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Phenotypic responses of invasive species to removals affect ecosystem functioning and restoration

Libor Závorka[1] | Rémy Lassus[1] | John Robert Britton[2] | Julien Cucherousset[1]

[1]Laboratoire Évolution & Diversité Biologique (EDB UMR 5174), CNRS, Université de Toulouse, IRD, Toulouse, France

[2]Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Poole, UK

Correspondence and present address
Libor Závorka, WasserCluster Lunz–Inter-University Centre for Aquatic Ecosystem Research, A-3293 Lunz am See, Austria.
Email: liborzavorka@email.cz

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Abstract

Reducing the abundances of invasive species by removals aims to minimize their ecological impacts and enable ecosystem recovery. Removal methods are usually selective, modifying phenotypic traits in the managed populations. However, there is little empirical evidence of how removal-driven changes in multiple phenotypic traits of surviving individuals of invasive species can affect ecosystem functioning and recovery. Overcoming this knowledge gap is highly relevant because individuals are the elemental units of ecological processes and so integrating individual-level responses into the management of biological invasions could improve their efficiency. Here we provide novel demonstration that removals by trapping, angling and biocontrol from lakes of the globally invasive crayfish *Procambarus clarkii* induced substantial changes in multiple phenotypic traits. A mesocosm experiment then revealed that these changes in phenotypic traits constrain recovery of basic ecosystem functions (decomposition of organic matter, benthic primary production) by acting in the opposite direction than the effects of reduced invader abundance. However, only minor ecological impacts of invader abundance and phenotypic traits variation remained a year after its complete eradication. Our study provides quantitative evidence to an original idea that removal-driven trait changes can dampen recovery of invaded ecosystems even when the abundance of invasive species is substantially reduced. We suggest that the phenotypic responses of invaders to the removal programme have strong effects on ecosystem recovery and should be considered within the management of biological invasions, particularly when complete eradication is not achievable.

KEYWORDS

aquatic invasions, ecosystem functioning, ecosystem management, eradication programmes, functional syndrome

1 | INTRODUCTION

The common goal of invasive species management is to restore the ecosystem properties and functions to their pre-invaded state, including native biodiversity and ecosystem services (Bellard, Cassey, & Blackburn, 2016; Kopf et al., 2017). Considerable resources are deployed globally to manage invasive species, yet the outcomes of this management remain equivocal, with numerous failures to permanently reduce or eradicate invaders and, ultimately, achieve biodiversity and ecosystem recovery (Kopf et al., 2017; Pluess et al., 2012). A central tenet of invasive species management is that removal programmes reduce the ecological impacts of invaders through decreasing their abundance (Hulme, 2006). However, this approach does not consider phenotypic responses to the removals in the surviving individuals.

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While the ecological impacts of biological invasions are determined by the number of individuals in the invasive populations, it is also affected by the per capita ecological effects of individuals (Dick et al., 2017; Parker et al., 1999). Removal efforts generally involve selective methods, including harvesting (e.g. fishing), and applications of biocides and biological agents (Britton, Gozlan, & Copp, 2011; Myers, Simberloff, Kuris, & Carey, 2000). This selective removal of individuals from populations can become a principal driver of rapid trait change (e.g. behaviour, morphology and life-history traits) as driven by phenotypic plasticity and selection (i.e. contemporary evolution; Fugère & Hendry, 2018; Mimura et al., 2017). This is important, because intraspecific phenotypic trait variability can have strong effects on ecosystem functioning (Des Roches et al., 2018; Palkovacs, Moritsch, Contolini, & Pelletier, 2018; Raffard, Santoul, Cucherousset, & Blanchet, 2019), and the distribution of phenotypic traits across invasive populations influences the rate, extent and impacts of their invasion (Britton et al., 2011; Evangelista, Cucherousset, & Lecerf, 2019). For example, a recent study revealed that harvest-induced reduction in the activity of gray snapper (*Lutjanus griseus*) decreased nutrient supply to the water column in a coastal ecosystem (Allgeier et al., 2020). Thus, it can be predicted that invaded ecosystems will suffer additional ecological impacts if removals induce strong trait changes in the surviving invasive individuals (Závorka, Lang, et al., 2018). However, there remains a considerable knowledge gap in how ecological impacts manifest from reduced invader abundances and any consequent removal-induced trait changes.

Evidence also suggests that historic variation in invader abundance can affect the dynamics of the ecosystem following eradication of invader, thus altering the long-term trajectory of ecosystem recovery (Marchante, Kjøller, Struwe, & Freitas, 2009; Reynolds et al., 2017). Therefore, it can also be expected that, should the removal-induced trait changes of an invader occur before its complete eradication from an ecosystem, these trait changes will affect the long-term trajectory of ecosystem recovery. However, there is scant knowledge on how historic intraspecific variation in phenotype and abundance within managed populations of invasive species affects the long-term trajectory of ecosystem recovery after eradication, despite this information being of high importance to managers whose aim is to reduce invasion impacts using removal methods.

The aim of this study was first to quantify the effects of removal programmes on the phenotypic traits of the invasive red swamp crayfish (*Procambarus clarkii*), a high impacting global invader (Souty-Grosset et al., 2016) that has been subjected to numerous control attempts (e.g. Aquiloni et al., 2010). We compared a suite of ecologically important traits among invasive populations from lakes with and without removal programmes, where removals are through trapping, angling and biocontrol. We then used experimental mesocosms to decouple the effects of reduced crayfish abundance from removal-induced phenotypic changes on macroinvertebrate community and ecosystem functioning (benthic and pelagic primary production, litter decomposition, ecosystem metabolism and nutrient cycling). The use of an experimental approach is important for the teasing apart of the two effects, given that removal programmes typically reduce abundance whilst simultaneously inducing pressure that can drive trait changes (caused by phenotypic plasticity and selection) in the target invasive species. Finally, we removed all crayfish from the mesocosms to simulate a successful and complete eradication, and re-evaluated the macroinvertebrate community and ecosystem functioning a year later to determine the long-term trajectory of ecosystem recovery. The three approaches enabled testing of the following hypotheses: (a) removal programmes induce changes in ecologically significant phenotypic traits of the invasive species; (b) the direction of the ecological effects induced by invader trait changes and abundance reduction are opposite, and can reduce the efficiency of removal programmes; and (c) historic variation in invader phenotype and abundance alters the long-term trajectory of ecosystem recovery.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted from May 2017 to August 2018. We used a well-studied model system of invasive populations of red swamp crayfish *P. clarkii* that have invaded gravel-pit lakes along the flood plain of the Garonne River in southwestern France (Alp, Cucherousset, Buoro, & Lecerf, 2016; Evangelista et al., 2019; Jackson et al., 2017; Raffard et al., 2017; Zhao, Grenouillet, Pool, Tudesque, & Cucherousset, 2016). Invasive red swamp crayfish was introduced into the study area in the mid-1990s and virtually all lakes in the area are now colonized by the species. Red swamp crayfish occur primarily in the littoral habitats of these lakes (Jackson et al., 2017). The present study was performed using invasive crayfish collected in six gravel pit lakes (mean ± *SD* water surface: 11 ± 7 ha and water depth: 2.8 ± 1.1 m) that were generally similar in their biotic and abiotic conditions, but differed in the presence/absence of a programme dedicated to remove invasive red swamp crayfish (i.e. removal programmes, Supporting Information S1). Three lakes (BID, BVI, LIN) have invasive crayfish being removed by a combination of fishing, trapping and the introduction of predatory fish (see Supporting Information S1), while the three other lakes (CEA, SAB, SOA) have never been subjected to any removal programmes. Fishing by hoop nets and introductions of predatory fish in the three lakes with removals programmes have been ongoing for more than 20 years prior to the experiment, while trapping commenced 10 and 1 year prior to the experiment in BID and LIN respectively. All other biotic and abiotic environmental factors related to lake hydro-morphology, water quality and crayfish density did not differ significantly between the two groups of lakes (Supporting Information S1), indicating that the main difference between these groups was the presence/absence of crayfish removal programmes. In addition, crayfish populations from lakes with and without removal programmes displayed very similar genetic characteristics in term of expected heterozygozities, allelic richness, private allelic richness and within-population genetic uniqueness value (J. Cucherousset, unpublished data). Invasive crayfish populations in the area were highly structured genetically, indicating that gene flow between lakes is extremely limited and that each lake represent a genetically distinct population, except for lakes BVI and LIN which belong to the same genetic cluster (J. Cucherousset, unpublished data). Consequently, phenotypic differences between populations were assumed to be the direct outcomes of phenotypic trait changes induced by the removal programme applied in the lakes.

2.2 | Crayfish phenotype scoring

Red swamp crayfish were collected between May 29 and June 2, 2017 using pairs of baited Promar mesh 503 and 501 traps set overnight (Alp et al., 2016). Trapping can be a selective method of crayfish sampling, reducing the variation of phenotypes among captured individuals compared to the variation in the whole population (Biro & Dingemanse, 2009). This results from issues such as trap selectivity arising from the mesh and entrance sizes used (Green, Bentley, Stebbing, Andreou, & Britton, 2018). However, the sampling method used here has recently been shown to be highly efficient (De Palma-Dow, Curti, & Fergus, 2020), and we used the same method and effort to collect crayfish in all lakes. For this reason, the chance of sampling bias across the lakes was minimized and was considered unlikely to increase phenotypic differences among the populations with and without removal programmes. A total of 238 individual crayfish were collected (BID: 40, BVI: 42, LIN: 40, CEA: 40, SAB: 40, and SOA: 36, respectively). These were then transported to the experimental facility and kept in aerated holding tanks (one population per tank, cattle tank: 550 L) containing shelters and covered by a mesh net.

On June 3, 2017, each individual crayfish was measured (carapace length to 0.01 mm, body mass to 0.01 g). Mean (±*SD*) of carapace length and body mass was 46.98 ± 4.75 mm and 24.75 ± 8.98 g respectively. Then, chelae strength was quantified with individual pinching a sensor (Magtrol SA), which recorded the maximum applied force (nearest 0.001 N). To induce the crayfish to pinch, individuals were held by the carapace and the sensor was placed between dactylopodite and the propodite of the left chelae (Malavé, Styga, & Clotfelter, 2018). We took a single measurement of pinch force of each individual, but each individual was given sufficient time to produce maximum pinching force. This enabled a relatively robust record of maximum pinch force to be measured across individuals, while limiting the potential negative effects of handling on individuals subsequently used in the mesocosm experiment. Finally, crayfish were individually tagged with a passive integrated transponder tag (8 × 1.4 mm and 12 × 2.15 mm, FDX-B tags; Oregon RFID), inserted at the base of the fifth pereiopod pair through an incision made with a sterile scalpel (Bubb, Lucas, Thom, & Rycroft, 2002). Individuals were then returned to their holding tank for recovery. From all tagged crayfish ($n = 238$), 144 individuals were subsequently used ($n = 12$ males and 12 females from the six populations, selected randomly) for further phenotypic scoring and in the mesocosm experiment. All individuals were sexually mature adults and the experiment was performed before the spawning season.

Three behavioural traits (boldness, activity and voracity) were quantified before the experiment commenced. Scoring was conducted from 08.00 until 17.00 under the natural light conditions and stable temperature (water ~20°C, air ~25°C). Crayfish were fasted in acclimation tanks for 24 hr prior to scoring to standardize their hunger levels. Movement of crayfish was quantified in contexts of terrestrial and aquatic environments. Movements of individuals were measured using open field tests conducted in barren white translucid rectangular plastic tanks (65 × 36.5 × 15 cm), with no refugia and that were positioned underneath a camera (HD Webcam C525; Logitech). The whole experimental set-up was placed under a translucid tent. When subjected to the trial, individuals were gently netted from the acclimation tank and placed into trial tanks (one per tank). Terrestrial movement was recorded in an empty tank for 10 min after 10 min of acclimation. Immediately after the terrestrial movement scoring, tanks were filled with 50 mm of tap water and aquatic movement was recorded for 10 min following acclimation for 10 min. Tanks were emptied and cleaned between each trial. Crayfish movements (measured as distance moved during the trial) were analysed using video tracking software (LoliTrack 4.0; Loligo Systems ApS). Terrestrial movement was assumed to correspond to boldness, given that red swamp crayfish rarely moves overland and only under certain climatic conditions (e.g. rain), and it has demonstrated that crayfish are at high predation and desiccation risk during movements in terrestrial environments (Ramalho & Anastácio, 2015). Aquatic movement was assumed to correspond to activity of individuals in a familiar environment (i.e. individuals had time to habituate to the environment of the tank during the scoring of terrestrial movement). Therefore, this represented relatively low-stress conditions, as suggested for measuring activity (Réale, Reader, Sol, McDougall, & Dingemanse, 2007).

Following the open field test, voracity (i.e. individual foraging linked to its behaviour and metabolism; Pintor, Sih, & Bauer, 2008) was quantified by placing each individual into a white translucid circular tank (18 cm deep, 21.5 cm diameter, covered by lid) with 15 live red maggots (Diptera), with the number consumed in 15 min determined. The measurement was repeated in three consecutive trials that followed immediately after one another. Maggot consumption rate (ind.min^{-1}) was decreasing over the three consecutive trials ($F_{2,401} = 33.77$; $p < .001$), but individual differences were significantly repeatable across the three trials ($R_{\text{adj}} = .389$, 95% CI [0.280, 0.493]). Therefore, we used the mean of the three trials as a measurement of voracity rate.

There was no difference in activity ($F_{1,121} = 0.708$; $p = .402$), voracity ($F_{1,104} = 1.913$; $p = .170$), and growth rate ($F_{1,112} = 0.008$; $p = .928$) between males and females. However, males were bolder than females ($F_{1,128} = 6.460$; $p = .012$). Body mass of individuals was negatively correlated to growth rate ($F_{1,128} = 50.80$; $p < .001$), but activity ($F_{1,121} = 0.016$; $p = .901$), boldness ($F_{1,128} = 1.732$; $p = .190$), and voracity ($F_{1,104} = 2.085$; $p = .152$) were not significantly correlated to body mass. The effect of body mass on phenotypic traits was controlled in the models testing the phenotypic divergence between the populations with and without removal programmes by adding body mass as co-variable (see details in Section 2.4).

At the end of the mesocosm experiment (see details below), all crayfish were collected from the mesocosms using traps and a small hand net on August 3, 2017, euthanized and body mass, carapace length (nearest 0.01 mm) measured with a caliper and dorsal pictures of body and right chela were taken. Specific growth rate (SGR) was then calculated as:

$$\mathrm{SGR} = \frac{\ln M_f - \ln M_i}{T} \times 100,$$

where M_f and M_i were the final and initial body mass, respectively and T the time interval between two measurements, expressed in days (i.e. 62).

Morphological analysis quantifying body and chela shape was performed using geometric morphometric analysis performed using the R package 'GEOMORPH' (Adams & Otárola-Castillo, 2013). Body and chela shape analyses were based on 17 and seven homologous landmarks respectively (Supporting Information S2). Partial warps, which represent the non-uniform components of the body and chela shape variation, were constructed and further examined by principal component analysis (PCA). Consequently, each component of the PCA corresponded to a component of the shape represented by partial warps (Adams & Otárola-Castillo, 2013). The first partial warp of body shape and the first partial warp of chelae shape explained substantial morphological variance (body shape PC1 = 20.5%, chela shape PC1 = 27.7%; Supporting Information S2) and were used for the analyses of morphological variation.

2.3 | Mesocosm experiment

The main mesocosm experiment lasted 7 weeks from June 8, 2017 (i.e. introduction of crayfish into the mesocosms) to July 31, 2017 (i.e. final measurement and sampling of the community and ecosystem metrics) using 36 outdoor mesocosms (circular tanks, 550 L, 0.63 m deep, 1.28 m diameter). From May 3 to 5, 2017, each mesocosm was provided with 5 cm of gravel substrate (to mimic the substrate in the lakes), 400 L of dechlorinated tap water, 30 ml of liquid fertilizer (N 3% and K 5%) and 20 L of unfiltered water from a gravel pit lake containing an inoculum of autotrophic and heterotrophic microorganisms. The mesocosms were also inoculated with periphyton and zooplankton collected from a nearby gravel pit lake. On May 9, 2017, benthic macroinvertebrates were introduced to each mesocosm from mesh bags containing 5 g of a leaf litter mixture that have been placed in a gravel pit lake for 20 days. In addition, seven freshwater snails (*Physa* sp.) collected from local ponds were added to each mesocosm. On May 16, 2017, in each mesocosm, seven pieces of drainpipe (three pieces 10 × 20 cm and four pieces 4 × 20 cm) and a half of an alveolar construction brick (50 × 15 × 15 cm) were added to provide crayfish shelters. On May 19, 2017, 20 g (wet mass) of macrophytes (*Ceratophyllum* sp.), collected from local ponds, were added to each mesocosm.

The experiment was based on a factorial design with two main treatments as follows: crayfish phenotype (two levels, i.e. crayfish with and without removal-induced phenotypic changes) and crayfish abundance (two levels, i.e. low abundance—two individuals per mesocosm—and high abundance—six individuals per mesocosm; Figure 1). The treatment combinations were—two individuals with removal-induced phenotypic changes, mean ± *SD* crayfish biomass: 56.3 ± 14.0 g (low abundance and removal programme), six individuals with removal-induced phenotypic changes, mean ± *SD* biomass: 153.0 ± 41.1 g (high abundance and removal programme), two individuals without removal-induced phenotypic changes, mean ± *SD* biomass: 43.2 ± 13.6 g (low abundance and no removal programme), and six individuals without removal-induced phenotypic changes, mean ± *SD* biomass: 123.6 ± 15.3 g (high abundance and no removal programme). Density of crayfish was chosen to simulate the range of typical densities that are apparent in invaded lakes (Evangelista et al., 2019; Jackson et al., 2017). Size of crayfish corresponded to the mean size of adult individuals in the invaded lakes. Crayfish were always stocked to the mesocosms with individuals from the same population and each treatment combination was replicated nine times, totalling 36 mesocosms (Figure 1). Sex ratio was 1 male:1 female in all mesocosms to control for the potential effect of sex ratio on ecosystem dynamics (Fryxell, Arnett, Apgar, Kinnison, & Palkovacs, 2015). Temperature loggers (HOBO

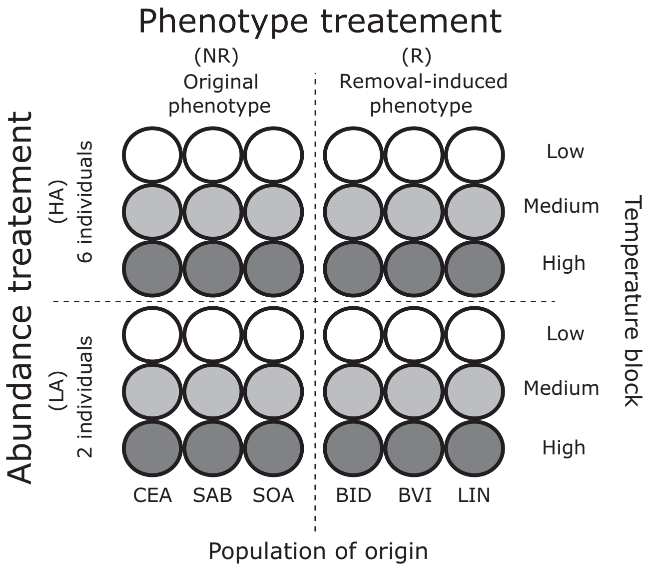

FIGURE 1 Design of the mesocosm experiment. Diagram of treatments distribution between the mesocosms

Temperature/Light Data Logger UA-002-64; Onset Computer Corporation) were placed in each mesocosm and temperature differences among mesocosms caused by the spatial structure of the mesocosms platform was measured during their set-up in May. Consequently, mesocosms were divided into three temperature blocks prior to crayfish introduction to account for temperature variability (Figure 1; Supporting Information S3). Overall, there was no difference throughout the experiment in mean water temperature (21.2°C ± 2.5 *SD*) between the mesocosms with different crayfish abundances ($F_{1,321} = 0.02$; $p = .90$) and with different crayfish phenotypes ($F_{1,321} = 0.02$; $p = .89$). Dechlorinated tap water was added to all mesocosms to balance the effect of evaporation on July 3, 2017 (15, 30, 45 L in the low, medium and high temperature block respectively).

At the end of the experiment (August 1, 2017), macroinvertebrates were sampled in each mesocosm using a hand-net pulled around the edge of the tank for two turns (Evangelista et al., 2019). Prior to sampling, macroinvertebrates were dislodged by disturbing bottom sediments and stirring round the water of the mesocosms. Samples were stored in 90% ethanol and subsequently identified to the lowest taxonomic level (mainly Family). In addition, individual snails (*Physa* sp.) attached to the wall of the mesocosm were counted at 5 cm above and 5 cm below the water surface around the mesocosm perimeter (i.e. the count was done without removing individuals from the mesocosms). A total of 10 macroinvertebrate taxa (*Physa*, Chironomidae, other Diptera, Corixidae, Ephemeroptera, Odonata, Oligochaeta, Coleoptera, Notonecta, and Hydra) were identified and counted (Supporting Information S6).

On July 31, 2017, we also quantified a total of nine response metrics related to ecosystem functioning. Gross and net primary productivity (GPP and NPP) and respiration (R) were estimated using diurnal changes in dissolved oxygen (DO) concentrations (mg/L; Harmon et al., 2009). These measurements were conducted using a DO probe (ProDSS Multiparameter Water Quality Meter; YSI) at dusk and dawn (July 31/August 1, 2017). Benthic algae production was measured as chlorophyll-*a* concentration (μg chl-*a*/cm^2) on ceramic tiles (10 × 10 cm) placed in the mesocosms on June 6, 2017 using a portable fluorometer (BenthoTorch; BBE moldaenke GmbH; Kahlert & McKie, 2014). Production of pelagic algae was assessed by measuring total chlorophyll-*a* concentration in the water column (μg chlo-*a*/L) using a portable fluorometer (AlgaeTorch; BBE moldaenke GmbH). The decomposition rate of leaf litter was quantified by measuring breakdown of 3 g bouquet of leaves of black poplar *Populus nigra* placed into the mesocosms on June 13 and retrieved on July 31, 2017. Decomposition rate (K, day^{-1}) was calculated following (Lecerf, Dobson, Dang, & Chauvet, 2005):

$$K = \frac{-\ln\frac{M_f}{M_i}}{T},$$

where M_f is final and M_i is initial oven-dried mass of leaf litter, T the duration of leaf exposure in mesocosms (48 days). Soluble reactive phosphorous (PO_4^{3-}), ammonium (NH_4^+) and dissolved organic carbon (DOC) were quantified from filtered water samples (50 ml) collected with a syringe fitted with a filter (Whatman GF/C, pore size 1.2 μm). Concentrations of PO_4^{3-} and NH_4^+ were quantified using the molybdenum blue and phenol-hypochlorite methods respectively, performed by an automated continuous-flow colorimetric analyzer (ALPKEM Corporation). DOC concentration was quantified by samples pacification using HCl and analyses using a TOC analyzer (TOC-L; Shimadzu).

Before crayfish introduction to the mesocosms, there were no significant differences in the nine metrics of ecosystem functioning between the mesocosms stocked with different crayfish abundance and phenotype (i.e. measurements at the beginning of the experiment; Supporting Information S4). At the end of the first part of the experiment (August 3, 2017), all crayfish were removed from the mesocosms to simulate the successful eradication of an invasive species. Nearly one year after this crayfish eradication (June 27, 2018), we assessed the effects of the historic treatments (i.e. abundance and phenotype of crayfish) on the ecosystem responses. This sampling was conducted following the same procedure as described above (for details, see Supporting Information S5). This aimed to determine how the ecological effects caused by abundance reduction and removal-induced phenotypic changes will affect the trajectory of ecosystem responses if complete eradication of invader is achieved.

2.4 | Statistical analyses

The effects of the removal programmes on eight phenotypic traits (i.e. activity, boldness, voracity, body and chelae shape, pinch force, SGR and body mass) were tested using multivariate analysis of variance (MANOVA), with the presence or absence of removal programme in the lake of origin as a response variable. The divergence of phenotypes was further tested by linear discriminant analysis (LDA), which evaluated the probability of correct assignment of individuals to the two classes (i.e. lakes with and without removal programme) based on a linear combination of the eight phenotypic traits. The missing data in the matrix of phenotypic traits were imputed using the regularized iterative PCA algorithm (Josse & Husson, 2012). The divergence between the groups in their single phenotypic traits was tested by linear models, with the removal programme as a response variable and body mass as covariate (note that the model for body mass did not include body mass as covariate). The divergence in body and chelae shape was tested with Procrustes ANOVA with 9,999-round randomized residual permutation procedures and controlled for the centroid size. P-values of the models for single phenotypic traits were adjusted by the false discovery rate method. Generalized linear models (GLM), with initial body mass and population of origin as covariates, were used to test the effect of sex and tag size on behaviour and growth rate of crayfish. Repeatability of maggot consumption rate across the three trials adjusted for body mass was quantified using the intraclass correlation coefficient extracted from linear mixed models with individual identity as a random factor (Nakagawa & Schielzeth, 2010).

The effects of the treatments on the macroinvertebrate community in the mesocosm experiment was assessed using non-metric multidimensional scaling (NMDS) ordinations based on Euclidean distance, calculated from untransformed abundances of each taxa in each mesocosm that resulted in two dominant axes, NMDS 1 and NMDS 2 (Supporting Information S6). We then used a multifunctional approach to quantify ecosystem response to treatments (Antiqueira, Petchey, & Romero, 2018) and quantified the distribution of ecosystem metrics (n = 9) between the mesocosms using PCA. This method allows quantification of dominant axes of multifunctionality, synergies and trade-offs among functions. Therefore, this approach provides a novel integrative perspective on how global change drivers, such as biological invasions, will impact the simultaneous provisioning of multiple ecosystem functions (Giling et al., 2019). All ecosystem metrics were centred, scaled and transformed if needed to approach normal distribution. This procedure resulted in three PC axes (eigenvalue > 1) that represented the majority of variation in the original nine metrics (74.4% in total, PC 1:42.0%, PC 2:19.6%, and PC 3:12.8%). We interpreted these three independent dimensions as ecosystem multifunctional components, related to and summarizing different and important ecosystem properties (Antiqueira et al., 2018; Supporting Information S7). Ecosystem metabolism was the first ecosystem multifunctional component and was positively related to GPP (r = .95), NPP (r = .94), R (r = .94) and pelagic algae production (r = .59). Decomposition of organic matter was the second ecosystem multifunctional component positively related to decomposition rate of leaf litter (r = .72) and concentration of DOC (r = .70) and reactive phosphorous (r = .51). Finally, benthic primary production was the third ecosystem multifunctional component positively related to the production of benthic algae (r = .68) and negatively to the concentration of ammonium in the water (r = −.59).

Hedges' g effect sizes compared the effects of crayfish phenotypic change (i.e. effect of phenotypes from lakes with and without removal programme) and abundance on macroinvertebrates community and ecosystem multifunctional components (Des Roches et al., 2018). They were calculated using the following formula:

$$\text{Hedges}'\ g = \frac{m_{imp} - m_{ctrl}}{\sqrt{\frac{(n_{imp}-1)SD_{imp}^2 + (n_{ctrl}-1)SD_{ctrl}^2}{n_{imp}+n_{ctrl}-2}}},$$

where m is the group mean and SD is the group standard deviation of a response variable determined as control $ctrl$ (n = 18) and impact imp (n = 18). When calculating the effects size of crayfish phenotype, variables measured in the mesocosms stocked with crayfish from lakes without a removal programme (i.e. crayfish with assumed original phenotypes) were used as control and variables measured in mesocosms stocked with crayfish from the lakes with a removal programme (i.e. crayfish with removal-induced phenotypic changes) were used as impact. When calculating the effect size of crayfish abundance, variables measured in mesocosms with six individuals were used as a control (i.e. high abundance before removal) and variables measured in mesocosms with two individuals (i.e. low abundance after removal) were used as impact. Following Des Roches et al. (2018), values of Hedges' g were interpreted as negligible if $|g| < 0.20$, small if $|g| < 0.30$, medium if $|g| < 0.80$ and large if $|g| \geq 0.80$.

We used GLMs to test the effects of crayfish phenotype, abundance, their interaction term and temperature block as covariables on macroinvertebrate communities and ecosystem multifunctional components. The interaction term between crayfish phenotype and abundance was not significant in any tested model and was thus removed from all final models. The difference between the absolute effect sizes (i.e. magnitude) of crayfish ecological impacts measured before (August 2017) and 1 year after (June 2018) crayfish removal was tested by a paired t test. All analyses were conducted in R v. 3.4.1 (R Core Development Team).

3 | RESULTS

3.1 | Removal-induced phenotypic changes in crayfish

Ecologically significant phenotypic traits of invasive crayfish differed between populations from lakes with and without removal programmes (MANOVA $F_{8,134} = 5.934$, $p > .001$). LDA indicated that individuals from lakes with and without removal programmes could be correctly identified with a mean probability of 76.9% based on the eight recorded phenotypic traits (i.e. activity, boldness, voracity, body and chelae shape, pinch force, SGR and body mass; Figure 2). Supporting Information S2 has further details on variation of phenotypic traits between individual lakes. At the single trait level, removal-induced phenotypic changes were most distinctively

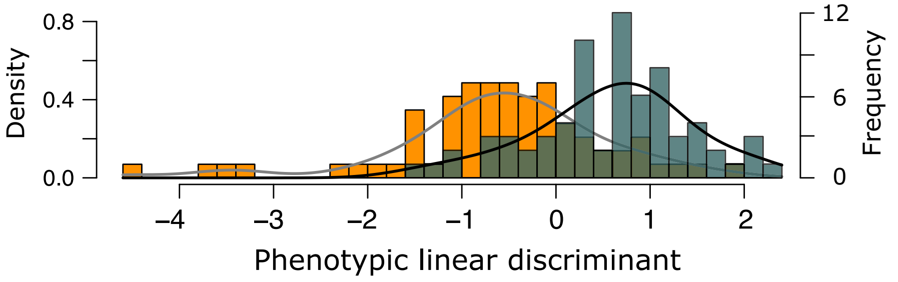

FIGURE 2 Frequency histogram and kernel density distribution of the phenotypic linear discriminant of individuals from the lakes with (orange bars and grey curve) and without (green bars and black curve) removal programme [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Differences in single phenotypic traits: Phenotypic traits mean (±*SD*) of individuals from lakes with (R) and without (NR) removal programme. For units and scoring methods, see Section 2. Difference between the groups is based on models controlled for body size. Significant differences (adjusted $p < .05$) are displayed in bold

	Activity	Boldness	Voracity	Body shape	Chelae shape	Pinch force	SGR mass	Body mass
R	549.57 (±247.86)	381.392 (±151.159)	0.007 (±0.004)	0.001 (±0.010)	0.005 (±0.024)	6.484 (±6.071)	0.065 (±0.180)	27.358 (±9.724)
NR	562.429 (±195.683)	329.668 (±137.154)	0.004 (±0.002)	−0.001 (±0.011)	−0.005 (±0.023)	7.097 (±5.742)	0.166 (±0.356)	22.029 (±7.038)
Difference between groups	NS	**R > NR**	**R > NR**	NS	NS	NS	NS	**R > NR**

demonstrated in higher body mass and a mass-independent increase of boldness and voracity in crayfish from lakes with removal programmes (Table 1).

3.2 | Ecological consequences of removal programmes

There were no significant effects of removal-induced phenotypic changes and abundance reduction of crayfish on macroinvertebrate abundance (crayfish phenotype: $F_{1,31} = 1.35$, $p = .25$; crayfish abundance: $F_{1,31} = 2.24$, $p = .14$; Figures 3a and 4a) and diversity (NMDS1—crayfish phenotype: $F_{1,31} = 0.20$, $p = .66$; crayfish abundance: $F_{1,31} = 0.83$, $p = .37$; Figure 3b and NMDS2—crayfish phenotype: $F_{1,31} = 0.72$, $p = .40$; crayfish abundance: $F_{1,31} = 1.04$, $p = .32$; Figures 3c and 4a; Supporting Information S6).

All ecosystem metrics were summarized by the three ecosystem multifunctional components that represented the synergies and tradeoffs among the important ecosystem functions of ecosystem metabolism, decomposition rate of organic matter and benthic primary production (Supporting Information S7). The cumulation of negative effects of removal-induced phenotypic changes ($F_{1,31} = 3.88$, $p = .06$; Figure 3d) and abundance reduction ($F_{1,31} = 6.20$, $p = .02$; Figure 3d) of crayfish on ecosystem metabolism resulted in a strong decrease in ecosystem metabolism (Hedges' $g = -1.00$; Figure 4b). The significantly increased decomposition rate due to removal-induced phenotypic changes ($F_{1,31} = 5.65$, $p = .02$; Figure 3e) contrasted with the significant decrease of decomposition rate caused by crayfish abundance reduction ($F_{2,31} = 5.01$, $p = .03$; Figure 3e), which resulted in a negligible overall effect of removal programmes on decomposition (Hedges' $g = 0.05$; Figure 4b). Removal-induced phenotypic changes of crayfish ($F_{1,31} = 5.97$, $p = .02$; Figure 3f), but not reduction of crayfish abundance ($F_{1,31} = 0.00$, $p = .95$; Figure 3f), led to a decrease in benthic primary production (Hedges' $g = -0.82$; Figure 4b).

3.3 | Ecological consequences of historic variation in invader phenotype and abundance

The magnitude of the ecological effects of crayfish invasion on macroinvertebrate community and ecosystem functioning was significantly lower 1 year after crayfish eradication ($|t| = 3.27$, $p < .01$; Supporting Information S5). The decrease in average magnitude of the ecological effects was similar for crayfish phenotype ($\Delta|\text{Hedges' } g| = -0.14$) and abundance ($\Delta|\text{Hedges' } g| = -0.23$). Overall, crayfish eradication resulted in a relatively small mean magnitude of the ecological effects of historical variation in invasive crayfish phenotype: $|\text{Hedges' } g| = 0.30$ and abundance: $|\text{Hedges' } g| = 0.21$ on macroinvertebrate community and ecosystem functioning.

4 | DISCUSSION

Our findings reveal that reductions in the population abundances of invasive species do not necessarily minimize their invasion impacts and facilitate restoration of the ecosystem back to its pre-invaded state, thus challenging a central tenet of invasive species management. Instead, these results indicate that ecosystems can instead suffer additional impacts if removals of invasive species induce strong phenotypic responses in the surviving individuals. We confirmed our first hypothesis that the removal programme induced changes in ecologically significant phenotypic traits of the invasive crayfish. Our findings were also consistent with our second hypothesis, as we demonstrated that the direction of the ecological effects of removal-induced phenotypic changes and abundance reduction can be opposite and result in the lower efficacy of removal programmes that aim to limit invader impacts. This result was strongest in the ecosystem multifunctional components that are susceptible to the consumptive effects of invasive crayfish that is, the decomposition of organic matter and benthic primary production (Twardochleb, Olden, & Larson, 2013). Conversely, the effects of phenotypic changes and abundance reduction acted in the same direction on the ecosystem multifunctional component susceptible to non-consumptive effects, that is, ecosystem metabolism. Finally, we found that, a year after crayfish eradication, the effects of historic variation in crayfish phenotype and abundance on ecosystem functioning were reduced and generally minor. This was inconsistent with our third hypothesis that historic variation in invader phenotype and abundance alters the long-term trajectory of ecosystem recovery. This is an encouraging result, as it indicates that ecological impacts of changes in

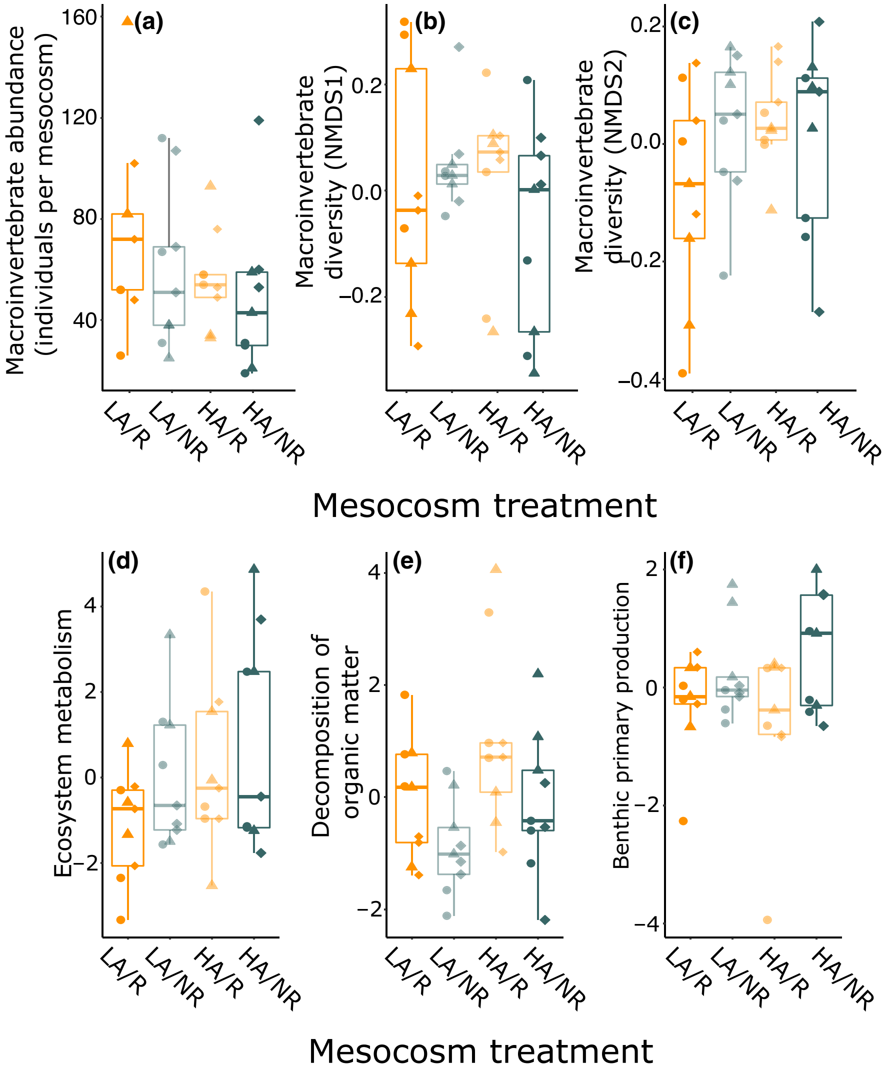

FIGURE 3 Ecological effects in the experimental mesocosms. Effects of crayfish phenotype and abundance on community of macroinvertebrates (a–c) and on ecosystem multifunctional components (d–f) in the experimental mesocosms. Boxplots show effects of treatment combinations (LA/R—two crayfish with removal-induced phenotypic changes, LA/NR—two crayfish with original phenotype, HA/R—six crayfish with removal-induced phenotypic changes, HA/NR—six crayfish with original phenotype). Orange and green boxplots represent mesocosms containing crayfish with and without removal-induced phenotypic changes respectively. Shape and colour of the points in the box plot also correspond to the specific lake of crayfish origin (orange circle—BVI [R], orange triangle—BID [R], orange diamond—LIN [R], green circle—SAB [NR], green triangle—CEA [NR] and green diamond—SOA [NR]) [Colour figure can be viewed at wileyonlinelibrary.com]

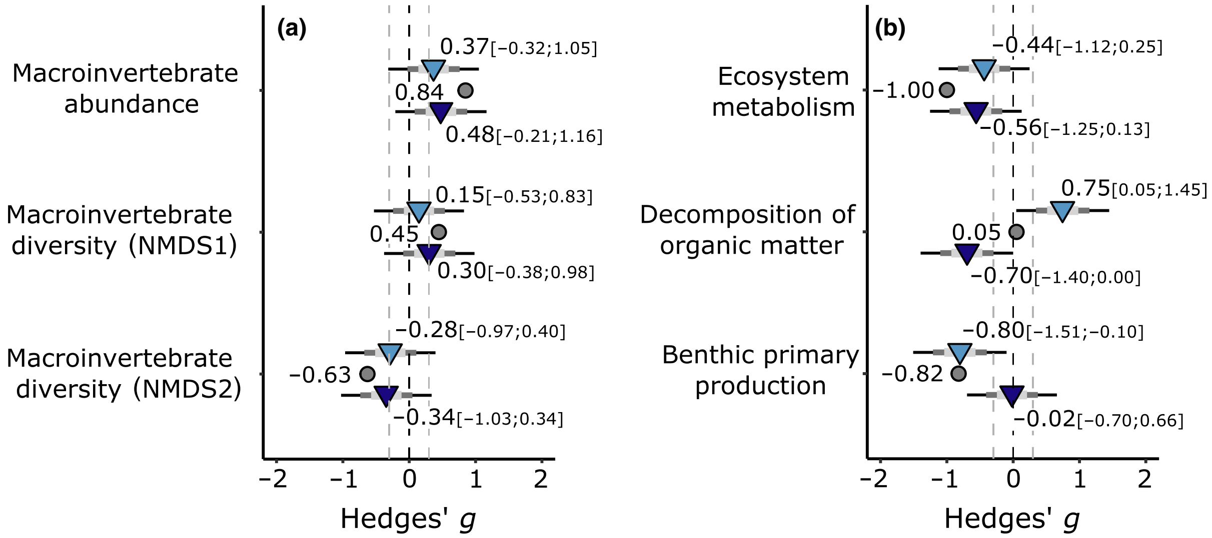

FIGURE 4 Effect size of ecological impacts of phenotypic changes and abundance reduction. Overall effect size (Hedges' *g*) and CI (95% black line, 75% dark grey line, 50% light grey line) of crayfish removal-induced phenotypic changes (light blue) and abundance reduction (dark blue) on ecological metrics of (a) community of macroinvertebrates and (b) ecosystem multifunctional components. Light grey vertical lines represent a range of small effect size that is, Hedges' *d* (−0.3, 0.3). Empty cycles illustrate the cumulative effect size of phenotypic change and abundance effects [Colour figure can be viewed at wileyonlinelibrary.com]

invaders phenotype and abundance caused by previously unsuccessful eradication programmes (Pluess et al., 2012; Zavaleta, Hobbs, & Mooney, 2001) might not constrain future ecosystem recovery providing that eradication is achieved, although this remains to be tested and quantified in more natural and complex settings.

Trapping, angling and stocking of fish predators used in the lakes of invasive crayfish origin have yet to result in significantly decreased invasive crayfish abundance (i.e. based on data from 2016 survey; Supporting Information S1). Nonetheless, we have already observed that crayfish from lakes with removal programmes were bolder, more voracious and had larger body mass. Previous studies suggest that large crayfish displaying bold behaviours are less likely to be consumed by fish predators (Roth & Kitchell, 2005; Stein & Magnuson, 1976). In contrast, large size, bold behaviours and voracity are the traits most likely to increase the probability of individuals being harvested by angling or trapping (Biro & Sampson, 2015; Green et al., 2018; Koeck et al., 2019). However, because harvest and stocking of predators were applied simultaneously in our study systems, it is not possible to decouple the relative effects of each removal method on invader phenotype. The selection pressure induced by harvesting may leave surviving individuals more susceptible to predation and vice versa (e.g. Olsen & Moland, 2011). As little is known about the effects of harvesting in truly natural contexts, future work should aim to identify the mechanisms driving phenotypic responses of invaders in removal programmes and the relative contributions of different control methods to observed phenotypic changes when multiple removal methods are used. Interestingly, while there were some differences among the lakes in the way the removals were performed (duration and effort) due to individual differences between managers in their methods, we found similar phenotypic trait changes across all lakes with removal programmes (Supporting Information S2). Individual differences in boldness and voracity have previously been shown to be highly consistent overtime and influence the trophic ecology of invasive crayfish (Raffard et al., 2017). Therefore, the removal-induced phenotypic changes of the invasive crayfish could magnify their consumptive effects, which are a dominant driver of their ecological impacts (Twardochleb et al., 2013).

Invasive crayfish are known to accelerate the decomposition rate of organic matter (Alp et al., 2016), and reduce macrophyte (Nyström & Strand, 1996) and standing algae (Rudnick & Resh, 2005) production through consumption. We revealed that the removal-induced phenotypic changes caused accelerated decomposition of organic matter and reduced benthic primary production, highlighting that, even if invader abundance was lowered, these important ecosystem functions do not recover. This could possibly be the consequence of increased consumption of benthic algae and leaf litter by the individual crayfish from the lakes that had a removal programme. Our results thus directly corroborate previous findings indicating that boldness and foraging rates (which were found to be higher in crayfish from lakes with removal programmes) are often associated within a functional syndrome that has direct impacts on consumptive effects of crayfish invasion (Pintor et al., 2008; Raffard et al., 2017). We also found that removal-induced phenotypic changes of crayfish reduced ecosystem metabolism, despite their consumption not directly impacting the pelagic ecosystem where most of the oxygen production and respiration occurs (Harmon et al., 2009). Previous work has revealed that crayfish can impact the pelagic components of ecosystems indirectly through nutrient recycling (Vanni, 2002) and bioturbation (Angeler, Sánchez-Carrillo, García, & Alvarez-Cobelas, 2001), which are the processes that could have been affected by removal-induced phenotypic changes in the surviving populations of invasive crayfish (Evangelista et al., 2019; Raffard et al., 2017). Our findings showed that the non-consumptive effects of phenotypic changes can combine with the effects of reduced crayfish abundance and lead to reduced ecosystem metabolism. This suggests that removal programmes could facilitate recovery of ecosystem metabolism. We observed only limited effects of crayfish abundance and phenotype on the macroinvertebrate communities, although the abundance of macroinvertebrates tended to increase in response to the effect of removal-induced phenotypic changes and crayfish abundance reduction. The limited response of macroinvertebrate communities indicates that effects of invasive crayfish on ecosystem functioning were unlikely to have been mediated by a trophic cascade (Souty-Grosset et al., 2016; Twardochleb et al., 2013). The lack of community response to crayfish phenotype and abundance could be at least partially due to relatively low taxonomic diversity of the macroinvertebrate community in the mesocosms (Supporting Information S6).

Our study provides the first direct quantitative evidence supporting the idea that removal methods cause complex changes in phenotype of invasive species that can alter the ecological impacts of invasion, even when the abundance of invasive species is substantially reduced (Palkovacs et al., 2018; Závorka, Lang, et al., 2018). While this finding is based on a single species, the novel concept we describe requires further attention, given that single species studies have been shown to provide key insights into dynamics of biological invasions (Pyšek et al., 2008). The density of invasive crayfish in the mesocosms was within the range occurring in invaded lakes (Evangelista et al., 2019; Jackson et al., 2017), but the scale of mesocosm studies can limit the complexity of ecological interactions therein (e.g. intimidation effect by predators; Stein & Magnuson, 1976; Aquiloni et al., 2010). The scale of the mesocosms can also affect ecosystem processes, but strong effects of phenotypic variability on ecosystem functioning have previously been shown in both mesocosms and larger, natural experiments (Des Roches et al., 2018; Raffard et al., 2019). We found that ecosystems can be highly resilient, as relatively small effects of historical variation in invasive crayfish phenotype and abundance were observed a year after complete eradication of crayfish from the mesocosms. However, caution is needed in the interpretation of these results, as ecosystem resilience depends on the intensity of ecological impacts of invasions and occur at time scales that are dependent upon the ecological context. For example, a previous study found that the negative ecological impacts of an invasive species decreased over time, probably due to the rapid response of native organisms (e.g. their adaptation), but also local extinction (Závorka, Buoro, & Cucherousset, 2018). These responses of native organisms could reduce the capacity of ecosystem to return to its pre-invaded state even after invasive species eradication. The removal-induced phenotypic changes of invasive species should thus be accounted for in ecosystem management planning, especially in cases where eradication of an invader is not possible and population containment via removals is the only practicable option. Previous studies have shown that rapid evolutionary responses depend not only on selection pressures, but also on genetic architecture and phenotypic plasticity of the target population (Kokko et al., 2017), which could limit the heritability of phenotypic changes induced by selective removal methods. However, removal programmes are often relatively short term (Britton et al., 2011), and thus are likely to primarily impact invader phenotype through phenotypic plasticity. Therefore, studies testing how phenotypic plasticity and genetic divergence of ecologically significant traits can affect ecosystem functioning requires further attention in context of invasion biology (Lundsgaard-Hansen, Matthews, Vonlanthen, Taverna, & Seehausen, 2013).

In conclusion, our field and experimental approaches provided novel results that revealed the phenotypic responses of invasive species can have fundamental implications for how recipient ecosystems respond to invader removals and eradication. The successful testing of our first two hypotheses demonstrated that invaded ecosystems can suffer additional ecological impacts via strong responses of phenotypic traits in the surviving invasive individuals and, whilst these phenotypic responses facilitate recovery of some ecosystem functions, they simultaneously constrain the recovery of others. Considering this trade-off should thus become an integral part of risk-benefit assessment of invasive species control efficiency in order to avoid negative consequences on recipient ecosystems and native biota.

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AUTHOR CONTRIBUTION

J.C. and L.Z. designed the experiment, L.Z., R.L. and J.C. collected the data, L.Z. analysed the data and drafted the first version of the manuscript. L.Z., R.L., J.C. and J.R.B. participated in manuscript editing and revision.

DATA AVAILABILITY STATEMENT

All data are archived at figshare.com (https://doi.org/10.6084/m9.figshare.7869413).

ORCID

Libor Závorka https://orcid.org/0000-0002-0489-3681
John Robert Britton https://orcid.org/0000-0003-1853-3086
Julien Cucherousset https://orcid.org/0000-0003-0533-9479

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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