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How biodiversity affects ecosystem processes: implications for ecological revolutions and benthic ecosystem function

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ABSTRACT: Current and projected rates of extinction provide impetus to investigate the consequences of biodiversity loss for ecosystem processes. Yet our understanding of present day biodiversity–ecosystem functioning relations contrasts markedly with our understanding of the responses of species to changes that have occurred in the geological record. Of the experiments that have explicitly tested the relationship between biodiversity and ecosystem functioning, few have attempted to reconcile whether the underlying process that gives rise to the observed response is affected by biodiversity in the same way as the observed response. In the present study, we use benthic macrofaunal invertebrates to examine and distinguish the effects of species richness and species identity on bioturbation intensity, a key mechanism that has been important on evolutionary timescales regulating ecosystem functioning in the marine benthos. Our study identifies significant effects of species richness that reflect species-specific impacts on particle reworking that, in turn, lead to elevated levels of nutrient generation. However, our findings also suggest that the consideration of only bioturbation intensity forms an incomplete evaluation of bioturbation effects because the way in which species interact with the benthic environment does not necessarily reflect organism traits only associated with particle transport. Our study emphasises the need for caution when extrapolating from assumed knowledge of organism traits to how changes in species composition associated with ecological crises may impact ecosystem function. Nonetheless, the empirically derived mechanistic effects of bioturbation on ecosystem functioning documented here are sufficiently general to seek correlations between diversity and function in natural systems, including those from the palaeoecological record.

KEY WORDS: Biodiversity · Ecosystem function · Bioturbation · Substrate revolution · Extinction · Marine benthos

INTRODUCTION

One of the most significant events in the history of marine life was the emergence and subsequent diversification of bilaterian invertebrate fauna, approximately 0.5 to 1.0 billion years ago (Wray et al. 1996, Droser et al. 2002). Many morphological features of these fauna suggest that they had a primarily benthic lifestyle (Valentine 1994, Budd & Jensen 2000), and their appearance in the fossil record correlates well with an increase in the depth and intensity of sediment mixing, especially during the Proterozoic–Phanerozoic transition (Droser & Bottjer 1988, McIlroy & Logan 1999). As these early metazoans began to interact with their environment (see, inter alia, Gray 1974, Rhoads 1974, Rhoads et al. 1977, Aller 1982, Rhoads & Boyer 1982, Krantzberg 1985), the sediment–water interface began to change from a distinct and effectively impermeable boundary to a more open and diffuse layer that was more habitable to life. Sequential changes in benthic community structure that coincided with this 'agronomic revolution' (sensu Seilacher & Pflüger 1994) resemble present day conceptual models of benthic macrofaunal succession (Pearson & Rosenberg

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1978, Rhoads et al. 1978, Rumohr et al. 1996); the colonisation of a few opportunistic species paved the way for a greater variety of species and dramatically transformed (i.e. Cambrian substrate revolution, sensu Bottjer et al. 2000) the Precambrian bacterial and algal planar mat systems (e.g. Gehling 1999) into diverse 4-dimensional benthic communities that were sated with complex biogeochemical cycles and novel ecospace (Seilacher 1999, Dornbos et al. 2005, Dornbos 2006). Such changes in community structure and function are expected to be reversible and linked with mass extinction events, a conclusion that corresponds to evolutionary patterns in the fossil record that reveal a close association between extinction forcing agents and the diversification of marine soft-bottom communities (Macleod 1994, Martin 1996, Jacobs & Lindberg 1998, Harper 2006, Pruss et al. 2006, Canfield et al. 2007).

Of relevance to contemporary ecology, the geological record provides confirmation that diversification and extinction events are intimately linked to the provision of ecosystem processes, particularly the biogeochemical cycles of carbon and nutrients (Logan et al. 1995, Martin 1996). Although the ecological impacts of even well-documented ecological revolutions in the geological record have recently been considered (e.g. Jenkins 1992, Dornbos et al. 2005, Seilacher et al. 2005, James & Price 2008), the focus has been on documenting faunal change rather than on examining how changes in biodiversity may have altered species interactions and the functioning of the marine ecosystem (exceptions include Clapham & Narbonne 2002, Vannier et al. 2007, Dornbos & Chen 2008). Many ecosystem processes are mediated by infaunal invertebrates, and concomitant changes in the rates and depths of bioturbation through the Phanerozoic (Thayer 1983) provide compelling evidence that benthic nutrient cycling was necessary to sustain the primary productivity of the marine biosphere (Martin et al. 2008). Given that these early faunal assemblages are ecologically similar to modern communities (Bottjer & Ausich 1986, Clapham et al. 2003, Narbonne 2005 and references therein), and that they appear to have been critical to the provision of ecosystem processes, much can be learnt by performing manipulative experiments with fauna from the present day (e.g. Emmerson et al. 2001, Marinelli & Williams 2003, Mermillod-Blondin et al. 2005, Norling et al. 2007) or by applying modelling approaches (e.g. Solan et al. 2004) to data in order to predict how extinctions have in the past, or will in the future, affect ecosystem processes.

In recent years, spurred by the anticipation that anthropogenic forcing (Worm et al. 2006, Halpern et al. 2008) is likely to have considerable ecological consequences within the next century (Sala et al. 2000), an extensive body of literature has emerged that focuses on the effects of biodiversity loss on key ecological processes (for reviews, see Covich et al. 2004, Hooper et al. 2005, Cardinale et al. 2006, Stachowicz et al. 2007). By adopting an experimental approach (see Raffaelli et al. 2003) that involves measuring pertinent ecosystem processes from simple model communities that differ only in the number of species, it has been possible to identify mechanisms that are important and underpin the biodiversity–ecosystem functioning relationship (e.g. complementarity and selection effects; Cardinale et al. 2002, Loreau & Hector 2001). These reflect how species interact with the environment, such that ecosystem processes can often be predicted from species composition or species traits, but not necessarily from species richness per se (see Stachowicz et al. 2007). Few studies, however, have attempted to reconcile whether the assumed mechanism that mediates the observed response is affected by biodiversity in the same way as the observed response. If the effects of biodiversity alter both the process responsible for generating an ecosystem response (e.g. bioturbation) as well as the response itself (e.g. nutrient generation) then it would be reasonable to assign causality and determine the relative contribution of a given process to the total observed yield.

In the present study, we build on previous empirically derived knowledge (species richness, identity and density effects on benthic nutrient cycling; Ieno et al. 2006) by distinguishing the effects of species richness and species identity on bioturbation intensity, a key process that underpins and regulates ecosystem functioning in the marine benthos. Our aim is: (1) to establish whether strong species richness and identity effects regulate bioturbation intensity and, if so, demonstrate that bioturbation forms the mechanistic link between the infauna and their effect on the benthic environment and (2) to demonstrate the utility of our approach to the palaeoecological research community who hold a valuable repository of information (species richness, evenness, morphological and behavioural information, proxies for ecosystem function; Clapham et al. 2003, Widdicombe et al. 2003) from times of major transitions in the evolutionary past that could be used to inform the biodiversity–ecosystem function agenda.

MATERIALS AND METHODS

Faunal and sediment collection. Sediment and 3 infaunal invertebrates, the deposit-feeding polychaete *Hediste diversicolor* (HD), the surficial grazing bivalve *Hydrobia ulvae* (HU) and the suspension-feeding bivalve *Cerastoderma edule* (CE) were collected from mud flats in the Ythan estuary, Aberdeenshire, Scotland (57° 20.085′ N, 02° 0.206′ W). Sediment was sieved

(0.5 mm mesh) in a seawater bath to remove macrofauna and then allowed to settle for 24 h to retain the fine fraction (<63 µm). The settled sediment was homogenised and added to each mesocosm 48 h prior to species addition. Seawater (UV-sterilised, 10 µm prefiltered, salinity ≈ 33) was replaced after 24 h to allow the removal of excess nutrients associated with disturbance during assembly.

Mesocosms. Replicate (n = 5) macrofaunal communities were assembled in monoculture and in mixtures of 2 and 3 species in Perspex cores (330 mm high, 100 mm internal diameter) containing 10 cm depth of sediment (785 cm^3) and 20 cm of overlying seawater (2.35 l). The mesocosms were maintained in environmental chambers (VC 4100, Vötsch Industrietechnik) that can control temperature (14.0 ± 0.1°C) and light period (12 h light:12 h dark cycle; 2 × 36 W fluorescent tube lights, Arcadia, Model FO-30) for 21 d. To minimise hidden treatment effects (sensu Huston 1997), eliminate pseudoreplication and allow the generality of any diversity effects to be evaluated, species richness treatments containing 1 and 2 species were replicated using unique species permutations (3 × 1 sp. [HD, CE, HU] and 3 × 2 spp. mixtures [CEHU, CEHD, HDHU]; Table 1). This is not possible for the 3-species mixture (CEHDHU) because of the limited available species pool (n = 3). Biomass was fixed at 2.0 g $mesocosm^{-1}$ (≈255 g m^{-2}) in all species richness treatments, a level consistent with that found at the study site. All mesocosms were continually aerated.

Tracers. Sediment particle reworking by benthic fauna was estimated using luminophore tracers (i.e. natural sediments treated with a dye that fluoresces in ultraviolet light; Mahaut & Graf 1987). The luminophores (sand-based, size class 125 to 250 µm diameter; Partrac) were pre-soaked (24 h) and vigorously shaken in seawater prior to addition to the mesocosms to prevent particle aggregation and other hydrophobic effects. For each mesocosm, 0.1 g dry weight of luminophores was added and evenly distributed across the sediment surface. Following experimental incubation (21 d), extruded cores were vertically sectioned at a resolution of 0.5 cm to a depth of 8.0 cm (i.e. 16 slices). Each slice was homogenised, and a subsample (2.5 cm^3) was taken. Each subsample was spread thinly on a 90 mm diameter Petri dish and illuminated by an ultraviolet light source (2 × 8 W tubes; Sylvania Blacklight). Luminophores were viewed via a ⅓ inch CCD colour camera (Genie C8706/240) linked to a television monitor and manually counted. Luminophore counts from each core slice were normalised to the total recovered from the cores.

Bioturbation model. The biodiffusion coefficient (D_b) was determined for the relative concentration of luminophores in each profile using the solution to the 1-dimensional diffusion model presented by Crank (1975):

$$C(z,t) = \frac{M}{\sqrt{\pi D_b t}} \exp\left(\frac{-z^2}{4D_b t}\right) \quad (1)$$

where $C(z,t)$ is the relative tracer (i.e. luminophores) concentration at depth z and time t, and M is the total amount of luminophores applied. D_b was derived by weighted least-squares regression of predicted profiles on observed tracer concentrations (François et al. 2002). This procedure calculates a squared residual between the observed (O) and predicted (P) concentrations for each depth horizon, which is weighted by the corresponding observed concentration + 1 to prevent a null denominator. By summing the residuals, a regression coefficient (r) is calculated as:

$$r = \sum_{i=1}^{n} \frac{(O_i - P_i)^2}{O_i + 1} \quad (2)$$

where the predicted profile forms an identical match with the observed profile, $r = 0$. Model profiles with the lowest r were selected to calculate D_b.

Statistical analyses. Statistical models were developed for the dependent variable bioturbation (D_b), and the independent nominal variables species richness (SR, n = 4) and, separately, species identity (SPID, n = 8) (Table 1). For the latter, the contribution of species mixture to bioturbation was assumed to be synergistic rather than additive (e.g. Ieno et al. 2006) and each species combination was treated as a unique 'species' identity.

Graphical exploratory techniques were used to check for outliers. As a first step we fitted a linear regression. A model validation was applied to check that underlying statistical assumptions were not violated; normality was assessed by plotting theoretical quantiles versus standardised residuals (quantile–quantile plots), homogeneity of variance was evaluated by plotting

Table 1. Summary of species combinations used in the assembled macrofaunal communities. Biomass represents target biomass (realised biomass accuracy, mean ± SE = 2.0082 ± 0.0196 g, n = 35). HD: *Hediste diversicolor*; CE: *Cerastoderma edule*; HU: *Hydrobia ulvae*

Species richness	Species identity	n	Biomass (g $mesocosm^{-1}$) HD	CE	HU	Total biomass (g $mesocosm^{-1}$)
0	0	5	0	0	0	–
1	1	5	2.0	0	0	2.00
1	2	5	0	2.0	0	2.00
1	3	5	0	0	2.0	2.00
2	4	5	0	1.0	1.0	2.00
2	5	5	1.0	1.0	0	2.00
2	6	5	1.0	0	1.0	2.00
3	7	5	0.67	0.67	0.67	2.00

residuals versus fitted values, non-linearity was evaluated by plotting residuals versus explanatory variables, and influential data points were identified using Cook's distance (Quinn & Keough 2002). Where there was evidence of unequal variance in the residuals, we used linear regression with a generalised least-squares (GLS) estimation procedure (Pinheiro & Bates 2000). Use of GLS means that a data transformation to stabilise the variance is not necessary because of the use of variance–covariate terms that allow for unequal variance. This has the added advantage that the original structure of the data can be retained.

To find the minimal adequate model, we adopted the approach outlined by Verbeke & Molenberghs (2000) and Diggle et al. (2002), i.e. the most appropriate structure in terms of random components is determined using a REML (restricted maximum likelihood) estimation; subsequently, the optimal fixed effects structure is determined using an ML (maximum likelihood) estimation. The optimal random structure was determined by starting with a model without any variance–covariate terms (equivalent to linear regression) and comparing this model with subsequent GLS models that contained specific variance structures (i.e. different spread per stratum for each nominal variable; see Table 5.2 in Pinheiro & Bates 2000). Comparisons of these models were made using the AIC (Akaike information criteria) and plots of residuals versus fitted values. The optimal fixed structure was established by applying a backward selection using the likelihood ratio test obtained by ML estimation. The importance of each explanatory factor in the minimum adequate model was assessed by comparing a reduced model (with all terms involving the factor of interest removed) with the full model, using the likelihood ratio test. The numerical output of the optimal model was obtained using REML estimation (West et al. 2007). All analyses were performed using the 'nlme' package (v3.1; Pinheiro et al. 2006) in the R (v2.6.1) statistical and programming environment (R Development Core Team 2005).

In order to assess whether there were any positive effects of species interactions on bioturbation intensity, we compared bioturbation intensity in species mixtures relative to monocultures using D_{max} (Loreau 1998):

$$D_{max} = \frac{D_{b\,mix} - \max(D_{b\,mono})}{\max(D_{b\,mono})} \quad (3)$$

where $D_{b\,mix}$ is the observed bioturbation intensity in the mixture and $\max(D_{b\,mono})$ is the maximum observed bioturbation intensity in the monocultures. When a mixture performs better than the corresponding monocultures (i.e. overyielding), $D_{max} > 0$.

RESULTS

Bioturbation

Use of luminophores allowed quantitative differences in infaunal bioturbation activity to be determined. The mean ± SD relative count in the uppermost layer (0 to 0.5 cm) of mesocosms containing no macrofauna was 99.4 ± 0.01 % (n = 5), indicating that any vertical displacement of particles was not related to the properties of the luminophores or to the method of recovery. The form of the vertical profile differed between species identity treatments, but varied little between replicates (Fig. 1). The maximum vertical displacement of luminophores was 1.0 to 1.5 cm for monocultures of *Hydrobia ulvae*, 1.5 to 2.0 cm when *Cerastoderma edule* was present (alone or in mixture with *H. ulvae*) or >7.5 cm in all treatments containing *Hediste diversicolor*. Despite differences between luminophore profiles (compare panels, Fig. 1), the transport of luminophore particles approximated a biodiffusive profile with depth in all mesocosms (r_{max} = 0.024, n = 40; Fig. 2a). Of the single species treatments, mean D_b ($\times 10^2$ cm^2 yr^{-1}, ±SD, n = 5) was greatest in *H. diversicolor* (4.73 ± 0.89), followed by *C. edule* (3.45 ± 0.86)

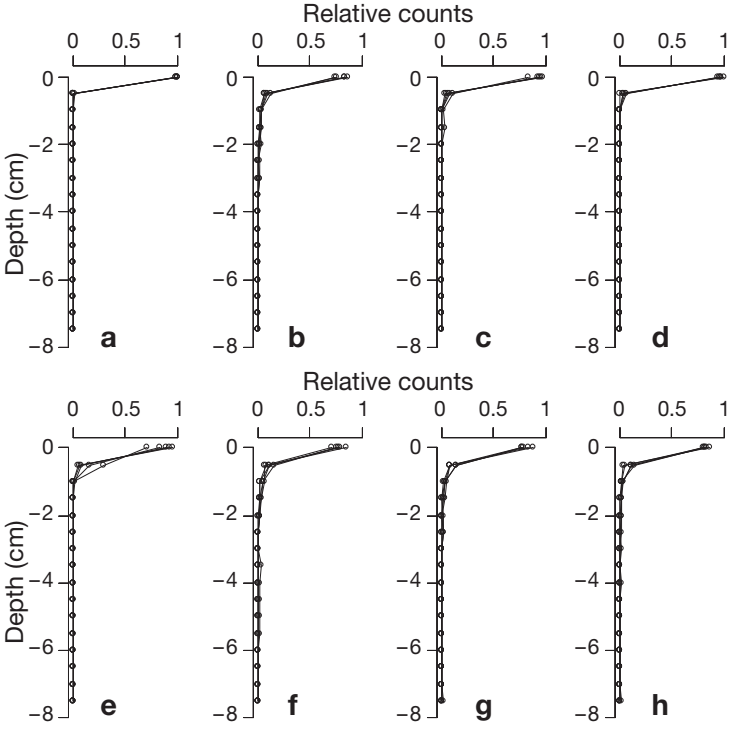

Fig. 1. Vertical distribution of luminophores in each mesocosm for replicate (n = 5) cores containing: (a) no macrofauna, (b) *Hediste diversicolor* (HD), (c) *Cerastoderma edule* (CE), (d) *Hydrobia ulvae* (HU), (e) CEHU, (f) CEHD, (g) HDHU and (h) CEHDHU. The numbers of luminophores from each sediment slice are expressed relative to the total number recovered from each core

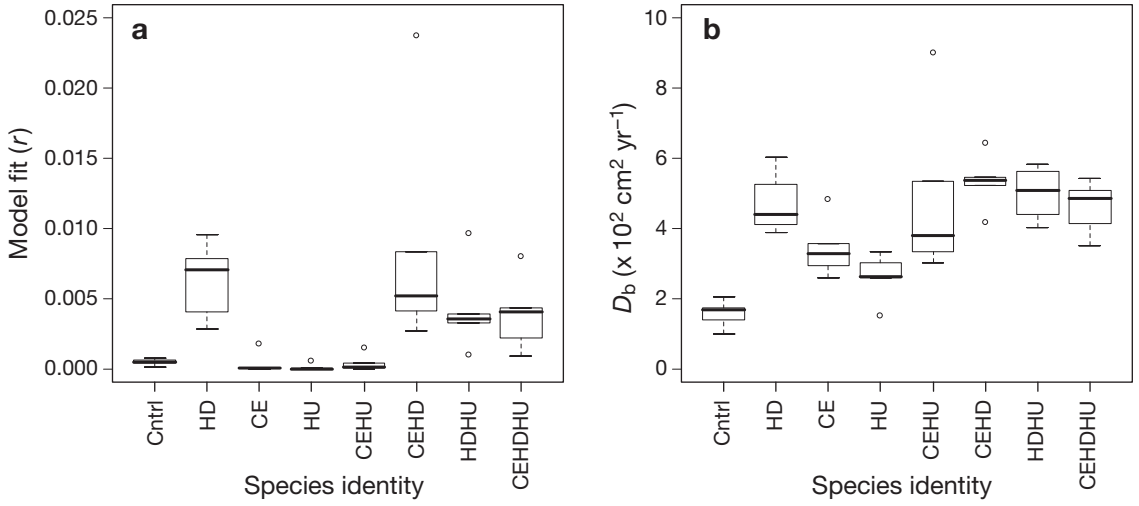

Fig. 2. (a) Weighted least-squares regression of predicted profiles on observed tracer concentrations (r) and (b) bioturbation intensity (D_b) for each species identity treatment (n = 5). In each case, the median is indicated at the midpoint, the upper and lower quartiles are indicated by the hinges, lines represent the spread and open circles indicate outliers. The species composition of each mixture is indicated on the x-axis, where species identity corresponds to: no macrofauna (Cntrl); *Hediste diversicolor* (HD), *Cerastoderma edule* (CE) and *Hydrobia ulvae* (HU)

and *H. ulvae* (2.62 ± 0.69). The mean D_b ($\times 10^2$ cm^2 yr^{-1}, ±SD, n = 5) of the multispecies mixtures tended to be high relative to the monocultures (except *H. diversicolor*), ranging from 4.60 ± 0.78 (3 spp. mixture) to 5.33 ± 0.81 (*C. edule* + *H. diversicolor*) (Fig. 2b).

Species richness effects

There were clear positive effects of species richness on bioturbation intensity (Fig. 3a). The minimal adequate model (Model 1 in Appendix 1) was a linear regression with a GLS extension incorporating species richness as a main term and as a variance–covariate. Although the influence of species richness on bioturbation intensity was significant (L-ratio = 30.14, df = 3, $p < 0.0001$), closer examination of the coefficient tables (Table A1) revealed that bioturbation intensity was greatest in treatments containing 2 species (coefficient = 3.50, $t = 8.42$, $p < 0.0001$) rather than in those containing the highest level of species richness (coefficient = 3.03, $t = 7.78$, $p < 0.0001$). Bioturbation intensity in both the 2-species (coefficient = 3.48, $t = 3.05$, $p = 0.0042$) and 3-species mixtures (coefficient = 1.01, $t = 2.18$, $p = 0.0359$) were greater than the corresponding monocultures (Table A1).

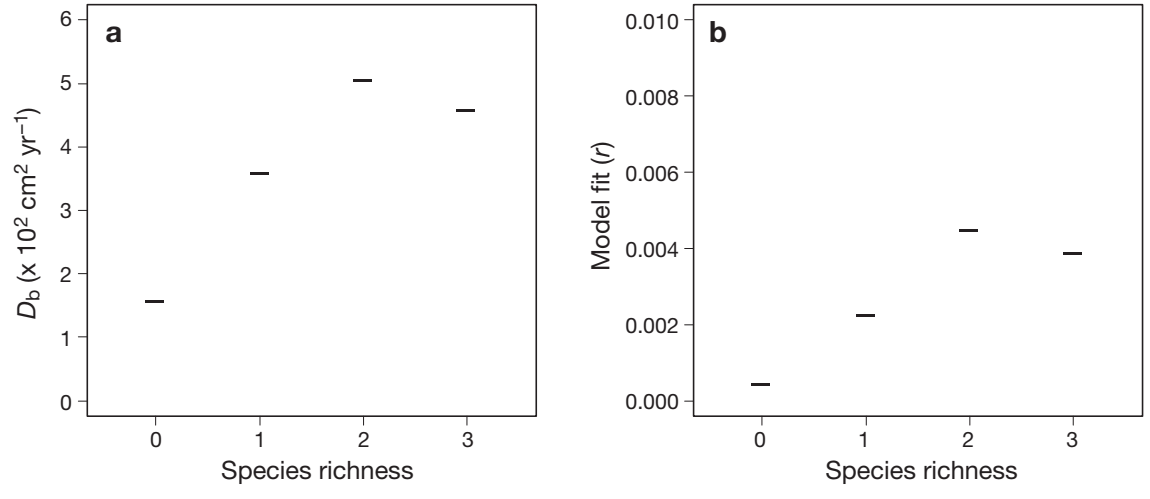

Fig. 3. Effect of species richness on: (a) bioturbation intensity (D_b) and (b) the weighted least-squares regression of predicted profiles on observed tracer concentrations (r). Horizontal bars represent predicted values from the optimal regression model for each species richness treatment. As the generalised least-squares (GLS) framework allows for different spreads in the data, individual data points are omitted to prevent misinterpretation

Although the fit of the biodiffusive model (Fig. 2a) to the observed vertical distribution of luminophores (Fig. 1) was acceptable in all cases, the mean square deviation between the experimental and simulated luminophore counts was affected by species richness (Fig. 3b). The minimal adequate model (Model 2 in Appendix 1), a linear regression with a GLS extension incorporating species richness as a variance–covariate, indicated a negative effect (increasing r) of species richness on model fit (L-ratio = 15.23, df = 3, p = 0.0016). Comparison of the regression model coefficients (Table A2) indicates that the greatest deviation between fitted and observed profile values occurred at intermediate levels of species richness (SR = 2, coefficient = 0.004, t = 2.56, p = 0.0149), followed by the 3-species mixture (coefficient = 0.003, t = 2.85, p = 0.0072) and the monocultures (coefficient = 0.002, t = 2.07, p = 0.0461), although there was no significant difference between the 2-species and 3-species mixtures (coefficient = 0.0006, t = 0.31, p = 0.762).

Species identity effects

The effects of species richness on bioturbation intensity were underpinned by strong effects of species composition (Fig. 4a). The minimal adequate model (Model 3 in Appendix 1), a linear regression, with a GLS extension, species identity as a main term and variance–covariate, highlighted clear differences in bioturbation intensity with species identity (L-ratio = 44.589, df = 7, p < 0.0001; Fig. 4a). Comparison of model coefficients (Table A3) revealed that the effects of *Hediste diversicolor* were greater than those of *Cerastoderma edule* (coefficient = 1.280, t = 2.31, p = 0.0275) and *Hydrobia ulvae* (coefficient = 2.113, t = 4.182, p = 0.0002). Whilst it is clear from the monocultures that the presence of *H. diversicolor* is influential in determining the intensity of bioturbation, the presence of this species did not necessarily dictate polyculture performance as mixtures containing *H. diversicolor* were not significantly higher than mixtures where *H. diversicolor* was absent (compare $SPID_{CEHU}$ to $SPID_{CEHD}$, $SPID_{HDHU}$ and $SPID_{CEHDHU}$ in Table A3; p = 0.7179 to 0.9394). As the lowest bioturbation intensity in monoculture occurred with *H. ulvae*, it is intuitive to assume that the role of *C. edule* is of importance in the mixtures; yet, the contribution of *C. edule* was not significantly higher (coefficient = 0.833, t = 1.691, p = 0.1005) than that of *H. ulvae* when in monoculture. However, when *H. diversicolor* and *C. edule* are in mixture (= $SPID_{CEHD}$), their combined contribution in terms of bioturbation intensity tends to be equivalent to mixtures that contain at least 1 of these species ($SPID_{HD}$, coefficient = –0.596, t = –1.108, p = 0.2763; $SPID_{CEHU}$, coefficient = –0.422, t = –0.364, p = 0.7179; $SPID_{HDHU}$, coefficient = –0.333, t = –0.665, p = 0.5108; $SPID_{CEHDHU}$, coefficient = –0.723, t = –1.446, p = 0.158; Table A3 & Fig. 4a). Thus, the relative contribution to bioturbation from *H. diversicolor* or *C. edule* is so dominant that it masks the contribution of other species present within the same mixture, irrespective of proportional representation.

The relative fit between the experimental and simulated luminophore counts (Fig. 4b) was also influenced by species identity (L-ratio = 29.59, df = 7, p < 0.0001; linear regression with a GLS extension, species identity as a main term and variance–covariate; Model 4 in Appendix 1). Evaluation of the model coefficients

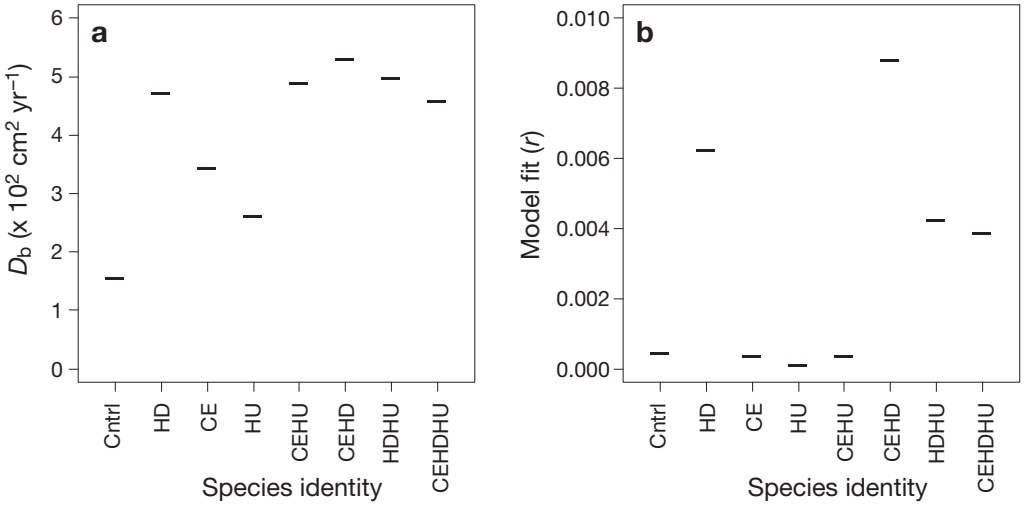

Fig. 4. Effect of species identity on: (a) bioturbation intensity (D_b) and (b) the weighted least-squares regression of predicted profiles on observed tracer concentrations (r). Horizontal bars represent predicted values from the optimal regression model for each species identity treatment. As the GLS framework allows for different spreads in the data, individual data points are omitted to prevent misinterpretation

(Table A4) reveal a marked difference in fit between treatments that contained *Hediste diversicolor* and those where the species was absent. Relative to treatments containing *H. diversicolor* in monoculture, the fit of the biodiffusive model was improved ($r \rightarrow 0$) when *H. diversicolor* was absent ($SPID_0$, coefficient = -5.80×10^{-4}, $t = -4.68$, $p < 0.0001$; $SPID_{CE}$, coefficient = -5.89×10^{-4}, $t = -4.59$, $p < 0.0001$; $SPID_{HU}$, coefficient = -6.16×10^{-4}, $t = -4.96$, $p < 0.0001$; $SPID_{CEHU}$, coefficient = -5.88×10^{-4}, $t = -4.65$, $p < 0.0001$; Table A4). In mixtures where *H. diversicolor* was present, irrespective of the composition of the mixture, the fit of the model was insignificantly different ($SPID_{CEHD}$, coefficient = 2.56×10^{-4}, $t = 0.63$, $p = 0.5317$; $SPID_{HDHU}$, coefficient = -2.01×10^{-4}, $t = -1.06$, $p = 0.2969$; $SPID_{CEHDHU}$, coefficient = 2.40×10^{-4}, $t = -1.38$, $p = 0.1762$; Table A4) to that derived for treatments containing *H. diversicolor* in monoculture. All treatments containing macrofauna, but excluding *H. diversicolor*, showed indistinguishable levels of model fit from one another (compare $SPID_{CE}$ to $SPID_{HU}$ and $SPID_{CEHU}$ in Table A4).

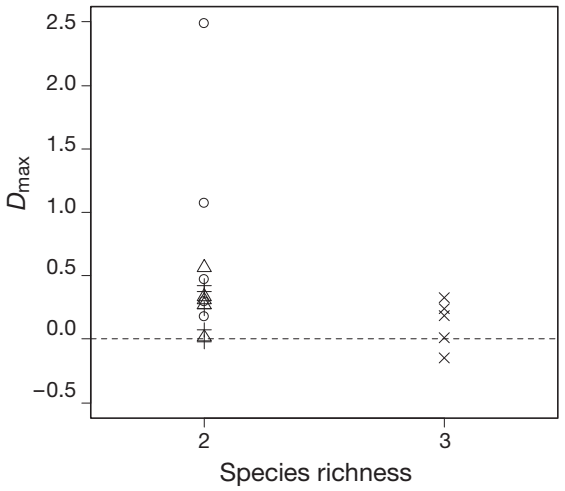

Fig. 5. Observed yield (D_{max}, see Eq. 3) in bioturbation intensity (D_b) relative to monocultures as a function of species richness. Overyielding determines whether a species mixture outperforms the best single species treatment for those species contained in that mixture and is distinguished when $D_{max} > 0$. The species composition of each mixture is indicated by plot symbols (○: CEHU; △: CEHD; +: HDHU; ×: CEHDHU). HD: *Hediste diversicolor*; CE: *Cerastoderma edule*; HU: *Hydrobia ulvae*

Overyielding

Bioturbation intensity in the majority (90%, n = 20) of the species mixtures showed evidence of overyielding ($D_{max} > 0$; Fig. 5), but the degree of overyielding (D_{max}, mean ± SD) reduced with increasing species richness (SR_2, 0.47 ± 0.62, n = 15; SR_3, 0.12 ± 0.19, n = 5). The variability in D_{max} was lower in the 3-species mixtures (range = 0.47, n = 5) relative to the 2-species mixtures (range = 2.51, n = 20), although there were 2 influential points within the $SPID_{CEHU}$ treatment that overemphasised this trend (when removed, range = 0.59, n = 18). Clear effects of species identity underpin the variability in overyielding, with the presence of *Cerastoderma edule* and *Hediste diversicolor* leading to elevated levels of bioturbation intensity.

Linking ecosystem process to ecosystem function

As species richness and species identity have significant effects on both nutrient generation (Ieno et al. 2006) and bioturbation intensity (present study), and as bioturbation forms the mechanistic link between the infauna and their effect on the benthic environment, it is inappropriate to simultaneously regress bioturbation intensity and species richness (or species identity) against nutrient generation, because species richness (or species identity) and bioturbation intensity are collinear. Whilst we recognise that care must be taken in inferring causality from correlation, correlations of bioturbation intensity from the present analysis with all 3 nutrients (NH_4-N, $\rho = 0.429$, $t = 2.93$, df = 38, $p = 0.0057$; NO_x-N, $\rho = -0.337$, $t = -2.21$, df = 38, $p = 0.0333$; PO_4-P, $\rho = 0.447$, $t = 3.08$, df = 38, $p = 0.0038$) previously reported in Ieno et al. (2006) suggest that the effects of biodiversity on nutrient generation are, at least partly, a function of the intensity of particulate bioturbation.

DISCUSSION

When considering the extent to which changes in biodiversity are linked to the provision of ecosystem services, it is important to distinguish between the supply of an ecosystem service (e.g. nutrient availability), the ecosystem function that contributes to that service (e.g. nutrient generation) and the mechanistic processes (e.g. bioturbation intensity) that regulate the observed level of functioning (de Groot 2006). Several studies have recently demonstrated clear effects of species richness (Emmerson et al. 2001, Marinelli & Williams 2003, Mermillod-Blondin et al. 2005, Ieno et al. 2006, Norling et al. 2007) and/or species density (Ieno et al. 2006, Rossi et al. 2008) on several measures of benthic ecosystem function, attributing such effects to inter-specific differences in sediment reworking (bioturbation intensity). Whilst the causal link between changes in biodiversity, bioturbation behaviour and nutrient generation are instinctive (e.g. Gray 1974, Rhoads 1974, Rhoads et al. 1977, Aller 1982, Rhoads & Boyer 1982, Krantzberg 1985) and have even been

implicated as being important over geological timescales (e.g. Martin et al. 2008), the present study is the first to demonstrate positive effects of species richness on the process of bioturbation rather than on ecosystem functions that are a consequence of bioturbation. These effects lead to elevated levels of bioturbation intensity relative to the best performing monocultures for the majority of multi-species treatments, although the proportion of mesocosms exhibiting overyielding declines with increasing species richness as the contributions of individual species become less prominent. The higher levels of bioturbation intensity, in turn, appear to stimulate the release of nutrients from the sediment (Ieno et al. 2006).

Although these findings support the view that nutrient generation is dependent on inter-specific differences in bioturbation intensity, the correlations between bioturbation intensity and nutrient generation were low. This implies that the relationship between ecosystem function and bioturbation is either not so straightforward or, more likely, that the consideration of only bioturbation intensity based on particle movement forms an incomplete evaluation of bioturbation effects (Mermillod-Blondin et al. 2005). Indeed, comparison of the findings from the present study with those of Ieno et al. (2006) reveal differences in the relative contribution of each species to individual response variables (nutrient generation, *Hediste diversicolor* > *Hydrobia ulvae* > *Cerastoderma edule*; bioturbation intensity, *H. diversicolor* > *C. edule* > *H. ulvae*). This observation has important implications for understanding how benthic species influence ecosystem processes, as not all species will interact with the benthic environment in the same way. For example, feeding, burrowing and tube construction tend to influence particle transport, whilst irrigation of burrow structures influences water and solute exchange, yet few studies have assessed the combined relative importance of these activities (Quintana et al. 2007). The results of Ieno et al. (2006), when combined with the present study, are consistent with those of others (e.g. Mermillod-Blondin et al. 2005). They indicate that *H. diversicolor* established deep (>7.5 cm), semi-permanent burrows, which it actively maintained and irrigated; *C. edule* mixed sediments in the upper 2 cm of sediment and actively suspension fed; whilst *H. ulvae* actively grazed the uppermost layers of the sediment–water interface. These differences in lifestyle led to dramatic variations in both particle and solute exchange between species. Whilst all species could be modelled as biodiffusors, the model fit data provided some evidence that the net effect of reworking activity may be more appropriately represented using an alternative bioturbation model when *H. diversicolor* is present. Failure to take into account such species interactions and differing lifestyle traits runs the risk of underestimating both the importance and relevance of individual species in space and in time, and the levels of biodiversity required to maintain multifunctional ecosystems (Hector & Bagchi 2007).

Despite common agreement amongst the biodiversity–function community that there is a need to address the shortcomings of an experimental methodology, little effort has been made to demonstrate and substantiate biodiversity–function relations using data from the real world. The considerable gaps that remain in our understanding of biodiversity–ecosystem functioning relations contrast markedly with our understanding of the responses of species to changes that have occurred in the geological record (e.g. Macleod 1994, Twitchett & Barras 2004, Pruss et al. 2006). The empirically derived mechanistic effects of diversity on ecosystem functioning from the present study are sufficiently general to seek correlations between diversity and function in natural systems (Stachowicz et al. 2007), including those from the palaeoecological record. The latter offers an opportunity to gain vital insights on how strong environmental forcing associated with several major extinction events caused changes in ecosystem function. In many cases, the causes and order of species extinction are well known (Twitchett & Barras 2004, Pruss et al. 2006), the nature of the ichnofabric has been documented, trace fossil preservation and morphological features are sufficient to provide an indication of species lifestyle traits (Dornbos 2006), and community composition can be reconstructed (Clapham et al. 2003, Seilacher et al. 2005). For syntheses attempting to examine biodiversity–ecosystem function relations, the difficulty of obtaining large inventories of species trait data along with suitable measures of ecosystem function is not trivial; however, it is possible to circumvent such problems. For example, trace fossil signatures can be used as surrogates for macrofaunal diversity and community structure (e.g. Widdicombe et al. 2003), and simple models can be parameterised to explore how various drivers of extinction influence infaunal activity in the absence of direct measures of bioturbation given certain circumstances (Solan et al. 2004). Informed by actual events from the palaeoecological record, this approach explicitly recognizes that species are likely to go extinct in a particular order, commensurate with the type of extinction driver and as a function of the susceptibility of each species to extinction. Using approaches such as these, it will be possible to converge current perspectives of biodiversity–ecosystem function relations with longer term evolutionary patterns (diversity gains and losses, functional shifts) that are associated with past ecological crises. In building such an evidence base, ecologists will be better able to

inform and more accurately predict what the likely consequences of future biodiversity change will be for human well-being.

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Appendix 1. Summary of the statistical analyses for our 4 GLS models (Models A1 to A4). For each model, we list the initial linear regression model, the minimal adequate linear regression model with GLS estimation, and a summary of the coefficient table. The coefficients indicate the relative performance of each treatment level relative to the relevelled baseline (first column of each table)

Model 1. Form of the initial linear regression model (Eq. A1) and the minimal adequate linear regression model with generalised least-squares (GLS) estimation (incorporating species richness as a variance covariate) (Eq. A2) for the effects of species richness (SR) on D_b.

$$D_b \sim \text{as.factor(SR)} \quad \text{(A1)}$$

$$D_b \sim \text{as.factor(SR)}, \text{weights} = \text{varIdent[form} = \sim 1|\text{as.factor(SR)}], \text{method} = \text{'ML'} \quad \text{(A2)}$$

Table A1. Coefficient table for Model 1. Intercept ± SE (when baseline = Cntrl): 1.574187 ± 0.1771883, t = 8.884258, p < 0.0001. Coefficients ± SE and t-values are presented. Significance values are in parentheses

	SR_0	SR_1	SR_2	SR_3
SR_0	–	2.02394 ± 0.351699 5.754744 (<0.0001)	3.49952 ± 0.415631 8.419761 (<0.0001)	3.028525 ± 0.389231 7.780786 (<0.0001)
SR_1	−2.02394 ± 0.351699 −5.754744 (<0.0001)	–	1.475577 ± 0.483374 3.052662 (0.0042)	1.004586 ± 0.460871 2.179756 (0.0359)
SR_2	−3.49952 ± 0.415631 −8.419761 (<0.0001)	−1.47558 ± 0.483374 −3.052662 (0.0042)	–	−0.470991 ± 0.511331 −0.921109 (0.3631)
SR_3	−3.02853 ± 0.389231 −7.780786 (<0.0001)	−1.00459 ± 0.460871 −2.179756 (0.0359)	0.47099 ± 0.511331 0.921109 (0.3631)	–

Model 2. Form of the initial linear regression model (Eq. A3) and the minimal adequate linear regression model with GLS estimation (incorporating species richness as a variance–covariate) (Eq. A4) for the effects of species richness (SR) on the fit of the D_b model to the observed luminophore profile.

$$\text{Fit} \sim \text{as.factor(SR)} \quad \text{(A3)}$$

$$\text{Fit} \sim \text{as.factor(SR)}, \text{weights} = \text{varIdent[form} = \sim 1|\text{as.factor(SR)}], \text{method} = \text{'ML'} \quad \text{(A4)}$$

Table A2. Coefficient table for Model 2. Intercept ± SE (when baseline = Cntrl): 0.000472 ± 0.000103, t = 4.582764, p < 0.0001. Coefficients ± SE and t-values are presented. Significance values are in parentheses

	SR_0	SR_1	SR_2	SR_3
SR_0	–	0.001784 ± 0.000863 2.065994 (<0.0461)	0.004023 ± 0.001573 2.557451 (0.0149)	0.003421 ± 0.001201 2.848089 (0.0072)
SR_1	−0.001784 ± 0.000863 −2.065994 (0.0461)	–	0.002239 ± 0.001789 1.251834 (0.2187)	0.001637 ± 0.001472 1.111813 (0.2736)
SR_2	−0.004023 ± 0.001573 −2.5574515 (0.0149)	−0.002239 ± 0.001789 −1.2518343 (0.2187)	–	−0.000602 ± 0.001974 −0.3051636 (0.762)
SR_3	−0.003421 ± 0.001201 −2.848089 (0.0072)	−0.001637 ± 0.001472 −1.111813 (0.2736)	0.000602 ± 0.001974 0.305164 (0.762)	–

Model 3. Form of the initial linear regression model (Eq. A5) and the minimal adequate linear regression model with GLS estimation (incorporating species identity as a variance covariate) (Eq. A6) for the effects of species identity (SPID) on D_b.

$$D_b \sim \text{as.factor(SPID)} \quad \text{(A5)}$$

$$D_b \sim \text{as.factor(SPID), weights = varIdent[form = } \sim 1|\text{as.factor(SPID)], method = 'ML'} \quad \text{(A6)}$$

Table A3. Coefficient table for Model 3. Intercept ± SE (when baseline = Cntrl): 1.574187 ± 0.1771883, t = 8.884258, p < 0.0001. Coefficients ± SE and t-values are presented. Significance values are in parentheses

	$SPID_0$	$SPID_{HD}$	$SPID_{CE}$	$SPID_{HU}$	$SPID_{CEHU}$	$SPID_{CEHD}$	$SPID_{HDHU}$	$SPID_{CEHDHU}$
$SPID_0$	–	3.155 ± 0.437 7.215 (<0.0001)	1.875 ± 0.423 4.436049 (<0.0001)	1.042 ± 0.356 2.924789 (0.0063)	3.330 ± 1.113 2.990346 (0.0053)	3.751 ± 0.401 9.34410 (<0.0001)	3.418 ± 0.391 8.744099 (<0.0001)	3.029 ± 0.389 7.780786 (<0.0001)
$SPID_{HD}$	−3.155 ± 0.437 −7.214680 (< 0.0001)	–	−1.280 ± 0.554 −2.309957 (0.0275)	−2.113 ± 0.505 −4.182115 (0.0002)	0.175 ± 1.170 0.149210 (0.8823)	0.596 ± 0.538 1.107648 (0.2763)	0.263 ± 0.530 0.495589 (0.6236)	−0.127 ± 0.529 −0.239115 (0.8125)
$SPID_{CE}$	−1.875 ± 0.423 −4.436049 (<0.0001)	1.280 ± 0.554 2.309957 (0.0275)	–	−0.833 ± 0.493 −1.691033 (0.1005)	1.455 ± 1.164 1.249343 (0.2206)	1.876 ± 0.526 3.564703 (0.0012)	1.543 ± 0.518 2.976835 (0.0055)	1.154 ± 0.517 2.230999 (0.0328)
$SPID_{HU}$	−1.042 ± 0.356 −2.924789 (0.0063)	2.113 ± 0.505 4.182115 (0.0002)	0.833 ± 0.493 1.691033 (0.1005)	–	2.288 ± 1.142 2.003537 (0.0536)	2.709 ± 0.475 5.708600 (<0.0001)	2.376 ± 0.466 5.102120 (<0.0001)	1.987 ± 0.464 4.278772 (0.0002)
$SPID_{CEHU}$	−3.330 ± 1.113 −2.990346 (0.0053)	−0.175 ± 1.170 −0.149210 (0.8823)	−1.455 ± 1.164 −1.249343 (0.2206)	−2.288 ± 1.142 −2.003537 (0.0536)	–	0.422 ± 1.157 0.364418 (0.7179)	0.088 ± 1.153 0.076561 (0.9394)	−0.301 ± 1.153 −0.261192 (0.7956)
$SPID_{CEHD}$	−3.751 ± 0.401 −9.344109 (<0.0001)	−0.596 ± 0.538 −1.107648 (0.2763)	−1.876 ± 0.526 −3.564703 (0.0012)	−2.709 ± 0.475 −5.708600 (< 0.0001)	−0.422 ±1.157 −0.364418 (0.7179)	–	−0.333 ± 0.501 −0.664998 (0.5108)	−0.723 ± 0.500 −1.445578 (0.1580)
$SPID_{HDHU}$	−3.418 ± 0.391 −8.744099 (<0.0001)	−0.263 ± 0.530 −0.495589 (0.6236)	−1.543 ± 0.518 −2.976835 (0.0055)	−2.376 ± 0.466 −5.102120 (0.0001)	−0.089 ± 1.153 −0.076561 (0.9394)	0.333 ± 0.501 0.664998 (0.5108)	–	−0.389 ± 0.491 −0.792261 0.4340
$SPID_{CEHDHU}$	−3.029 ± 0.389 −7.780786 (<0.0001)	0.127 ± 0.529 0.239115 (0.8125)	−1.154 ± 0.517 −2.230999 (0.0328)	−1.987 ± 0.464 −4.278772 (0.0002)	0.301 ± 1.153 0.261192 (0.7956)	0.723 ± 0.500 1.445578 (0.1580)	0.389 ± 0.491 0.792261 (0.4340)	–

Model 4. Form of the initial linear regression model (Eq. A7) and the minimal adequate linear regression model with GLS estimation (incorporating species identity as a variance covariate) (Eq. A8) for the effects of species identity (SPID) on the fit of the D_b model to the observed luminophore profile.

Fit ~ as.factor(SPID) (A7)

Fit ~ as.factor(SPID), weights = varIdent[form = ~ 1|as.factor(SPID)], method = 'ML' (A8)

Table A4. Coefficient table for Model 4. Intercept ± SE (when baseline = Cntrl): $0.472 \times 10^{-4} \pm 0.103 \times 10^{-4}$, t = 4.582664, p < 0.0001. [a]Coefficients ± SE ($\times 10^{-4}$); t-values and significance values (in parentheses) are also presented

	$SPID_0$	$SPID_{HD}$	$SPID_{CE}$	$SPID_{HU}$	$SPID_{CEHU}$	$SPID_{CEHD}$	$SPID_{HDHU}$	$SPID_{CEHDHU}$
$SPID_0$	–	5.799 ± 1.239[a] 4.678804 (<0.0001)	−0.092 ± 0.368[a] −0.249746 (0.8044)	−0.355 ± 0.153[a] −2.323676 (0.0267)	−0.08 ± 0.294[a] −0.273502 (0.7862)	8.356 ± 3.852[a] 2.169415 (0.0376)	3.793 ± 1.436[a] 2.640891 (0.0127)	3.421 ± 1.201[a] 2.848068 (0.0076)
$SPID_{HD}$	−5.799 ± 1.239[a] −4.678804 (<0.0001)	–	−5.891 ± 1.285[a] −4.585449 (<0.0001)	−6.155 ± 1.24[a] −4.961955 (<0.0001)	−5.88 ± 1.266[a] −4.646012 (<0.0001)	2.557 ± 4.044[a] 0.63229 (0.5317)	−2.006 ± 1.892[a] −1.060448 (0.2969)	−2.379 ± 1.72[a] −1.383109 (0.1762)
$SPID_{CE}$	0.092 ± 0.368[a] 0.249746 (0.8044)	5.891 ± 1.285[a] 4.585449 (<0.0001)	–	−0.263 ± 0.371[a] −0.709835 (0.4829)	0.012 ± 0.448[a] 0.025674 (0.9797)	8.448 ± 3.866[a] 2.184883 (0.0363)	3.885 ± 1.476[a] 2.632941 (0.0129)	3.513 ± 1.248[a] 2.815198 (0.0083)
$SPID_{HU}$	0.355 ± 0.153[a] 2.323676 (0.0267)	6.155 ± 1.24[a] 4.961955 (<0.0001)	0.263 ± 0.371[a] 0.709835 (0.4829)	–	0.275 ± 0.298[a] 0.92321 (0.3628)	8.711 ± 3.852[a] 2.261501 (0.0307)	4.149 ± 1.437[a] 2.886744 (0.0069)	3.776 ± 1.202[a] 3.141542 (0.0036)
$SPID_{CEHU}$	0.08 ± 0.294[a] 0.273502 (0.7862)	5.88 ± 1.266[a] 4.646012 (<0.0001)	−0.012 ± 0.448[a] −0.025674 (0.9797)	−0.275 ± 0.298[a] −0.92321 (0.3628)	–	8.436 ± 3.86[a] 2.185494 (0.0363)	3.874 ± 1.459[a] 2.655206 (0.0122)	3.501 ± 1.228[a] 2.851232 (0.0076)
$SPID_{CEHD}$	−8.356 ± 3.852[a] −2.1694153 (0.0376)	−2.557 ± 4.044[a] −0.6322899 (0.5317)	−8.448 ± 3.866[a] −2.1848828 (0.0363)	−8.711 ± 3.852[a] −2.2615008 (0.0307)	−8.436 ± 3.86[a] −2.1854938 (0.0363)	–	−4.563 ± 4.108[a] −1.1106207 (0.275)	−4.935 ± 4.032[a] −1.2240458 (0.2299)
$SPID_{HDHU}$	−3.793 ± 1.436[a] −2.6408909 (0.0127)	2.006 ± 1.892[a] 1.0604481 (0.2969)	−3.885 ± 1.476[a] −2.6329414 (0.0129)	−4.149 ± 1.437[a] −2.8867439 (0.0069)	−3.874 ± 1.459[a] −2.6552064 (0.0122)	4.563 ± 4.108[a] 1.1106207 (0.275)	–	−0.373 ± 1.867[a] −0.1996348 (0.843)
$SPID_{CEHDHU}$	−3.421 ± 1.201[a] −2.848068 (0.0076)	2.379 ± 1.72[a] 1.383109 (0.1762)	−3.513 ± 1.248[a] −2.815198 (0.0083)	−3.776 ± 1.202[a] −3.141542 (0.0036)	−3.501 ± 1.228[a] −2.851232 (0.0076)	4.935 ± 4.032[a] 1.224046 (0.2299)	0.373 ± 1.867[a] 0.199635 (0.843)	–

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