1 **Response of benthic assemblages to multiple stressors: comparative effects of nutrient**

2 **enrichment and physical disturbance**

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4 Joseph M. Kenworthy[1,2]*, David M. Paterson[1] and Melanie J. Bishop[2]

5 [1]Sediment Ecology Research Group; Scottish Oceans Institute, University of St Andrews, East

6 Sands, St. Andrews, Fife, KY16 8LB, UK

7 [2]Department of Biological Sciences, Macquarie University, North Ryde NSW, 2109, Australia

8 *Corresponding author: joseph.kenworthy@sb.roscoff.fr

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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 (Lindegarth & 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 Woolooware 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-aground, 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 m^2,

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 mm^2 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 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-weigh 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 [0N], low [LN], high [HN]), physical disturbance (fixed; 3 levels: zero [0D], 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.

Results

Microphytobenthic component

Prior to experimental manipulations there were no differences in organic content or chlorophyll *a*

content of sediments between the two sites, although NDVI was significantly greater at Lane Cove

than Botany Bay (main effect of site PERMANOVA: $p < 0.05$; see supplemental materials). Across

the two sites, there were no pre-existing differences in NDVI among plots that corresponded to

treatment assignments (PERMANOVA $p > 0.05$, see supplemental materials; insufficient plots

were sampled to address this question for organic content and chlorophyll *a*). At none of the

sampling times following application of stressors were there experimental artefacts of the

method of fertiliser burial on any of the three variables, organic content, chlorophyll *a* or NDVI

(PERMANOVA $p > 0.05$; see supplemental material).

Organic content did not respond to the addition of stressors at either site (Table 1).By contrast,

site-specific effects of stressors on the two measures of MPB biomass, chlorophyll *a*

concentration and NDVI were observed (Table 1). Neither variable displayed interactive effects

between the two stressors so additive effects could be interpreted. Chlorophyll *a* concentration

displayed a significant interaction between physical disturbance, site and month (Table 1; Figures

1A,B), whereas there was no effect of physical disturbance on NDVI at either site (Table 1). In

Botany Bay (Figure 1A), although a slight negative impact of high physical disturbance on

chlorophyll *a* was observed in August (month 3), prior to September (month 4) the effects of

disturbance were non-significant (*a posteriori* pairwise comparisons $p > 0.05$; 0D = LD = HD). In

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(perm) =

269 0.048). There were no differences between the undisturbed and low disturbance treatments (t =

270 1.59, p(perm) = 0.113), or the low and high disturbance treatments (t = 0.14, p(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; HD < LD, t = 3.42, p(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(perm) = 0.022) or high disturbance treatment (t = 2.97, p(perm) =

277 0.004), but there was no difference between the high disturbance and undisturbed treatments (t

278 = 0.71, p(perm) = 0.497) (LD > 0D = 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(perm) = 0.032) or high nutrient addition (t = 2.75, p(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(perm) = 0.557) (0N < LN = 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(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(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(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(perm) = 0.031), with no differences

313   between the low and the highly enriched treatments (t = 1.36, p(perm) = 0.077) or between the

314   low enrichment and unenriched treatments (t = 1.04, p(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, 0N < LN = HN; PERMDISP: $F_{(2,58)}$ = 8.94, p(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, 0N > 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 (0N > LN,

323   HN; PERMDISP: $F_{(2,58)}$ = 7.06, p(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(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(perm) = 0.013), with no significant difference between plots receiving high or low physical

348   disturbance(t = 0.40, p(perm) = 0.829) and between plots receiving high or no physical

349   disturbance (t = 1.94, p(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 > 0D; July: t

359 = 21.30, p(MC) = 0.037; September: t = 19.32, p(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(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 > 0D; t = 31.87, p(MC) = 0.017), whereas in

364 September, highly disturbed plots contained fewer individuals than those that were undisturbed

365 (HD < 0D; t = 12.33, p(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(0N >

369 LN; t = 51.61, p(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(MC) = 0.33; high: t = 41.83, p(MC) = 0.017) but did not significantly

373   differ from one another (0N > LN = HN; t = 0.04, p(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(perm) = 0.039; Figure 5A). Among plots subjected to zero physical

380   disturbance, plots receiving low (t = 2.23, p(perm) = 0.034) and high (t = 2.13, p(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 (0N > 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 (0D > HD; t = 2.46, p(perm) = 0.030) whereas

387   there were no differences between plots receiving low and no disturbance (t = 2.38, p(perm) =

388   0.064) or low and high disturbance (t = 0.12, p(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(perm) = 0.045; Figure 5B) whereby abundance

392   decreased with increasing nutrient enrichment (0N > 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 m^2, 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 i*n 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 JMJ, 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   subsequentutilization 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   (*Callianassa 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 *a* concentration and Normalised**
738 **Difference Vegetation Index (NDVI) in Botany Bay and Lane Cove estuaries. Plot (P) was nested**
739 **within NxDxS. 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**

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

Factor	df	*Mediomastus australiensis* Pseudo-*F*	P(perm)	*Prionospio* sp. Pseudo-	P(perm	*Nephtys australiensis* Pseudo-*F*	P(perm)	*Mysella* sp. 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								

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

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

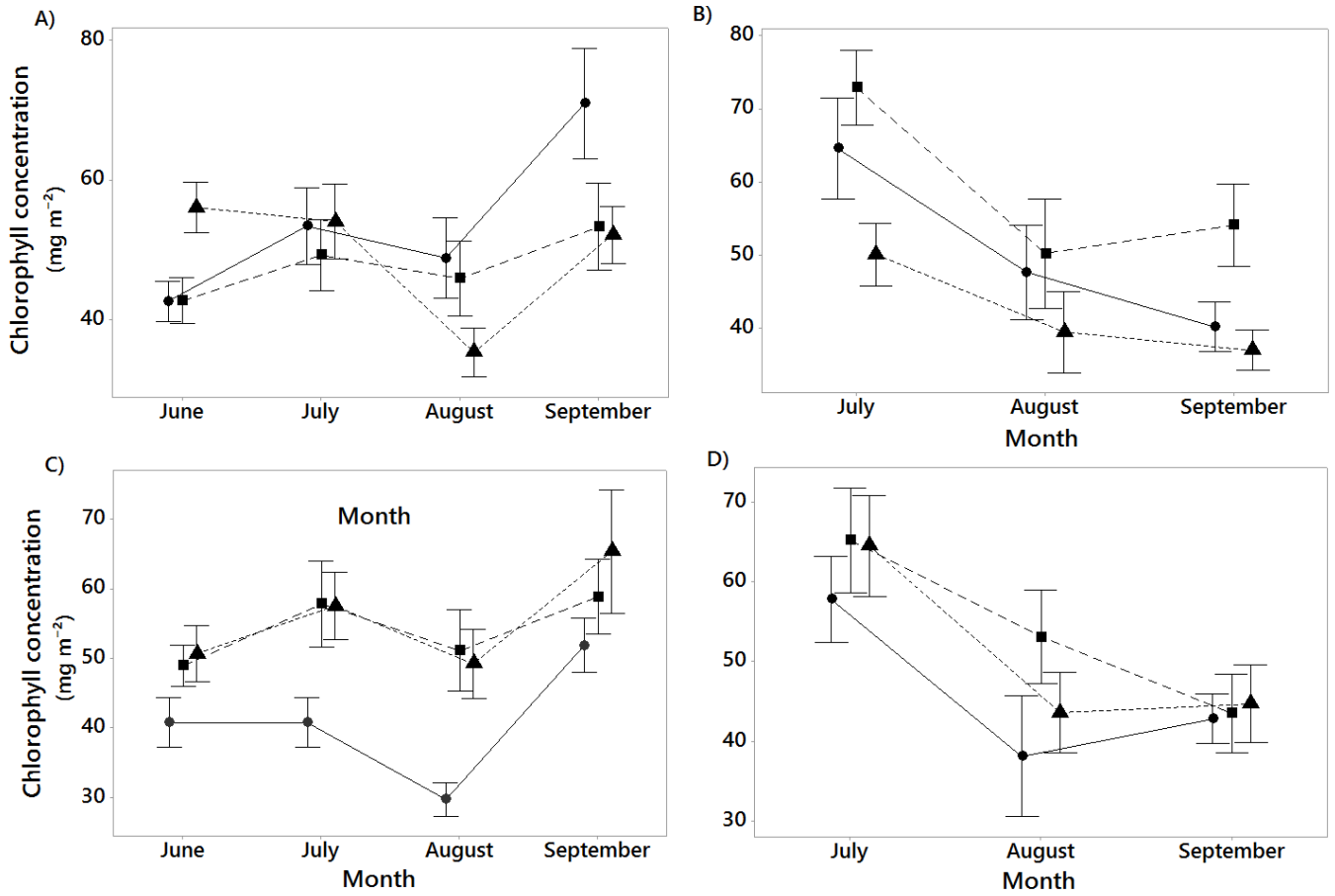779   transformed and matrix based upon Bray Curtis similarity. N = 5-7

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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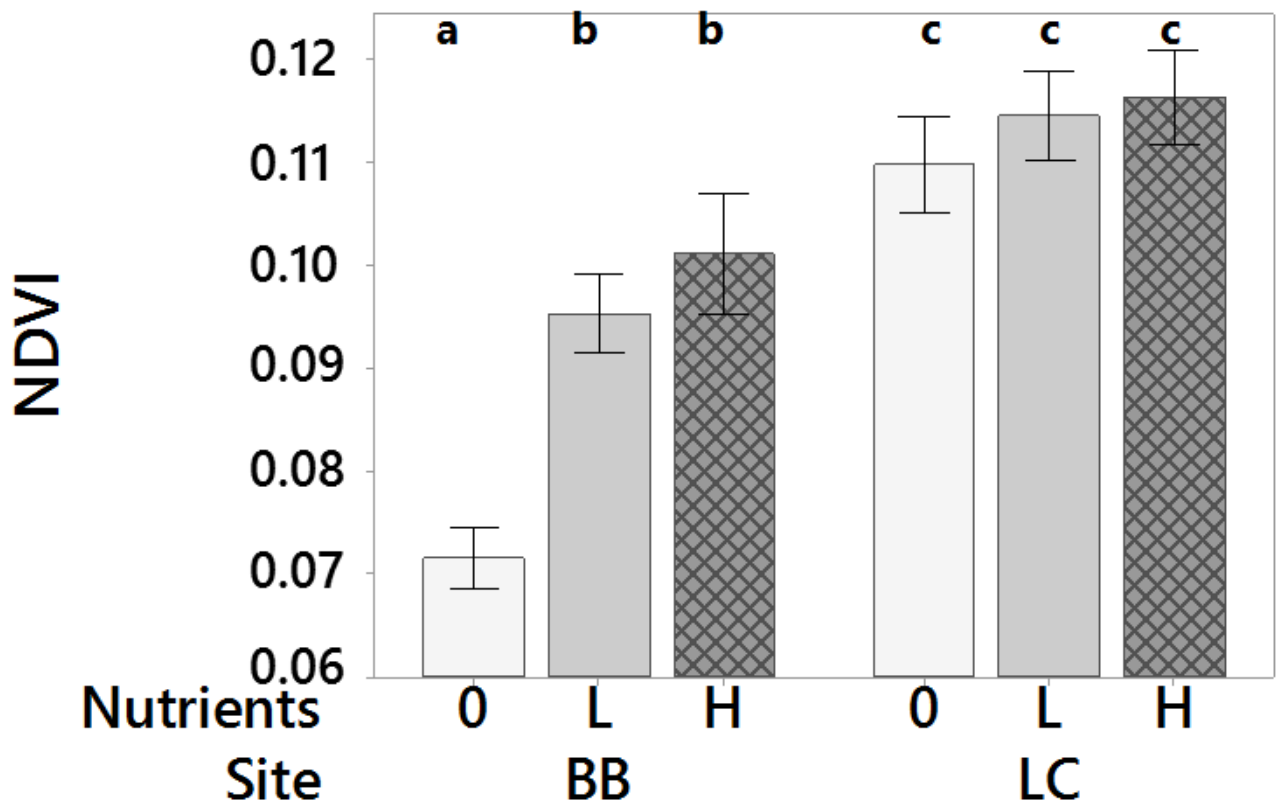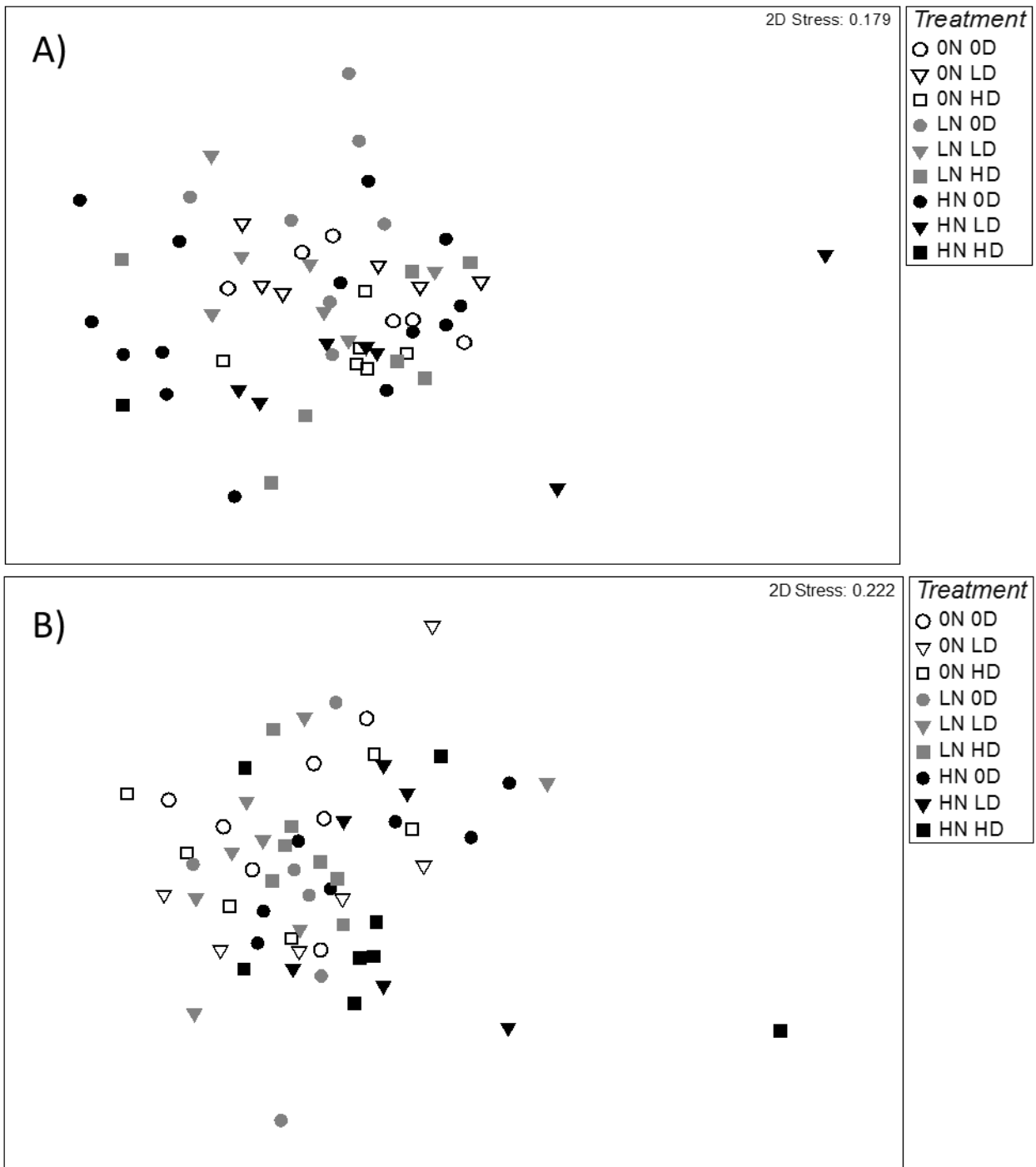
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790 **Figure 1:**
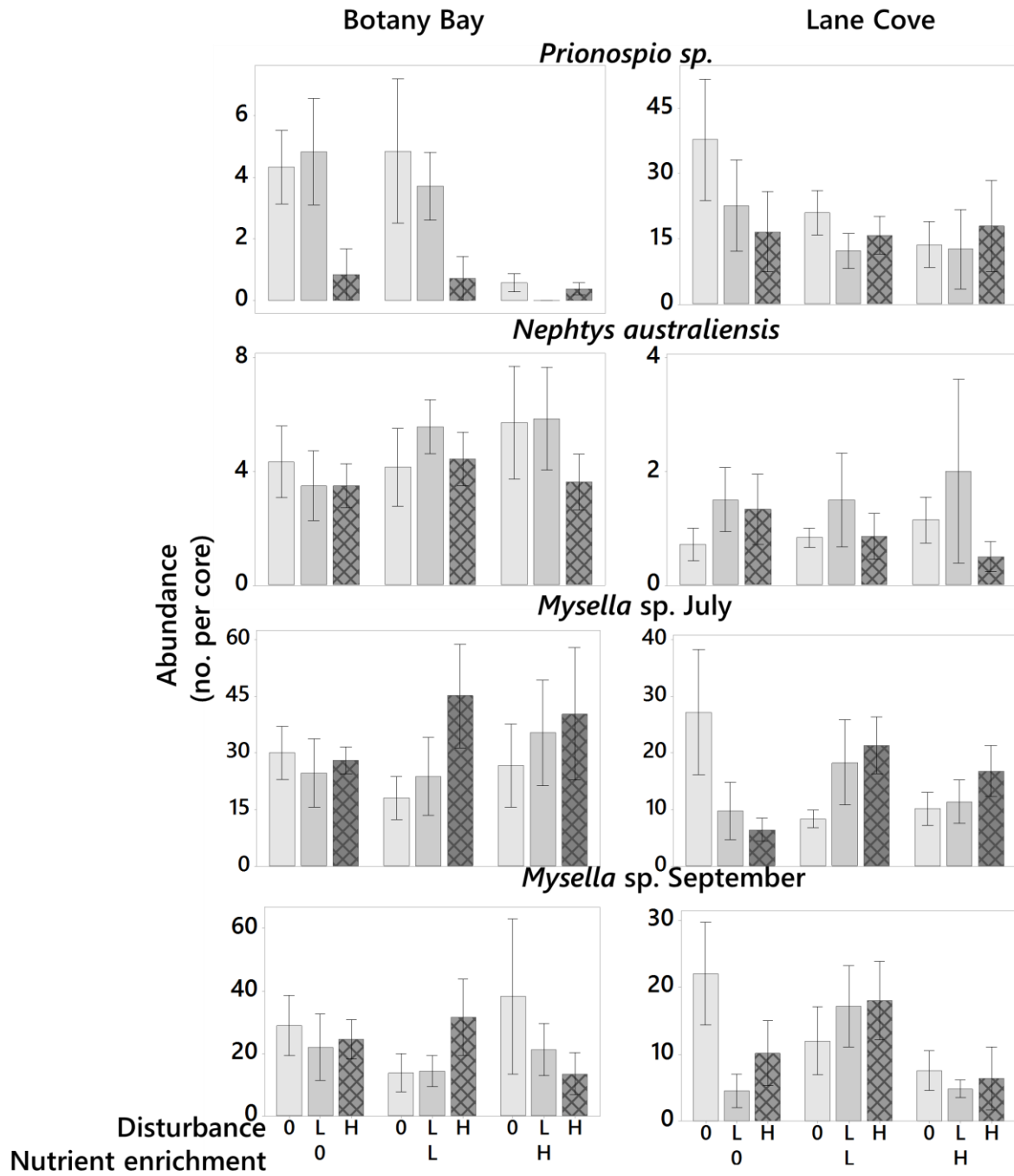
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Figure 2:

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Figure 3:

Figure 4:

A)

B)

Disturbance

Nutrient enrichment

Figure 5: