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Effects of two lubricant oils on marine nematode assemblages in a laboratory microcosm experiment

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26 species could be categorized as “resistant” to mineral oil contamination and intolerant to
27 synthetic lubricant contamination. *Terschellingia longicaudata* increased only in synthetic
28 lubricant treatments (clean” and used) and appeared to be a “synthetic oil- resistant” species.

29

30 *Keywords:* Benthos; Free-living marine nematodes; Bioassay; Hydrocarbons; Lubricant oils;
31 Sediment; Assemblage composition; Lagoons.

32

33

34 **1. Introduction**

35 Many coastal marine ecosystems are well recognized to be contaminated with
36 anthropogenically introduced petroleum hydrocarbons (Kennish, 1992; Louati et al., 2001;
37 Zaghden, 2005). In marine systems, the major sources of hydrocarbon contamination are oil
38 exploration and production, natural seeps, atmospheric input, tanker accidents, industrial
39 discharge, and urban run-off (NAS, 1985 ; AMSA, 1993). In addition to these sources of
40 hydrocarbon contamination, engine lubricants are another source of oil pollution. For
41 example, in Europe, the fate of around 1.1 million tonnes per annum (20% total market) of
42 used lubricating oils is not known (Anonymous, 1997). While not all of this will enter the
43 environment, significant amounts do, particularly ‘total loss’ lubricants which are released
44 through normal use. These products include: chain saw oils, concrete mould-release
45 (‘shuttering’) oils, two-stroke engine oils, chassis greases, railway wheel flange greases,
46 lubricants for ski lifts and railway points greases (Battersby, 2000). Each year approximately
47 600 000 tonnes of total loss lubricants are released into the environment in the European
48 Union (Stempf el al., 1993).

49 The main lubricant oils used in vehicles and heavy machinery are mineral-based
50 lubricating oils and synthetic lubricants. Synthetic lubricants are created from a base fluid

51 (e.g. esters, alpha-olefins, alkylated naphthalenes) in which additives are blended to create
52 and enhance engine performance (Thompson et al., 2007). Additive compounds within
53 lubricants (e.g. di-phenylamines), are known to be highly toxic to certain aquatic organisms
54 (Drzyzga, 2003) and are often more recalcitrant to biodegradation than the base oil (Powell et
55 al., 2005). Therefore, additives are the most limiting components in synthetic lubricant oils
56 (Willing, 2001). Used lubricants also contain metals and additional polycyclic aromatic
57 hydrocarbons that are introduced via engine operation and oil combustion (Thompson et al.,
58 2007). Such used lubricants are known to be highly mutagenic and more toxic to aquatic
59 organisms than their unused counterparts (e.g. Vazquez-Duhalt, 1989).

60 Pollution of the marine environment with oil is potentially one of the most devastating
61 impacts on coastal, estuarine, and inter-tidal regions (Mercurio, 2004). The effects of crude
62 and refined petroleum hydrocarbons on benthic fauna have been extensively studied
63 following real oil spills (Krebs and Burns, 1977; Boucher et al., 1980; Bodin, 1988;
64 McGuinness, 1990; Teal et al., 1992; Agard et al., 1993; Moore and Stevenson, 1997; Smith
65 and Simpson, 1998; Jewett et al., 1999; Michel and Hayes, 1999), in field experiments
66 (Alongi et al., 1983; Decker and Fleeger, 1984; Plante-Cuny et al., 1993; Christie and Berge,
67 1995; Feder and Blanchard, 1998), experimental mesocosms (Farke et al., 1985; Frithsen et
68 al., 1985) and laboratory microcosms (Grassle et al., 1980; Carman et al., 2000, Mahmoudi et
69 al., 2005), but the influence of lubricant oils on natural communities is poorly understood.
70 The few studies concerning engine lubricants and benthic organisms (Powell et al., 2005;
71 Thompson et al., 2007) have been conducted using Antarctic assemblages, and there are no
72 studies reporting the impacts to Mediterranean species or under temperate environmental
73 conditions.

74 Among benthic organisms, nematode assemblages are well suited to microcosm
75 experiments. They have short generation time, high density and continuous reproduction

76 (Suderman and Thistle, 2003). These small animals are also easily maintained and sensitive to
77 many toxicants (Coull and Chandler, 1992; Long, 1992; Guo et al., 2001). Furthermore, the
78 nematode assemblage-level approach to the detection and monitoring of the biological effects
79 of pollutants has been validated in many studies (Warwick et al., 1988; Sundelin and
80 Elmgren, 1991; Austen et al., 1994; Austen and McEvoy, 1997; Austen and Somerfield, 1997;
81 Mahmoudi et al., 2005; Gyedu-Ababio and Baird, 2006).

82 In the present study, we present the results of a microcosm experiment designed to
83 compare the response of Mediterranean meiobenthic nematode assemblages in term of
84 abundance, diversity and species composition to two different lubricants before and after their
85 use in vehicles.

86 **2. Materials and methods**

87 *2.1. Collecting site*

88 Natural meiobenthic assemblages were collected from Ghar El Melh lagoon (Tunisia)
89 on October 2006. Hand-cores of 10 cm² were used to a depth of 15 cm to transfer sediment
90 into a bucket. At the prospected site (37° 09.10' N 10° 13.01' E), depth was 1.30 m and
91 salinity was 36 PSU. Sediments were rich in silts (40%) and clays (21%) with a median grain
92 size of 39 µm. Sediment organic carbon content was 1.5%.

93 On return to the laboratory, sediments were homogenised by gentle hand stirring with
94 a large spatula before they were used for lubricant sediment contamination or microcosms
95 filling.

96 *2.2. Lubricant contamination of sediment*

97 Sediment used for contamination was first alternately frozen and thawed three times to
98 defaunate it (Austen et al., 1994, Gyedu-Ababio and Baird, 2006), and then it was wet sieved
99 to remove the larger particles (>63 µm). Next, quantities of 100 g (wet weight) of this
100 sediment were contaminated with appropriate doses of one of four lubricants in order to

101 obtain final concentrations of 4.29 mg each lubricant kg^{-1} after being mixed with 200 g of
102 natural (uncontaminated) sediment. Treatments comprised of either Mobil 20W-50 (mineral
103 lubricant treatment), the same lubricant (Mobil 20W-50) after use in a vehicle (used mineral
104 lubricant treatment), Mobil 0W-40 (synthetic lubricant treatment) or the same lubricant
105 (Mobil 0W-40) after use in a vehicle (used synthetic lubricant treatment). Used oils (mineral
106 and synthetic) were taken directly out of two cars after they were used for 80 engine hours
107 over 2 months. The doses used (4.29 mg lubricant kg^{-1} of wet sediment) were comparable to
108 those (~150 ml of lubricants to ~35 L of wet sediment) employed by Thompson et al. (2007)
109 and that had revealed significant effect on the benthic infauna. Each lubricant was mixed into
110 the sediment with a food mixer and the amended sediment was left to equilibrate for 2 weeks
111 at 5°C before microcosms were assembled.

112 2.3. *Experimental set-up*

113 Microcosms consisted of 570 ml glass bottles. One control and four treatments with
114 four replicates each were set up. Treated microcosms were gently filled with 300 g of
115 homogenised sediment (200 g of natural sediment and 100 g contaminated sediment) topped
116 up with filtered (1 μm) natural lagoon water at 36 PSU. In control microcosm, the
117 contaminated sediment was replaced by 100 g (wet weight) of the defaunated sediment. Each
118 microcosm bottle was stoppered with a rubber bung with two holes and aerated via an air
119 stone diffuser. The same kind of experiment set-up was successfully used by Schratzberger
120 and Warwick (1998) to examine the effects of intensity and frequency of organic enrichment
121 on two estuarine nematode communities. A control and 4 lubricant treatments were used
122 (Table 1). There were 4 replicate control microcosms which remained uncontaminated and 4
123 treatments with 4 replicates each. The microcosms were kept in the dark, initially at the field
124 temperature on the day of collection but this was gradually raised at a rate of 1-2°C per day
125 until a steady experimental temperature of 20°C was reached. Experimental temperatures

126 were higher than field temperatures to stimulate and optimise conditions for nematode
127 reproduction and growth. All experiments were terminated after 5 weeks and the sediments
128 fixed in 4 % formalin.

129 *2.4. Sample processing*

130 Meiofauna samples were rinsed with a gentle jet of freshwater over a 1 mm sieve to
131 exclude macrofauna, decanted over a 40 μm sieve, centrifuged three times with LudoxTM
132 HS40 (specific density 1.18) and stained with Rose Bengal. Meiofauna was counted and
133 identified at the higher taxon level using a stereomicroscope. Nematodes, comprising
134 approximately two thirds of total meiofauna abundance, were identified to genus or species
135 using the pictorial keys of Platt and Warwick (1983, 1988), and Warwick et al. (1998).

136 *2.5. Data processing*

137 The majority of data analysis followed standard community analysis methods
138 described by Clarke (1993) and Clarke and Warwick (2001) using the PRIMER (Plymouth
139 Routines in Multivariate Ecological Research) software package.

140 Since the main objective of the study was to assess the response of nematofauna to in
141 vitro lubricant contamination of sediment, statistical analyses focused on the faunal data.
142 Univariate indices were computed: total nematode abundance (I), number of species (S),
143 diversity (Shannon-Wiener index H'), species richness (Margalef's d) and evenness (Pielou's
144 J') were calculated for each microcosm to describe nematode assemblage structure. Structure
145 of the data was first tested in order to assess normality (Kolmogorov-Smirnov test) and
146 equality of variance (Bartlett test). One-way analysis of variance (ANOVA or Kruskal-Wallis
147 tests) was used to test for overall differences between these indices between controls and
148 treatments and the Tukey HSD multiple comparisons test was used in pairwise comparisons
149 of treatments and control. In all the above statistical significance testing a significant
150 difference was assumed when $p < 0.05$.

151 Multivariate data analysis was by non-parametric multi-dimensional scaling (MDS)
152 ordination with the Bray-Curtis similarity measure performed on square-root transformed
153 species abundance data to determine whether the nematode assemblages responded to the
154 contaminations by changes in the relative abundance of species. Pairwise analysis of
155 similarities (ANOSIM) was carried out to determine if there were any significant differences
156 between nematode assemblages in different treatments. The nature of the community
157 groupings identified in the MDS ordinations was explored further by applying the similarity
158 percentages programme (SIMPER) to determine the contribution of individual species to the
159 average dissimilarity between treatments and control.

160 Throughout the text, the shorthand codes given in Table 1 are used to identify
161 microcosms.

162 **3. Results**

163 *3.1. Univariate indices*

164 The graphical summary of univariate indices for nematode assemblages from each
165 microcosm and the results of significance testing using the 1-way ANOVA (Fig. 1) illustrate
166 significant differences between nematode assemblages from undisturbed controls and those
167 from lubricant treatments for most univariate measures. Total nematode abundance (I),
168 species richness (d) and number of species (S) decreased significantly in all lubricant
169 contaminated microcosms. However, evenness was not affected in all treated replicates except
170 in used mineral lubricant treatment where it was significantly higher than in the control.
171 Diversity (H') was only altered in mineral lubricant treatments.

172 *3.2. Multivariate indices*

173 In the MDS ordination for all the microcosms (Fig. 2) the effects of both lubricants
174 are obvious: all the treated samples are distinct from the control.

175 ANOSIM results reveal a significant effect of both lubricants, "clean" or used, at the
176 dose tested on nematode assemblages. All treatments were significantly different from the
177 controls and from each other (Table 2).

178 SIMPER results show that the lowest value of average dissimilarity was recorded
179 between control and unused mineral lubricant amended sediment microcosms (Table 3).

180 A total of 17 nematode species were recorded in all the microcosms (Table 4). The
181 control microcosms (C) were mainly dominated by *Daptonema trabeculosum*, *Araeolaimus*
182 *bioculatus*, *Bathylaimus australis*, *Oncholaimus campylocercoides*, *Ascolaimus elongatus*
183 and *Spirinia gerlachi*. The treatment CML was dominated by *Araeolaimus bioculatus*,
184 *Marylynnia stekhoveni*, *Spirinia gerlachi*, *Bathylaimus australis*, *Mesacanthion hirsutum* and
185 *Ascolaimus elongatus*. The microcosms UML were mainly dominated by *Bathylaimus*
186 *australis*, *Spirinia gerlachi*, *Metalinhomoeus numidicus*, *Neochromadora trichophora*,
187 *Ascolaimus elongatus* and *Prochromadorella neapolitana*. The synthetic lubricant amended
188 sediment microcosms (CSL and USL) were mainly dominated by *Terschellingia*
189 *longicaudata* (Table 4).

190 Significant differences between control and treated microcosms mainly resulted from
191 changes in the abundances of the dominant species (Table 5). Elimination of *Daptonema*
192 *trabeculosum* and *Oncholaimus campylocercoides*, increasing numbers of *Marylynnia*
193 *stekhoveni* and *Spirinia gerlachi* were responsible for significant difference between C and
194 CML. Elimination of *Daptonema trabeculosum*, decreasing abundance of *Araeolaimus*
195 *bioculatus* and increasing numbers of *Spirinia gerlachi* and *Bathylaimus australis* were
196 responsible for significant difference between C and UML. Synthetic lubricant contamination
197 led to an elimination of *Daptonema trabeculosum*, an increase in the abundances of
198 *Terschellingia longicaudata* and *Metalinhomoeus numidicus*, and a decrease in the

199 abundance of *Araeolaimus bioculatus* causing significant difference between control and the
200 microcosm CSL and USL.

201 **4. Discussion**

202 Although we have analysed the nematode assemblages after 5 weeks, an exposure
203 period long enough to allow nematode assemblages to adapt to the presence of the
204 contaminants, lubricant oils (mineral and synthetic, "clean" or used) seemed to have severe
205 effects on the Ghar El Melh lagoon nematofauna at the dose tested. Univariate analysis
206 showed that lubricant contamination led to significant effects on free-living nematode
207 assemblages. Total nematode abundance (I), species richness (d) and number of species (S)
208 decreased significantly in the contaminated microcosms. The toxicity and longevity of
209 lubricants in the environment is not known because very few experiments of this type have
210 been done. Haus et al. (2001) linked the chemical and physical properties of mineral oils to
211 their biodegradability and found that as the relative amount of aromatic and polar compounds
212 increased, the biodegradability decreased. However, Eisentraeger et al. (2002) found that
213 neither usage nor the presence of additives such as antioxidants affected the biodegradability
214 of oils. Powell et al. (2005) demonstrated that the Antarctic benthic microbial communities in
215 the "clean" and used lubricant treatments were not significantly different. By chemical
216 analysis of the sediments, these authors found that the same components were lost from both
217 the "clean" and used Mobil 0W-40 and they concluded that in the short-term the effect of
218 used oil on microbes is not appreciably different to that of the "clean", unused product.
219 According to Thompson et al. (2007), lubricants contain many toxic compounds such as
220 diphenylamines, alky naphthalenes, and other minor phenol additives that are not readily
221 biodegradable. Such compounds are very toxic to many aquatic organisms such as
222 bioluminescent bacteria (*Photobacterium phosphoreum*), amphipods (*Gammarus fasciatus*)
223 and daphnia (*Daphnia magna*) (Drzyzga, 2003).

224 Decreased abundance of dominant nematode species in the replicates treated with used
225 mineral lubricant resulted in a significantly higher evenness of nematode assemblages in these
226 microcosms compared to those in the controls. Schratzberger et al. (2003) have also observed
227 an increase of evenness of benthic assemblages in response to oil contamination and had
228 considered this univariate indice as the potentially most sensitive biological response for
229 nematodes and macrofauna communities.

230 Multivariate analysis of the data revealed significant differences between control and
231 all treated microcosms. This indicates that the response of the free-living nematode
232 assemblages to lubricant contamination is dependent on the type of lubricant used (mineral or
233 synthetic, "clean" or used) for the sediment contamination. In the MDS plot for the nematode
234 assemblages, all replicates were separated from each other (Fig. 2) indicating that the
235 dominance relationship among species changed in all treatments compared to the control.

236 Sediment toxicity alone may not be responsible for assemblage differences observed
237 between the contaminated and 'control' sediments. Other factors such as organic enrichment
238 and anoxia may also contribute to impacts to infaunal communities following an oil spill (e.g.
239 Elmgren et al. 1983). Direct correlations between high levels of sediment organics and
240 reduced infaunal abundances have been well established (Pearson and Rosenberg, 1978; Spies
241 et al., 1988, Mahmoudi et al., 2003).

242 Lubricant oils as other contaminants may have more direct impact on associated
243 microbial communities in the sediment than the meiobenthos. This may be reflected in
244 meiobenthic community structure because of alteration of food supply or altered
245 decomposition processes (Austen and McEvoy 1997). Without microbial analysis in parallel
246 with meiobenthos analysis it will not be possible to distinguish which group of organisms are
247 the most sensitive to the pollutant.

248 Conditions in microcosms may have influenced the manifestation of contaminant
249 effects (sensu Carpenter 1996). However, faunal responses in microcosms exposed to
250 lubricant differed significantly from changes that occurred in control microcosms. Thus, the
251 causative mechanisms of population change were strongly influenced by the presence of
252 contaminant. While we acknowledge that microcosms of natural assemblages can never
253 completely mimic natural conditions, we feel that the observed responses of benthic fauna in
254 microcosms provides insight into the complex direct and indirect effects that contaminant can
255 have on natural communities.

256 It is manifested in the present study that the effects of the lubricants used could be
257 easily distinguished from one another as far as the structuring of the nematode assemblage is
258 concerned. The nematode assemblage in the four lubricant treatments was different from the
259 control and from one another ($p < 0.05$). Thus, the effect of "clean" mineral lubricant on the
260 structuring of the nematode assemblage is different from that of used mineral lubricant and
261 from those of synthetic lubricant ("clean" and used). This differential response was not
262 observed for copepod assemblages in antarctic sediments with a "biodegradable" synthetic
263 lubricant TITAN GT1 (0W/20) and a synthetic lubricant (Mobil 0W-40, "clean" and used) as
264 contaminants. In fact, in their study of the contamination effects by a "conventional" and a
265 'biodegradable' lubricant oil on infaunal recruitment to Antarctic sediments, Thompson et al.
266 (2007) analysed meiofauna communities in field experiment bioassays and found that
267 copepods dominated the benthic assemblage in contaminated and "control" sediments and the
268 recruitment of these crustaceans was largely unaffected by the presence of lubricants.
269 Following an oil spill, copepods have been suggested to become opportunistic because of
270 their potential for rapid reproduction which allows them to dominate the initial stages of
271 faunal succession after a disturbance (Grassle et al., 1981). In general, recolonisation of

272 populations of smaller and short-lived organisms, such as copepods, is expected to be more
273 rapid than longer lived species (Driskell et al., 1996).

274 Many other factors can explain the variability in response of different communities to
275 pollutants. Species vary broadly in their response to contaminants (Austen and McEvoy,
276 1997; Millward et al., 2004); the nature of the sediment and seawater characteristics from
277 which the communities are derived affect contaminant bioavailability (Depledge et al. 1994,
278 Langston and Spence 1994); Bioavailability and hence toxicity of pollutants depends on their
279 partitioning between the sediment, pore water and overlying water (Austen and McEvoy,
280 1997) and this can also be dependent on sediment organic carbon content (Di Toro et al.,
281 1991).

282 Nematode assemblages seemed to be more affected by synthetic lubricant treatments
283 (Fig. 2, Table 3). Synthetic oils contain additives (Thompson et al., 2007) that are known to
284 be highly toxic to many aquatic organisms (Drzyzga, 2003) and are often more recalcitrant to
285 biodegradation than the base oil (Powell et al., 2005).

286 *Daptonema trabeculosum* was eliminated in all lubricant treatments (Table 4) and
287 seemed to be an intolerant species to oil contamination. *Spirinia gerlachi* increased in
288 mineral lubricant treatments ("clean" and used) but was eliminated in all synthetic lubricant
289 treatments. This species could be categorized as "resistant" to mineral oil contamination and
290 intolerant to synthetic lubricant contamination. *Terschellingia longicaudata* increased only in
291 synthetic lubricant treatments ("clean" and used) and appeared to be a "synthetic oil-
292 resistant" species.

293

294 **5. Conclusions**

295 The results from our study demonstrate a clear differential response of free-living
296 nematode assemblages to lubricant contamination. After only a short in situ incubation we

297 were able to measure differences in the nematode assemblages due to the effects of different
298 oil treatments. The synthetic lubricant appears to have caused the biggest change in nematode
299 assemblage composition.

300 Whilst the methods used here were useful in showing broad changes to the nematode
301 assemblages, a more detailed examination of the benthic nematode ecology is needed to
302 better understand the impacts oil contamination. Further studies are planned to evaluate the
303 potential impact of lubricants on the most sensitive species and to elucidate the mechanisms
304 of resistance of the most tolerant species living in the Ghar El Melh lagoon.

305

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311

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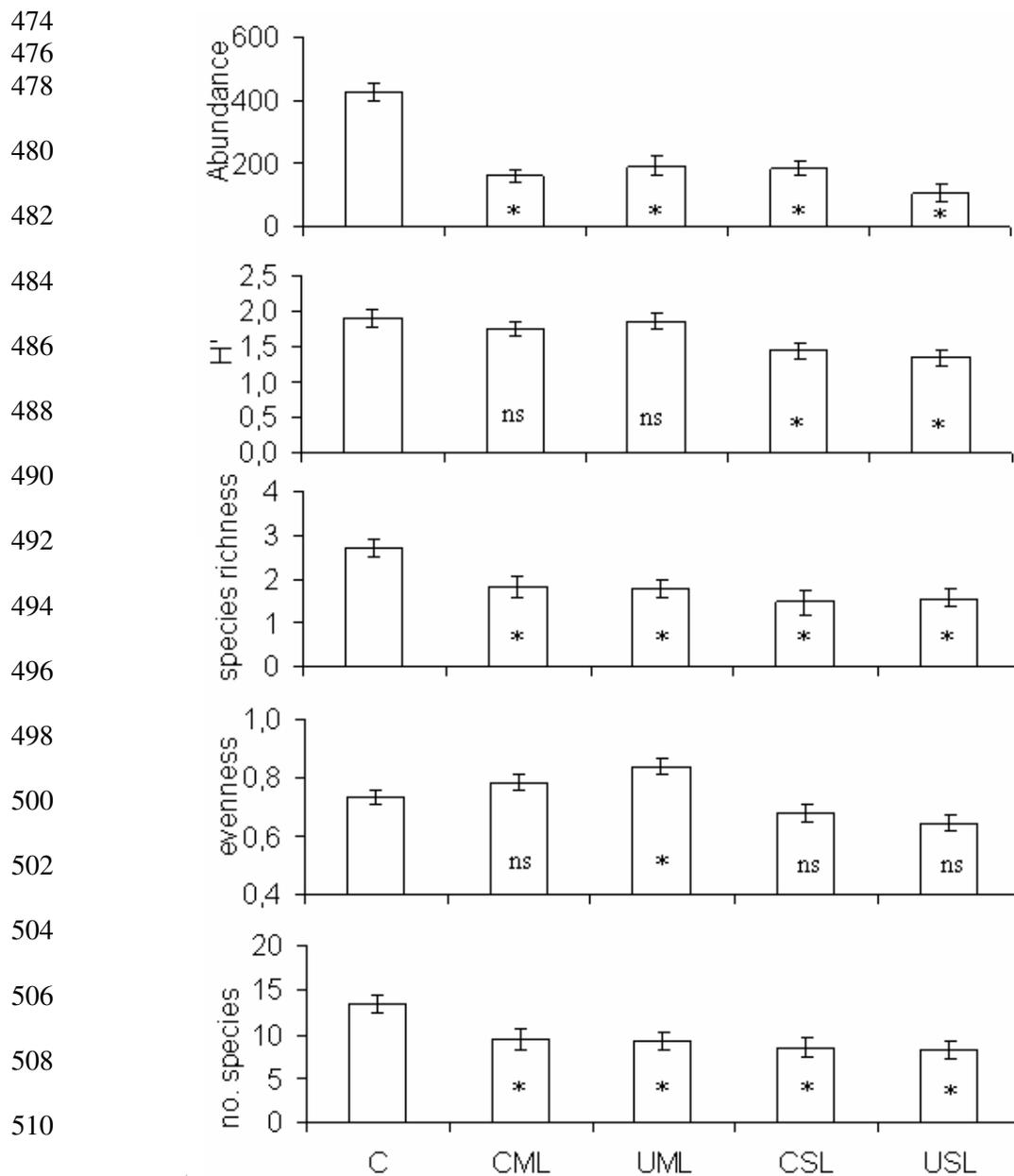
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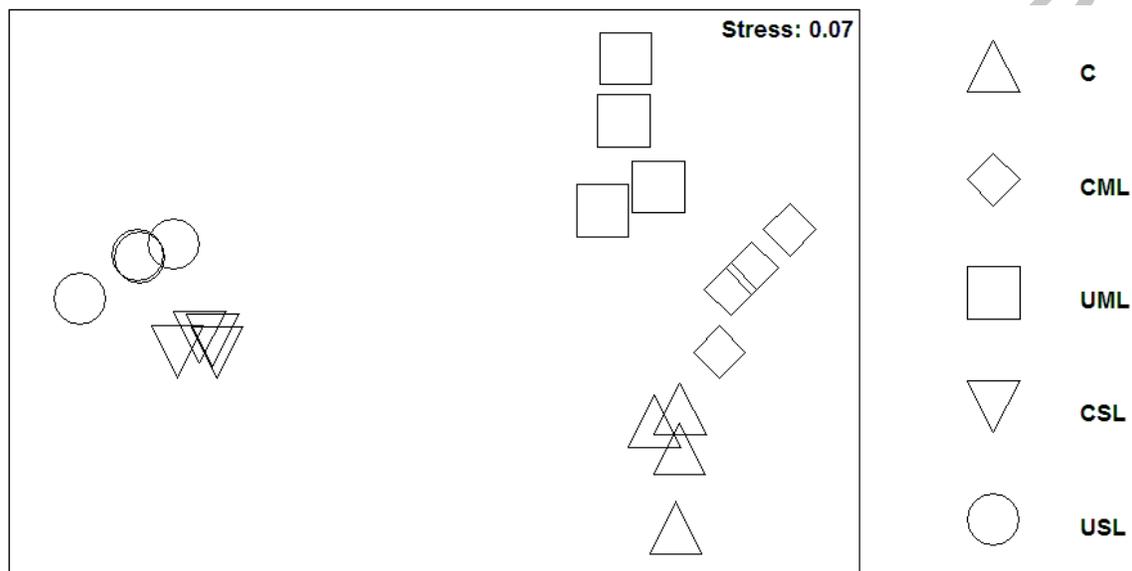
513 Fig. 1. Graphical summary of means and 95% pooled confidence intervals of univariate
 514 indices for nematode assemblages from each microcosm. Abundance = absolute abundance in
 515 individuals per microcosm; H' = Shannon-Wiener index; species richness = Margalef's d ;
 516 evenness = Pielou's J ; no. species = number of species (S); ns: no significant difference of the
 517 univariate index in the contaminated microcosm when compared to the control; *: significant
 518 difference of the univariate index.

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524 Fig. 2. Non-metric MDS ordination of square-root transformed nematode species absolute
525 abundance data from uncontaminated sediment control microcosm (C) and lubricant
526 contaminated sediment treatments [CML: "clean" mineral lubricant treatment, UML: used
527 mineral lubricant treatment, CSL: "clean" synthetic lubricant treatment, USL: used synthetic
528 lubricant treatment].

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552 Table 1. Codes used to identify microcosms.

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| Treatment | Code |
|---|------|
| Uncontaminated control | C |
| Sediment with a final "clean" mineral lubricant concentration of 4.29 mg kg ⁻¹ | CML |
| Sediment with a final used mineral lubricant concentration of 4.29 mg kg ⁻¹ | UML |
| Sediment with a final "clean" synthetic lubricant concentration of 4.29 mg kg ⁻¹ | CSL |
| Sediment with a final used synthetic lubricant concentration of 4.29 mg kg ⁻¹ | USL |

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569 Table 2. Anosim results (R statistic and significance level) of pairwise tests for pairwise
570 differences between treatments and control using square-root transformed nematode
571 abundance data.

572

| Groups | R value | Significance level |
|----------|---------|--------------------|
| C, CML | 0.870* | 0.029 |
| C, UML | 0.913* | 0.024 |
| C, CSL | 0.924* | 0.024 |
| C, USL | 0.942* | 0.024 |
| CML, UML | 0.895* | 0.023 |
| CML, CSL | 0.964* | 0.022 |
| CML, USL | 0.978* | 0.022 |
| UML, CSL | 0.918* | 0.024 |
| UML, USL | 0.936* | 0.024 |
| CSL, USL | 0.816* | 0.029 |

573 * Denotes significant differences when $p < 0.05$.

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586 Table 3. Average dissimilarity between microcosms.

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| Average Dissimilarity (%) | C | CML | UML | CSL | USL |
|------------------------------|-------|-------|-------|-------|-----|
| CML | 63,56 | | | | |
| UML | 68,60 | 65,25 | | | |
| CSL | 82,27 | 81,60 | 74,20 | | |
| USL | 90,87 | 88,84 | 79,02 | 36,49 | |

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601 Table 4. Absolute abundance (individuals per microcosm) and standard deviation of nematode

602 species identified in the control microcosm (C) and lubricants amended sediment treatments

603 [CML, UML, CSL and USL].

604

| | C | CML | UML | CSL | USL |
|-------------------------------------|---------|---------|---------|---------|--------|
| <i>Ascolaimus elongatus</i> | 18 ± 5 | 6 ± 1 | 15 ± 2 | 0 | 2 ± 1 |
| <i>Araeolaimus bioculatus</i> | 119 ± 6 | 53 ± 13 | 9 ± 5 | 9 ± 1 | 6 ± 1 |
| <i>Bathylaimus australis</i> | 44 ± 6 | 13 ± 11 | 55 ± 14 | 18 ± 2 | 3 ± 1 |
| <i>Calomicrolaimus honestus</i> | 4 ± 4 | 2 ± 2 | 0 | 0 | 3 ± 2 |
| <i>Daptonema trabeculosum</i> | 144 ± 7 | 0 | 0 | 0 | 0 |
| <i>Hypodontolaimus colesi</i> | 4 ± 3 | 0 | 4 ± 5 | 0 | 0 |
| <i>Marylynnia stekhoveni</i> | 8 ± 1 | 42 ± 6 | 4 ± 4 | 6 ± 3 | 2 ± 2 |
| <i>Metalinhomoeus numidicus</i> | 2 ± 2 | 0 | 19 ± 2 | 14 ± 1 | 23 ± 2 |
| <i>Mesacanthion hirsutum</i> | 15 ± 9 | 7 ± 3 | 2 ± 2 | 0 | 0 |
| <i>Monhystera parva</i> | 8 ± 4 | 0 | 0 | 13 ± 2 | 0 |
| <i>Neochromadora trichophora</i> | 4 ± 3 | 2 ± 1 | 16 ± 4 | 0 | 0 |
| <i>Odontophora wieseri</i> | 1 ± 2 | 8 ± 4 | 0 | 0 | 0 |
| <i>Oncholaimus campylocercoides</i> | 35 ± 4 | 0 | 0 | 8 ± 4 | 6 ± 3 |
| <i>Paracomesoma dubium</i> | 2 ± 2 | 0 | 5 ± 6 | 1 ± 1 | 2 ± 2 |
| <i>Prochromadorella neapolitana</i> | 4 ± 4 | 7 ± 3 | 12 ± 8 | 8 ± 3 | 0 |
| <i>Spirinia gerlachi</i> | 16 ± 6 | 21 ± 7 | 50 ± 16 | 0 | 0 |
| <i>Terschellingia longicaudata</i> | 1 ± 2 | 1 ± 3 | 0 | 108 ± 6 | 62 ± 4 |

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607 Table 5. Species responsible for differences between control and treated microcosms based
 608 on similarity percentages (SIMPER) analysis of square-root transformed data. (+): more
 609 abundant; (-): less abundant; (elim): elimination. Species accounting for ~ 70% of overall
 610 dissimilarity between treatment groups are ranked in order of importance of their contribution
 611 to this dissimilarity.

612

| CML | UML | CSL | USL |
|---|---|--|--|
| <i>Daptonema</i> | <i>Daptonema</i> | <i>Terschellingia</i> | <i>Terschellingia</i> |
| <i>trabeculosum</i> (elim) | <i>trabeculosum</i> (elim) | <i>longicaudata</i> (+) | <i>longicaudata</i> (+) |
| <i>Marylynnia stekhoveni</i> (+) | <i>Araeolaimus</i> <i>bioculatus</i> (-) | <i>Daptonema</i> <i>trabeculosum</i> (elim) | <i>Daptonema</i> <i>trabeculosum</i> (elim) |
| <i>Spirinia gerlachi</i> (+) | <i>Spirinia gerlachi</i> (+) | <i>Araeolaimus</i> <i>bioculatus</i> (-) | <i>Araeolaimus</i> <i>bioculatus</i> (-) |
| <i>Oncholaimus</i> <i>campylocercoides</i> (elim) | <i>Bathylaimus</i> <i>australis</i> (+) | <i>Metalinhomoeus</i> <i>numidicus</i> (+) | <i>Metalinhomoeus</i> <i>numidicus</i> (+) |

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