondence analysis (CoCA; ter Braak and Schaffers 2004). CoCA estimates the covariance in species composition of the two taxonomic groups, predicting the species composition of one group (zooplankton) based on the species composition of the other group (plants as the explanatory matrix). The CoCA ordination axes with site scores that produced the maximum prediction accuracy were used as plant-composition variables in the zooplankton RDA (see Viana et al. 2016 for a similar approach). These analyses were performed using the R (R Development Core Team 2014) packages *vegan* (Oksanen et al. 2013) and *cocorresp* (Simpson 2009).

Results

Effect of biotic resistance on immigration success

In aquatic plant communities, we found a significant interaction between the number of resident species and inoculation time (Fig. 2a; $\chi^2 = 5.848$, $df = 1$, $P = 0.016$), indicating that the effect of resident-species richness on colonization success became stronger (i.e. the slope of their relationship became significantly steeper) for late (I4) as compared to early (I2) inoculations. The result was robust against possible outliers, as confirmed by leave-one-out

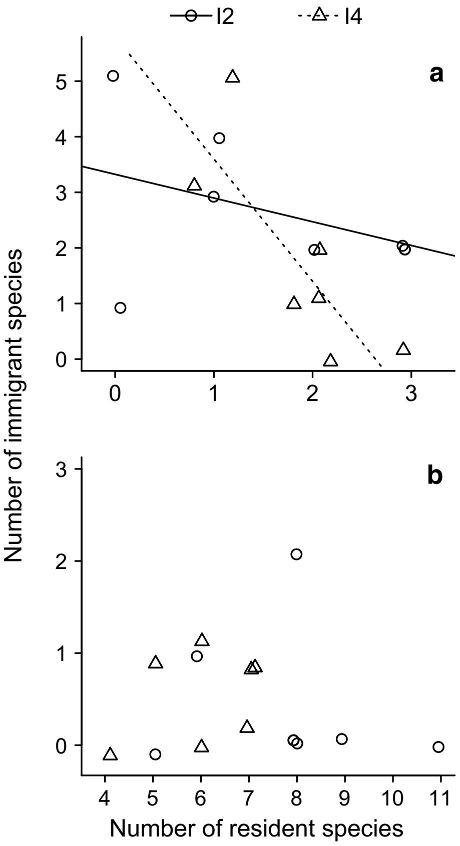

Fig. 2 Effects of immigrants' inoculation time (test for priority effects), after 2 months (I2) and 4 months (I4), and number of resident species (test for diversity resistance) on the colonization success of aquatic plant (**a**) and zooplankton (**b**) immigrant species (number of established immigrant species in each mesocosm; $n = 14$). The slopes of the regression lines are statistically different, as tested by the interaction between inoculation time and number of resident species (see "Results"). Note that some "jitter" has been added to the data points to avoid symbol overlap

cross-validation, and even when the most influential data pair was removed, the interaction remained significant. Competing models that included plant density as a predictor had higher AICc (>2; Table 1), meaning that the model with the interaction between inoculation time and number of resident species explained more variation in immigration success. For zooplankton, neither interactive nor additive models were significant (Table 1); thus the inoculation time, number of resident species and zooplankton density did not affect the establishment of immigrant species (Fig. 2b). The latter result was the same when all species, including the 11 "accidental immigrants" found in the noS-noI control tank, were included in the immigrants count (see Online Resource 3, Table S1). We did not detect any statistically significant effect of abiotic variables on the colonization success of both plant and zooplankton immigrants (see results in Online Resource 1, Table S3).

Table 1 Statistical results from the analysis of the effect of biotic resistance on the colonization success of immigrants (GLM with Poisson error distributions and log link functions)

Taxon	Model	AICc	χ^2	*df*	*p*
Plants	IT + SR + IT*SR	52.32	11.06	3	0.011
	IT + D + IT*D	57.501	5.88	3	0.118
	D + SR + D*SR	56.626	6.751	3	0.08
	IT + D + SR	56.816	6.561	3	0.08
Zooplankton	IT + SR + IT*SR	36.903	0.632	3	0.889
	IT + D + IT*D	37.069	0.465	3	0.926
	D + SR + D*SR	33.772	3.762	3	0.288
	IT + D + SR	37.309	0.226	3	0.973

Statistical tests correspond to likelihood ratio tests (LRT) between the null model (model with only an intercept term) and the defined model

IT inoculation time, *SR* number of resident species, *D* community density (summed abundance of all species)

Effect of biotic resistance on community diversity

In plant communities, the inoculation time had a negative effect on total species richness (Fig. 3a; $F_{1,11} = 9.45$, $P = 0.011$). As the season progressed, inoculation resulted in smaller increases in total species richness, i.e. fewer immigrants became established (Fig. 3a; I0 > I2 > I4 = noI; pairwise comparisons were assessed by post hoc *t* tests with Holm's sequential Bonferroni correction). The growing number of emerging resident species throughout the season (Fig. 3c) tended to reinforce the negative effect of a delayed inoculation on the establishment of immigrants, though this relationship was only marginally significant ($F_{1,11} = 4.61$, $P = 0.055$). Variation in species richness was mediated by the fraction of immigrant species ($F_{1,19} = 16.94$, $P < 0.01$, $R^2 = 0.44$) rather than the fraction of resident species that

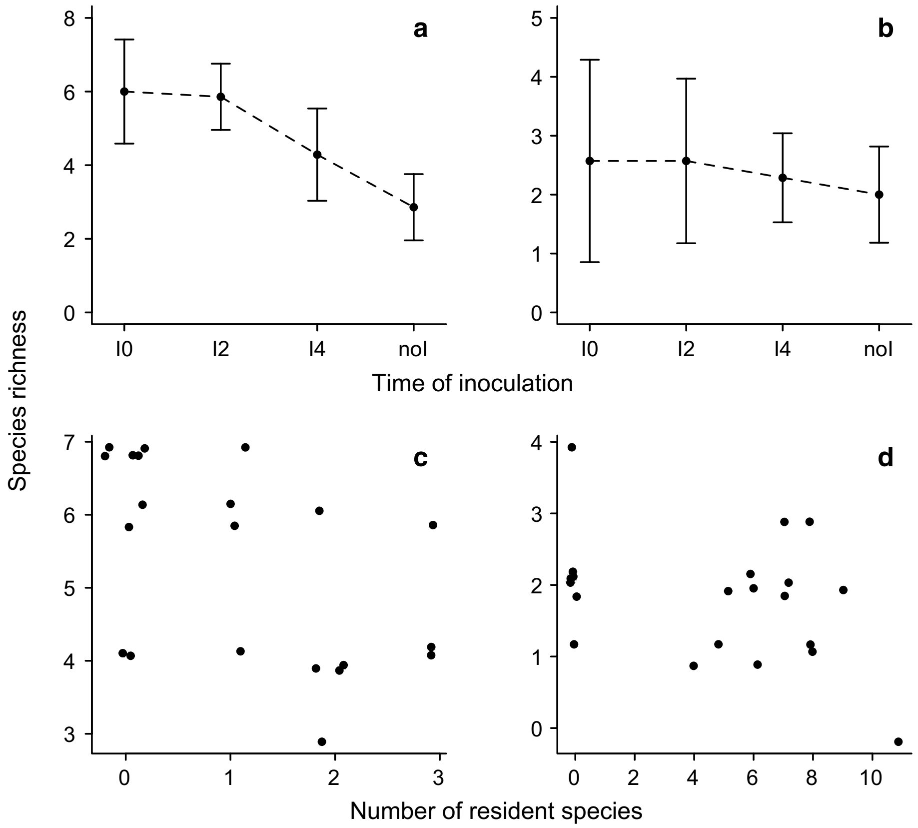

Fig. 3 Effect of time of inoculation (I0, I2, I4, noI; **a**, **b**; *n* = 7) and number of resident species (**c**, **d**; *n* = 21; note that some "jitter" has been added to visualize overlapping points), on the species richness of aquatic plants (*left panels*) and zooplankton (*right panels*). Species richness (mean ± SE in the *upper panels*) was based on data from the last survey (April 2008). *I0* inoculation at the beginning of the growing season (i.e. moment of tank filling); *I2* inoculation 2 months later; *I4* inoculation 4 months later; *noI* control (no inoculation). *Letters* above *error bars* in the *upper panels* indicate statistically significant differences

emerged throughout the growing season ($F_{1,19} = 0.62$, $P = 0.44$, $R^2 = -0.02$), indicating that biotic resistance ultimately limited species richness in mature communities. These results did not vary across resident communities, as tested by the LMM that included the source ponds with different diversity and composition as a random effect ($\chi^2 = 25.938$, $df = 3$, $P < 0.001$).

In contrast to plants, the richness of zooplankton communities (excluding accidental invaders) was neither affected by the inoculation time ($F_{1,11} = 0.83$, $P = 0.38$; Fig. 3b), even when accounting for variation in the identity of source ponds (LMM; $\chi^2 = 4.843$, $df = 3$, $P = 0.184$), nor the number of resident species ($F_{1,11} = 0.01$, $P = 0.91$; Fig. 3d). Similar qualitative results were obtained when running the analysis including the accidental zooplankton invaders (see Online Resource 3, Table S2). The fraction of both immigrant species ($F_{1,19} = 22.76$, $P < 0.01$, $R^2 = 0.52$) and resident species emerging throughout the season ($F_{1,19} = 12.76$, $P < 0.01$, $R^2 = 0.37$) contributed significantly to the total species richness.

Effects of biotic resistance and environment on community composition

Plant communities showed less compositional variation (i.e. lower beta-diversity) in communities invaded at the beginning of the growth season (I0 and I2), i.e. these communities tended to cluster, as compared to non-invaded communities (noI) and those invaded later (I4) (Fig. 4a). In contrast, zooplankton communities showed the inverse result, i.e. more compositional variation in early-invaded communities (Fig. 4b). The composition of plant communities was not affected by the abiotic environment ($F_{5,22} = 1.03$, $P = 0.43$), whereas the composition of zooplankton communities was related to the plant assemblage ($F_{2,25} = 1.03$, $P < 0.01$, $R^2 = 0.43$). Similar qualitative results were obtained when running the analysis including the accidental zooplankton invaders (see Online Resource 3, Fig. S1).

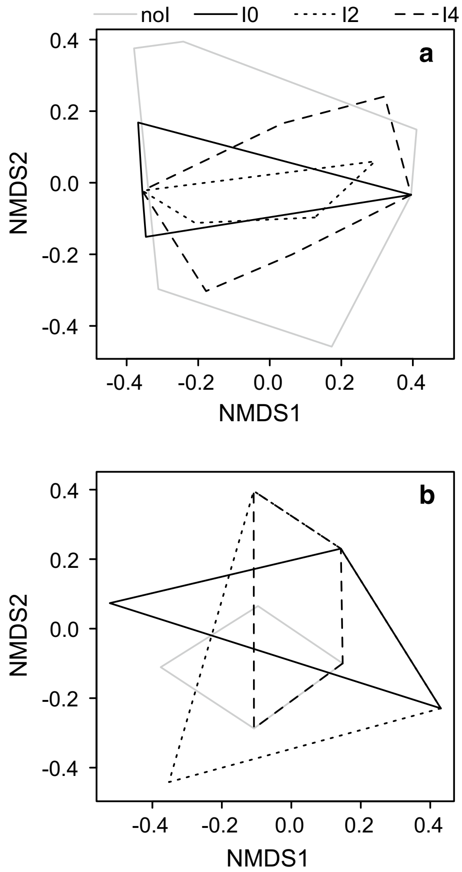

Fig. 4 Effect of inoculation time (*I0*, *I2*, *I4*, *noI*) on the compositional variation of aquatic plants (**a**) and zooplankton (**b**) measured by non-metric multidimensional scaling (NMDS) analysis. *Polygons* represent convex hulls encircling the score data points for each treatment ($n = 7$; time of inoculation *I0*, *I2*, *I4* and the control *noI*). *I0* inoculation at the beginning of the growing season (i.e. moment of tank filling); *I2* inoculation 2 months later; *I4* inoculation 4 months later; *noI* control (no inoculation)

Discussion

Our results show that while immigrant species successfully colonized resident communities, biotic resistance constrained the invasibility of aquatic plant communities and eventually determined their diversity. On the other hand, species sorting imposed by environmental conditions, rather than biotic resistance, determined the assembly of zooplankton communities. The contrasting results between aquatic plants and zooplankton might be explained by their different trophic status, since in aquatic habitats primary producers such as plants and phytoplankton mediate trophic processes that govern the entire ecosystem (e.g. Scheffer and Nes 2007). Indeed, our analyses show that the species composition of the aquatic plant community affected the species composition of the zooplankton community, and we propose that such influence represents a key environmental filter that controls the establishment of immigrant zooplankton species and thus determines zooplankton community assembly (see below for further discussion).

Notwithstanding these different mechanisms, both plants and zooplankton seem not to show specific functional traits associated with resistance or colonization capacity (see Online Resource, Tables S1, S2). The apparent lack of functional associations might be explained to some extent by stochastic species compositions resulting from chance colonization and biotic resistance hampering deterministic competitive outcomes. It also suggests that biotic resistance might be largely exerted by the diversity of resident species that may occupy more niche space potentially suited to immigrants (niche complementarity), rather than containing (a few) invasion-resistant species showing particular traits related to a competitive dominance effect (Fargione and Tilman 2005).

On the other hand, competition and other biotic interactions within and between-trophic levels that were allowed to act in the study of mesocosms, but were not controlled for, also complicated the interpretation of the precise mechanisms underlying the results obtained, including those of biotic resistance in plants and species sorting in zooplankton. Interspecific competition, for example, is best evaluated with controlled invasions that include single or few pairs of species (e.g. one resident vs. one immigrant), such as in Jiang et al. (2011). In addition, uncontrolled invasions in zooplankton communities might have produced noise in the results that further limit interpretation, particularly the generation of abiotic and biotic interactions that could somehow influence immigration success. Although the precise mechanisms behind the results could not be identified, we showed that the interaction between diversity resistance and priority effects is an assembly mechanism that might generate high beta-diversity such as that observed in several aquatic communities (e.g. Heino 2011; Viana et al. 2016). This interactive mechanism of biotic resistance might lead to alternative compositional states and explain a considerable amount of unexplained compositional variation in observational studies. Identifying this and other community assembly mechanisms, such as pure stochastic processes that are difficult to infer from observational studies but contribute to explain patterns of beta-diversity, is a necessary task to understand biodiversity patterns in space and time, as discussed in Vellend et al. (2014).

Aquatic plants

Biotic resistance in aquatic plant communities appeared to be mediated by community buildup: as the growing season progressed, species richness and abundance increased (Spearman correlation rho $= 0.63$) and in turn limited the number of successful immigrant species. However, the number (rather than the abundance) of resident species was more important to explain immigration success, suggesting that diversity per se is an important resistance mechanism (sensu Elton 1958). Moreover, the effect of diversity resistance was stronger in communities invaded later in the season, i.e. diversity resistance increased as priority effects became stronger. This interaction might be explained to some extent by plant growth in early stages of community development, as a more stable and clear water column might facilitate the establishment of immigrant species (Hao et al. 2013). The interplay between diversity resistance and priority effects can occur during community assembly in newly colonized habitat patches and in seasonal communities where the number of resident species increases throughout the growing season.

According to our expectations, communities in which biotic resistance was stronger (I4) had ultimately lower species richness. Although the decrease in species richness was hypothesized to be the direct result of biotic resistance, owing to reduced establishment by immigrant species, it could also be explained by a richness saturation threshold caused by the emergence of resident species, over which immigrant species could no longer establish. However, the number of immigrant species that actually colonized low-resistance communities (I0 and I2) was higher than in high-resistance communities (I4) and did not differ from the total number of species in the immigrant (regional) pool (Online Resource 1, Fig. S2; the immigrant pool corresponds to the species present in the inoculum that are not present in the resident community), meaning that a saturation in the number of species was not reached. In addition, the number of immigrant species, but not the number of resident species, was positively correlated to the richness of mature communities, supporting the importance of biotic resistance in determining community diversity.

The fact that virtually all species in the immigrant pool could establish in low-resistance communities can also explain the observed compositional homogenization (i.e. lower beta-diversity), in contrast to the higher compositional variation under stronger biotic resistance. Because the arrival order is often stochastic (at least over spatial extents in which dispersal is not a limiting factor), biotic resistance may ultimately result in alternative stable states of community composition (Drake 1991; Chase 2003; Jiang et al. 2011) and thus contribute to larger differences in compositional variation among local communities—high beta-diversity among aquatic plant communities has been found in different continents over spatial extents of tens of kilometres (>0.75), even though dispersal limitation seems a less important assembly process in these communities (Capers et al. 2010; Viana et al. 2016). Under the influence of recurrent disturbances (e.g. inter-annual variation in flooding regimes of temporary aquatic habitats), biotic resistance can also induce alternative transient states (Fukami and Nakajima 2011), where immigrant species use transient windows of opportunity to colonize resident communities.

Zooplankton

Unlike aquatic plants, neither the inoculation time nor the species richness of resident communities affected the invasibility of zooplankton communities. Rather than biotic resistance, the composition of zooplankton communities was driven by the composition of plant communities. We cannot exclude the possibility of priority effects in zooplankton communities, as observed in other studies (Louette and De Meester 2007; Symons and Arnott 2014). Nevertheless, we propose that the assembly and composition of zooplankton communities likely depended primarily on (1) functional interactions with other trophic groups in the habitat patch, (2) abiotic conditions, which may be mediated by other taxa, as in clear vs. turbid states, and/or (3) common responses of zooplankton and other taxa to abiotic conditions not measured in this study. In fact, it has been shown that environmental heterogeneity might supersede priority effects (Tucker and Fukami 2014), especially when strong environmental filters cause high selection pressures on species (Chase 2007). In this study, plant-driven environmental conditions might underlie the lack of observed priority effects in zooplankton communities. For example, zooplankton might have benefitted from the structuring effect of plants in terms of microhabitat provision (Jeppesen et al. 1997; De Meester et al. 2006; Declerck et al. 2007) and/or from environmental conditions determined by bottom-up effects produced by macrophyte and phytoplankton abundance and composition and associated productivity levels (Declerk et al. 2007; Declerck et al. 2011). In particular, charophytes, whose presence was recorded in most mesocosms at relatively large densities, have been found to impact planktonic food webs, having an effect on the structure and dynamics of phyto- and zooplankton communities (Van Donk and Van de Bund 2002).

The species sorting mechanism observed in zooplankton communities might also explain the lower compositional variation observed in control mesocosms (noI) and those inoculated later (I4), as the number of plant species was lower in these communities. As the number of plant species and associated habitat heterogeneity grows, they may act as structuring agents for the zooplankton assemblage (Davidson et al. 2011; Declerck et al. 2011; Viana et al. 2016). If most immigrant species out of the total regional pool are sorted out as a result of environmental filtering, these will not be able to establish, as observed in this experiment (Online Resource 1, Fig. S2), and the identity of those that eventually establish will vary largely across mesocosms differing in environmental conditions, owing in this experiment to variation in the composition of the plant community and the biotic and abiotic conditions imposed therein. Indeed, Louette et al. (2006) showed that species sorting is an important mechanism in determining zooplankton composition during the early phases of community assembly. Alternatively or complementarily, the low proportion of established immigrants might be caused by strong priority effects exerted by the pioneer community (as observed in other studies; e.g. Louette et al. 2008), which could hamper immigration independently of the immigrants' arrival order and resident diversity. In such a case, the higher compositional variation of inoculated communities could reflect mere stochasticity in the identity of immigrant species succeeding to establish and caused solely by the low odds of such establishment.

Conclusions

The interplay between diversity resistance and priority effects determined the assembly and diversity of aquatic plant communities. In contrast, biotic resistance was not observed during the assembly of zooplankton communities. Instead, their diversity was shaped by species sorting mechanisms mediated mostly by the biotic conditions imposed by the plant community. Our results show for the first time in semi-natural communities that diversity resistance interacts with priority effects to form a complex mechanism of biotic resistance. This mechanism is not, however, universal across functional groups, as the influence of the environment can supersede the effect of biotic resistance mechanisms for certain taxonomic groups (such as zooplankton). Overall, this experiment showed that multiple interactive mechanisms of biotic resistance and species sorting determine the assembly of local communities. Therefore, historical processes must be taken into account to understand how communities are assembled. In particular, we should understand how diversity resistance and priority effects interact during community assembly, and how abiotic and trophic conditions modulate it. This milieu of interactions throughout the history of community assembly, together with metacommunity dynamics mediated by dispersal, selection and ecological drift (Leibold et al. 2004; Vellend 2010), ultimately determine the assembly and diversity of biological communities.

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