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PERPECTIVES AND PARADIGMS

To include or not to include (the invader in community analyses)? That is the question

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Abstract An increasing number of studies report impacts from invasive species on community metrics or ecosystem functions. We draw attention to an issue arising whenever impact is measured on a community where the invader is an integrated part: should or shouldn't the attributes of the invader itself be included in the data-analysis? We identify many examples from the published literature showing inconsistency in whether or not data for the invader is included or excluded, and discuss potential implications for ecological interpretations. We also provide a case study to show that the invasive seaweed *Undaria pinnatifida* can be interpreted to have strong or no impact on seaweed communities, depending on its inclusion or exclusion in the data analysis. We conclude that it is critical for studies to (1) clearly state in the methods section, if the invaders are included or excluded from the data-analysis, (2) acknowledge potential differences in outcomes when comparing results based on different methods, and (3) analyze, if possible, impacts both with and without the invader. Finally, we note that this 'inclusion versus exclusion' conundrum is not only relevant to invasion biology, but to *any field where the test-object of interest can be an integrated part of the response*, such as when impact of seaweed blooms are analysed on community productivity or community effects are quantified over time from ecological pulse-perturbation experiments.

Keywords Invasion impact · Data inclusion criteria · Mensurative experiment · Manipulative experiment

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Invasive species have impacted communities and ecosystem functioning across the globe, and an increasing number of studies therefore report impacts on community metrics (e.g., richness, diversity, similarity) or ecosystem functions (e.g., nutrient fluxes; Powell et al. 2011; Thomsen et al. 2014; Vilà et al. 2011). Invasion impact can be quantified using mensurative experiments, where researchers have no control over invader abundances, or manipulative experiments, where researchers have at least partial control over invader abundances (Hurlbert 1984). These approaches have contrasting advantages and disadvantages but, when used complementarily, can bridge analyses across taxa, spatiotemporal scales and ecological hierarchies (Sol et al. 2008). Here we draw attention to an important but neglected methodological question arising whenever impact is measured on a community of which the invader is an integrated part (cf. Fig. 1): *should or shouldn't the attributes of the invader itself be included in the data-analysis?*

Many mensurative experiments include the invader, sampling all organisms in invaded and non-invaded plots (quadrats, sites, locations, time-periods), reporting everything present in the species-sample matrices used to calculate impacts on diversity, total abundances and community structures (community 1 in Fig. 1; examples across ecosystems include Aguilera et al. 2010; Angeloni et al. 2006; Christian and

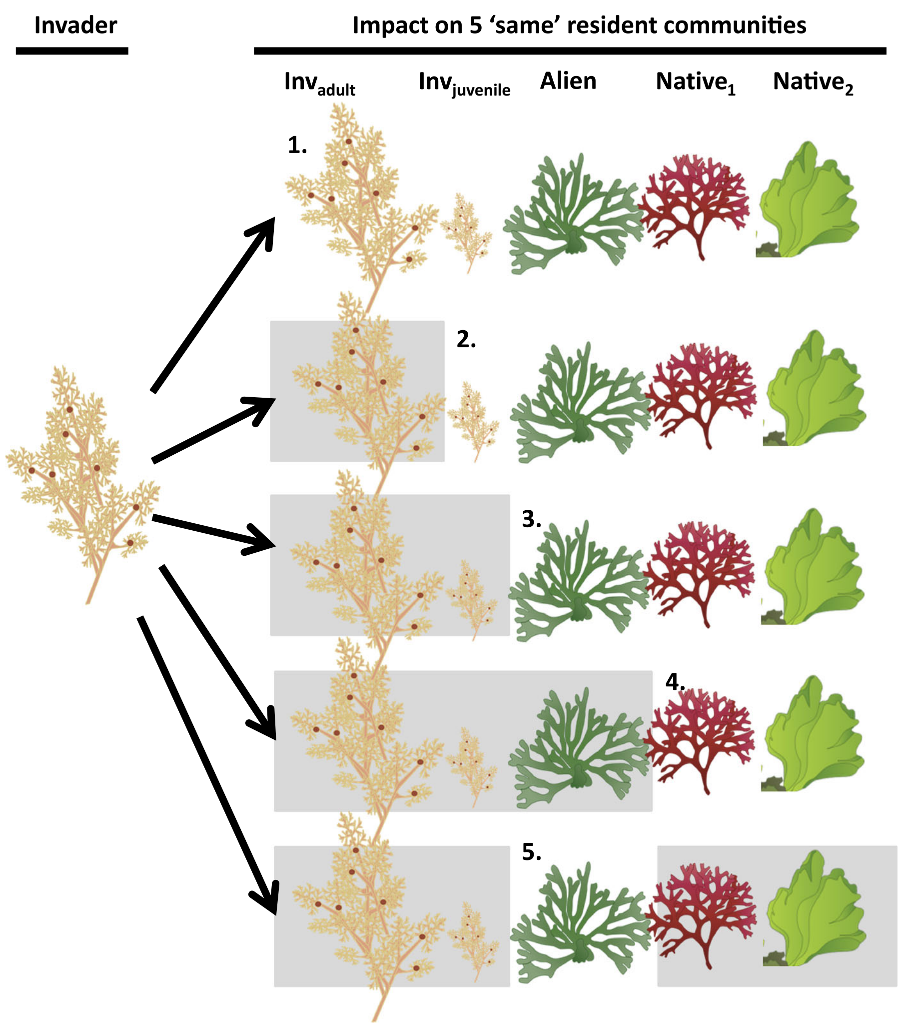

Fig. 1 To include or not to include? Common ways to report invasion impacts on communities within which the invader has become an integrated part, for example, when impacts are reported on the same trophic level or functional group as the invader itself. Impact reported on: *1*. Entire resident community—including adults and new recruits of the invasive species of interest (the brown macrophyte *Sargassum muticum*) and other alien (the green macrophyte *Codium fragile)* and native (the red and green macrophytes *Chondrus crispus* and *Ulva lactuca*) species (Klein and Verlaque 2011; Staehr et al. 2000). *2*. Resident community including new recruits of the invasive species of interest and other alien and native species (Lang and Buschbaum 2010; Werner and Rothhaupt 2007). *3*. Resident community including other alien and native species (Balata et al. 2004; Blum et al. 2007; Thomsen et al. 2010; Truscott et al. 2008), *4*. Native community (Flory and Clay 2009; Stiers et al. 2011). *5*. Alien community (often shown as a contrast to impact reported on native communities, e.g., Andreu et al. 2010; Cushman and Gaffney 2010). Impact reported on community 1, and to a lesser extent community 2, may result in higher richness, total abundances, system-wide primary productivity and multivariate community similarity but lower evenness, compared to impacts reported on community 3–5. *Grey boxes* represent species in the community that were excluded from statistical analysis

Wilson 1999; Clarke et al. 2005; Forrest and Taylor 2003; Harries et al. 2007; Klein and Verlaque 2011; Olden et al. 2008; Schooler et al. 2006; Staehr et al. 2000). By contrast, *manipulative experiments typically exclude the invader* from the species-sample matrix, because here it is self-evident that the manipulated species of interest (the invader) should not be an integrated part of (i.e., confound) the measured response (e.g., diversity or similarity; community 3–5 in Fig. 1; examples across ecosystems include Albins 2013; Andreu et al. 2010; Blum et al. 2007; Cushman and Gaffney 2010; Hejda and Pyšek 2006; Piazzi and Ceccherelli 2006; Sánchez and Fernández 2005; Schreiber et al. 2002; Truscott et al. 2008; Werner and Rothhaupt 2007). Nevertheless, it is also possible to find examples of *manipulative experiments that include the invader* (Klein and Verlaque 2011; Morrison 2002) as well as *mensurative experiments that exclude the invader* (Cushman and Gaffney 2010; Piazzi and Ceccherelli 2006; Scharfy et al. 2009; Smith et al. 2014) from the analyses of impact. In short, it would appear that 'everything goes' and we are not aware of any general discussion, consensus or recommendations in the invasion literature about the implications of using these different analytical approaches.

Including or excluding the invader in the analyses is likely to influence interpretations of invasion impacts because presence of the invader automatically adds one taxon to total richness, and a high value to total community abundance in the invaded plots. For example, in a recent meta-analysis of manipulative experiments that reported impacts of invasive marine macrophytes, we found that taxonomic richness and total macrophyte abundances in invaded plots were *negative or zero depending on whether or not the invaders were excluded or included in the analysis* (cf. Fig. 2, redrawn from Thomsen et al. 2015). In other words, invaders had negative impact on other macrophytes but no effects on the total macrophyte abundances, suggesting substitution, rather than addition or removal, of the overall ecosystem functioning (assuming that ecosystem function depend on macrophytes abundances). For the same analytical reasons, it might not be surprising that multivariate techniques, such as ordinations (e.g., nMDS plots), can visualize both strong or absent 'homogenization' of communities, i.e., with small or large dispersion between invaded samples in the plots, respectively (cf. Fig. 3, redrawn from Fig. 5 in Staehr et al. 2000 and Fig. 3 in Sánchez and Fernández 2005). To further illustrate this conundrum we here compare impacts from the invasive seaweed *Undaria pinnattifida* (included on a list of 100 most invasive species worldwide, Lowe et al. 2000) on the resident seaweed community at Moeraki Point (Southern New Zealand), by including and excluding the abundance of the invader, in parallel analyses. Seaweed communities were

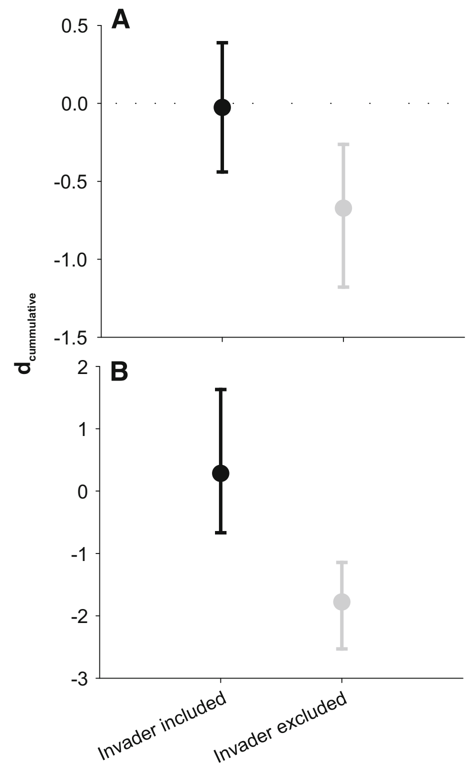

Fig. 2 Meta-analysis documenting impacts of invasive seaweeds on resident seaweed communities (re-drawn from Fig. 2 in Thomsen et al. 2015). *Grey color* represents hedges $d_{\mathrm{cumulative}}$ effect size with 95 % bias corrected confidence limits calculated from published manipulative impact-experiments where the invader attributes was excluded from analysis (=community 3–5 in Fig. 1). Each analyzed paper also presented data on invader abundance in separate graphs, tables or text. *Black color* represent hedges d calculated when the attribute of the invader (its taxonomic status and abundance) was added to the reported community impacts (=community 1 in Fig. 1.). A negative $d_{\mathrm{cumulative}}$ corresponds to negative effects on community richness or abundance. $d_{\mathrm{cumulative}}$ can be interpreted to be significantly different from zero if the 95 % CL did not overlap zero. Note that the conclusion about invasion impact changes from negative (*grey*) to neutral (*black*) depending on whether the invasive species' attributes are excluded or included in the data-analysis. **a** Richness, **b** community abundance

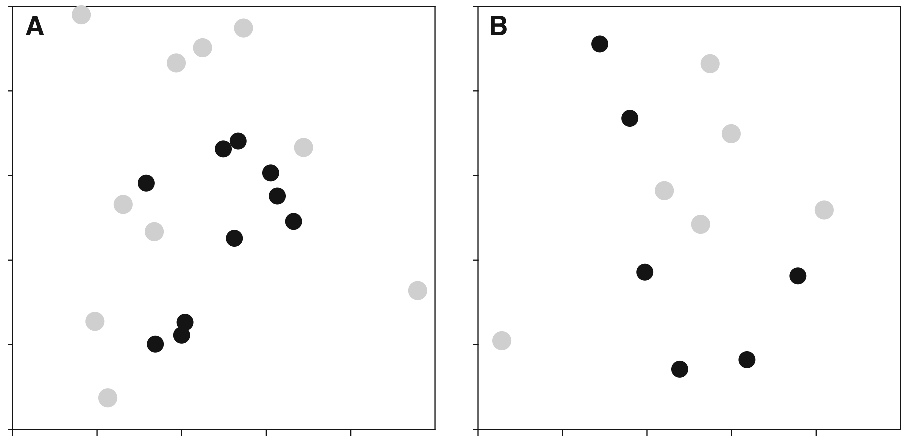

Fig. 3 Examples of published MDS plots visualizing invasion impact on community structures where the abundance of the invader was included (**a** redrawn from Fig. 5 in Staehr et al. 2000, a mensurative experiment, cf. Fig. 1A) and excluded (**b** redrawn from Fig. 3A March 2004 in Sánchez and Fernández 2005, a manipulative experiment, cf. Fig. 1C). *Black* = invaded samples, *grey* = non-invaded samples. Staehr et al. concluded from their mensurative experiment that the brown seaweed *Sargassum muticum* had increased community similarity in Limfjorden, Denmark (including the invaders attribute in analysis; grey pre-invasion samples are more dispersed that black post-invasion samples). By contrast, Sánchez and Fernández, concluded that *Sargassum* had not affected seaweed community structure in Aramar, northern Spain, based on a manipulative removal experiment where the attributes of the invader were excluded from data analysis (excluding the invader's attributes in analysis; **b** *grey* invader-removal samples are interspersed with *black* invaded samples)

quantified in ten 1 m^2 plots in September 2012, one year after a press-removal experiment was established (comparing 5 invaded plots vs. 5 invader removal plots, South et al. 2016). We compared the impact of *Undaria* on both multivariate community structure and multivariate dispersion (cf. Fig. 4). This analysis showed that when the invader's abundance was included in the species-sample matrix the centroid position of the multivariate community structure changed (Permanova $F_{1,9} = 6.29$, $p = 0.01$) and increased multivariate community dispersion (mean deviation from centroids; removal = 19.5 ± 1.6 vs.

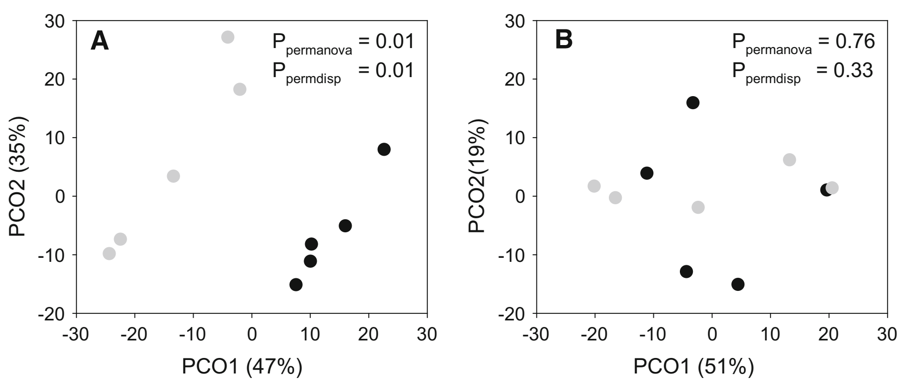

Fig. 4 Example of invasion impact, visualised with PCO plots, that show 5 invaded (*black*) and 5 non-invaded (*grey*; invader removed) samples either including (**a**) or excluding (**b**) the abundance of the invasive seaweed *Undaria pinnatifida* from the data-analysis. Details about the methods and results from this press-invader removal experiment are described in detail in South et al. (2016). Abundance data (percent cover) were square-root transformed and similarity matrices calculated with the Bray–Curtis coefficient. Statistical analysis showed that if the invaders abundance was included in the data-analysis, *Undaria* changed the centroid position of the multivariate community structure (**a** $p_{permanova} = 0.01$) and decreased multivariate community dispersion (**a** $p_{permdisp} = 0.01$). By contrast, if the abundance of *Undaria* was excluded from the data-analysis, the invader had no effect on either community structure or community dispersion (**b** $p > 0.05$)

control = 12.6 ± 0.4; Permdisp $F_{1,9} = 16.52$, $p = 0.01$). By contrast, if the abundance of *Undaria* was excluded, the invader had no effect on either community structure (Permanova $F_{1,9} = 0.512$, $p = 0.76$) or community dispersion (Permdisp $F_{1,9} = 0.602$, $p = 0.33$). Clearly, the interpretations of invasion impact can therefore depend on whether or not the invader's attributes are included in analysis, at the same time making it possible to conclude that an invader can change, increase, decrease, or have no effects on resident community richness, abundances, structure and dispersion. We also note that how much including or excluding the invader will affect the impact-analysis will depend on the choice of transformation (increasing the severity of transformation reduces the importance of dominant organisms)—and for multivariate analysis—on the choice of similarity matrices (ranging from compositional presence-absence to linear Euclidean metrics; Anderson et al. 2008; Clarke and Warwick 1994).

Importantly, *just like mensurative and manipulative experiments complement one another, so does including and excluding the invader in analyses*, each representing what the invader does to the entire aggregated 'new' community (=community 1–2 in Fig. 1; potentially of more interest to ecosystem ecologists and biogeographers) versus what the invader does to 'everything else'—including both native and other alien species (=community 3–5 in Fig. 1; potentially of more interest to conservation ecologists). Furthermore, the underpinning mechanisms that are interpreted to drive observed patterns would likely also differ when impact is assessed based on different data inclusion criteria. For example, it could be relevant to discuss addition or substitution of Biodiversity-Ecosystem-Functioning theories (community 1 vs. 2 in Fig. 1), intra-specific competition processes (community 2 vs. 3 in Fig. 1) or the importance of co-evolution and shared histories (community 4 vs. 5 in Fig. 1), depending on what components of the community that was analyzed.

Our main take home message here is that invasion impact studies should, when evaluated on the 'same' type of responses, clearly emphasize the analytical approach used and the implications for ecological interpretations. This is particularly important in light of the growing number of reviews and meta-analyses that aim to identify and test for impact generalities, because effect sizes will likely differ in magnitude—and perhaps even direction—depending on whether the invader of interest (and other alien species in the same community) is included or excluded in the response. Importantly, this 'inclusion versus exclusion' conundrum is not only relevant to invasion biology, but to *any field where the test-object of interest can be an integrated part of the response*. For example, similar analytical and interpretational problems arise when impact of macroalgal blooms are evaluated on community productivity, where, not surprisingly, studies that include the alga find massive positive effects (e.g., Cacabelos et al. 2012; Dalsgaard 2003; Qu et al. 2003) whereas studies that exclude the alga find significant negative effects (e.g., Corzo et al. 2009; Sundbäck et al. 1996; for meta-analysis of these results—see Maggi et al. 2015). Other broader examples include impact studies of valued species in conservation sciences or unwanted (but native) species in pest-management where the species of interest is an integral part of the measured community (Cléments et al. 1994; Grey et al. 1998), or ecological pulse-perturbation experiments where an initially removed organism, in contrast to press-perturbation, can recover and, over time, become an increasingly important part of the community (Bender et al. 1984; Glasby and Underwood 1996).

In conclusion we suggest that future studies (1) clearly state in the introduction section, the research question that is asked (which will guide the choice of analytical methods), (2) clearly state in the methods section, if the invader is included or excluded in data analysis (cf. Fig. 1) along with a rationale for this decision (Klein and Verlaque 2011), (3) acknowledge potential differences when comparing results based on different methods, e.g., in meta-analysis (Thomsen et al. 2015), and, (4) if possible, analyze impact both with and without the invaders to test if and how much results differed (Fig. 4; Werner and Rothhaupt 2007), or alternatively show the supplementary analyses in an online appendix.

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