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1 **Response of benthic assemblages to multiple stressors: comparative effects of nutrient**
2 **enrichment and physical disturbance**

3

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9

10 **Abstract**

11 Stressors to ecological communities often overlap in time and space and may have additive,
12 synergistic or antagonistic effects. Nutrient enrichment and physical disturbance are two
13 commonly co-occurring stressors to estuarine ecosystems, but their combined effects have mainly
14 been investigated in mesocosm experiments of unknown relevance to field scenarios. Here, the
15 interacting effects of these two stressors were examined at two field locations – Botany Bay and
16 Lane Cove, New South Wales, Australia – using a fully orthogonal manipulative experiment. All
17 possible combinations of zero, low and high intensities of nutrient enrichment and physical
18 disturbance on macrofaunal and microphytobenthic communities were examined. Effects of
19 stressors were generally site-specific and additive, differing in terms of magnitude of effects,

20 although some idiosyncratic interactive effects were seen on selected species. Where effects of
21 stressors were observed, nutrient enrichment generally increased microphytobenthic biomass
22 and altered the macrofaunal community structure while physical disturbance produced limited
23 impacts. The divergent results of this and previous mesocosm experiments, which found primarily
24 interactive effects of the stressors, highlights the importance of undertaking field experiments
25 that offer a greater element of realism. Furthermore, this study, in finding differing responses to
26 stressors at the two sites, highlights the importance of environmental context in mediating
27 effects.

28 **Key words:**

29 Multiple Stressors; Context dependence; Nutrient Enrichment; Disturbance; Macrobenthos,
30 Microphytobenthos, Field Experiments

31 **Introduction**

32 How organisms respond to naturally occurring and anthropogenic stressors has been a subject
33 that has dominated the marine ecological literature for many decades (Dayton 1971, Connell
34 1978, Paine & Levin 1981, Menge & Sutherland 1987, Hall 1994, Crain et al. 2008). Early studies
35 considered the effects of stressors independently from one another and on individual species, yet
36 stressors to natural ecological systems rarely occur singularly (Crain et al. 2008, Halpern et al.
37 2008) and species rarely occur alone. Effects of multiple stressors cannot easily be predicted from
38 the effects of singular stressors because, when combined, they are rarely neutral but have
39 additive, subtractive or synergistic effects (Crain et al. 2008, Darling & Côté 2008, Bijma et al.
40 2013). Yet many studies continue to examine the effects of stressors individually and on single

41 species or small sub groups of taxa (Crain et al. 2008). As coastal development and climate change
42 continue to increase the frequency and intensity of stressors (Halpern et al. 2008, Bijma et al.
43 2013) there is need to understand the combined effects of stressors, so as to develop appropriate
44 policy and management strategies to manage and perhaps minimise their impact.

45 Of the studies that have considered the effects of multiple stressors on organisms, many have
46 been conducted in the controlled environment of experimental mesocosms, with species isolated
47 from their habitats and communities (Hicks et al. 2011, Godbold et al. 2011). Although such
48 studies allow the effects of stressors to be examined in the absence of other confounding
49 influences, the extent to which results can be applied to natural systems is unclear because they
50 fail to take into consideration the role of the environmental and biotic context in mediating
51 stressor impacts. For example, organisms can make use of microhabitats to minimise exposure to
52 stressors (Jones & Boulding 1999) and, under natural conditions, ecological interactions such as
53 competition, facilitation and predation may dampen or exacerbate stressor impacts (Christensen
54 et al. 2006, Hicks et al. 2011). *In situ* mesocosms have been used effectively to add an element of
55 realism to assessments of stressor impacts (e.g. Christensen et al. 2006). Nevertheless, while
56 studies such as these offer interesting insights and explore important relationships between
57 changes in ecosystem function and biodiversity under scenarios of environmental change, they do
58 not represent natural conditions and field studies are still needed as part of the experimental
59 framework (for a review of the topic see Crain et al. 2008; Przeslawski et al. 2015 and references
60 therein).

61 Estuarine environments are among the most heavily impacted in the world, with greater than
62 40% of the world's human population living within 100 km of the coast. Among the plethora of
63 stressors affecting these systems on a daily basis, nutrient enrichment and physical disturbances
64 of sediments are often singled out as the most severe (Gray 1997). Leaching of nutrients from the
65 terrestrial environment, atmospheric deposition and decomposition are natural sources of
66 nutrient loading in these environments but these can become exacerbated by human influences
67 (Nixon 1995). For example, the discharge of sewage into aquatic environments, as well as run-off
68 of nutrient-rich fertilisers, cleaning products and animal wastes from the land can enhance
69 delivery of nitrogen and phosphorus to estuarine systems (Nixon 1995, Vitousek et al. 1997a).
70 Where nitrogen and/or phosphorous is a limiting resource, addition of these nutrients has the
71 potential to greatly stimulate primary productivity (Vitousek et al. 1997a, Cloern 2001). Excess
72 nutrients have been linked to eutrophication events worldwide whereby algal blooms are
73 facilitated, ultimately leading to hypoxic and anoxic conditions through over-stimulation of
74 oxygen-consuming microbial decomposition (Vitousek et al. 1997b).

75 High human population densities around estuaries also facilitate many small scale disturbances to
76 estuarine sediments through recreation – e.g. boat wake, anchorage, propeller scarring, coastal
77 walking (Bishop 2005, Rossi et al. 2007) – and as a consequence of livelihood exploitation – e.g.
78 bait digging, fishing, dredging (Wynberg & Branch 1994, Somerfield et al. 1995, Brown & Wilson
79 1997). These physical disturbances have been widely studied and can lead to the disturbance and
80 redistribution of sediments (Hall 1994). Such disturbances are likely to cause physical damage to
81 sediment-dwelling organisms or burrows (Hall & Harding 1997). This physical disturbance has the

82 potential to alter the distribution, abundance and diversity of macrofaunal assemblages
83 (Fraterrigo & Rusak 2008). At a larger scale, natural events such as storms also cause physical
84 disturbances to sediments although less frequently, offering the potential for recovery of
85 sediment communities between events. These events loosen sediment, making it more
86 susceptible to erosion and further stimulating damaging scour impacts (Yeo & Risk 1979). While
87 sediment grain size can mediate the effect of disturbance on communities (Lindegarh & Hoskin
88 2001), grain size itself can also become altered by intense disturbance effects (Bishop 2005)
89 thereby facilitating a change in community structure.

90 The aim of this study was to explore how the stressors, nutrient enrichment and physical
91 disturbance, interact under natural field conditions. Nutrient enrichment and physical
92 disturbances often overlap in time and space. For example, nutrient enrichment influences whole
93 catchments, within which physical disturbances may also occur at smaller scales. Additionally,
94 storm events that flush nutrient pollution into estuarine and coastal areas may also cause physical
95 disturbance (Harris 2014). Despite this, most studies that have examined effects of these stressors
96 on sediment communities have done so independently, and those that have examined interacting
97 effects have done so in small-scale mesocosm experiments (see Widdicombe & Austen 2001,
98 Austen & Widdicombe 2006). According to Huston's (1979) dynamic equilibrium model, that
99 posits that larger disturbances will be required to disrupt competitive dominance when
100 productivity is high, the two stressors are predicted to display non-additive effects. Mesocosm
101 experiments provide support for this hypothesis (Widdicombe & Austen 2001, Austen &
102 Widdicombe 2006). In buckets, experimental manipulations of nutrient enrichment (via addition

103 of dried and powdered *Ascophyllum*) and physical disturbance (via surface raking) revealed that
104 diversity was lower than expected when low frequencies of physical disturbance were applied in
105 combination with high levels of organic enrichment or vice versa (Widdicombe & Austen 2001,
106 Austen & Widdicombe 2006). Diversity was higher than expected when both disturbance and
107 enrichment were either high or low (Widdicombe & Austen 2001). Despite these results, it is
108 unknown whether these non-additive effects also occur in field systems in which environmental
109 conditions may buffer effects and recruitment of fauna is possible. Here, manipulative field
110 experiments were conducted in two estuaries of eastern Australia to test the hypotheses that (1)
111 the two stressors would display non-additive effects on microphytobenthic and macrofaunal
112 communities, that are not easily predicted from their independent effects and (2) the nature of
113 interactions would vary across field sites that differ in biotic and abiotic conditions.

114 **Materials and Methods**

115 **Study site**

116 Field experiments were conducted between May and September 2012 at two locations within
117 Sydney, New South Wales, Australia: Tambourine Bay within Lane Cove River (33°49'39"S
118 151°09'38"E); and Woollooware Bay within Botany Bay (34°01'11"S, 151°07'46"E). Sites were un-
119 vegetated intertidal mud flats, adjacent to mangrove habitat. Each was within 15 km of the
120 estuary mouth, was situated in a highly urbanised catchment, and had sandy-mud sediments.
121 Sites were away from storm-water drains, which transport nutrients into estuaries. Although the
122 sites may be subject to some, minimal, trampling and the occasional boat run-against, neither
123 was subject to background raking or substantial bait-digging prior to experimental intervention.

124 At an intertidal elevation of ~0.5 m above mean low water springs, 70 square plots (0.5 x 0.5 m in
125 size) were randomly established, and marked by a single post in the upper left corner of the plot.
126 Each plot was separated by approximately 3 metres.

127 **Experimental approaches**

128 The interactive effect of stressors was determined using a fully orthogonal experimental design
129 with two factors, nutrient enrichment and physical disturbance. Each factor had three levels of
130 disturbance intensity: zero, low or high. Seven plots were randomly assigned to each of the
131 resulting nine experimental treatments. The remaining seven plots were procedural controls for
132 the method of nutrient enrichment (see below).

133 Physical disturbance was manipulated by raking sediments (50 cm wide rake) to a depth of 2–4 cm.
134 Raking is one of the most commonly used methods for physically disturbing sediments (e.g. Cowie
135 et al. 2000, Whomersley et al. 2010), and mimics cockle hand raking (Kaiser et al. 2001, Mistri et al.
136 2004). For plots assigned to the low or high physical disturbance, an area of approximately 1 m²,
137 centring on but extending beyond the experimental plot was raked in a cross-hatched pattern. The
138 low treatment consisted of two strokes of the rake, perpendicular to one another. This level of
139 disturbance has previously been shown to illicit a response from intertidal communities
140 (Whomersley et al. 2010). The high treatment consisted of 6 strokes, each stroke perpendicular to
141 the previous. This disturbance was applied at the start of experiment and then monthly, over a
142 four-month period.

143 Nutrient plots were enriched using Scotts Osmocote Pro, 8-9 month coated fertiliser pellets (N:P:K
144 ratio of 16:4.8:8.3). These pellets which gradually release nutrients provide a controlled method of

145 testing the effects of chronic nutrient enrichment in soft sediment benthic environments without
146 need for replenishment (Worm et al. 2000). Plots were given 0 g (zero), 500 g (low) or 1000 g (high)
147 of fertiliser. The high nutrient treatment was based on the level of nutrient loading that might be
148 experienced in the vicinity of the discharge point of a moderately sized sewage treatment plant
149 (Morris & Keough 2002, 2003a, O'Brien et al. 2010) and the low treatment was set at half of this.
150 For each of the plots assigned to the high or low nutrient treatment, nutrients were dispensed via
151 five bags per plot, made from nylon panty-hose, among which the fertiliser was evenly distributed.
152 These bags were spread evenly within each plot and were buried approximately 2–4 cm below the
153 surface of the sediment. The nylon provided a fine permeable membrane through which nutrients
154 could leach out into the plots. A procedural control tested for any experimental artefacts associated
155 with burying the fertiliser; for this treatment nylon bags contained sediment in place of fertiliser
156 and were deployed in the same manner as the nutrient treatments. Nylon bags containing slow
157 release fertiliser or sediment (for the procedural control) were added to plots at the start of the
158 experiment.

159 **Sampling**

160 Prior to initial manipulation of stressors, the chlorophyll and organic content of a subset ($n = 25$)
161 of plots was sampled to determine background conditions at each site. Additionally, the spectral
162 reflectance of the sediment and the macrofaunal community were sampled in each plot
163 immediately prior to the manipulation of stressors. All variables were subsequently sampled
164 monthly, for four months, in the case of sediment properties and at 2 (July) and 4 (September)
165 months for macrofauna. Sampling of chlorophyll *a* content and spectral reflectance were together

166 used as proxies for the biomass of microphytobenthos (Tolhurst et al. 2005, Kromkamp et al.
167 2006). Sampling was conducted immediately prior to each re-application of the physical
168 disturbance treatment. Sampling at Lane Cove was not possible in the first month following initial
169 disturbance due to large amounts of overlying water caused by a sizable low pressure system. The
170 location of sample collection within each plot at each sampling time was noted, so as to avoid
171 repeated collection of samples from the same area.

172 The sediment organic content and chlorophyll *a* concentration was assessed through the contact
173 coring method (Ford & Honeywill 2002) whereby the top 2 mm of the sediment surface was flash-
174 frozen with liquid nitrogen and then freeze dried. A single randomly-positioned 2463 mm² core
175 was collected from each plot at each sampling time. Over a period of 48 hours, photosynthetic
176 pigments were extracted from a 200 mg subsample of sediment from each core using 1.5 ml of
177 90% acetone under dark conditions within a -80 °C freezer. Samples were agitated after 24 hours
178 for 10 seconds by using a vortex mixer. The chlorophyll *a* concentration of the acetone solution
179 was determined spectrophotometrically using the method of Jeffrey & Humphrey (1975) and the
180 chlorophyll *a* content of sediment was calculated per unit area (mg m⁻²). A second 2 g subsample
181 of sediment was taken from each contact core to determine the organic content through loss-on-
182 ignition (450 °C for 4 hours).

183 An Ocean Optics USB2000 spectroradiometer was used to measure the spectral reflectance of the
184 sediment surface *in situ*. Reflectance values (R) were made in the visible (675 nm) and infra-red
185 (750 nm) parts of the spectra, and used to calculate the Normalised Difference Vegetation Index

186 (NDVI), a measure of the photosynthetically-related biomass based upon reflectance of
187 chlorophyll *a* in the sediment (Kromkamp et al. 2006):

$$188 \text{ NDVI} = (R750 - R675) / (R750 + R675)$$

189 Three measurements were collected per plot allowing an average NDVI to be calculated.

190 Reflectance measurements were discarded where there was interference from surface water.

191 Nevertheless, at each sampling time measurements from at least $n = 3$ plots per treatment could
192 be included in the analyses.

193 One large core (10 cm diameter, 10 cm depth) was collected from each plot to quantify
194 macrofaunal biodiversity each of the sampling times indicated above. Sediment cores were taken
195 from between nylon bags, so as not to disrupt dispense of nutrients. Sediment core samples were
196 sieved through a 500 μm diameter mesh and material retained on the sieve was fixed in 10%
197 buffered formalin. Retained material was subsequently transferred into 70% ethanol and
198 examined under a dissecting microscope to separate fauna from other remaining material. Fauna
199 were identified to species level or morphospecies where this was not possible.

200 **Statistics**

201 Permutational analyses of variance (PERMANOVA: Anderson 2001, Anderson et al. 2008) were
202 used to analyse univariate and multivariate data. Although initially developed for multivariate
203 application, PERMANOVA can also be run on univariate data (Anderson et al. 2008) and has the
204 advantage over ANOVA that it does not have assumptions regarding the underlying distribution of
205 the data and can be used on any distance matrix (Anderson et al. 2008). Multivariate analyses,

206 using Bray Curtis dissimilarities, were run on macrofaunal composition data. Macrofaunal
207 composition data was square-root transformed prior to PERMANOVA in order to down-weight the
208 effect of species dominance and ordinated using two-dimensional non-metric multidimensional
209 scaling (nMDS). Univariate tests, using Euclidean distance matrices, were run on each of sediment
210 organic content, chlorophyll a content, NDVI, Shannon's diversity of macrofauna, total abundance
211 of macrofauna, species richness of macrofauna, as well as the abundance of macrofaunal species
212 that were key discriminators of multivariate differences among treatments.

213 First, for macrofauna and NDVI variables, three-way analyses on time 0 data, collected prior to
214 application of perturbations, and including the factors nutrient enrichment (fixed; 3 levels: zero
215 [ON], low [LN], high [HN]), physical disturbance (fixed; 3 levels: zero [OD], low [LD], high [HD]) and
216 site (fixed; 2 levels) tested for any pre-existing differences in variables of interest among plots and
217 between sites that coincided with their treatment assignment. Due to only a subset of contact
218 core samples taken in the first month we were unable to run a complete analysis testing
219 differences between treatments for chlorophyll *a* and organic content, however a one-way
220 analysis was conducted testing for differences between sites. Second, four-way analyses, with the
221 factors time (4 levels for most variables [1, 2, 3, 4 months after perturbation], and 2 levels for
222 macrofauna [2, 4 months]), site (2 levels, random), treatment (2 levels: undisturbed, disturbance
223 control) and plot (random; nested within site and treatment) tested for experimental artefacts of
224 the method of nutrient application. Third, five-factor analyses with the factors site (random, 2
225 levels); nutrient enrichment (fixed; 3 levels); physical disturbance (fixed; 3 levels); plot (random, 7
226 levels, nested within site x nutrients x disturbance) and time (random, 2 or 4 levels) tested for

227 interacting effects of the two stressors across both sites and all time periods. The inclusion of plot
228 as a nested factor enabled time to be treated as a repeated measure. Following these analyses
229 pairwise *post hoc* tests were conducted to identify significant differences between factors. Where
230 the number of possible permutations was lower than 100, Monte Carlo (MC) testing was run to
231 generate p-values from constructed asymptotic permutation distributions for the pseudo-*F*
232 statistic. All analyses were conducted using the PRIMER v6 statistical program with the
233 PERMANOVA+ addition (Clarke & Gorley 2006, Anderson et al. 2008).

234 The macrofaunal multivariate analysis revealed large spatial and temporal differences in
235 communities resulting in strong interactions between month and site. These prevented the
236 factors of interest (nutrient enrichment and physical disturbance) from being appropriately tested
237 and so separate tests were run for each month and site using a reduced model including only the
238 factors nutrient enrichment and physical disturbance. Key discriminating species, contributing to
239 multivariate differences in macrofaunal community structure, were identified by SIMPER analysis
240 and were defined as those having a dissimilarity/standard deviation ratio > 1 between nutrient or
241 physical disturbance treatments. Of these, species that were present at both sites were analysed
242 using the complete 5-factor model, with abundances 4th root transformed. Discriminating species
243 that were unique to a site were analysed under the reduced 2-factor model. Permutational
244 multivariate dispersions (PERMDISP) were analysed alongside the multivariate PERMANOVAs to
245 assess the extent to which any treatment effects were driven by differences in dispersion
246 between treatments and unless otherwise indicated were non-significant.

247 **Results**

248 **Microphytobenthic component**

249 Prior to experimental manipulations there were no differences in organic content or chlorophyll *a*
250 content of sediments between the two sites, although NDVI was significantly greater at Lane Cove
251 than Botany Bay (main effect of site PERMANOVA: $p < 0.05$; see supplemental materials). Across
252 the two sites, there were no pre-existing differences in NDVI among plots that corresponded to
253 treatment assignments (PERMANOVA $p > 0.05$, see supplemental materials; insufficient plots
254 were sampled to address this question for organic content and chlorophyll *a*). At none of the
255 sampling times following application of stressors were there experimental artefacts of the
256 method of fertiliser burial on any of the three variables, organic content, chlorophyll *a* or NDVI
257 (PERMANOVA $p > 0.05$; see supplemental material).

258 Organic content did not respond to the addition of stressors at either site (Table 1). By contrast,
259 site-specific effects of stressors on the two measures of MPB biomass, chlorophyll *a*
260 concentration and NDVI were observed (Table 1). Neither variable displayed interactive effects
261 between the two stressors so additive effects could be interpreted. Chlorophyll *a* concentration
262 displayed a significant interaction between physical disturbance, site and month (Table 1; Figures
263 1A,B), whereas there was no effect of physical disturbance on NDVI at either site (Table 1). In
264 Botany Bay (Figure 1A), although a slight negative impact of high physical disturbance on
265 chlorophyll *a* was observed in August (month 3), prior to September (month 4) the effects of
266 disturbance were non-significant (*a posteriori* pairwise comparisons $p > 0.05$; OD = LD = HD). In
267 September, plots receiving the high physical disturbance treatment had a significantly lower

268 chlorophyll *a* concentration than those not receiving physical disturbance ($t = 1.99$, $p(\text{perm}) =$
269 0.048). There were no differences between the undisturbed and low disturbance treatments ($t =$
270 1.59 , $p(\text{perm}) = 0.113$), or the low and high disturbance treatments ($t = 0.14$, $p(\text{perm}) = 0.898$). At
271 Lane Cove (Figure 1B), lower levels of chlorophyll were observed in plots receiving high than low
272 physical disturbance in July (month 2; $\text{HD} < \text{LD}$, $t = 3.42$, $p(\text{perm}) = 0.002$), but in August these two
273 treatments did not significantly differ, and there were no significant differences between other
274 pairs of treatments in either July or August (*a posteriori* pairwise comparisons: $p > 0.05$). In
275 September, low disturbance resulted in significantly greater concentrations of chlorophyll *a* than
276 in the undisturbed ($t = 2.34$, $p(\text{perm}) = 0.022$) or high disturbance treatment ($t = 2.97$, $p(\text{perm}) =$
277 0.004), but there was no difference between the high disturbance and undisturbed treatments (t
278 $= 0.71$, $p(\text{perm}) = 0.497$) ($\text{LD} > \text{OD} = \text{HD}$).

279 Despite chlorophyll *a* displaying a significant interaction between nutrient addition and month (N
280 x M interaction; Table 1) and a weak trend for a greater concentration of in plots receiving a low
281 or high nutrient addition than no nutrients (Figure 1C, D), in none of the months were significant
282 differences among nutrient treatments found (*a posteriori* pairwise comparisons: $p > 0.05$). By
283 contrast, NDVI displayed a significant interaction between nutrient enrichment and site (Table 1),
284 with effects of nutrient enrichment apparent in Botany Bay only (Figure 2). In Botany Bay, plots
285 receiving either a low ($t = 2.16$, $p(\text{perm}) = 0.032$) or high nutrient addition ($t = 2.75$, $p(\text{perm}) =$
286 0.011) had a significantly greater NDVI than unenriched plots. There were no differences between
287 the low and the high treatment ($t = 0.63$, $p(\text{perm}) = 0.557$) ($\text{ON} < \text{LN} = \text{HN}$).

288 **Macrofauna**

289 Analysis of macrofaunal community structure prior to manipulations revealed that multivariate
290 community composition differed significantly between the sites (Pseudo- $F_{(1,108)} = 31.63$, $p(\text{perm})$
291 $= 0.001$), with a greater abundance of invertebrates per plot at Lane Cove than Botany Bay
292 (Pseudo- $F_{(1,108)} = 3.70$, $p(\text{perm}) = 0.031$). Sixty-five species were identified, of which 28 were
293 common between the two sites, 19 species were unique to Botany Bay and 18 were unique to
294 Lane Cove. Shannon Diversity and total plot species richness did not significantly differ between
295 the two sites (PERMANOVA $p > 0.05$; see supplemental materials). At neither of the sites were
296 there pre-existing differences among plots that corresponded to treatment assignments (see
297 supplemental materials). Furthermore, at none of the sampling times were there experimental
298 artefact associated of the method of nutrient addition on macrofauna (PERMANOVA $p > 0.05$; see
299 supplemental materials).

300 In the analysis comparing stressor impacts to macrofauna between the two sites, and across all
301 sampling times, there was no effects of nutrient enrichment or physical disturbance on
302 community composition or biodiversity indices, however there was a significant interaction
303 between month and site (PERMANOVA: $p(\text{perm}) < 0.05$). When sites and times were considered
304 separately, stressor effects on macrofauna could not be distinguished in July (PERMANOVA, $p >$
305 0.05) and in September, following four months of continued exposure to stressors, there was no
306 interacting effect of nutrients and physical disturbance on any of the measures of macrofaunal
307 community structure, at either site, allowing interpretation of main effects (Table 2). Whereas the
308 September analysis revealed no effect of physical disturbance on any of the measures of

309 macrofaunal community structure, nutrient enrichment affected multivariate community
310 composition at Botany Bay and at Lane Cove it had a weak, though non-significant effect on this
311 variable (Table 2; Figure 3). At Botany Bay, the highly enriched treatment was significantly
312 different to the unenriched treatment only ($t = 1.63$, $p(\text{perm}) = 0.031$), with no differences
313 between the low and the highly enriched treatments ($t = 1.36$, $p(\text{perm}) = 0.077$) or between the
314 low enrichment and unenriched treatments ($t = 1.04$, $p(\text{perm}) = 0.386$). Additionally at Botany Bay
315 the multivariate dispersions were significantly greater in the enriched plots in comparison to the
316 controls (PERMANOVA, *a posteriori* tests, $ON < LN = HN$; PERMDISP: $F_{(2,58)} = 8.94$, $p(\text{perm}) =$
317 0.002). There were no treatment effects on Shannon diversity for either Botany Bay or Lane Cove
318 (Table 2). However, for Lane Cove, there was a treatment effect of nutrient enrichment on the
319 total macrofaunal abundance whereby increasing enrichment caused a decrease in total
320 abundance (Table 2; PERMANOVA, *a posteriori* tests, $ON > LN > HN$). Analysis of dispersions also
321 revealed increased nutrient enrichment significantly decreased the dispersion of abundances at
322 Lane Cove with no differences in dispersion of either low or highly enriched treatment ($ON > LN,$
323 HN ; PERMDISP: $F_{(2,58)} = 7.06$, $p(\text{perm}) = 0.027$).

324 SIMPER analysis identified six discriminating species contributing most to dissimilarity between
325 nutrient treatments in Botany Bay, four of which coincided with those found in Lane Cove. These
326 were *Mysella* sp., *Mediomastus australiensis*, *Prionospio* sp., and *Nephtys australiensis*. These
327 species were among the most abundant species found at each site. The additional discriminating
328 species at Botany Bay were *Salinator fragilis* and *Owenia australis*. Lane Cove had a total of 12
329 discriminating species which also included two species of *Gammarus* amphipods, Oedicerotidae

330 sp. amphipods, *Scoloplos* sp., *Laternula* sp., *Platynereis uniseris*, *Australonereis ehlersi* and large
331 nematodes.

332 In repeated measures analyses that included both sites, *M. australiensis* did not display any
333 significant effects of nutrient enrichment or physical disturbance at either of the sites or at any of
334 the sampling times (Table 3). Nutrient enrichment caused a significant alteration of the
335 abundance of *Prionospio* sp. at Botany Bay only (nutrient enrichment x site interaction; Table 3; *a*
336 *posteriori* pairwise comparisons: $p < 0.05$; Figure 4). Pairwise comparisons revealed that at Botany
337 Bay, the high nutrient enrichment plots had significantly lower *Prionospio* sp. abundances than
338 the unenriched plots ($t = 2.42$, $p(\text{perm}) = 0.016$) whereas there were no significant differences
339 between the unenriched and low enrichment plots, or the low and high enrichment plots ($p >$
340 0.05).

341 Across both sites and all sampling times, there was a significant interaction between nutrient
342 enrichment and physical disturbance for the species *N. australiensis* (Table 3; Figure 4). There
343 were no significant differences due to the impact of disturbance among plots that received either
344 zero or high enrichment (*a posteriori* pairwise comparisons: $p > 0.05$). Among plots that received
345 low nutrient enrichment, abundances of *N. australiensis* were significantly greater in plots that
346 also received low physical disturbance than those that were not disturbed (LD > 0D; $t = 16.64$,
347 $p(\text{perm}) = 0.013$), with no significant difference between plots receiving high or low physical
348 disturbance ($t = 0.40$, $p(\text{perm}) = 0.829$) and between plots receiving high or no physical
349 disturbance ($t = 1.94$, $p(\text{perm}) = 0.223$). By contrast, among plots receiving high or zero nutrient
350 enrichment, there was no significant effect of physical disturbance on *N. australiensis* abundance

351 (*a posteriori* pairwise comparisons: $p > 0.05$). When the interaction was examined around the
352 other way around, there was no significant effect of nutrient enrichment, within any of the three
353 levels of physical disturbance (*a posteriori* pairwise comparisons: $p > 0.05$).

354 *Mysella* sp. displayed a three-way interaction between nutrient enrichment, physical disturbance
355 and month, with no differences between sites (Table 3; Figure 4). Among unenriched plots, there
356 was no significant effect of physical disturbance in either of the months (*a posteriori* pairwise
357 comparisons: $p > 0.05$). Among plots subjected to low nutrient enrichment, abundances were
358 greater in plots receiving high than no physical disturbance in each of the months (HD > OD; July: t
359 = 21.30, $p(\text{MC}) = 0.037$; September: $t = 19.32$, $p(\text{MC}) = 0.031$) and in July, there was also a
360 significant difference between the high and low disturbance treatment (HD > LD; $t = 22.42$, $p(\text{MC})$
361 = 0.037) but all other pairwise comparisons were non-significant ($p > 0.05$). Among plots receiving
362 high nutrient enrichment, plots receiving high physical disturbance had greater abundances of
363 *Mysella* sp. than undisturbed plots in July (HD > OD; $t = 31.87$, $p(\text{MC}) = 0.017$), whereas in
364 September, highly disturbed plots contained fewer individuals than those that were undisturbed
365 (HD < OD; $t = 12.33$, $p(\text{MC}) = 0.049$), with no other pairwise comparisons between levels of
366 physical disturbance significant for either of the sampling times ($p > 0.05$). Examining the
367 interaction around the other way, in July, plots receiving no physical disturbance had lower
368 abundances of *Mysella* sp. when they received low as compared to no nutrient enrichment (ON >
369 LN; $t = 51.61$, $p(\text{MC}) = 0.013$), but other pairwise contrasts revealed no other significant
370 differences (*a posteriori* pairwise comparisons: $p > 0.05$). In September, both the low and high
371 nutrient treatments had lower abundances of *Mysella* sp. in comparison to the unenriched

372 treatment (low: $t = 17.51$, $p(\text{MC}) = 0.33$; high: $t = 41.83$, $p(\text{MC}) = 0.017$) but did not significantly
373 differ from one another (ON > LN = HN; $t = 0.04$, $p(\text{MC}) = 0.977$). There was no effect of nutrient
374 enrichment on *Mysella* sp. among plots receiving low or high physical disturbance (*a posteriori*
375 pairwise comparisons: $p > 0.05$).

376 Few of the species that were key discriminating taxa at only one of the two sites displayed
377 significant responses to stressors (see supplemental materials: Table S4). At Lane Cove, *Scoloplos*
378 sp. displayed a significant interaction between nutrient enrichment and physical disturbance
379 (Pseudo- $F_{(2,52)} = 2.91$, $p(\text{perm}) = 0.039$; Figure 5A). Among plots subjected to zero physical
380 disturbance, plots receiving low ($t = 2.23$, $p(\text{perm}) = 0.034$) and high ($t = 2.13$, $p(\text{perm}) = 0.050$)
381 nutrient contained fewer *Scoloplos* sp. than those receiving zero enrichment, with no differences
382 between the low and high nutrient treatments (ON > LN = HN). By contrast, among plots receiving
383 low or high physical disturbance, there was no effect of nutrient enrichment (PERMANOVA *a*
384 *posteriori* tests: $p > 0.05$). When the interaction was examined around the other way, among plots
385 subjected to zero nutrient enrichment, high physical disturbance resulted in significantly fewer
386 *Scoloplos* sp. as compared to undisturbed controls (OD > HD; $t = 2.46$, $p(\text{perm}) = 0.030$) whereas
387 there were no differences between plots receiving low and no disturbance ($t = 2.38$, $p(\text{perm}) =$
388 0.064) or low and high disturbance ($t = 0.12$, $p(\text{perm}) = 0.99$). Among plots receiving low or high
389 nutrient enrichment, there was no significant effect of disturbance on *Scoloplos* sp. (PERMANOVA
390 *a posteriori* tests: $p > 0.05$). At Lane Cove, nematodes displayed a significant main effect of
391 nutrient enrichment only (Pseudo- $F_{(2,52)} = 3.25$, $p(\text{perm}) = 0.045$; Figure 5B) whereby abundance

392 decreased with increasing nutrient enrichment (ON > LN > HN; PERMANOVA *a posteriori* tests: $p <$
393 0.05).

394 **Discussion**

395 The limited number of mesocosm studies focussing on the interaction between physical
396 disturbance and nutrient enrichment have highlighted the potential for interactions between
397 these two stressors (Widdicombe & Austen 2001, Austen & Widdicombe 2006). This study sought
398 to expand upon this earlier work by examining the interaction between nutrient enrichment and
399 physical disturbance, under larger-scale field conditions that offer an element of ecological
400 realism To our knowledge, it represented the first field-based study to examine the multiple
401 effects of these two stressors in an intertidal sedimentary habitat (but see Rossi & Underwood
402 2002 for a discussion on the impacts of organic matter burial that result both from physical
403 disturbance of sediments and nutrient enrichment during decay). It found site-specific effects of
404 nutrient enrichment and physical disturbance, with limited interactions between stressors. The
405 study thereby demonstrates the role environmental context plays on mediating the impact of
406 multiple stressors, and raises the possibility that communities may be more resilient to increased
407 stress than can be predicted from laboratory studies alone.

408 Of the two stressors examined by this study, nutrient enrichment generally elicited stronger
409 ecological responses than physical disturbance. Eastern Australian estuaries are, in general,
410 oligotrophic and phosphorus-limited (Bishop et al. 2006, Scanes et al. 2007, Kelaher et al. 2013).
411 In nutrient-limited systems such as these, nutrient enrichment typically stimulates bottom-up
412 responses of the MPB (O'Brien et al. 2010, Pascal et al. 2013). This growth may then lead to

413 cascading positive effects on higher trophic levels (York et al. 2012). Nevertheless, because excess
414 primary production can also result in deterioration of sediment conditions through over-
415 stimulation of oxygen-consuming bacteria, the effects of nutrient enrichment on macrofaunal
416 abundance and richness can range from positive (Morris & Keough 2003a, b) to negative (Fitch &
417 Crowe 2010, Botter-Carvalho et al. 2014), depending on the level of enrichment and
418 environmental conditions (Pearson & Rosenberg 1978).

419 In the present study, an effect of nutrient enrichment on MPB was identified at both study sites
420 (although effects were stronger in Botany Bay), and the effect was positive, in agreement with
421 mesocosm studies (e.g. Sundback et al. 2010). While nutrient additions acted to alter macrofaunal
422 community structure at each of the sites, effects were weaker than expected, with few species
423 significantly affected by stressors, and the nature of effects variable between these. It has been
424 hypothesised that estuarine communities have an inherent ability to resist stress at levels that
425 would push other aquatic systems over a threshold, and which are above and beyond that which
426 they are subjected to daily (e.g. salinity fluctuations, tides, and emersion periods; see Elliott &
427 Whitfield 2011). Within nature, biotic interactions may reduce the impacts of nutrient
428 enrichment. The limited effects of nutrient enrichment observed in this study indicate there could
429 be a mix of top down and bottom up effects impacting the community. Although in nutrient-
430 limited systems, nutrient enrichment facilitates autotrophic growth, this effect can become
431 masked by top down processes where increased productivity is matched by increased grazing
432 pressure (Pascal et al. 2013). Alternatively, the weak effects of nutrient enrichment observed in
433 the present study may be a function of other abiotic and biotic characteristics of the sites, an

434 aspect that would be controlled for in closed systems. Sedimentary grain size and starting
435 community composition can play an important role in interpreting how communities will respond
436 to stressors (Lindegarth & Hoskin 2001, Rossi & Underwood, 2002, Whomersley et al. 2010). The
437 relatively weak effects of nutrient enrichment detected in this study are consistent with larger-
438 scale surveys that indicate that nutrient enrichment is potentially a weak driver of change in
439 benthic community composition in comparison to environmental characteristics, such as grain
440 size (Nicastro & Bishop 2013). Alternatively, the weak effects of nutrient enrichment seen in this
441 study may reflect the small scale of manipulations. Larger-scale disturbances alter system
442 dynamics ultimately impacting recovery and community resilience (Ellis et al. 2000).

443 Both MPB and macrofauna displayed a limited to neutral response to physical disturbance despite
444 predictions, based on previous field studies, of negative effects of this stressor from destruction
445 of sediment structure, burrows and damage to softer-bodied species (Hall 1994, Brown & Wilson
446 1997, Hall & Harding 1997, Dernie et al. 2003, Rossi et al. 2007). Community or species-specific
447 responses to physical disturbance have been previously noted to vary according to environmental
448 context (Whomersley et al. 2010) and previous mesocosm experiments have found that, in line
449 with predictions of the Dynamic Equilibrium Hypothesis (Huston 1979), the effects of physical
450 disturbance vary across productivity gradients caused by nutrient enrichment (Widdicombe &
451 Austen 2001, Austen & Widdicombe 2006). Consistent with the Dynamic Equilibrium Hypothesis,
452 a number of macrofaunal species in this study displayed responses to physical disturbance that
453 varied according to the prevailing levels of nutrient enrichment. Overall, however, the number of

454 species exhibiting such a non-additive responses was few, and nutrient enrichment was the
455 dominant stressor impacting community composition in these systems.

456 While small scale disturbances such as raking and trampling sediments have been shown to
457 negatively impact sediment communities in other studies (Rossi et al 2007; Whomersley et al
458 2010), the spatial scale and frequency of raking may simply not have been great enough to elicit a
459 strong response in the communities examined here. In this experiment, plots were less than 1 m
460 in diameter, allowing species to rapidly recolonise from outside affected areas (see Lee et al.
461 2011). This small scale of physical disturbance might be analogous to that caused by clam raking,
462 by boat-run up on intertidal sediments or by propeller scaring. However, for disturbances, such as
463 storms, that cause physical disturbance at the scale of the entire site, movement of species
464 between affected areas would not be possible. Treatment effects at this level are likely to
465 facilitate the selection for more resilient organisms that are able to tolerate stressors (Sanford &
466 Kelly 2011). The relatively (low) monthly frequency of raking may also have contributed to the
467 weak to absent responses of taxa to physical disturbance. The frequency of disturbance is an
468 important factor determining the magnitude of impact (Connell 1978, White & Pickett 1985), with
469 infrequent disturbances providing opportunity for organisms to recolonise in between,
470 particularly if the spatial scale of the disturbance is small, but frequent disturbances preventing
471 recovery before the next perturbation. Nevertheless, in Northern Europe, a single raking event
472 covering an area of 36 m², that perturbed large benthic species, had ecological impacts that
473 extended a year (Kaiser et al. 2001). Impacts are, therefore, likely to be dependent on the size and
474 scale of the raking event, as well as the species present. Although the physical disturbance

475 imposed by repeatedly sampling of sediments may have complicated results by leading to small
476 scale disturbance across all experimental plots, irrespective of disturbance treatment (Lindegarth
477 and Underwood 2000), we were careful to sample different areas on each sampling date.

478 Within the literature, there are many examples of context dependent effects of manipulations,
479 even across geographically proximate and/or environmentally similar locations (e.g Whomersley
480 et al. 2010, Bishop & Kelaher 2013, Gladstone-Gallagher et al. 2014). In this study, the differing
481 response between the two sites to environmental perturbations may be a direct effect of
482 environmental differences between sites, or an indirect effect arising from differences in their
483 community composition. Although the two sites did not differ in background sediment organic
484 content or chlorophyll concentration and were selected to be of similar grain size, other
485 environmental variables are likely to have differed. Botany Bay is a large industrial area with a
486 history of contamination whereas Lane Cove is surrounded by bushland and is used for
487 recreational purposes such as bushwalking, boating and fishing. Habitat characteristics such as
488 grain size, organic content and water content mediate the speed of benthic recovery from
489 perturbations (Dernie et al. 2003). Additionally, under natural conditions, habitat heterogeneity
490 can buffer against treatment effects (Godbold et al. 2011) or lead to substantial background
491 variation, against which treatment effects are hard to detect (Bulling et al. 2008). Community
492 structure may mediate stressor impacts by dictating the functional capability of the ecosystem,
493 and the ways in which species may interact to buffer the effects of stressors (Bulling et al. 2008,
494 Godbold & Solan, 2009).

495 Additionally, differences between the sites in background stressors may have contributed to the
496 differing sensitivity of their communities to the experimental perturbations. Background stress
497 can mediate how a system will respond to further stress via two main mechanisms. First,
498 prevailing stress may select for organisms that have enhanced resistance to local stressors and so
499 are able to withstand further disturbance (Sanford & Kelly, 2011). Second, prevailing stressors can
500 determine how far away a system is from a tipping-point beyond which the application of
501 additional stress pushes the system over a threshold value, resulting in a system collapse (Pearson
502 & Rosenberg, 1978; Whomersley et al., 2010). How far away systems are from tipping points is
503 related to the conditions to which fauna are adapted and the trade-offs they have made either
504 through evolutionary or local adaptations (Sanford & Kelly 2011, Botero et al. 2015). In dynamic
505 environments subjected to multiple stressors such as estuaries, it would be expected that
506 communities are dominated by species that are resilient to perturbation (Sanford & Kelly 2011).
507 Only where stressors exceed the threshold of resilience is there system collapse or change to
508 another stable state.

509 Although previous mesocosm studies have identified interactions between nutrient enrichment
510 and physical disturbance (e.g. Widdicombe & Austen 2001), our study demonstrates that the
511 results of such studies cannot necessarily be used to infer responses of benthic communities to
512 stressors in the field. While some studies have found agreement between the outcomes of field
513 and mesocosm experiments (e.g. Sundback et al. 2010), mesocosm studies can never completely
514 replicate the natural environment and conclusions about the way in which multiple stressors
515 interact can differ between these approaches (Przelawski et al. 2005; Crain et al. 2008, Alsterberg

516 et al. 2014). For example, Cowie et al (2000) observed a negative effect of physical disturbance on
517 the macrofauna in both experimental mesocosms and *in situ* treatments; however, the strength
518 of the decline of species was greater in mesocosm experiments. Adverse effects are likely to be
519 amplified in mesocosm studies due to the stress imposed on organisms as a consequence of
520 handling and containment in confined conditions, and because processes such as recruitment that
521 act to weaken impacts in field scenarios cannot always occur (see Cowie et al. 2000). The
522 heterogeneity of environments such as mudflats and the patchiness of species can contribute to
523 the differing responses observed between laboratory and mesocosm studies (Crain et al. 2008), or
524 even between different field sites (Norkko et al. 2010). While mesocosms provide a controlled
525 environment, there is a growing evidence that environmental context (such as prevailing nutrient
526 status) are often the determinant factors governing how systems will respond to stress (Sundback
527 2007; Bishop & Kelaher 2013; O'Connor & Donohue, 2013). Thus it follows that while mesocosm
528 and laboratory experiments are often a quick and simple tool in understanding the consequences
529 of change, these experiments should be utilised to formulate theories to be tested empirically
530 (see Benton et al. 2007), field experiments must be used to determine true impacts of stressors
531 under naturally occurring conditions. Furthermore, the implications of multiple stressor impacts
532 are well known to vary depending upon the experimental protocol (laboratory/mesocosm; see
533 Crain et al. 2008 and references therein) or the responses measured (Alsterberg et al. 2014). The
534 differing method of nutrient enrichment between our study, which applied fertiliser to plots, and
535 the previous mesocosm studies that enriched sediments via application of dried and ground-up
536 *Ascophyllum* (Widdicombe & Austen 2001, Austen & Widdicombe 2006), may have contributed to
537 differences in the conclusions of these. Similarly, the differing frequency of physical disturbance

538 between our study, which raked plots monthly, and the mesocosm experiments, that raked plots
539 daily to monthly (Widdicombe & Austen 2001, Austen & Widdicombe 2006) may also have led to
540 differences.

541 **Conclusions**

542 This study analysed the combined impact of selected multiple stressors –nutrient enrichment and
543 physical disturbance – in a field setting. While both stressors have been previously identified to be
544 detrimental to communities, and in mesocosm experiments produce non-additive effects, the
545 combined impacts observed in this study were weak, mainly additive (at least at the chosen levels
546 of stress), and mediated by environmental context. The differing results of this *in situ* study to
547 those of previous mesocosm experiments, and the site-specificity of effects, highlight the
548 important role that abiotic and biotic features of the environment play in mediating stressor
549 effects. Furthermore, the resilience of benthic communities will determine their response to
550 multiple stressors. The minor responses observed within this study highlight that benthic
551 sedimentary communities are more resilient to increased stress than previous studies would
552 indicate. Studies are now needed that examine which aspects of the environment or community
553 are most important in mediating stressor impacts.

554 **References**

- 555 Alsterberg C, Sundbäck K, Gamfeldt L (2014) Multiple stressors and multifunctionality: limited
556 effects on an illuminated benthic system. *Biol Lett* 10:20140640
- 557 Anderson M (2001) A new method for non-parametric multivariate analysis of variance. *Austral*
558 *Ecol* 26:32–46

- 559 Anderson M, Gorley RN, Clarke RK (2008) Permanova+ for Primer: Guide to Software and
560 Statistical Methods.
- 561 Austen MC, Widdicombe S (2006) Comparison of the response of meio- and macrobenthos to
562 disturbance and organic enrichment. *J Exp Mar Bio Ecol* 330:96–104
- 563 Benton TG, Solan M, Travis JM, Sait SM (2007) Microcosm experiments can inform global
564 ecological problems. *Trends Ecol Evol* 22:516–521
- 565 Bijma J, Pörtner H-O, Yesson C, Rogers AD (2013) Climate change and the oceans-what does the
566 future hold? *Mar Pollut Bull* 74:495–505
- 567 Bishop MJ (2005) Compensatory effects of boat wake and dredge spoil disposal on assemblages of
568 macroinvertebrates. *Estuaries* 28:510–518
- 569 Bishop MJ, Kelaher BP, Smith MPL, York PH, Booth DJ (2006) Ratio-dependent response of a
570 temperate Australian estuarine system to sustained nitrogen loading. *Oecol* 149:701-708
- 571 Bishop MJ, Kelaher BP (2013) Context-specific effects of the identity of detrital mixtures on
572 invertebrate communities. *Ecol Evol* 3:3986–3999
- 573 Botero CA, Weissing FJ, Wright J, Rubenstein DR (2015) Evolutionary tipping points in the capacity
574 to adapt to environmental change. *Proc Natl Acad Sci USA* 112:184–189
- 575 Botter-Carvalho ML, Carvalho PVVC, Valença APMC, Santos PJP (2014) Estuarine macrofauna
576 responses to continuous in situ nutrient addition on a tropical mudflat. *Mar Pollut Bull*
577 83:214–23
- 578 Brown B, Wilson WH (1997) The role of commercial digging of mudflats as an agent for change of
579 infaunal intertidal populations. *J Exp Mar Bio Ecol* 218:49–61
- 580 Bulling MT, Solan M, Dyson KE, Hernandez-Milian G, Luque P, Pierce GJ, Raffaelli D, Paterson DM,
581 White PCL (2008) Species effects on ecosystem processes are modified by faunal responses
582 to habitat composition. *Oecologia* 158:511–520
- 583 Christensen MR, Graham MD, Vinebrooke RD, Findlay DL, Paterson MJ, Turner M a. (2006)
584 Multiple anthropogenic stressors cause ecological surprises in boreal lakes. *Glob Chang Biol*
585 12:2316–2322
- 586 Clarke K, Gorley R (2006) PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.
- 587 Cloern J (2001) Our evolving conceptual model of the coastal eutrophication problem. *Mar Ecol*
588 *Prog Ser* 210:223–253

- 589 Connell JH (1978) Diversity in Tropical Rain Forests and Coral Reefs High diversity of trees and
590 corals is maintained. *Science* 199:1302–1310
- 591 Cowie PR, Widdicombe S, Austen MC (2000) Effects of physical disturbance on an estuarine
592 intertidal community: field and mesocosm results compared. *Mar Biol* 136:485–495
- 593 Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human
594 stressors in marine systems. *Ecol Lett* 11:1304–1315
- 595 Darling ES, Côté IM (2008) Quantifying the evidence for ecological synergies. *Ecol Lett* 11:1278–
596 1286
- 597 Dayton PK (1971) Competition, disturbance and community organization: the provision and
598 subsequent utilization of space in a rocky intertidal community. *Ecol Monograph* 41:351–389
- 599 Dernie KM, Kaiser MJ, Warwick RM (2003) Recovery rates of benthic communities following
600 physical disturbance. *J Anim Ecol* 72:1043–1056
- 601 Elliott M, Whitfield AK (2011) Challenging paradigms in estuarine ecology and management.
602 *Estuar Coast Shelf Sci* 94:306–314
- 603 Ellis JI, Norkko A, Thrush SF (2000) Broad-scale disturbance of intertidal and shallow sublittoral
604 soft-sediment habitats; Effects on the benthic macrofauna. *J Aquat Ecosyst Stress Recover*
605 7:57–74
- 606 Fitch JE, Crowe TP (2010) Effective methods for assessing ecological quality in intertidal soft-
607 sediment habitats. *Mar Pollut Bull* 60:1726–33
- 608 Ford RB, Honeywill C (2002) Grazing on intertidal microphytobenthos by macrofauna: is
609 pheophorbide *a* a useful marker? *Mar Ecol Ser* 229:33–42
- 610 Fraterrigo JM, Rusak JA. (2008) Disturbance-driven changes in the variability of ecological patterns
611 and processes. *Ecol Lett* 11:756–770
- 612 Gladstone-Gallagher RV., Lundquist CJ, Pilditch CA. (2014) Response of temperate intertidal
613 benthic assemblages to mangrove detrital inputs. *J Exp Mar Bio Ecol* 460:80–88
- 614 Godbold JA, Bulling MT, Solan M (2011) Habitat structure mediates biodiversity effects on
615 ecosystem properties. *Proc Biol Sci* 278:2510–8
- 616 Godbold JA, Solan M (2009) Relative importance of biodiversity and the abiotic environment in
617 mediating an ecosystem process. *Mar Ecol Prog Ser* 396:273–282

- 618 Gray JS (1997) Marine biodiversity patterns, threats and conservation needs. *Biodivers Conserv*
619 6:153–175
- 620 Hall SJ (1994) Physical disturbance and marine benthic communities: life in unconsolidated
621 sediments. *Oceanogr Mar Biol An Annu Rev Vol* 32:179–239
- 622 Hall SJ, Harding MJC (1997) Physical disturbance and marine benthic communities: The effects of
623 mechanical harvesting of cockles on non-target benthic infauna. *J Appl Ecol* 34:497–517
- 624 Halpern BS, Walbridge S, Selkoe KA, Kappel C V, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C,
625 Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M,
626 Steneck R, Watson R (2008) A Global Map of Human Impact on Marine Ecosystems. *Science*
627 319:948–952
- 628 Harris PT (2014) Shelf and deep-sea sedimentary environments and physical benthic disturbance
629 regimes: A review and synthesis. *Mar Geol* 353:169–184
- 630 Hicks N, Bulling MT, Solan M, Raffaelli D, White PCL, Paterson DM (2011) Impact of biodiversity-
631 climate futures on primary production and metabolism in a model benthic estuarine system.
632 *BMC Ecol* 11:Article No.: 7
- 633 Huston M (1979) A general hypothesis of species diversity. *Am Nat* 113:81–101
- 634 Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls
635 *a*, *b*, *c*1 and *c*2 in higher-plants, algae and natural phytoplankton. *Biochem Und Physiol Der*
636 *Pflanz* 167:191–194
- 637 Jones KMM, Boulding EG (1999) State-dependent habitat selection by an intertidal snail: the costs
638 of selecting a physically stressful microhabitat. *J Exp Mar Bio Ecol* 242:149–177
- 639 Kaiser MJ, Broad G, Hall SJ (2001) Disturbance of intertidal soft-sediment benthic communities by
640 cockle hand raking. *J Sea Res* 45:119–130
- 641 Kelaher BP, Van Den Broek J, York PH, Bishop MJ, Booth DJ (2013) Positive responses of a seagrass
642 ecosystem to experimental nutrient enrichment. *Mar Ecol Prog Ser* 487:15–25
- 643 Kromkamp JC, Morris EP, Forster RM, Honeywill C, Hagerthey S, Paterson DM (2006) Relationship
644 of intertidal surface sediment chlorophyll concentration to hyperspectral reflectance and
645 chlorophyll fluorescence. *Estuaries and Coasts* 29:183–196
- 646 Lee KM, Lee SY, Connolly RM (2011) Short-term response of estuarine sandflat trophodynamics to
647 pulse anthropogenic physical disturbance: Support for the Intermediate Disturbance
648 Hypothesis. *Estuar Coast Shelf Sci* 92:639–648

- 649 Lindegarth M, Hoskin M (2001) Patterns of distribution of macro-fauna in different types of
650 estuarine, soft sediment habitats adjacent to urban and non-urban areas. *Estuar Coast Shelf*
651 *Sci* 52:237–247
- 652 Lindegarth M, Underwood A (2002) A manipulative experiment to evaluate predicted changes in
653 intertidal macro- faunal assemblages after contamination by heavy metals. *J Exp Mar Biol*
654 *Ecol* 274:41–64
- 655 Menge BA, Sutherland JP (1987) Community regulation: Variation in disturbance, copetition, and
656 predation in relation to environmental stress and recruitment. *Am Nat*:730–757
- 657 Mistri M, Cason E, Munari C, Rossi R (2004) Disturbance of a soft-sediment meiobenthic
658 community by clam hand raking. *Ital J Zool* 71:131–133
- 659 Morris L, Keough MJ (2002) Organic pollution and its effects : a short-term transplant experiment
660 to assess the ability of biological endpoints to detect change in a soft sediment environment.
661 *225*:109–121
- 662 Morris L, Keough MJ (2003a) Testing the effects of nutrient additions on mudflat macroinfaunal
663 assemblages in the presence and absence of shorebird predators. *Mar Freshw Res* 54:859–
664 874
- 665 Morris L, Keough MJ (2003b) Variation in the response of intertidal infaunal invertebrates to
666 nutrient additions: field manipulations at two sites within Port Phillip Bay, Australia. *Mar Ecol*
667 *Ser* 250:35–49
- 668 Nicastro A, Bishop MJ (2013) Weak and habitat-dependent effects of nutrient pollution on
669 macrofaunal communities of Southeast Australian estuaries. *PLoS One* 8:e65706
- 670 Nixon SW (1995) Coastal marine eutrophication - a definition, social causes, and future concerns.
671 *Ophelia* 41:199–219
- 672 Norkko J, Norkko A, Thrush SF, Valanko S, Suurkuukka H (2010) Conditional responses to
673 increasing scales of disturbance, and potential implications for threshold dynamics in soft-
674 sediment communities. *Mar Ecol Prog Ser* 413:253–266
- 675 O'Brien AL, Morris L, Keough MJ (2010) Multiple sources of nutrients add to the complexities of
676 predicting marine benthic community responses to enrichment. *Mar Freshw Res* 61:1388–
677 1398
- 678 O'Connor N, Donohue I (2013) Environmental context determines multi-trophic effects of
679 consumer species loss. *Glob Change Biol* 19:431–440

- 680 Paine RT, Levin SA (1981) Intertidal landscapes : disturbance and the dynamics of pattern. *Ecol*
681 *Monogr* 51:145–178
- 682 Pascal PY, Fleeger JW, Boschker HTS, Mitwally HM, Johnson DS (2013) Response of the benthic
683 food web to short- and long-term nutrient enrichment in saltmarsh mudflats. *Mar Ecol Prog*
684 *Ser* 474:27–41
- 685 Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and
686 pollution of the marine environment. *Oceanogr Mar Biol Annu Rev* 16:229–311
- 687 Przeslawski R, Byrne M, Mellin C. (2015) A review and meta-analysis of the effects of multiple
688 abiotic stressors on marine embryos and larvae. *Glob Change Biol* 21:2122–2140
- 689 Rossi F, Forster RM, Montserrat F, Ponti M, Terlizzi a., Ysebaert T, Middelburg JJ (2007) Human
690 trampling as short-term disturbance on intertidal mudflats: effects on macrofauna
691 biodiversity and population dynamics of bivalves. *Mar Biol* 151:2077–2090
- 692 Rossi F, Underwood A (2002) Small-scale disturbance and increased nutrients as influences on
693 intertidal macrobenthic assemblages: Experimental burial of wrack in different intertidal
694 environments. *Mar Ecol Prog Ser* 241:29–39
- 695 Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. *Ann Rev Mar Sci* 3:509–35
- 696 Scanes P, Coade G, Doherty M, Hill R (2007) Evaluation of the utility of water quality based
697 indicators of estuarine lagoon condition in NSW, Australia. *Estuar Coast Shelf Sci* 74:306–319
- 698 Somerfield PJ, Rees HL, Warwick RM (1995) Interrelationships in community structure between
699 shallow-water marine meiofauna and macrofauna in relation to dredgings disposal. *Mar Ecol*
700 *Prog Ser* 127:103–112
- 701 Sundback K, Alsterberg C, Larson F (2010) Effects of multiple stressors on marine shallow-water
702 sediments: Response of microalgae and meiofauna to nutrient-toxicant exposure. *J Exp Mar*
703 *Bio Ecol* 388:39–50
- 704 Sundback K, Peterson DG, Dahllöf I, Larson F (2007) Combined nutrient-toxicant effects on a
705 shallow-water marine sediment system:sensitivity and resilience of ecosystem functions.
706 *Mar Ecol Prog Ser* 330:13–30
- 707 Tolhurst TJ, Underwood AJ, Perkins RG, Chapman MG (2005) Content versus concentration:
708 Effects of units on measuring the biogeochemical properties of soft sediments. *Estuar Coast*
709 *Shelf Sci* 63:665–673

- 710 Vitousek P, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman
711 GD (1997a) Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol*
712 *Appl* 7:737–750
- 713 Vitousek P, Mooney HA, Lubchenco J, Melillo JM (1997b) Human domination of Earth's
714 ecosystems. *Science* 277:494–499
- 715 White PS, Pickett STA (1985). Natural disturbance and patch dynamics: an introduction. In Pickett
716 STA, White PS (Eds.), *The ecology of natural disturbance and patch dynamics*. (pp. 3-13).
717 Academic Press.
- 718 Whomersley P, Huxham M, Bolam S, Schratzberger M, Augley J, Ridland D (2010) Response of
719 intertidal macrofauna to multiple disturbance types and intensities - an experimental
720 approach. *Mar Environ Res* 69:297–308
- 721 Widdicombe S, Austen MC (2001) The interaction between physical disturbance and organic
722 enrichment: An important element in structuring benthic communities. *Limnol Oceanogr*
723 46:1720–1733
- 724 Worm B, Reusch TBH, Lotze HK (2000) In situ nutrient enrichment: Methods for marine benthic
725 ecology. *Int Rev Hydrobiol* 85:359–375
- 726 Wynberg RP, Branch GM (1994) Disturbance associated with bait-collection for sandprawns
727 (*Callinassa kraussi*) and mudprawns (*Upogebia africana*): Long-term effects on the biota of
728 intertidal sandflats. *J Mar Res* 52:523–558
- 729 Yeo RK, Risk MJ (1979) Intertidal catastrophes: effect of storms and hurricanes on intertidal
730 benthos of the Minas Basin, Bay of Fundy. *J Fish Res Board Canada* 36:667–669
- 731 York P, Kelaher B, Booth D, Bishop M (2012) Trophic responses to nutrient enrichment in a
732 temperate seagrass food chain. *Mar Ecol Prog Ser* 449:291–296
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736 **Table 1: PERMANOVA analyses examining the interacting effects of Nutrients (N), Disturbance**
 737 **(D), Month (M), and Site (S) on organic content, chlorophyll α concentration and Normalised**
 738 **Difference Vegetation Index (NDVI) in Botany Bay and Lane Cove estuaries. Plot (P) was nested**
 739 **within Nx DxS. Nutrient enrichment and physical disturbance factors contained 3 levels of**
 740 **applied stress (Zero, Low and High). The three months (2 – July, 3 – August, 4 – September)**
 741 **during which both sites were sampled are included in the analysis. n=3-7 for all treatments**
 742 **levels**

743

Factor	Organic content			Chlorophyll α			NDVI		
	Df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)
N	2	1.68	0.255	2	3.95	0.070	2	1.22	0.406
D	2	3.47	0.076	2	2.49	0.143	2	0.34	0.916
M	2	1.60	0.371	2	0.84	0.492	3	0.58	0.693
S	1	7.62	0.002	1	0.12	0.747	1	2.59	0.116
NxD	4	0.54	0.859	4	1.10	0.422	4	0.75	0.696
NxM	4	0.42	0.821	4	27.40	0.002	6	1.45	0.336
NxS	2	0.54	0.828	2	1.25	0.282	2	3.42	0.039
DxM	4	0.58	0.730	4	0.08	0.981	6	0.79	0.615
DxS	2	0.42	0.916	2	1.11	0.323	2	2.33	0.104
MxS	2	2.37	0.078	2	9.88	0.001	3	25.91	0.001
NxDxM	8	1.24	0.363	8	1.80	0.221	12	0.53	0.855
NxDxS	4	1.46	0.166	4	0.47	0.945	4	1.23	0.262
NxMxS	4	0.78	0.572	4	0.05	0.998	6	1.54	0.161
DxMxS	4	2.10	0.054	4	3.05	0.015	6	1.55	0.174
P(NxDxS)	107	1.83	0.002	112	1.56	0.005	110	1.93	0.001
NxDxMxS	8	1.30	0.230	8	0.83	0.577	12	1.69	0.083
Res	179			187			301		

744 Key: **Bold** = significant effect at $p < 0.05$

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747 **Table 2: Two-way PERMANOVA analyses examining the interacting effects of Nutrients (N) and**
 748 **Disturbance (D) on the macrofaunal community at sites within Botany Bay and Lane Cove**
 749 **estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied**
 750 **stress (zero, low or high) over 4 months (M). n=5-7 for all treatments levels**

	df	Species richness		Abundance		Shannon Diversity		Multivariate	
		Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)
Botany Bay:									
N	2	0.67	0.562	0.51	0.618	1.20	0.311	1.92	0.035
D	2	0.36	0.702	0.19	0.846	0.64	0.523	1.31	0.181
NXD	4	0.48	0.767	0.52	0.766	0.55	0.699	0.98	0.476
Res	52								
Lane Cove:									
N	2	1.22	0.270	3.66	0.027	0.47	0.604	1.48	0.059
D	2	0.34	0.727	0.70	0.493	0.59	0.548	0.68	0.882
NXD	4	0.46	0.750	0.50	0.714	1.91	0.108	0.85	0.863
Res	52								

751 Key: **Bold** = significant effect at $p < 0.05$

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754 **Table 3: PERMANOVA analysis examining the interacting effects of Nutrients (N), Disturbance**
 755 **(D), Month (M), and Site (S) on the key discriminating species common to both sites within**
 756 **Botany Bay and Lane Cove estuaries. Plot (P) was nested within Nx Dx S. Nutrient enrichment**
 757 **and physical disturbance factors contained 3 levels of applied stress (zero, low or high), with**
 758 **sampling after 2 and 4 months (i.e. July, September). n=5-7 for all treatment levels**

Factor	df	<i>Mediomastus australiensis</i>		<i>Prionospio sp.</i>		<i>Nephtys australiensis</i>		<i>Mysella sp.</i>	
		Pseudo-F	P(perm)	Pseudo-	P(perm)	Pseudo-F	P(perm)	Pseudo-	P(perm)
N	2	0.69	0.629	0.56	0.697	0.46	0.758	1.17	0.509
D	2	0.78	0.586	0.66	0.631	1.62	0.324	0.17	0.949
M	1	4.22	0.326	0.96	0.629	0.11	0.506	35.18	0.343
S	1	13.98	0.001	2.90	0.086	14.03	0.001	7.15	0.010
NxD	4	0.21	0.977	1.87	0.197	4.66	0.026	2.87	0.087
NxM	2	1.04	0.497	8.67	0.104	1.90	0.355	483.73	0.003
NxS	2	0.68	0.530	2.64	0.045	2.13	0.138	1.15	0.310
DxM	2	2.73	0.264	1.45	0.375	0.79	0.565	6.32	0.132
DxS	2	0.80	0.466	1.11	0.397	0.48	0.637	0.69	0.512
MxS	1	4.73	0.037	8.06	0.007	11.36	0.002	0.58	0.455
NxDxM	4	4.22	0.092	0.92	0.516	0.27	0.893	6.81	0.045
NxDxS	4	0.92	0.544	0.81	0.681	0.68	0.772	0.60	0.867
NxMxS	2	1.69	0.193	0.25	0.769	0.20	0.833	0.01	0.989
DxMxS	2	0.47	0.639	0.89	0.429	1.25	0.299	0.07	0.933
P(NxDxS)	11	1.46	0.033	1.12	0.295	0.97	0.570	3.08	0.001
NxDxMxS	4	0.79	0.538	1.30	0.290	0.74	0.564	0.10	0.983
Res	10								

759 Key: **Bold** = significant effect at $p < 0.05$

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763 Figure 1: Mean (+/- SE) chlorophyll *a* concentration (mg m^{-2}) of surface sediments in
764 experimental plots. Differences among disturbance treatments through time at Botany Bay
765 (A) and Lane Cove (B). Differences among nutrient treatments through time at Botany Bay (C)
766 and Lane Cove (D). Sampling within Lane Cove could not be done in June due to large
767 amounts of overlying water. For all graphs, 0 treatment = circle, solid line; Low treatment =
768 square, long dashed line; High treatment = triangle; short dashed line. $n = 3-7$ for all
769 treatment levels.

770 Figure 2: Mean (+/- SE) normalised difference vegetation index (NDVI) at sites in Botany Bay (BB)
771 and Lane Cove (LC) following 4 months of continued nutrient enrichment and physical
772 disturbance crossed at 3 levels (0, L –Low, and H – High). Data averaged across all months
773 and physical disturbance treatments as there was no month x nutrient or disturbance x
774 nutrient enrichment interaction. $n = 3-7$ for all treatment levels. Letters indicate statistically
775 significant differences between treatments (PERMANOVA post hoc tests).

776 Figure 3: Two-dimensional nMDS configuration displaying macrofaunal composition at Botany Bay
777 (A) and Lane Cove (B) following 4 months of zero (0) low (L) and high (H) nutrient enrichment

778 (N) and physical disturbance (D). Points represent individual plots. Data square root
779 transformed and matrix based upon Bray Curtis similarity. N = 5-7

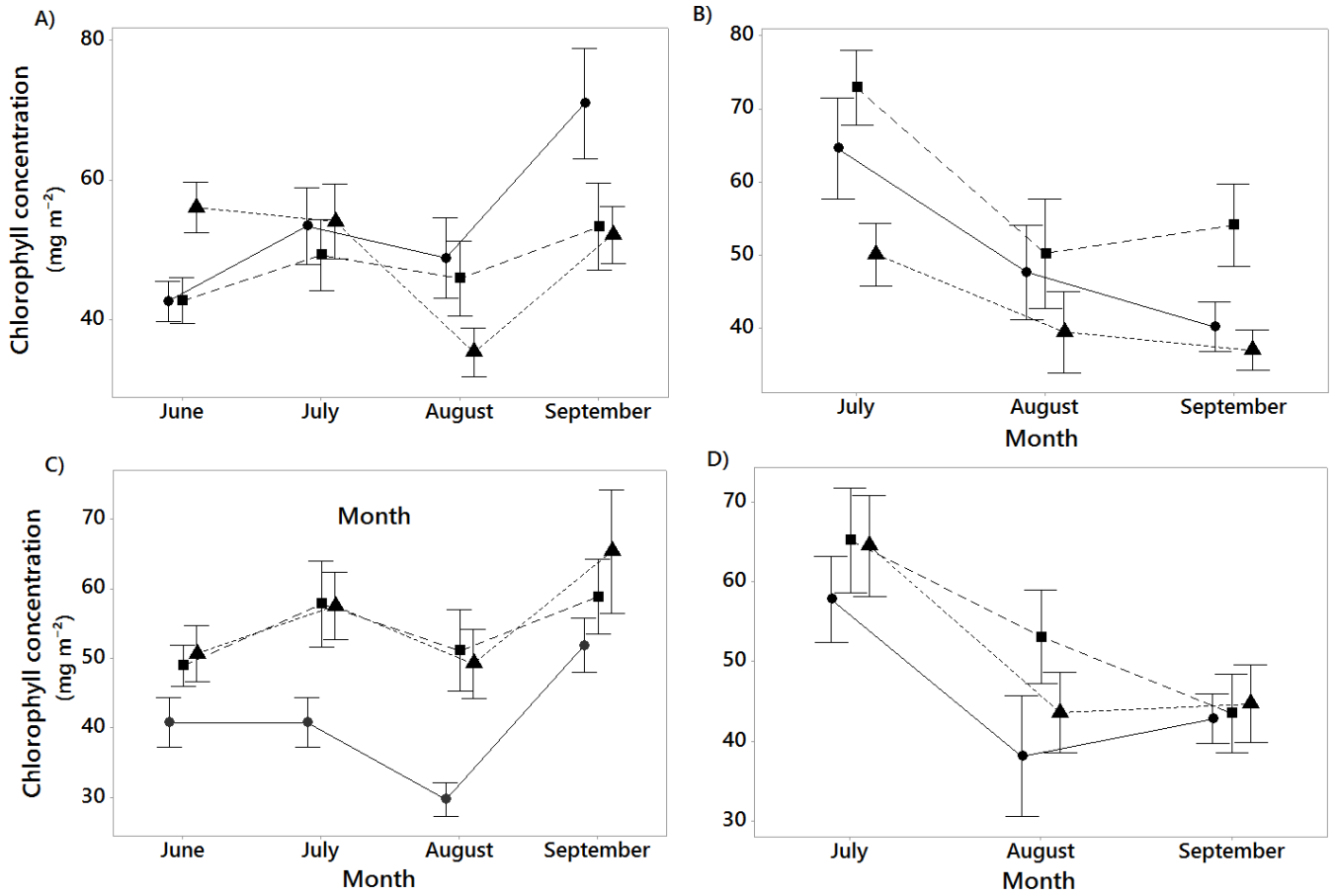
780 Figure 4: Mean (+/- SE) abundance of common macrofaunal species in experimental plots at
781 Botany Bay and Lane Cove following 4 months of nutrient enrichment and physical
782 disturbance crossed at 3 levels (0, L – Low, and H – High) each. N = 5-7.

783 Figure 5: Mean (+/- SE) abundance of A) *Scoloplos* sp. and B) nematodes in experimental plots at
784 Lane Cove following 4 months of nutrient enrichment and physical disturbance crossed at 3
785 levels (0, L – Low, and H – High) of each factor. N = 5-7

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790 **Figure 1:**

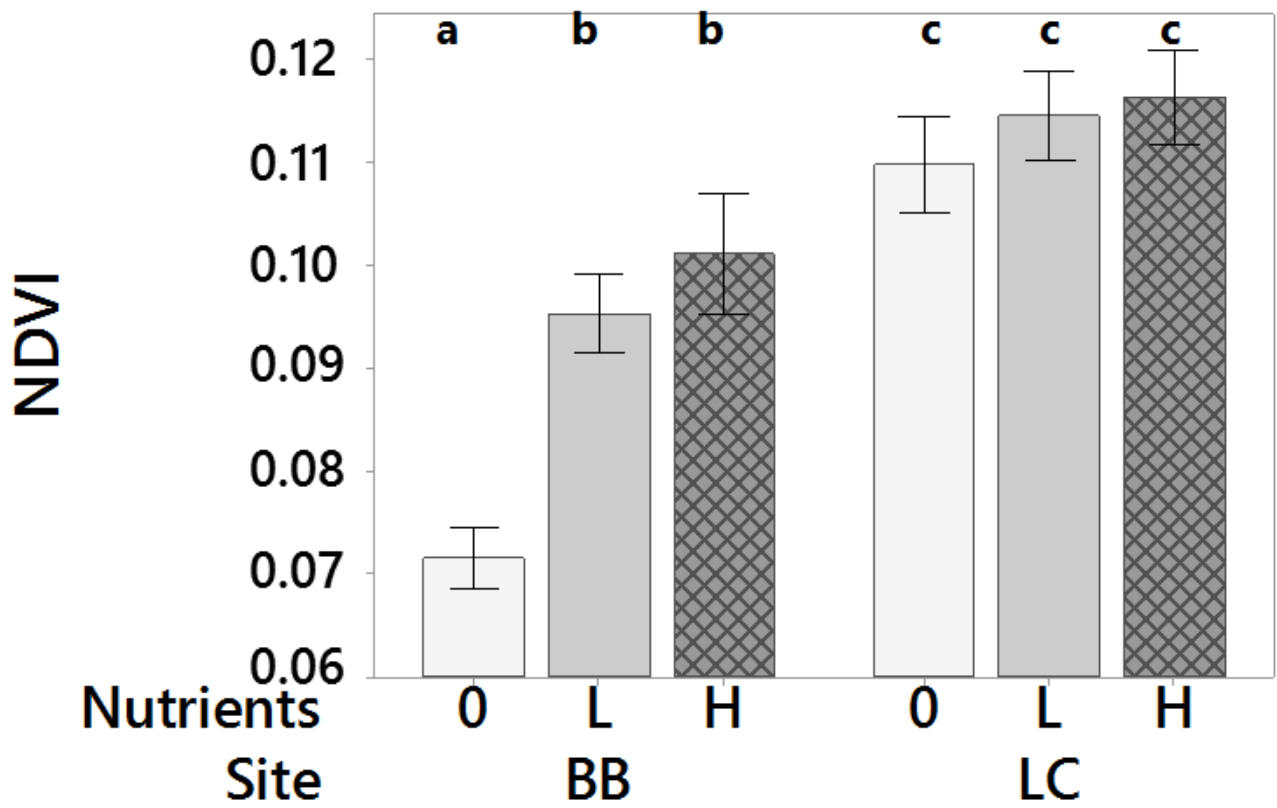
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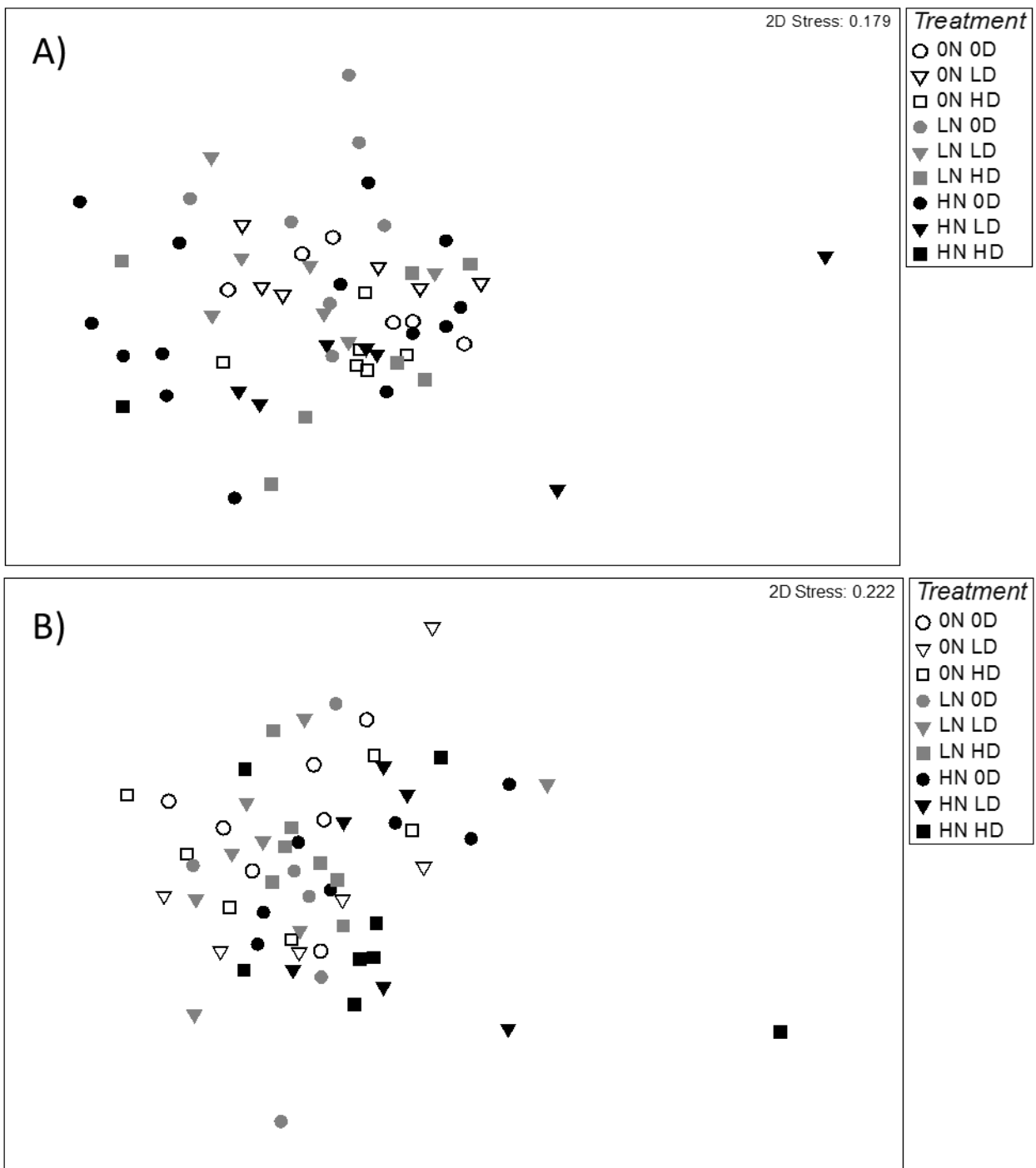
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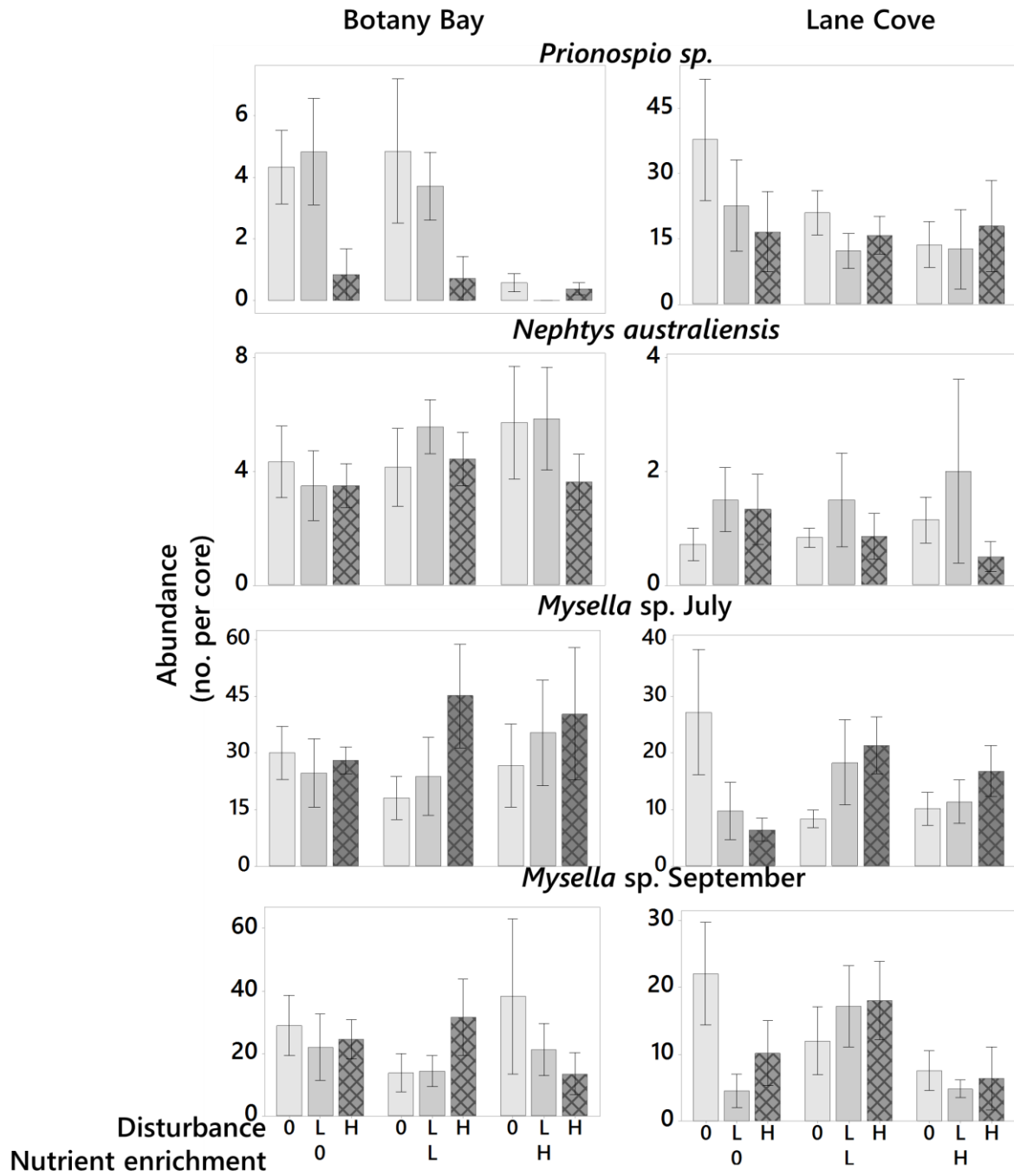
797 **Figure 2:**

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Figure 3:



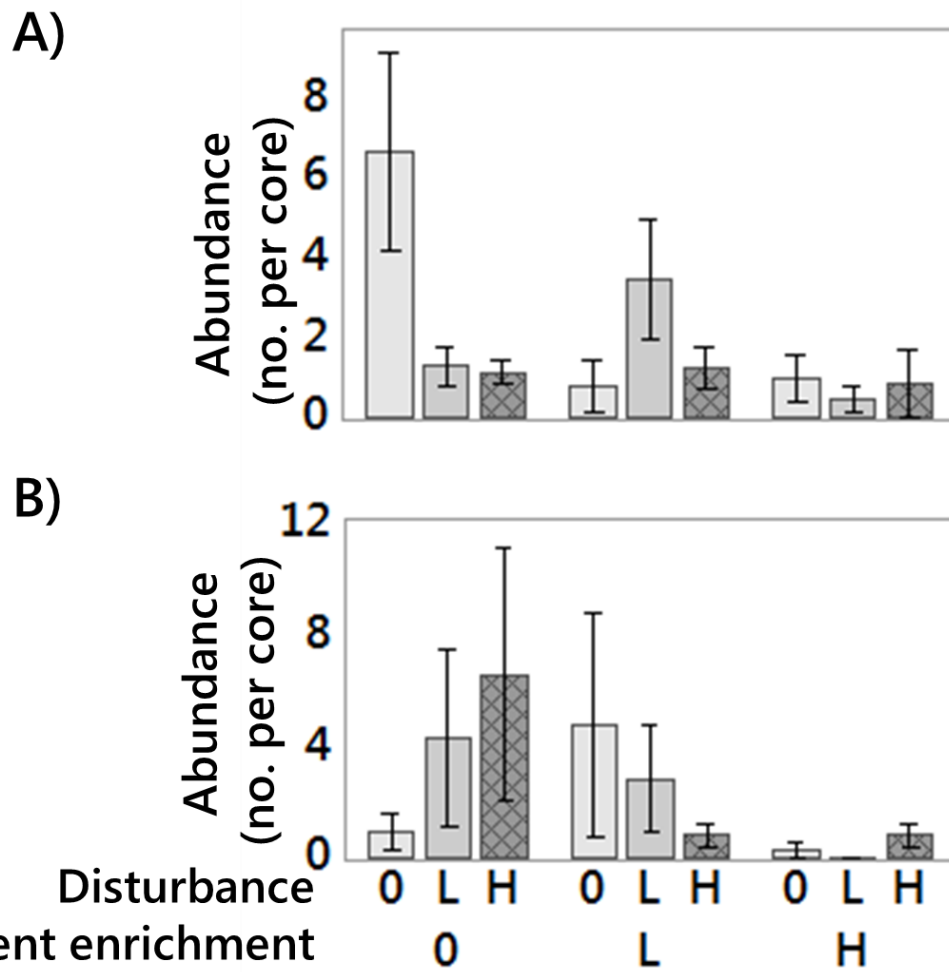
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803 **Figure 4:**

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808 **Figure 5:**

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