Invasive crayfish alter the long-term functional biodiversity of lotic macroinvertebrate communities

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Abstract

1. Invasive species represent one of the primary threats to global biodiversity. Despite acknowledged implications for taxonomic properties of ecological communities following invasion, functional measures remain poorly understood.

2. We examined the long-term implications of invasion by a crayfish species, Pacifastacus leniusculus, on functional alpha and beta diversity properties of lotic macroinvertebrate communities in three English regions. The dataset comprised 477 samples collected predominantly between 1990 and 2013. Unlike many invasion studies, we employed before and after invasion comparisons, in association with control rivers not invaded by crayfish.

3. Functional richness and divergence metrics displayed the strongest responses to invasion, highlighting that P. leniusculus may alter the range of functional niches and extreme trait values (likely reflecting resistance and resilience mechanisms) displayed by macroinvertebrate communities. Interestingly, in one region, biodiversity gains seen in control rivers were not mirrored in invaded rivers, potentially suggesting that invasion inhibited ecological improvements.

4. Invasion by signal crayfish resulted in functional compositional changes in two of the three English regions studied, with these rivers yielding greater ecological differences between pre- and post-invasion periods relative to control rivers. This change was primarily driven by higher nestedness values in invaded rivers, with communities containing functional subsets relative to control rivers. The third region demonstrated no functional compositional changes or alterations in functional beta diversity associated with invasion.

5. Our findings indicated that in most instances, the functional properties of macroinvertebrate communities responded to crayfish invasion and may therefore represent a complementary means to monitor the ecological condition of lotic...
ecosystems. Given their spatial transferability, functional traits could provide a platform to guide regional management practices in the face of biological invasions.

**KEYWORDS**
alpha diversity, beta diversity, biological invasions, freshwater ecosystems, functional indices, non-native species, signal crayfish

# 1 | INTRODUCTION

Freshwater ecosystems represent some of the most threatened habitats world-wide, with biodiversity losses far exceeding marine and terrestrial environments (Reid et al., 2019; Tickner et al., 2020). Invasive species are widely recognized as one of the primary threats to biodiversity globally due to their wide-ranging ecological effects for individual populations and communities of native taxa, through to entire ecosystem functioning (Carbonell et al., 2017; Flood, Duran, Barton, Mercado-Molina, & Trexler, 2020). However, managing the threat of invasive species remains an open challenge for biological conservation, biodiversity assessments, biomonitoring and restoration practices (Bruno et al., 2019; Guareschi & Wood, 2019).

Detecting the ecological implications of the arrival of non-native species can be difficult, being contextually dependent and variable between taxa, space and time (Thomsen, Wernberg, Olden, Griffin, & Silliman, 2011). Many non-native species will not establish successfully and cannot survive in their new habitat, or establish but have negligible effect on the wider ecosystem, while only a few establish and spread as invasive species (Colautti & Maclsaac, 2004). As a result, many invasion studies focus on effects once the invader is established (Seeley, Eastwood, Pattison, Wilby, & Bull, 2019), employ a space-for-time substitution (Mathers, Rice, & Wood, 2018) or undertake isolated experimental studies (Zenni et al., 2019). Long-term biological invasion studies remain rare (but see Mathers, Chadd, Dunbar, et al., 2016; Mathers, Chadd, Extenze, Rice, & Wood, 2016; Mathers, White, Forcarioli, & Chadd, 2020; Ruokonen, Ercoli, & Hämäläinen, 2016), but are vital to enable accurate and detailed understanding of how successful invaders affect and potentially alter community structure and ecosystem functioning under various environmental conditions (e.g. Mathers et al., 2020).

Functional measures (e.g. trait-based metrics) and their relationships with ecosystem processes have been widely studied in terrestrial ecology and may complement species diversity metrics in biological conservation and ecosystem management (Ahluhtta et al., 2019; Kraft, Godoy, & Levine, 2015). At the community level, functional measures go beyond a strictly taxonomic approach and have been shown to be associated with ecosystem functioning (Menezes, Baird, & Soares, 2010; Statzner & Beche, 2010). Functional properties of biological communities may respond to ecological change which would otherwise remain undetected using traditional taxonomic methods (e.g. Guareschi, Bilton, Velasco, Millán, & Abellán, 2015; Mouillot, Graham, Villéger, Mason, & Bellwood, 2013) and enable more spatially transferable responses at regional scales, as traits are not confined to the biogeographic constraints associated with species identity (Heino, Schmera, & Erős, 2013).

Functional diversity refers to the biological and ecological traits (e.g. feeding group, life-cycle duration, locomotion) expressed by organisms present in a biotic community which governs ecosystem functioning (Hill, Heino, White, Ryves, & Wood, 2019; Petchey & Gaston, 2006). It can be defined at the individual site scale (functional alpha diversity) and according to variation among samples (functional beta diversity). Functional beta diversity can further be partitioned into turnover and nestedness components. Nestedness refers to sites with low functional diversity representing a subset of diversity in functionally rich sites, while turnover refers to the replacement of functional properties between sites (Legendre, 2014). Examining both alpha and beta diversity facilitates a greater understanding of local and landscape biodiversity patterns. However, the application of functional beta diversity approaches remains underdeveloped for riverine invertebrates relative to other organism groups (Rocha et al., 2019). Moreover, very little is known about freshwater invasions and their impacts on functional aspects of invertebrate communities (but see fish-based studies: Colin, Villéger, Wilkes, de Sostoa, & Maceda-Veiga, 2018; Ruhi, Holmes, Rinne, & Sabo, 2015). Consequently, further studies are urgently required to complement taxonomic-based approaches to more effectively quantify the implications of freshwater biological invasions.

Crayfish represent one of the most successfully translocated faunal groups globally (Twardochleb, Olden, & Larson, 2013). Where invasive crayfish successfully establish they can have significant repercussions across all trophic levels including algal cover (Stenroth & Nyström, 2003), macrophytes (Nyström & Strand, 1996), macroinvertebrates (Mathers et al., 2018) and fish recruitment (Edmonds, Riley, & Maxwell, 2011), as well as impacting on wider ecosystem functioning (Doherty-Bone, Dunn, Liddell, & Brown, 2018). *Pacifastacus leniusculus* (signal crayfish, Dana, 1852), a North American species, is currently considered to be one of the most successful invaders in Europe (Kouba, Buřič, & Petrusek, 2015). *Pacifastacus leniusculus* was first introduced to the United Kingdom for the purposes of aquaculture in the 1970s, but escaped and is now widely established in many UK rivers (Holdich, James, Jackson, & Peay, 2014). Invasion by *P. leniusculus* represents a significant perturbation, with long-lasting
modifications in community structure evident over a decade after introduction (Mathers, Chadd, Dunbar, et al., 2016); however, their implications for functional aspects of communities remains poorly understood.

To better understand the poorly studied relationship between biological invasions and functional community metrics, we examined the long-term effects of the invasive signal crayfish on functional properties of macroinvertebrate communities within a number of rivers in England (United Kingdom). Aspects of functional beta and alpha diversity (coupled with taxonomic richness to exemplify underlying structural community responses) were explored within a Before-After-Control-Impact (BACI) design. Specifically, we addressed the following questions.

1. Does the invasion of *P. leniusculus* alter the long-term functional composition and beta diversity dynamics of macroinvertebrate communities inhabiting lotic ecosystems?

2. Does invasion by *P. leniusculus* alter functional alpha diversity measures unmasking hidden effects not detected by taxonomic richness?

2 | MATERIALS AND METHODS

2.1 | Macroinvertebrate data characterized

Data employed in this study were derived from the Environment Agency of England (EA) ‘BIOSYS’ database. Macroinvertebrate data from three regions (derived from geological and topographic catchment boundaries to provide environmental context) across England formed the basis for the analysis, and comprised of seven ‘invaded’ and seven ‘control’ lowland rivers, with a single sample site located on each river (Figure 1). Rivers in South East England are predominately characterized by chalk deposits (a fine-powdered limestone) resulting in groundwater-dominated flow regimes, those in Anglian are underlain by mudstones with a mixture of groundwater-dominated and precipitation-driven flow regimes, and those in the North West by sedimentary sandstone and igneous rocks with highly responsive precipitation driven flow regimes. North West rivers represent an upland setting while South East and Anglian are lowland systems. Consequently, the rivers employed were selected to provide a range of known geology, hydrology and topography environmental settings. However, to enable comparison between sites invaded by *P. leniusculus* and control sites, those selected for the study shared similar biogeographical characteristics within each region apart from the presence of signal crayfish including channel size, discharge, water chemistry and altitude.

Sites had to satisfy the following criteria: (a) records extended 5 years prior to invasion (or in case of control prior to average regional invasion date – see below); (b) sites did not support native crayfish *Austropotamobius pallipes* during the study period; (c) sites did not support any other invasive macroinvertebrate species (relaxed for historic Gastropoda invader *Potamopyrgus antipodarum* which has no known ecological effects in freshwaters; Múrria, Bonada, & Prat, 2008); and (d) sites were not subject to significant anthropogenic influences (e.g. water abstraction, flow impairment or water quality issues). All benthic macroinvertebrate samples were collected by the EA standard sampling protocol comprising a 3-min ‘kick-sample’ (mesh size 1 mm), which encompasses all available habitats, with an additional 1-min, detailed hand search (Murray-Bligh, 1999). Sampling was conducted in spring (March–May) and autumn (September–November). Community abundances were standardized to presence/absence due to variations in how macroinvertebrate abundances were recorded over time and between regions.

The dataset comprised 477 samples from 14 rivers (209 and 268 from invaded and control rivers respectively), with the majority of samples (443) collected between 1990 and 2013 (three sites had data series extending back to the 1970s and an additional three sites included data from the mid-1980s). A total of 101 macroinvertebrate taxa were recorded predominantly to species and genus level. Some taxa were resolved to family level, including various Diptera larvae and some aquatic insects that could not be consistently resolved beyond this taxonomic resolution (notably early instars without identifiable features; e.g. Leptoceridae and Limnephilidae).

Data were categorized into four groups within a BACI study design: (a) ‘before control’; (b) ‘after control’; (c) ‘before invasion’; and (d) ‘after invasion’. For invaded sites, the approximate date of invasion

FIGURE 1 Map of study sites employed with regional catchment boundaries delineated for environmental context. Note the clustering of sites within each region allows for changes between control and invaded sites to be closely interrelated within tight catchment terms. Map credit Environment Agency.
was determined by the first occurrence of *P. leniusculus* in the historical faunal series (sensu Mathers, Chadd, Dunbar, et al., 2016; Mathers, Chadd, Extence, et al., 2016) with kick samples representing an effective means of determining crayfish presence at moderate densities in the absence of trapping which is not a standard EA practice (Gladman et al., 2010). Control sites were divided into two periods (before invasion and after invasion) based on the mean date of invasion for the invaded rivers in the respective region (1997 for Anglian and North West; and 2003 for South East). As introductions may occur in conjunction with other stressors (Byers, 2002; Mathers et al., 2020), the use of control sites is crucial to enable the influence of invasion to be isolated. The assignment therefore of an invasion date for the control sites allowed a direct comparison between the control and invaded rivers over the same time period. This also provided a means of assessing whether there were temporal shifts in invertebrate community composition not associated with crayfish invasion, thereby enabling a complete BACI design.

2.2 | Data analysis

2.2.1 | Assignment of functional traits

Macroinvertebrates were assigned to 13 ‘grouping features’ comprising 76 functional ‘traits’ from the Tachet, Bournaud, Richoux, and Usseglio-Polatera (2010) European trait database (Table S1). Herein, functional properties are reported by their ‘grouping features’ (a functional trait category—e.g. ‘maximum body size’) and ‘traits’ (modalities residing within grouping features—e.g. ‘≤0.25 cm’, ‘≥8 cm’; see Schmera, Podani, Heino, Erős, & Poff, 2015). In total, 63 biological traits (11 grouping features) and 13 ecological traits (two grouping features: substrate and velocity preferences) were employed; the latter being selected a priori because *P. leniusculus* feed on taxa with specific sedimentological and hydraulic preferences (Mathers, Chadd, Dunbar, et al., 2016; Usio & Townsend, 2004). Moreover, recent research has shown that substrate composition (Beatty, Mathers, Patel, Constable, & Wood, 2020) and hydrological conditions (Mathers et al., 2020) in particular can influence the ecological implications of signal crayfish.

A fuzzy-coding procedure is adopted within the trait database with faunal affinities to individual traits ranging from zero (indicating no affinity) to three or five (indicating high affinity—the upper limit depending on existing scientific certainty—Tachet et al., 2010). Trait values were therefore standardized for all samples so that each grouping feature summed to 1 (to ensure trait affinities had an equal weighting between taxa). Taxa resolved to a higher resolution (e.g. species) than available in the trait database (e.g. genus) were aggregated (but maintained in a presence/absence format). All taxa recorded to family-level were excluded. A total of 62 taxa were assigned functional traits. Functional compositions were derived by multiplying standardized trait values with presence/absence data of the respective taxa (i.e. a trait-taxa array). These values were then averaged across all taxa and standardized across all grouping features.

2.2.2 | Influence of *P. leniusculus* invasion on the functional composition and beta diversity of macroinvertebrate communities

Differences in the functional composition of macroinvertebrate communities were analysed via principal coordinate analysis (PCoA) plots performed on a Sørensen dissimilarity matrix using the ‘cmdscale’ function in the stats package in the R environment (R Development Core Team, 2019). Ordinations were performed on a regional basis to explore differences in functional compositions between BACI levels. The total variation explained by each PCoA axis were calculated by dividing its eigenvalue by the sum of all eigenvalues. These plots enabled the shift in functional composition to be identified (via the convex hull—minimum possible area enclosing all functional community values). To identify the functional traits driving differences in functional composition, Pearson correlation tests were performed between individual traits and PCoA axes of the data for each region.

Functional beta diversity was calculated on a regional basis using the ‘functional.beta.pair’ function in the betapart package (based on Sørensen dissimilarity). This approach requires taxonomic and functional trait data inputs and as such the presence/absence dataset of the 62 taxa with trait information was used alongside two PCoA trait vectors (to reduce dimensionality sensu Hill et al., 2019); the latter was processed using the cmdscale function (as above), but using a Sørensen dissimilarity matrix constructed from standardized trait values (a PCoA of functional compositions was not used here as functional.beta.pair accounts for taxonomic inputs separately). Subsequently, three pairwise distance matrices were produced for each region which accounted for total functional beta diversity, and the functional turnover and nestedness-resultant components. Pairwise comparisons between ‘before control’ versus ‘after control’, and ‘before invasion’ versus ‘after invasion’ samples were subsequently identified to examine temporal changes (i.e. before vs. after invasion) in functional total beta diversity, turnover and nestedness separately for control and invaded rivers. These values were then used as a response variable in a linear model using treatment (invaded or control) as a covariate, which was tested in a one-way analysis of variance to identify the significance of differences between control and invaded rivers. Data were unbalanced with greater numbers of before-after comparisons of invaded rivers. To ensure that this did not account for any statistical differences, 100 random samples from control and invaded rivers (i.e. each treatment level) were selected and analysed as above within 30 simulations (see Tables S4–S6).

2.2.3 | Influence of *P. leniusculus* invasion on alpha diversity of macroinvertebrate communities

A number of functional alpha diversity metrics were derived for each sample site (sensu Mason, Mouillot, Lee, & Wilson, 2005; Villéger, Mason, & Mouillot, 2008) from the standardized trait
values. Metrics were calculated via the ‘dbFD’ function within the fd package (Laliberté & Legendre, 2010), which calculates properties of the multidimensional trait space based on a functional trait dissimilarity matrix (Sørensen was utilized on the aforementioned 76 traits), and a dataset characterizing the community composition (a presence/absence matrix of the 62 taxa utilized in the traits analyses). Subsequently, the functional richness (the minimum convex hull encompassing all species); functional evenness (the regularity of distances between taxa connected by a minimum spanning tree); functional divergence (the distribution of taxa in relation to the functional centroid, or the centre of gravity); and Rao’s quadratic entropy (RaoQ; the product of the pairwise distances between taxa) were calculated. In addition, functional alpha diversity was calculated from the functional composition dataset, whereby the inverse Simpson’s index was employed which accounts for the fixed number of traits and their lack of independence (White, Hill, Bickerton, & Wood, 2017). Taxonomic richness was also calculated as a measure of community biodiversity to aid interpretation of some functional measures (e.g. functional richness and functional nestedness; Villéger, Grenouillet, & Brosse, 2013). In summary, a total of six macroinvertebrate community diversity metrics were employed in subsequent analyses: taxonomic richness, functional diversity, functional richness, functional evenness, functional divergence and RaoQ.

To assess differences in the temporal responses (i.e. before vs. after invasion) of functional alpha diversity metrics between control and invaded rivers, Generalized Estimating Equations (GEEs) were fitted to each community response metric using the ‘geeglm’ function in the geepack package (Halekoh, Højsgaard, & Yan, 2006) on a regional basis. For this, treatment (invaded or control), time period (before or after) and their interactive effects (testing the influence of crayfish) were modelled as covariates, alongside a three-way interaction between treatment, time period and season (assessing whether the effect of crayfish differed between seasons). GEE models were established using each river identity as a clustering parameter to account for potential temporal autocorrelation between samples collected from each monitoring site (Liang & Zeger, 1986; Royan, Hannah, Reynolds, Noble, & Sadler, 2014). A fully iterated jackknife variance estimator was employed (associated with the small number of survey locations n = 14) and a first-order autoregressive correlation structure applied (Royan et al., 2014). Pearson residuals were visually inspected to check for heteroscedasticity and outliers (up to five points were removed from a single model).

3 | RESULTS

3.1 | Influence of P. leniusculus invasion on the functional composition and beta diversity of macroinvertebrate communities

PCoA highlighted a clear shift in functional compositions following invasion compared to control rivers in the South East and Anglian regions (Figure 2a,b). In the South East region, crayfish effects were positively associated with axis 1 and in the Anglian region were negatively associated with axis 1, which explained 26.2% and 21.8% of the statistical variation respectively. Functional communities demonstrated reductions in multivariate dispersion following crayfish invasion in the South East region, while increases in multivariate dispersion were evident in Anglian (Figure 2a,b). In contrast, invaded rivers in North West (Figure 2c) demonstrated minor differences in functional composition compared to control rivers even before the invasion, and temporal changes in composition broadly mirrored those seen in control rivers post-invasion (as a result no PCoA correlations are described below for North West but see Tables S2 and S3 for values). Seasonal plots are provided for information in Figure S1.

In the South East, taxa which respire via teguments and possess one brood per year were negatively associated with crayfish invasion, in addition to aquatic active dispersers, taxa that consume living macroinvertebrates and whose life cycles take longer than a year. In the same region, taxa that produce more than one brood per year, which consume dead plants (>1 mm) or are shredders, and...
| Region        | Direction                              | Grouping feature | Trait                           | r    |
|---------------|----------------------------------------|------------------|---------------------------------|------|
| South East    | Negative (negative association with crayfish) | Dispersal        | Aquatic active                  | -0.81|
|               |                                        | Food consumed    | Living macroinvertebrates        | -0.76|
|               |                                        | Voltnism         | 1                               | -0.76|
|               |                                        | Respiration mode | Tegument                        | -0.75|
|               |                                        | Life-cycle duration | >1 year                     | -0.63|
| Positive (positive association with crayfish) | Food consumed | Dead plant > 1 mm |                                   | 0.82 |
|               |                                        | Voltnism         | >1                              | 0.79 |
|               |                                        | Respiration mode | Gill                            | 0.77 |
|               |                                        | Feeding group    | Shredder                        | 0.69 |
|               |                                        | Food consumed    | Living microphytes              | 0.65 |
| Anglian       | Negative (positive association with crayfish) | Food consumed    | Detritus < 1 mm                 | -0.81|
|               |                                        | Substrate preference | Sand                      | -0.75|
|               |                                        | Substrate preference | Mud                       | -0.72|
|               |                                        | Dispersal strategy | Aerially active                | -0.71|
|               |                                        | Locomotion       | Burrower                        | -0.71|
| Positive (negative association with crayfish) | Substrate preference | Flags, boulders, cobbles, pebbles |                                   | 0.91 |
|               |                                        | Respiration mode | Tegument                        | 0.81 |
|               |                                        | Voltnism         | 1                               | 0.80 |
|               |                                        | Locomotion       | Crawler                        | 0.65 |
|               |                                        | Aquatic stage    | Adult                           | 0.62 |

**TABLE 1** Five strongest positive and negative Pearson correlation values between PCoA axis 1 and individual functional traits by region. The association with crayfish invasion provided is based on Figure 1. For correlation values between PCoA2 see Table S2.

**FIGURE 3** Violin plots showing the distribution of temporal changes (before vs. after) in total functional beta diversity, turnover and nestedness-resultant components for control and invaded rivers for each region.
that respire via gills were positively associated with crayfish invasion (Table 1). In the Anglian region, traits associated with habitat preferences of sand, mud and burrowers demonstrated a positive association with crayfish invasion, in addition to aerially active dispersers and taxa that consume detritus. In contrast, taxa associated with larger substrates (e.g. cobbles, pebbles) and expressing higher affinities for tegumental respiration, crawling locomotion behaviours and 1 brood per year demonstrated a negative association with crayfish invasion (Table 1). Functional traits associated with PCoA2 are presented in supplementary material (Table S3).

Total macroinvertebrate functional beta diversity was significantly higher after crayfish invasion in rivers from the South East and Anglian regions (Figure 3; Table 2). Despite a statistically significant difference in temporal changes (before vs. after invasion) in total beta diversity between control and invaded sites in the North West region, the statistical variation explained was negligible ($F = 7.6$, $r^2 = 0.00$, $p < 0.001$) and total beta diversity values were similar (Figure 3). Temporal differences in the functional nestedness-resultant component were significantly higher in invaded rivers than control in South East and Anglian regions, but displayed the opposite trend in the North West (Figure 3; Table 2). Functional turnover over time was significantly higher in invaded sites than in control sites in the North West and South East regions, but was similar for control and invaded sites among the Anglian region despite a significant difference being calculated (Figure 3; Table 2).

3.2 | Influence of *P. leniusculus* invasion on alpha diversity of macroinvertebrate communities

Functional richness values were lower in invaded rivers following invasion for all regions (Figure 4; Table 3). In the Anglian region, functional evenness demonstrated reductions following invasion compared with control rivers, while in the North West, evenness remained stable in invaded rivers but demonstrated an increase in control rivers. In the South East region, there was no change in

**TABLE 2** Summary values of linear model testing temporal changes (before vs. after) in functional beta diversity (dissimilarity values) between treatment groups (control vs. invaded rivers)

| Region    | Total beta $r^2$ | $F$ value | $p$ value | Turnover $r^2$ | $F$ value | $p$ value | Nestedness $r^2$ | $F$ value | $p$ value |
|-----------|------------------|-----------|-----------|---------------|-----------|-----------|------------------|-----------|-----------|
| South East| 0.23             | 1,485.6   | <0.001    | 0.12          | 651.1     | <0.001    | 0.07             | 395.4     | <0.001    |
| Anglian   | 0.23             | 557.0     | <0.001    | 0.04          | 77.13     | <0.001    | 0.13             | 285.7     | <0.001    |
| North West| 0.00             | 7.6       | <0.001    | 0.07          | 145.6     | <0.001    | 0.08             | 169.2     | <0.001    |

**FIGURE 4** Macroinvertebrate community responses (mean ± 2 SE) derived from GEE model for functional richness, functional evenness and functional divergence for each region for each before, after, invaded and control factor. Invaded = grey and control = black. For global plots of taxonomic richness, functional diversity and Rao’s quadratic entropy see Figures S2–S4.
TABLE 3  Summary values from the Generalized Estimating Equations (GEEs) for each English region. Significant terms are denoted in bold. Note: Treatment refers to invaded or control rivers and time period as before or after invasion.

| South East | Anglian | North West |
|------------|---------|------------|
| Treatment × Time period × Season | Treatment × Time period × Season | Treatment × Time period × Season |
| $\chi^2$ | $p$ value | $\chi^2$ | $p$ value | $\chi^2$ | $p$ value |
| Taxonomic richness | 11.36 | 0.022 | 12.24 | <0.001 |
| Functional diversity | 11.95 | 0.012 | 0.17 | 0.681 |
| Functional richness | 1.06 | 0.900 | 10.54 | 0.001 |
| Functional evenness | 2.76 | 0.599 | 6.22 | 0.013 |
| Functional divergence | 55.99 | <0.001 | 11.23 | <0.001 |
| RaoQ | 1.23 | 0.873 | 2.58 | 0.108 |

DISCUSSION

Macrobenthic communities demonstrated altered functional compositions following crayfish invasion compared with control (non-invaded) rivers over the same time period in two of the three regions studied (South East and Anglian). In marked contrast, changes in functional composition were less evident in the North West. These findings are in contrast to the taxonomic changes in all three regions studied (but see Mathers et al., 2020). The effects of crayfish predation on functional properties of macrobenthic communities were highly context-dependent as the three regions studied responded differently to invasion. For functional alpha diversity metrics, the North West control sites remained largely unaltered. These increases in invaded rivers were not observed in invaded rivers, indicating that whatever initiated these biodiversity increases (regional biomonitoring teams are unsure of any management interventions that may have prompted these increases) was restricted or inhibited in rivers invaded by crayfish. In South East and Anglian regions, we found strong evidence that different functional alpha metrics were negatively influenced by crayfish invasion. Functional richness consistently demonstrated significant reductions in invaded rivers while control rivers demonstrated significant increases (Figures 4c,d; Table 3). In contrast, evidence of invasion promoting functional divergence was significant in 11 (out of 18) models (Table 3). However univariate variations in taxonomic richness, functional diversity and RaoQ are not presented in the main text. The former metric yielded comparable results as functional richness (see Figure S2b), while the latter two displayed some trends comparable to functional evenness (Figures S2b,c). The treatment × time two-way interaction testing whether the influence of crayfish invasion varied seasonally was significant in 13 (out of 18) models (Table 3). However, univariate functional evenness within invaded rivers while control rivers demonstrated significant reductions (Figure 4; Table 3). In contrast, evidence of invasion promoting functional divergence (Figure 4; Table 3) was evident in all three regions. Overall, these findings extend and amplify our understanding of functional biodiversity responses to crayfish predation, indicating that crayfish invasion is a cause of widespread functional ecological responses. The combination of invaded functional diversity values increased considerably following invasion while control river values remained largely unchanged over time. However, univariate RaoQ reductions were not significant in invaded rivers (although a slight decrease was evident in the latter), indicating that whatever initiated these biodiversity increases (regional biomonitoring teams are unsure of any management interventions that may have prompted these increases) was restricted or inhibited in invaded rivers. These increases in invaded rivers were not observed in invaded rivers, indicating that whatever initiated these biodiversity increases (regional biomonitoring teams are unsure of any management interventions that may have prompted these increases) was restricted or inhibited in invaded rivers.

While this study, alongside others (e.g. Crawford, Yeomans, & Adams, 2006; Ruokonen et al., 2016), have examined taxonomic responses to signal crayfish invasion, there remains a paucity of studies that have examined the functional responses of benthic communities. This is despite the growing body of literature highlighting the importance of functional biodiversity in ecological communities (but see Mathers et al., 2020). Crawford, Yeomans, & Adams, 2006; Ruokonen et al., 2016), have examined taxonomic responses to signal crayfish invasion, there remains a paucity of studies that have examined the functional responses of benthic communities. This is despite the growing body of literature highlighting the importance of functional biodiversity in ecological communities (but see Mathers et al., 2020).
strong reductions following \textit{P. leniusculus} invasion, while taxonomic richness displayed reductions or remained stable relative to temporal increases in control rivers. Interestingly, while taxonomic and functional richness were positively correlated, the latter displayed stronger effects in the Anglian region demonstrating the ‘hidden’ effects that may be missed by employing purely taxonomic metrics. This highlights how species losses associated with crayfish invasion can reduce the range of functional trait niches displayed by macroinvertebrate communities, with implications for wider ecosystem functioning (Flood et al., 2020). For instance, \textit{P. leniusculus} selectively predate on sedentary taxa such as leeches (Hirudinea) and snails (Gastropoda) (Nyström & Strand, 1996), which possess distinct functional niches within macroinvertebrate communities (Usseglio-Polatera, Bournaud, Richoux, & Tachet, 2000). Such effects of crayfish predation may also result in the establishment of taxa possessing extreme trait combinations, such as taxa that display resistance strategies, becoming more prominent as shown in the higher functional divergence values within invaded rivers in general.

In South East and Anglian regions, voltinism and, in the South East region, life-cycle traits, appeared to be influenced by crayfish invasion, with taxa that have one brood per year and/or that take over a year to complete a life cycle being negatively affected. In contrast, macroinvertebrate taxa able to complete their life cycle in less than a year may be more resilient to invasion by enabling communities to respond when crayfish are less active (such as over winter) or following flood events when crayfish implications for biodiversity are dampened (Mathers et al., 2020). Similarly, multiple broods per year may also enhance the likelihood that a population persists at a site.

Greater functional total beta diversity was recorded over time (i.e. before vs. after periods) in invaded rivers compared with control rivers in the South East and Anglian regions. This was predominantly driven by higher nestedness values in invaded rivers, which may reflect the following mechanisms (which are not mutually exclusive): (a) selective predation of crayfish on prey macroinvertebrates resulting in a loss of functional richness among sample sites and subsequent increases in nestedness; (b) a change of abiotic environmental conditions driven by crayfish activity (e.g. increases in fine sediment), which may be unsuitable for many macroinvertebrate taxa and lead to their loss from invaded sites; and (c) low functional redundancy in the macroinvertebrate communities resulting in the loss of functional traits as single species are removed via direct and indirect effects of crayfish.

\textit{P. leniusculus} have been shown to engineer riverine environments, increasing suspended sediment levels and potentially enhancing the input of fine sediment into rivers associated with their burrowing behaviour (Faller et al., 2016; Rice, Johnson, Mathers, Reeds, & Extence, 2016). Such behaviour can have subsequent feedbacks in altering predator-prey dynamics (Mathers, Rice, & Wood, 2019) and may also lead to the selective exclusion of certain functional traits. Evidence from the Anglian region (which contains predominantly clay rivers) suggests that taxa which burrow into sand/mud substrates were positively associated with crayfish invasion, while crawling taxa inhabiting cobble and pebble substrata displayed the opposite trend.

We also found a strong association of feeding strategies/food sources and the presence of crayfish in both the South East and Anglian regions. Taxa that feed on organic matter (e.g. shredders) were positively associated with crayfish invasion, while taxa that predate on living macroinvertebrates were negatively affected. It is likely that some crayfish species may increase the production of detritus or dead plant material through directly increasing the breakdown of leaves (Creed & Reed, 2004; Jackson et al., 2014) and by damaging macrophytes (Nyström & Strand, 1996). However, it is also likely that the effects of crayfish are invader specific with contrasting responses documented for different crayfish species (Dunoyer, Dijoux, Bollache, & Lagrue, 2014) with shredder densities also being reduced via direct predation by some crayfish species (Lagrue, Podgorniak, Lecerf, & Bollache, 2014). The presence of invasive crayfish is likely to affect functional feeding groups, particularly predatory taxa, as crayfish will outcompete other macroinvertebrate species while simultaneously reducing prey taxa directly and may also consume larger predatory macroinvertebrates (Ruokonen et al., 2016; Usio, Suzuki, Konishi, & Nakano, 2006).

### 4.1 Implications for river monitoring and management

Our findings suggest that, in most instances, the functional properties of macroinvertebrate communities responded significantly to signal crayfish invasion. The responses were highly variable, however, and likely to be dependent on the environmental context within regions. Application of functional traits, which are not confined to the same biogeographic constraints as taxa, may therefore provide a platform to guide regional management strategies (see White, Hannah, et al., 2017). In this regard, Mathers et al. (2020) demonstrate how flow-management strategies might influence the functional biodiversity of lotic communities following crayfish invasion. Building on this work, by identifying patterns in functional diversity and the ecological processes and functional traits driving these trends on a regional basis, restoration practices could be targeted to achieve maximum benefit. Within our study, macroinvertebrate communities inhabiting Anglian rivers, broadly dominated by clay geology, became functional subsets of control rivers. This was driven primarily through reduced functional richness and an increased dominance of taxa that burrow into mud/sand substrates. Here, restoration strategies aimed at enhancing coarse substrate habitats and reducing fine sediment deposition could provide greatest gains (e.g. Sear, Newson, Hill, Old, & Branson, 2009). In contrast, rivers in the North West which are dominated by hard geology displayed impeded biodiversity gains which were otherwise seen in control rivers, where invasive crayfish were present. As such, restoration efforts in this region focused on enhancing local habitat heterogeneity to provide refugia from predation may provide beneficial outcomes by offsetting the additional
pressures caused by invasion (Barrios-O’Neill, Dick, Emmerson, Ricciardi, & MacIsaac, 2015; Beatty et al., 2020).

We call for further research that explores the ecological mechanisms behind invasion success and subsequent consequences on both functional and taxonomic properties of biological communities which could offer unique insights into managing and conserving freshwater ecosystems. Many biomonitoring metrics are derived from ecological guilds, such as fine sediment (EPSI—Empirically weighted Proportion of Sediment-sensitive Invertebrates; Turley et al., 2016) and flow (LIFE—Lotic-invertebrate Index for Flow Evaluation; Exteute, Balbi, & Chadd, 1999) and have been shown to highly sensitive to modifications in community structure associated with selective predation by invasive crayfish, leading to inaccurate inflation (Mathers, Chadd, Exteute, et al., 2016; Turley et al., 2017). By going beyond taxonomic approaches and examining functional aspects, potential implications for national biomonitoring programs may become evident.

Finally, our study highlights the importance of employing long-term, BACI designs when evaluating the ecological implications of stressors, such as biological invasions. By comparing ecological responses at invaded sites to temporal changes exhibited at control sites, the implications of crayfish invasion could be effectively drawn in each of the three English regions studied. Assessing biological invasions is inherently difficult, but the use of regulatory agency data that are often collected over multiple years represents a valuable approach (Dafforn et al., 2016) that can inform the monitoring and future protection of rivers and other ecosystems from biological invasions across the globe.

ACKNOWLEDGEMENTS

The authors thank Russ Barber, Judy England, Andy Goodwin, Katy Lee, Will Olsen and Mitch Perkins from the Environment Agency for kindly providing the data from the BIOSYS database to undertake the research and for providing environmental context. S.G. was supported by a Royal Society-Newton International Fellowship at Loughborough University, UK (NIF.RI.180346) while undertaking this research. Mark Szegner is gratefully acknowledged for the preparation of Figure 1. We thank two anonymous reviewers, the associate editor and editor for their constructive comments which have greatly improved the clarity and presentation of the study outcomes. The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTIONS

K.L.M. and J.C.W. conceived the ideas; J.C.W. analysed the data; all the authors contributed to conceptualization and interpretation. K.L.M. led the writing of the manuscript with support from J.C.W. and S.G. All the authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

All biological data can be obtained freely from the Freshwater river macroinvertebrate surveys (BIOSYS) at https://data.gov.uk/dataset/3fafa10d7-04bc-49e0-8377-6175186d21d/freshwater-river-macroinvertebrate-surveys-biosys using the site codes found in Table S7.

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**SUPPORTING INFORMATION**

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

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**How to cite this article:** Mathers KL, White JC, Guareschi S, Hill MJ, Heino J, Chadd R. Invasive crayfish alter the long-term functional biodiversity of lotic macroinvertebrate communities. Funct Ecol. **2020**, 00:1–12. https://doi.org/10.1111/1365-2435.13644