

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

BIOLOGICAL SCIENCES

Do all roads lead to Rome? Exploring community trajectories in response to anthropogenic salinisation and dilution of rivers

Journal:	<i>Philosophical Transactions B</i>
Manuscript ID	RSTB-2018-0009.R1
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	<p>Gutierrez-Cánovas, Cayetano; Universitat de Barcelona, Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio)</p> <p>Sánchez-Fernández, David; Instituto de Ciencias Ambientales (ICAM), Universidad de Castilla-La Mancha; Universidad de Murcia, Departamento de Ecología e Hidrología</p> <p>Cañedo-Argüelles, Miguel; Universitat de Barcelona, Grup de Recerca Freshwater Ecology, Hydrology and Management (FEHM-Lab), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de l'Aigua (IdRA)</p> <p>Millán, Andrés; Universidad de Murcia, Ecología e Hidrología</p> <p>Velasco, Josefa; Universidad de Murcia, Ecología e Hidrología</p> <p>Acosta, Raúl; Universitat de Barcelona, Grup de Recerca Freshwater Ecology and Management (FEM), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de l'Aigua (IdRA)</p> <p>Fortuño, Pau; Universitat de Barcelona, Grup de Recerca Freshwater Ecology and Management (FEM), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia</p> <p>Otero, Neus; Universitat de Barcelona, Grup de recerca MAiMA, Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra)</p> <p>Soler, Albert; Universitat de Barcelona, Grup de recerca MAiMA, Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra)</p> <p>Bonada, Núria; Universitat de Barcelona, , Grup de Recerca Freshwater Ecology and Management (FEM), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio)</p>
Issue Code (this should have already been entered but please contact the Editorial Office if it is not present):	SALT
Subject:	Ecology < BIOLOGY

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Keywords:	aquatic insects, community assembly, functional traits, global change, Mediterranean rivers, osmotic stress



Do all roads lead to Rome? Exploring community trajectories in response to anthropogenic salinisation and dilution of rivers

Cayetano Gutiérrez-Cánovas^{1*}, David Sánchez-Fernández², Miguel Cañedo-Argüelles³, Andrés Millán⁴, Josefa Velasco⁵, Raúl Acosta⁶, Pau Fortuño⁷, Neus Otero⁸, Albert Soler⁹ & Núria Bonada¹⁰

1. tano.gutierrez@ub.edu, Grup de Recerca Freshwater Ecology, Hydrology and Management (FEHM-Lab), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), 08028 Barcelona, Catalonia, Spain, ORCID: 0000-0002-6785-4049

2. David.SFernandez@uclm.es, Instituto de Ciencias Ambientales (ICAM), Universidad de Castilla-La Mancha, Toledo, Spain; Departamento de Ecología e Hidrología, Universidad de Murcia, 30100 Murcia, Spain, ORCID: 0000-0003-1766-0761

3. mcanedo-arguelles@ub.edu, Grup de Recerca Freshwater Ecology, Hydrology and Management (FEHM-Lab), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de l'Aigua (IdRA), Universitat de Barcelona (UB), 08028 Barcelona, Catalonia, Spain, ORCID: 0000-0003-3864-7451

4. acmillan@um.es, Departamento de Ecología e Hidrología, Universidad de Murcia, 30100 Murcia, Spain, ORCID: 0000-0003-0036-363X

5. jvelasco@um.es, Departamento de Ecología e Hidrología, Universidad de Murcia, 30100 Murcia, Spain, ORCID: 000-002-7457-2017

6. racosta@ub.edu, Grup de Recerca Freshwater Ecology, Hydrology and Management (FEHM-Lab), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de l'Aigua (IdRA), Universitat de Barcelona (UB), 08028 Barcelona, Catalonia, Spain

7. pfortuno@ub.edu, Grup de Recerca Freshwater Ecology, Hydrology and Management (FEHM-Lab), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona (UB), 08028 Barcelona, Catalonia, Spain, ORCID: 0000-0002-2198-3486

8. notero@ub.edu, Grup de recerca MAiMA, Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra, Universitat de Barcelona (UB), Barcelona, Catalonia, Spain, ORCID: 0000-0001-6553-7958

9. albertsolergil@ub.edu, Grup de recerca MAiMA, Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra, Universitat de Barcelona (UB), Barcelona, Catalonia, Spain, ORCID: 0000-0003-3140-182X

10. bonada@ub.edu, Grup de Recerca Freshwater Ecology, Hydrology and Management (FEHM-Lab), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), 08028 Barcelona, Catalonia, Spain, ORCID: 0000-0002-2983-3335

Keywords: aquatic insects; community assembly; functional traits; global change; Mediterranean rivers; osmotic stress

SUMMARY

Abiotic stress shapes how communities assemble and support ecological functions. However, it remains unclear whether artificially increasing or decreasing stress levels would lead to communities assembling predictably along a single axis of variation or along multiple context-dependent trajectories of change. In response to stress intensity alterations, we hypothesise that a single trajectory of change occurs when trait-based assembly prevails, while multiple trajectories of change arise when dispersal-related processes modify colonisation and trait-filtering dynamics. Here, we test these hypotheses using aquatic macroinvertebrates from rivers exposed to gradients of natural salinity and artificially diluted or salinised ion contents. Our results showed that trait-filtering was important in driving community assembly in natural and diluted rivers, while dispersal-related processes seem to play a relevant role in response to salinisation. Salinised rivers showed novel communities with different trait composition, while natural and diluted communities exhibited similar taxonomic and trait compositional patterns along the conductivity gradient. Our findings suggest that the artificial modification of chemical stressors can result in different biological communities depending on the direction of the change (salinisation or dilution), with trait-filtering, and organism dispersal and colonisation dynamics having differential roles in community assembly. The approach presented here provides both empirical and conceptual insights that can help anticipating the ecological effects of global change, especially for those stressors with both natural and anthropogenic origins.

INTRODUCTION

Abiotic stress, defined as the harmful environmental conditions exceeding the normal range experienced by organisms, is a key force determining how communities assemble and support ecosystem functions and services [1–3]. Chronic abiotic stress may arise naturally or be caused by ongoing global change [4,5], as occur with fire disturbance frequency, flow intermittence or water salinity. However, it remains unclear whether anthropogenically modified stress levels would result in communities assembling along a single axis based on organism's stress tolerance or along multiple

*Author for correspondence (tano.gutierrez@ub.edu).

1
2
3
4 32 trajectories of change depending on differential dispersal and colonisation abilities and population
5 33 dynamics [6,7].
6
7 34

8
9 35 Empirical and theoretical evidence suggests that abiotic filtering and interspecific competition are the
10 36 most important mechanisms driving the predictable assembly of communities in response to
11 37 environmental change [2,7] (trait-filtering processes). At a given stress level, abiotic filtering would
12 38 select the organisms showing the most suitable response traits (i.e. physiological, morphological and
13 39 life-history adaptations conferring resistance to environmental stress) within the regional species pool.
14 40 Among them, the proportion of organisms able to cope with stress depends on each lineage's
15 41 evolutionary context [8,9] and the stressor historical persistence within the region [10]. Besides having
16 42 a suitable set of traits, organisms showing a better ability to compete for resources or exploit
17 43 underutilised alternatives would show greater abundances [11]. However, other factors, such as
18 44 population dynamics and organism's dispersal and colonisation capacities, can influence community
19 45 assembly leading to multiple trajectories of change along stress gradients [6,12] (dispersal-related
20 46 processes). For example, organisms may have optimum environmental conditions in habitats that are
21 47 difficult to reach because of their low dispersal ability or landscape barriers [6]. In contrast, organisms
22 48 may establish in a newly disturbed habitat despite suboptimum conditions, when they are good
23 49 dispersers and have multiple population sources well connected through the landscape [13]. When
24 50 these first colonisers have appropriate resources to thrive, they may act as a barrier impeding further
25 51 colonisation (founder effect), shaping historically contingent communities [7,12].
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 52
41 53 Rivers offer a promising avenue to identify the mechanisms driving community responses to stress, as
42 54 they exhibit marked natural and anthropogenic environmental gradients [14]. For example, as a result
43 55 of global change, the salt concentration of most rivers is changing worldwide [15,16]. Many freshwater
44 56 rivers are being salinised by anthropogenic salt inputs (e.g. salt mining, de-icing salt), whereas many
45 57 other naturally saline rivers are being diluted by human activities that reduce their salt concentration
46 58 (e.g. agricultural drainages). Furthermore, climate change may intensify both processes as a result of
47 59 altered rainfall and evapotranspiration regimes (either increasing or decreasing effective precipitation)
48 60 or because of sea level rise or intrusion [17]. It is already well known that aquatic organisms show a
49 61 strong specificity along the salinity gradient [14] and that both impacts are driving strong detrimental
50 62 changes in the diversity and community composition of rivers [13,16] and their ecosystem functioning
51 63 [18,19]. Studies performed so far have investigated biological responses along a limited span of the
52
53
54
55
56
57
58
59
60

64 salinity gradient, considering only salinisation or dilution, but not both processes. Therefore, it remains
65 unclear if artificially modifying river salinity could yield similar or novel communities respect to
66 naturally assembled communities.

67
68 Here, we test three competing hypotheses of community assembly (Fig. 1) by exploring if
69 macroinvertebrate diversity and composition of natural, diluted and salinised rivers follow a single or
70 multiple trajectories of change along a wide salinity gradient (electrical conductivity: 0.3 to >100 mS
71 cm⁻¹). Firstly (H₁), we hypothesise that community assembly in response to dilution and salinisation
72 will be explained by abiotic filtering and competition. Taxa showing the most suited traits to cope with
73 a given salinity will be selected (trait-based assembly). As a result, communities with altered salt
74 concentrations will resemble natural communities at the same salinity. Secondly (H₂), dispersal-related
75 processes will influence community assembly in response to dilution and salinisation. Opportunistic
76 colonisation by good dispersers followed by trait-based abiotic and biotic filtering will result in novel
77 communities and multiple trajectories of change (dispersal-based assembly). Thirdly (H₃), trait-based
78 assembly and dispersal-related processes have differential importance depending on the direction of
79 change along the stress gradient (directional-dependent assembly).

81 **METHODS**

83 *Identifying diluted and salinised rivers*

84
85 A total of 107 sampling sites were surveyed in rivers across the Eastern part of the Iberian Peninsula
86 (Fig S1). Each site was sampled up to four times (170 samples), mostly during spring and summer
87 (period 2000-2018). Sampling sites included lowland and mid-mountain rivers (<1,200 m a.s.l.) of
88 Mediterranean climate, which exhibit a substantial variation in mean annual precipitation (250 to 900
89 mm) and different combinations of natural, semi-natural and anthropogenic land-uses.

90
91 These rivers have a varying concentration of salts, which depends on natural and anthropogenic factors.
92 Natural saline rivers were found in areas with increasing aridity and evaporitic outcrops [16]. However,
93 some naturally freshwater rivers were salinised due to potash and sodium chlorine mining activities
94 [20], whereas some of the naturally saline rivers were diluted mainly due to irrigation agriculture [19].
95 We performed dissolved sulphate isotopic analyses ($\delta^{34}\text{S}_{\text{SO}_4}$ and $\delta^{18}\text{O}_{\text{SO}_4}$), and compiled bibliographic

1
2
3
4 96 data [20–22] to distinguish if salinity was due to natural water-rock interaction or to the influence of
5
6 97 potash mine tailings (Supplementary Information S1 and Table S1); other important sources of sulphate
7
8 98 in the basin (fertilizers, urban effluents) [20] were also considered. On the other hand, to establish
9
10 99 which naturally saline rivers (flowing over evaporitic outcrops) can be anthropogenically diluted, we
11
12 100 selected those showing $\geq 20\%$ of irrigating agriculture at the entire catchment scale or $\geq 50\%$ at the
13
14 101 local scale. Local land-use estimation was carried out through the intersection between the entire
15
16 102 catchment and a 1-km-radius buffer centred on the sampling point. Anthropogenically reduced levels
17
18 103 of salinity have been previously reported in rivers exposed to intensive irrigation agriculture due to
19
20 104 freshwater inputs from agriculture drainages and from irrigation channels leaks [16,19]. When
21
22 105 classifying rivers as anthropogenically diluted, we focused on rivers of potentially high mineralisation
23
24 106 (historically exceeding 5 mS cm^{-1}), excluding freshwater rivers draining calcareous-dominated
25
26 107 catchments without evaporitic outcrops.

26 109 *Environmental data*

27
28 110
29
30 111 Electrical conductivity and pH were determined using a multiparametric probe. Water samples were
31
32 112 filtered through glass fibre filters (GF/F) (Whatman, Maidstone, UK), transported to the laboratory on
33
34 113 ice, and finally frozen for subsequent analysis. Major anions (chloride, sulphate, nitrite and nitrate)
35
36 114 were analysed by HP liquid chromatograph, alkalinity was measured by titration, and ammonium and
37
38 115 soluble reactive phosphorous concentrations were estimated using standard colorimetric methods [23].
39
40 116 As water chemistry, and especially salinity [24], may vary over time, we compiled water chemistry
41
42 117 data covering previous months or years from own data, water agencies and published papers [25,26]
43
44 118 to estimate a time-integrated value for each variable and site, when possible (Table S2).

45 119
46 120 We also delineated basins and compiled information to characterise other key environmental features
47
48 121 at site and catchment scales that can influence river biological composition such as site elevation,
49
50 122 latitude, longitude, basin area, basin mean annual precipitation and land-use. Major land-uses (natural,
51
52 123 non-irrigated agriculture, irrigated agriculture and urban zones, Corine Land Cover 2012) were
53
54 124 characterised for the whole river basin and just for 1-km-basin upstream sampling point. To control for
55
56 125 other anthropogenic impacts beyond salinisation or dilution, we estimated the number of pressures
57
58 126 affecting each river through the Mediterranean Reference Criteria (MRC) [27], which ranges from zero

1
2
3
4 127 (reference undisturbed condition) to 20 pressures (extremely disturbed). The number of pressures
5 128 affecting our sites ranged from zero to 12 pressures.

6
7 129

8
9 130 ***Taxonomic and functional metrics***

10
11 131

12 132 To assess biological responses along natural and anthropogenic gradients, we estimated several
13 133 taxonomic and functional metrics.

14
15
16 134

17 135 All macroinvertebrate samples were collected following a multi-habitat semiquantitative kick-sample.
18 136 Samples were preserved in 70%-96% ethanol and specimens were identified and counted to species
19 137 for Coleoptera, Hemiptera and Trichoptera, genus for most taxa or family in some few cases (e.g.
20 138 Diptera). Abundances were aggregated into classes to avoid distortions in statistical analysis due to
21 139 large abundance differences (0: 0; 1: 1-3; 2: 4-10; 3: 11-100; 4: >100 individuals). To estimate
22 140 taxonomic-based metrics, some genera and subfamilies belonging to the same family were aggregated
23 141 into families when part of them were identified at family-level to avoid distortions and double-counting
24 142 (taxonomic *sites x taxa* matrix with 166 taxa). For trait-based metrics, we used a *sites x taxa* matrix
25 143 with the finest taxonomic units, resulting in 220 taxa. For community composition analysis, we used a
26 144 matrix including species, genus and family levels (261 taxa).

27
28 145

29 146 To characterise macroinvertebrate traits, we compiled a database including four fuzzy-coding response
30 147 traits for the 220 taxa [28–30]: lifespan, number of generations per year, reproduction and respiration
31 148 modes (Table S3). These response traits, that include 18 trait categories, are potentially related to
32 149 resilience and resistance to osmotic stress [31–33]: short life-span is linked to reduced time and energy
33 150 to reach adult stage and reproduce earlier, and enhanced microevolutionary processes; multivoltinism
34 151 provides higher capacity to increase population and recover after disturbances; ovoviparity and
35 152 terrestrial reproduction give more independence from the aquatic environmental conditions; and aerial
36 153 respiration (i.e. spiracle and hydrostatic vesicle modes) are mechanisms which allow coping with low
37 154 dissolved oxygen concentrations, which may occur at high salinities. For each invertebrate genus, a
38 155 degree of affinity (i.e. ranging from 0 up to a total of 3, 5, 7) was assigned to each trait category,
39 156 according to the frequency of occurrence within the genus. Prior to analysis, fuzzy coded data were
40 157 converted into percentages of affinity for each trait.

41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57 158

1
2
3
4 159 Firstly, as taxonomic-based metrics, we estimated taxonomic, the proportion of Ephemeroptera,
5 160 Plecoptera and Trichoptera genera (EPT) taxa, Odonata, Coleoptera and Hemiptera (OCH) taxa and
6 161 non-native taxa for each sample, using the *sites x taxa* matrix with 166 taxa. Secondly, as functional
7 162 metrics, we calculated response trait diversity and response trait richness based on a functional space
8 163 built on a Gower's pair-wise functional dissimilarity matrix that included 220 taxa (fuzzy-coding-
9 164 adapted Gower's index [34], see Table S3 for further info on functional metric estimation). Pairwise
10 165 dissimilarity matrix was based on a *taxon x trait* category matrix, including the 18 trait categories. We
11 166 selected a 7D functional space because it represented well the original Gower dissimilarity matrix
12 167 (mean SD error=0.017) [35], and functional spaces with more dimensions did not substantially
13 168 improved such representation (10D, mean squared deviance error=0.013) (Table S3). Response trait
14 169 diversity, which represents the functional dispersion of taxa with respect to the average response trait
15 170 values, was estimated as the mean distance of each taxon to the mean abundance-weighted centroid in
16 171 the 7D functional response space [36]. Response trait richness, which represents the response trait
17 172 range variation for the community [37], was estimated as the ratio (from 0 to 1) between the 3D
18 173 functional space volume of the convex hulls enclosing all the taxa occurring at each sample and that
19 174 encompassing the 233 taxa studied. Despite the 7D functional space better represented the original trait
20 175 matrix [35], we used a 3D functional space to estimate response trait richness to avoid multiple non-
21 176 defined values at sites with low diversity (see methodological details in Table S3).

22 177
23 178 Furthermore, we estimated the community weighted means for four trait categories and combinations
24 179 of them that could be positively related to higher resilience and resistance to osmotic stress: the
25 180 proportion of organisms with short life-span, multivoltinism, ovoviparity and terrestrial reproduction
26 181 and aerial respiration (combining spiracle and hydrostatic vesicle modes).

27 182 28 183 **Data analysis**

29 184
30 185 To assess if biological responses to salinity differed between communities exposed to natural and
31 186 altered salt concentrations, we used Boosted Regression Trees (BRT), Linear Mixed-effect Models
32 187 LMM, *lme4* R package [38] and multivariate generalised linear models (*manyglm* function, *mvabund*
33 188 R package) [39]. Before analyses, we applied a log-transformation to family richness and response trait
34 189 richness and a square-root transformation to the proportion of organisms showing aerial respiration.
35 190 Besides, logit-, log- or square-root-transformations were applied to quantitative environmental

1
2
3
4 191 predictors to reduce their distribution skewness and improve linearity, when necessary. In all models,
5 192 we used mean conductivity for each site to have a time-integrated measure of osmotic stress.

6 193
7
8
9 194 To summarise the main axes of biological variation, we also performed a non-metric MultiDimensional
10 195 Scaling (MDS) based on a Bray-Curtis dissimilarity matrix, built upon the matrix of macroinvertebrate
11 196 family, genus and species abundances excluding rare taxa (i.e. taxa occurring in less than 5 sampling
12 197 sites). The first MDS axis represented well the original Bray-Curtis similarities ($r=0.88$).

13 198
14
15
16 199 We used BRT to rank predictor importance for taxonomic richness, response trait diversity and
17 200 response trait richness, community mean trait categories and MDS axis 1. BRT is a non-parametric
18 201 regression technique able to handle heterogeneous predictors, non-linear relationships and missing
19 202 values. As predictors, we included water chemistry (conductivity, pH, ion contents, and nutrients),
20 203 catchment descriptors (basin area, climate, elevation, and geographic coordinates), land-uses (irrigation
21 204 agriculture and urban intensity) and multiple anthropogenic impacts (alteration salinity class, MRC).
22 205 The results of these analyses showed that mean conductivity was the most important predictor across
23 206 response variables (median rank=2) (Table S4). In addition, mean conductivity was highly correlated
24 207 with sulphate ($r=0.91$), alkalinity : salinity ratio ($r=-0.88$), chloride ($r=0.90$) and chloride : salinity ratio
25 208 ($r=0.75$), suggesting that ionic composition is well represented by mean conductivity.

26 209
27
28
29 210 Using LMM we modelled salinity effects on taxonomic richness, response trait diversity and response
30 211 trait richness, community mean trait categories and MDS axis 1. Each model included conductivity, a
31 212 factor indicating alteration class (levels: natural control, diluted and salinised), and the pairwise
32 213 interaction between conductivity and alteration class. To control for other environmental covariates
33 214 and assisted by BRT results, we also included sampling site elevation, latitude, catchment area, mean
34 215 catchment annual precipitation and percentage of irrigation agriculture and urban land-uses as fixed
35 216 factors. LMM are ideal statistical techniques to cope with residual dependent structures caused by
36 217 repeated measures in the same location [40], through the use of a random intercept factor (Site code).
37 218 To quantify predictor's effect sizes and significance, we adopted a multi-model inference approach
38 219 [41], using the *MuMIn* R package [42]. This statistical technique ranks all the models generated using
39 220 all the possible combination of predictors using the Akaike's Information Criterion (AIC). Then, a set
40 221 of top models is selected to produce an average model only if the model ranking first is ambiguously
41 222 supported (model weight<0.90). We chose top models differing in no more than four AIC units

1
2
3
4 223 ($\Delta \leq 4$) from the model ranked first (minimum AIC). We adopted a natural average method to
5
6 224 conduct the model averaging, which consists in averaging predictors only over models in which the
7
8 225 predictor appears and weighting predictor's effect size by the summed weights of these models [43].
9
10 226 For each LMM model, two measures of goodness-of-fit were estimated [44]: marginal goodness-of-fit
11
12 227 (r^2_m) indicates the variance explained only by the fixed factors, while conditional goodness-of-fit (r^2_c)
13
14 228 shows the variance accounted for by both fixed and random terms.
15

16 230 Given that taxonomic and trait diversity and richness can be correlated by chance (selection probability
17
18 231 effect) [45], we performed null models to confirm whether the observed response diversity and richness
19
20 232 patterns were not simply a consequence of the underlying taxonomic variation. Methodological details
21
22 233 are described in Table S7.
23

24
25 235 Multivariate generalised linear models (*manyglm* function [39], *mvabund* R package) were fitted to
26
27 236 explore community composition changes (matrix at family, genus and species levels, 220 taxa) and
28
29 237 taxon-specific univariate responses to natural and altered salinity and other covariates, using a
30
31 238 negative-binomial error distribution (after exploring mean-variance assumption), excluding rare taxa
32
33 239 and selecting one sample per site ($n=107$). As predictors, we selected mean conductivity, alteration
34
35 240 class and their interaction. We also added a mean conductivity quadratic term to capture bell-shaped
36
37 241 responses around species' osmotic optimums. Additionally, we tested if including more environmental
38
39 242 covariates, such as those used in the LMM, could reduce model AIC, resulting in the inclusion of
40
41 243 precipitation and geographical longitude. Predictors were tested for significance with a Likelihood
42
43 244 Ratio Test (LRT) and a PIT-trap resampling with 999 iterations [39]. We also used Wald tests to
44
45 245 examine model terms significance and corrected univariate taxon-specific p-values for multiple testing.
46
47 246

48
49 247 All models were validated by visually checking their residuals for normality and homoscedasticity.
50
51 248 The code and functions used to run all these analyses are available in Supplementary Information 2,
52
53 249 which were conducted using the R version 3.4.1 [46].
54
55 250

56 251

57 252

58 253 **RESULTS**

59

60

1
2
3
4 254 In sites showing natural salinity, communities at low conductivity ($0.3\text{-}1.0\text{ mS cm}^{-1}$) have similar
5 255 proportions of the salt-sensitive EPT taxa and salt-tolerant OCH taxa (ranging from 20%-60%) (Fig.
6 256 S4). However, these groups followed opposing trends in response to increasing conductivity so that
7 257 the proportion of EPT taxa declined sharply while the proportion of OCH taxa increased up to the
8 258 complete domination of communities at higher conductivities.

9 259
10
11
12
13
14 260 Our LMM models showed that conductivity effects differ between natural and salinised sites for
15 261 taxonomic richness ($r^2_m=56.7\%$) and response trait diversity ($r^2_m=37.8\%$), as observed by the
16 262 significant interactions (Table 1, Fig. 2a-c; see Tables S5 and S6 for detailed results). Sites under
17 263 natural (control) conditions showed a significantly stronger reduction of taxonomic richness and
18 264 response trait diversity with conductivity compared to salinised sites (Fig. 2a,b), while the response of
19 265 diluted sites did not differ from the natural and salinised sites (Table 1). Response trait richness
20 266 declined similarly with increasing conductivity across alteration classes ($r^2_m=38.1\%$; (Fig. 2c).
21 267 Remarkably, while taxonomic and response trait richness declined in salinised sites of higher
22 268 conductivities, response trait diversity increased along the conductivity gradient. Null models showed
23 269 that response trait richness patterns were linked to trait filtering rather than simply to taxonomic
24 270 variation. However, in the case of response trait diversity, only one model parameter (*salinisation*) was
25 271 indistinguishable from the null distributions (Table S7).

26 272
27
28
29
30
31
32
33
34
35
36 273 Traits responded differently to conductivity, showing in some cases distinct responses among the
37 274 salinity alteration classes, as revealed by their significant interactions with conductivity (Table 1; Fig.
38 275 2d-g; see Tables S5 and S6 for detailed results). The most evident case was the proportion of organisms
39 276 with short lifespan, which was higher at salinised sites over the whole gradient, and increased with
40 277 conductivity, while natural and diluted rivers show progressively a lower proportion of short lifespan
41 278 organisms along the conductivity gradient (Fig 2d, $r^2_m=67.9\%$; see differences in effect size 95%
42 279 confidence intervals in Table 1). Salinised sites were dominated by short lifespan organisms such as
43 280 the dipterans Ceratopogonidae, Chironomidae, Ephydriidae and Simuliidae. The proportion of
44 281 organisms with multivoltine life cycle showed contrasting patterns across alteration classes, showing
45 282 great disparities at low conductivities but converging progressively at higher salinities ($>100\text{ mS cm}^{-1}$)
46 283 (Fig 2e, $r^2_m=40.4\%$). In this case, the response of the diluted sites was significantly different from
47 284 that of the natural sites, but indistinguishable from salinised sites' response. The proportion of
48 285 organisms with ovoviviparous and terrestrial reproduction increased with conductivity across salinity

1
2
3
4 286 alteration classes (Fig 2f, $r^2_m=25.8\%$). However, the proportion of organisms with aerial respiration
5
6 287 showed a more complex trend in response to conductivity for the three alteration classes (Fig 2g,
7
8 288 $r^2_m=46.8\%$), showing a greater increase at salinised sites compared to natural sites, which decreased
9
10 289 with higher conductivity. The response of diluted sites was statistically indistinguishable from the other
11
12 290 alteration classes. Finally, we did not observe any significant trend for the proportion of non-native
13
14 291 taxa (Fig 2h).

15
16 293 Community composition changes in response to conductivity also differed among natural and salinised
17
18 294 sites. The MDS ordination showed a first axis along which communities were distributed in relation
19
20 295 with increasing conductivity towards the positive side of this axis (Fig. 3a). When aggregated by
21
22 296 conductivity classes (from freshwaters to hypersaline waters, Fig. 3b), salinised samples were generally
23
24 297 placed far from the natural and diluted community centroids. Natural and diluted samples showed a
25
26 298 greater overlap, although diluted sites displayed substantial differences at mesosaline conductivities.
27
28 299 The variation of the MDS axis 1 values in response to conductivity revealed significantly different
29
30 300 compositional change trajectories for salinised communities relative to natural and diluted
31
32 301 communities, as showed by the significantly less pronounced slope in salinised communities (Fig 2i,
33
34 302 $r^2_m=85.6\%$; see differences in effect size 95% confidence intervals in Table 1), while diluted and
35
36 303 natural sites' responses did not differ. The results of the multivariate GLM (Tables S8 and S9) indicated
37
38 304 that macroinvertebrate responses to conductivity differed across alteration classes (LRT=193.4,
39
40 305 $p<0.001$). However, such differences seemed to be a result of organisms responding differently
41
42 306 between salinised and natural sites ("conductivity x salinised" coefficient significance, Wald test value
43
44 307 = 8.21; $p<0.001$), while organisms' response to salinity was statistically indistinguishable between
45
46 308 diluted and natural sites ("conductivity x diluted" coefficient significance, Wald test, value = 6.37;
47
48 309 $p<0.711$). Furthermore, taxon-specific responses to conductivity varied across natural and altered
49
50 310 classes (Fig. S5), including salt-intolerant responses, wide range of conductivity tolerance
51
52 311 irrespectively of the alteration class, preference for naturally saline and salinised rivers, and preference
53
54 312 for natural saline and diluted rivers.

50 313

51 314

54 315 **DISCUSSION**

55 316

1
2
3
4 317 Our results suggest that different ecological processes may shape community assembly in response to
5
6 318 anthropogenic alterations, depending on the direction of change along the same environmental
7
8 319 gradient. Trait filtering processes seemed to drive community assembly in natural and diluted rivers,
9
10 320 whereas dispersal-related processes were more relevant in response to salinisation. Communities at
11
12 321 salinised rivers tended to show strong differences in diversity and composition respect to natural
13
14 322 communities. However, we found a much evident overlap between the taxonomic composition of
15
16 323 diluted and natural communities, despite observing significant differences in other aspects of diversity
17
18 324 and trait composition. Overall, these results support our third hypothesis (H₃), indicating divergent
19
20 325 trajectories of change between salinised rivers, which resulted in novel communities [13], and diluted
21
22 326 rivers, whose communities resembled those of natural rivers with the same conductivity.

23 328 Abiotic filtering is often considered one of the major forces determining biological responses to stress
24
25 329 [4]. In our study, we observed a consistent richness and diversity decline, and strong compositional
26
27 330 changes as salinity increases, which can be attributed to increasing abiotic filtering. However, these
28
29 331 changes arose in divergent trajectories when comparing natural and salinised rivers, which supports
30
31 332 the role of dispersal-related processes in structuring salinised communities [12]. Generally, organisms
32
33 333 show a differential capacity to tolerate osmotic stress as a result of specialised physiological and life
34
35 334 history traits [47,48], confirmed by experiments in absence of competition [8,49]. Thus, while
36
37 335 organisms inhabiting freshwaters need to actively uptake ions to maintain metabolic functions, taxa
38
39 336 tolerating mineralised waters need to match their internal concentrations to external salinity or even
40
41 337 actively excrete ions when salinity is sufficiently high. Nonetheless, salt-tolerant organisms are also
42
43 338 able to cope with freshwater conditions, as observed experimentally [50]. Diversity declined sharply
44
45 339 after conductivities 5-20 mS cm⁻¹, which could be reflecting a critical physiological threshold after
46
47 340 which most organisms are unable to thrive. Considering that aquatic insect haemolymph is roughly 15
48
49 341 mS cm⁻¹ [47], only few saline specialists with appropriate physiological mechanisms can live above
50
51 342 this threshold. Our data reflected such pattern in natural and altered communities, matching the results
52
53 343 of previous studies finding stronger salinity effects after conductivities ranging 5-30 mS cm⁻¹
54
55 344 [15,51,52].

56 345
57 346 Besides, our results showed that conductivity tends to select organisms with multivoltine life cycle,
58
59 347 aerial respiration and ovoviviparous or terrestrial reproduction across alteration classes, as previously
60
348 observed in natural [32,53] and salinised rivers [54,55]. These trait categories are probably key to cope

1
2
3
4 349 with the stressful environment of saline rivers [31]. However, we found shorter lifespan organisms in
5
6 350 salinised rivers which contrast not only with our patterns for diluted and natural rivers but also with
7
8 351 previous studies performed on natural and salinised locations [53,55]. In our study, salinisation could
9
10 352 have favoured short lifespan organisms in absence of better suited organisms because of their rapid
11
12 353 reproductive cycles. Alternatively, it could have been reflecting a trait syndrome, were other correlated
13
14 354 trait is explaining the success of the organisms successfully colonising salinised rivers
15

16 356 The fact that the studied salinised sites were relatively distant from naturally saline rivers could have
17
18 357 favoured their colonisation by a mix of opportunistic species and saline specialist with good dispersion
19
20 358 capacities. Our analyses revealed that some taxa which are highly abundant in naturally saline rivers
21
22 359 (genera *Ochthebius* and *Nebrioporus*) were absent from salinised rivers, perhaps due to limited
23
24 360 dispersion [56,57] or other biotic factors [50]. However, although it remains unknown if organisms
25
26 361 already established at salinised sites could act as also as biological barrier for saline specialists, this
27
28 362 possibility seems unlikely given the reduced competition pressure at meso- and hypersaline
29
30 363 environments [2]. Contrary to previous findings [52,54,55], we did not find estuarine taxa or invasive
31
32 364 species frequently occurring at salinised rivers. This could suggest that saline tolerant species available
33
34 365 within the inland species pool are preventing invasions from other habitats via competition or estuarine
35
36 366 taxa could not reach the salinised spots. On the other hand, dilution led to communities assembling
37
38 367 along the same axis of variation than natural communities. Although reducing stress levels allowed a
39
40 368 greater number of organisms to colonise diluted rivers, a great extent remain too saline for most
41
42 369 opportunistic colonisers (ca. 60% of diluted rivers have conductivities >20 mS cm⁻¹). In addition,
43
44 370 naturally saline rivers are typically clustered within arid regions with evaporitic outcrops, which
45
46 371 constitute abundant sources of potential saline specialist colonisers.

47
48 372
49 373 Our study is the first in comparing the ecological effects of bidirectional anthropogenic salinity
50
51 374 modifications along a wide osmotic gradient, and one of the few examples in a wider context of
52
53 375 stressors and systems [58]. Nonetheless, our data are limited due to the reduced available number of
54
55 376 rivers primarily affected by dilution or salinisation, and not by other human impacts, and because
56
57 377 quantifying the precise magnitude of the salinity change requires pre-disturbed data, which is typically
58
59 378 unavailable. Particularly, the fact that all salinised sites were concentrated within the same basin
60
379 (Llobregat River basin), could have affected our capacity to detect biological changes in response to

1
2
3
4 380 salinisation. Future research using manipulative experiments should be performed to overcome these
5 381 limitations and compare against the observational patterns presented here.

6 382
7
8 383 Two major management implications arise from this study. Firstly, salinisation may cause less
9 384 predictable biological responses compared to dilution, and would depend on the proportion of salt-
10 385 tolerant species in the regional pool and their dispersal and colonisation capacities [13]. However,
11 386 reducing osmotic pressure in salinised rivers (e.g. diverting brine effluents) might be an effective
12 387 restorative measure when rivers are easily accessible by close, abundant sources of freshwater
13 388 colonisers. On the other hand, dilution reduces the habitat availability for a substantial fraction of meso-
14 389 and hypersaline specialists, which can threaten their populations [59]. Therefore, the conservation of
15 390 this unique fraction of biodiversity requires preserving sufficient natural habitats along the full
16 391 conductivity gradient. Furthermore, these results can be also relevant to predict the impacts of other
17 392 anthropogenic stressors that also occur naturally and whose frequency and intensity are expected to
18 393 increase with global change, such as flow intermittence or fire disturbance [60]. In this case, ecological
19 394 responses would depend on the proportion of tolerant organisms within the regional species pool and
20 395 their capacity to colonise such novel environments [61].

21 396
22
23 397 In conclusion, we showed how the artificial modification of chemical stressors can result in directional-
24 398 dependent effects along the same stress gradient, where trait-filtering and dispersal-related processes
25 399 have differential roles in community assembly. The approach presented here provides empirical and
26 400 conceptual insights that can help anticipating ecological effects of global change and improving
27 401 environmental management.

28 402

29 403 **ACKNOWLEDGEMENTS**

30 404

31 405 We would like to thank the water agencies of Catalonia (Agència Catalana de l'Aigua) and Segura
32 406 River (Confederación Hidrográfica del Segura) for providing sampling site information and water
33 407 chemistry data for most of the studied rivers. We are grateful to Verónica Granados-Pérez, Rebeca
34 408 Arias del Real, Daniel Castro and Sara Campillo for their kind assistance in field sampling and water
35 409 chemistry analyses. We are also grateful to Daniel Bruno, Óscar Belmar, Simone Guareschi and José
36 410 Antonio Carbonell for generously providing data from freshwater rivers of the Segura river watershed.

37 411

1
2
3
4 412 **DATA ACCESSIBILITY**

5
6 413 Supplementary Information S2 includes the R code and data to reproduce the analysis presented here.
7

8 414
9

10 415 **AUTHOR'S CONTRIBUTIONS**

11 416 CG-C, DS-F, MC-A, NB, AM and JV conceived the ideas and designed methodology; All authors
12 417 collected macroinvertebrate and water samples; NO and AS performed isotopic analysis to identify
13 418 sites affected by salinisation. JV performed water chemistry analysis. RA, PF, AM and NB identified
14 419 macroinvertebrate taxa; CG-C analysed the data; CG-C led the writing of the manuscript. All authors
15 420 contributed critically to data interpretation and manuscript reviewing, and gave final approval for
16 421 publication.
17
18
19
20
21

22 422

23
24 423 **COMPETING INTERESTS**

25
26 424 We have no competing interests.
27

28 425
29

30 426 **FUNDING**

31 427 CG-C is supported by a "Juan de la Cierva-Formación" research contract (MINECO, FJCI-2015-
32 428 25785) and DS-F by two post-doctoral contracts funded by the Universidad de Castilla-La Mancha and
33 429 the University of Murcia. This research was also partially supported by the Catalan Government
34 430 project of Mineralogía Aplicada, Geoquímica i Geomicrobiología research group (2017SGR-1733) and
35 431 MECODISPER project (Advancing meta-community ecology in intermittent rivers to improve their
36 432 conservation and management; MINECO, CTM2017-89295-P).
37
38
39
40
41

42 433
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

434 REFERENCES

1. Chase JM. 2003 Community assembly: When should history matter? *Oecologia* **136**, 489–498. (doi:10.1007/s00442-003-1311-7)
2. Weiher E, Freund D, Bunton T, Stefanski A, Lee T, Bentivenga S. 2011 Advances, challenges and a developing synthesis of ecological community assembly theory. *Philos. Trans. R. Soc. B Biol. Sci.* **366**, 2403–2413. (doi:10.1098/rstb.2011.0056)
3. Baert JM, Janssen CR, Sabbe K, De Laender F. 2016 Per capita interactions and stress tolerance drive stress-induced changes in biodiversity effects on ecosystem functions. *Nat. Commun.* **7**, 12486. (doi:10.1038/ncomms12486)
4. Badyaev A V. 2005 Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proc. R. Soc. B Biol. Sci.* (doi:10.1098/rspb.2004.3045)
5. Côté IM, Darling ES, Brown CJ. 2016 Interactions among ecosystem stressors and their importance in conservation. *Proc. R. Soc. B Biol. Sci.* **283**, 20152592. (doi:10.1098/rspb.2015.2592)
6. Chase JM, Myers JA. 2011 Disentangling the importance of ecological niches from stochastic processes across scales. *Philos. Trans. R. Soc. B Biol. Sci.* **366**, 2351–2363. (doi:10.1098/rstb.2011.0063)
7. Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM. 2015 Community assembly, coexistence and the environmental filtering metaphor. *Funct. Ecol.* **29**, 592–599. (doi:10.1111/1365-2435.12345)
8. Pallarés S, Botella-Cruz M, Arribas P, Millán A, Velasco J. 2017 Aquatic insects in a multistress environment: cross-tolerance to salinity and desiccation. *J. Exp. Biol.* (doi:10.1242/jeb.152108)
9. Buchwalter DB, Cain DJ, Martin CA, Xie L, Luoma SN, Garland T. 2008 Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 8321–6. (doi:10.1073/pnas.0801686105)
10. Taylor DR, Aarssen LW, Loehle C. 1990 On the Relationship between r/K Selection and Environmental Carrying Capacity: A New Habitat Templet for Plant Life History Strategies. *Oikos* **58**, 239. (doi:10.2307/3545432)
11. Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E. 1997 The influence of functional diversity and composition on ecosystem processes. *Science (80-.)*. **277**, 1300–1302. (doi:10.1126/science.277.5330.1300)
12. Li Y, Shipley B. 2018 Community divergence and convergence along experimental gradients of stress and disturbance. *Ecology* **99**, 775–781. (doi:10.1002/ecy.2162)
13. Kefford BJ, Buchwalter D, Cañedo-Argüelles M, Davis J, Duncan RP, Hoffmann A, Thompson R. 2016 Salinized rivers: degraded systems or new habitats for salt-tolerant faunas? *Biol. Lett.* **12**, 20151072. (doi:10.1098/rsbl.2015.1072)
14. Gutiérrez-Cánovas C, Millán A, Velasco J, Vaughan IP, Ormerod SJ. 2013 Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. *Glob. Ecol. Biogeogr.* **22**, 796–805.
15. Cañedo-Argüelles M, Kefford BJ, Piscart C, Prat N, Schäfer RB, Schulz CJ. 2013 Salinisation of rivers: An urgent ecological issue. *Environ. Pollut.* **173**, 157–167. (doi:10.1016/j.envpol.2012.10.011)
16. Millán A, Velasco J, Gutiérrez-Cánovas C, Arribas P, Picazo F, Sánchez-Fernández D, Abellán P. 2011 Mediterranean saline streams in southeast Spain: What do we know? *J. Arid Environ.* **75**, 1352–1359. (doi:10.1016/j.jaridenv.2010.12.010)
17. Herbert ER *et al.* 2015 A global perspective on wetland salinization: Ecological consequences

- of a growing threat to freshwater wetlands. *Ecosphere* **6**, 1–43. (doi:10.1890/ES14-00534.1)
18. Cañedo-Argüelles M, Bundschuh M, Gutiérrez-Cánovas C, Kefford BJ, Prat N, Trobajo R, Schäfer RB. 2014 Effects of repeated salt pulses on ecosystem structure and functions in a stream mesocosm. *Sci. Total Environ.* **476–477**, 634–642.
19. Gutiérrez-Cánovas C, Hernández J, Millán A, Velasco J. 2012 Impact of chronic and pulse dilution disturbances on metabolism and trophic structure in a saline Mediterranean stream. *Hydrobiologia* **686**, 225–239.
20. Otero N, Soler A, Canals À. 2008 Controls of $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ in dissolved sulphate: Learning from a detailed survey in the Llobregat River (Spain). *Appl. Geochemistry* **23**, 1166–1185. (doi:10.1016/j.apgeochem.2007.11.009)
21. Otero N, Soler A. 2002 Sulphur isotopes as tracers of the influence of potash mining in groundwater salinisation in the Llobregat Basin (NE Spain). *Water Res.* **36**, 3989–4000. (doi:10.1016/S0043-1354(02)00125-2)
22. Viladés M. 2013 Estudi de les aigües salines a la conca del riu Llobregat i Cardener: Implicacions ambientals. Universitat Autònoma de Barcelona, Final-degree project.
23. APHA. 2005 Standard methods for the examination of water and wastewater. *Am. Public Heal. Assoc. Washington, DC, USA* (doi:30M11/98)
24. Timpano AJ, Zipper CE, Soucek DJ, Schoenholtz SH. 2018 Seasonal pattern of anthropogenic salinization in temperate forested headwater streams. *Water Res.* **133**, 8–18. (doi:10.1016/j.watres.2018.01.012)
25. Gómez R, Asencio AD, Picón JM, Del Campo R, Arce MI, del Mar Sánchez-Montoya M, Suárez ML, Vidal-Abarca MR. 2016 The effect of water salinity on wood breakdown in semiarid Mediterranean streams. *Sci. Total Environ.* **541**, 491–501. (doi:10.1016/j.scitotenv.2015.09.040)
26. Moreno JL, Angeler DG, De las Heras J. 2010 Seasonal dynamics of macroinvertebrate communities in a semiarid saline spring stream with contrasting environmental conditions. *Aquat. Ecol.* **44**, 177–193. (doi:10.1007/s10452-009-9251-x)
27. Sánchez-Montoya MM *et al.* 2009 Defining criteria to select reference sites in Mediterranean streams. *Hydrobiologia* **619**, 39–54. (doi:10.1007/s10750-008-9580-0)
28. Bonada N, Dolédec S. In press. Does the Tachet trait database report voltinism variability of aquatic insects between Mediterranean and Scandinavian regions? *Aquat. Sci.* (doi:10.1007/s00027-017-0554-z)
29. Bonada N, Dolédec S. 2011 Do mediterranean genera not included in Tachet *et al.* 2002 have mediterranean trait characteristics? *Limnetica* **1**, 129–142.
30. Picazo F, Millán A, Dolédec S. 2012 Are patterns in the taxonomic, biological and ecological traits of water beetles congruent in Mediterranean ecosystems? *Freshw. Biol.* **57**, 2192–2210. (doi:10.1111/j.1365-2427.2012.02859.x)
31. Odum EP. 1985 Trends Expected in Stressed Ecosystems. *Bioscience* **35**, 419–422. (doi:10.2307/1310021)
32. Díaz AM, Alonso MLS, Gutiérrez MRVA. 2008 Biological traits of stream macroinvertebrates from a semi-arid catchment: Patterns along complex environmental gradients. *Freshw. Biol.* **53**, 1–21. (doi:10.1111/j.1365-2427.2007.01854.x)
33. Higgins JK, MacLean HJ, Buckley LB, Kingsolver JG. 2014 Geographic differences and microevolutionary changes in thermal sensitivity of butterfly larvae in response to climate. *Funct. Ecol.* **28**, 982–989. (doi:10.1111/1365-2435.12218)
34. Pavoine S, Vallet J, Dufour AB, Gachet S, Daniel H. 2009 On the challenge of treating various types of variables: Application for improving the measurement of functional diversity. *Oikos* **118**, 391–402. (doi:10.1111/j.1600-0706.2008.16668.x)
35. Maire E, Grenouillet G, Brosse S, Villéger S. 2015 How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of

- functional spaces. *Glob. Ecol. Biogeogr.* **24**, 728–740. (doi:10.1111/geb.12299)
36. Laliberte E, Legendre P. 2010 A distance-based framework for measuring functional diversity from multiple traits. *Ecology* **91**, 299–305. (doi:10.1890/08-2244.1)
37. Villéger S, Mason NWH, Mouillot D. 2008 New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* **89**, 2290–2301. (doi:10.1890/07-1206.1)
38. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* (doi:10.18637/jss.v067.i01)
39. Wang Y, Naumann U, Wright ST, Warton DI. 2012 Mvabund - an R package for model-based analysis of multivariate abundance data. *Methods Ecol. Evol.* **3**, 471–474. (doi:10.1111/j.2041-210X.2012.00190.x)
40. Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM, Ebooks Corporation. 2009 *Mixed effects models and extensions in ecology with R*. (doi:10.1007/978-0-387-87458-6)
41. Grueber CE, Nakagawa S, Laws RJ, Jamieson IG. 2011 Multimodel inference in ecology and evolution: Challenges and solutions. *J. Evol. Biol.* **24**, 699–711. (doi:10.1111/j.1420-9101.2010.02210.x)
42. Bartoń K. 2016 MuMIn: Multi-model inference. R package version 1.15.6. *Version 1*, 18.
43. Burnham KP, Anderson DR. 2002 *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach (2nd ed)*. (doi:10.1016/j.ecolmodel.2003.11.004)
44. Nakagawa S, Schielzeth H. 2013 A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142. (doi:10.1111/j.2041-210x.2012.00261.x)
45. Huston MA. 1997 Hidden treatments in ecological experiments: Re-evaluating the ecosystem function of biodiversity. *Oecologia*. **110**, 449–460. (doi:10.1007/s004420050180)
46. R Core Team. 2016 R Development Core Team. *R A Lang. Environ. Stat. Comput.* **55**, 275–286.
47. Bradley T. 2009 *Animal osmoregulation*. Oxford, UK: Oxford University Press.
48. Arribas P, Andújar C, Abellán P, Velasco J, Millán A, Ribera I. 2014 Tempo and mode of the multiple origins of salinity tolerance in a water beetle lineage. *Mol. Ecol.* (doi:10.1111/mec.12605)
49. Carbonell JA, Millán A, Velasco J. 2012 Concordance between realised and fundamental niches in three Iberian *Sigara* species (Hemiptera: Corixidae) along a gradient of salinity and anionic composition. *Freshw. Biol.* **57**, 2580–2590. (doi:10.1111/fwb.12029)
50. Arribas P, Gutiérrez-Cánovas C, Botella-Cruz M, Cañedo-Argüelles M, Carbonell JA, Millán A, Pallarés S, Velasco J, Sánchez-Fernández D. In press. Insect communities in saline waters consist of realised but not fundamental niche specialists. *Philos. Trans. R. Soc. B*
51. Dunlop JE, Horrigan N, McGregor G, Kefford BJ, Choy S, Prasad R. 2008 Effect of spatial variation on salinity tolerance of macroinvertebrates in Eastern Australia and implications for ecosystem protection trigger values. *Environ. Pollut.* **151**, 621–630. (doi:10.1016/j.envpol.2007.03.020)
52. Pinder AM, Halse SA, McRae JM, Shiel RJ. 2005 Occurrence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. *Hydrobiologia* **543**, 1–24. (doi:10.1007/s10750-004-5712-3)
53. Gutiérrez-Cánovas C, Sánchez-Fernández D, Velasco J, Millán A, Bonada N. 2015 Similarity in the difference: changes in community functional features along natural and anthropogenic stress gradients. *Ecology* **96**, 150310120641001. (doi:10.1890/14-1447.1)
54. Piscart C, Moreteau JC, Beisel JN. 2005 Biodiversity and structure of macroinvertebrate communities along a small permanent salinity gradient (Meurthe River, France). *Hydrobiologia* **551**, 227–236. (doi:10.1007/s10750-005-4463-0)
55. Szöcs E, Coring E, Bäche J, Schäfer RB. 2014 Effects of anthropogenic salinization on

- 1 biological traits and community composition of stream macroinvertebrates. *Sci. Total Environ.*
2 **468–469**, 943–949. (doi:10.1016/j.scitotenv.2013.08.058)
- 3
4 56. Arribas P, Velasco J, Abellán P, Sánchez-Fernández D, Andújar C, Calosi P, Millán A, Ribera
5 I, Bilton DT. 2012 Dispersal ability rather than ecological tolerance drives differences in range
6 size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *J. Biogeogr.* **39**, 984–
7 994. (doi:10.1111/j.1365-2699.2011.02641.x)
- 8 57. Abellán P, Millán A, Ribera I. 2009 Parallel habitat-driven differences in the
9 phylogeographical structure of two independent lineages of Mediterranean saline water
10 beetles. *Mol. Ecol.* **18**, 3885–3902. (doi:10.1111/j.1365-294X.2009.04319.x)
- 11 58. Ormerod SJ, Durance I. 2009 Restoration and recovery from acidification in upland Welsh
12 streams over 25 years. *J. Appl. Ecol.* **46**, 164–174. (doi:10.1111/j.1365-2664.2008.01587.x)
- 13 59. Arribas P, Abellán P, Velasco J, Millán A. 2015 Evolutionary ecology, biogeography and
14 conservation of water beetles in Mediterranean saline ecosystems. *Limnetica* **34**, 481–494.
15 (doi:10.23818/limn.34.36)
- 16 60. Bonada N, Resh VH. 2013 Mediterranean-climate streams and rivers: Geographically
17 separated but ecologically comparable freshwater systems. *Hydrobiologia.* **719**, 1–29.
18 (doi:10.1007/s10750-013-1634-2)
- 19 61. Hobbs RJ, Higgs E, Harris JA. 2009 Novel ecosystems: implications for conservation and
20 restoration. *Trends Ecol. Evol.* **24**, 599–605. (doi:10.1016/j.tree.2009.05.012)
- 21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

TABLES

Table 1.

Metric	cond	diluted	salinised	cond x diluted	cond x salinised	cond x diluted 95% CI	cond x salinised 95% CI	r^2_m	r^2_c
Taxonomic richness	-0.343***	-1.243	-1.559	0.146	0.231*	(-0.046, 0.338)	(0.165, 0.297)	56.7	82.1
Response diversity	-0.031***	-0.11	-0.309**	0.015	0.038**	(-0.010, 0.040)	(0.014, 0.062)	37.8	63.2
Response richness	-1.015***	0.81	-0.763	0.003	0.687	(-0.821, 0.827)	(-0.127, 1.501)	38.1	74.9
% short lifespan	-0.031***	0.27	-0.224	-0.028	0.046*	(-0.064, 0.008)	(0.008, 0.084)	67.9	71.8
% multivoltine	0.013**	0.548***	0.206	-0.048***	-0.018	(-0.070, -0.026)	(-0.041, 0.005)	40.4	40.4
% ovoviviparous & terrestrial rep.	0.076***	-0.094	0.033	0.019	-0.049	(-0.040, 0.078)	(-0.106, 0.008)	25.8	48.6
% aerial respiration	-0.007	-0.131	-0.942***	0.018	0.114***	(-0.043, 0.079)	(0.055, 0.173)	46.8	78.4
% non-native taxa	-0.003	0.017	-0.003	-0.004	0.001	(-0.013, 0.005)	(-0.008, 0.010)	7.2	51.0
MDS axis 1	0.385***	-0.442	1.485**	0.037	-0.201***	(-0.080, 0.154)	(-0.311, -0.091)	85.6	96.0

FIGURE AND TABLE CAPTIONS

Fig. 1. Hypothetical community change trajectories in response to anthropogenic dilution and salinisation. Natural communities: blue squares; Diluted: orange circles; Salinised: red triangles H₁: communities with anthropogenically altered salinity could resemble natural communities with the same salinity (trait-filtering). H₂: communities with altered salinity could differ from natural communities following convergent trajectories (dispersal related-processes). H₃: communities of salinised and diluted rivers would follow divergent trajectories of change (trait-filtering and dispersal-related processes would have different importance depending on the salinity change direction).

Fig. 2. Plots of the models examining the effect of conductivity, altered salinity classes (*diluted* and *salinised*) and their interactions (*cond x diluted*, *cond x salinised*) on taxonomic richness, response trait diversity, response trait richness, community weighted mean traits (proportion of organisms with short life-span, multivoltinism, ovoviviparity and terrestrial reproduction and aerial respiration), proportion of non-native taxa and MDS axis 1. Conductivity axis is represented in log-scale. Control: blue filled squares; Diluted: orange empty circles; Salinised: red empty triangles.

Fig. 3. Plot showing the first two axes of the MDS ordination for all samples (a) and grouped by conductivity classes (b) to show the degree of overlap between control, diluted and salinised communities. Control: blue filled squares; Diluted: orange empty circles; Salinised: red empty triangles. Conductivity classes: fresh: < 0.1 mS cm⁻¹; sub: ≥0.1 – >5.0 mS cm⁻¹; hypo: ≥5 – >30 mS cm⁻¹; meso: ≥30->100 mS cm⁻¹; hyper: ≥ 100 mS cm⁻¹. Parameter d represents the proportion of the representation scale between MDS 1 and 2 axes (e.g. d=0.5 means that the scale of MDS axis 2 is magnified by 2 respect to MDS 1).

Table 1. Results of the models examining the effect of conductivity (*cond*), altered salinity classes (*diluted* and *salinised*) and their interaction (*diluted x cond*, *salinised x cond*) on taxonomic richness, response diversity, response richness, community weighted mean traits and MDS axis 1. Predictor effect sizes and their significance are shown for each model (Significant terms are showed in bold: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). 95% Confidence Intervals (CI) were also showed for the interaction terms to allow SES comparison. Information for the effect sizes and significance of the other environmental variables can be found in Table S5. Goodness-of-fit is also shown for the fixed factors (r^2_m) and fixed plus the random factor for each site (r^2_c).

SUPPLEMENTARY MATERIAL

Fig. S1. Geographical location of the sampling sites.

Table S1. Results of the $\delta^{34}\text{S}_{\text{SO}_4}$ and $\delta^{18}\text{O}_{\text{SO}_4}$ analysis and sulphate concentrations for rivers potentially affected by salinisation.

Table S2. Description of the environmental variables used in the study.

Table S3. Pearson correlation coefficients between functional space (PCoA) axes and original response trait categories.

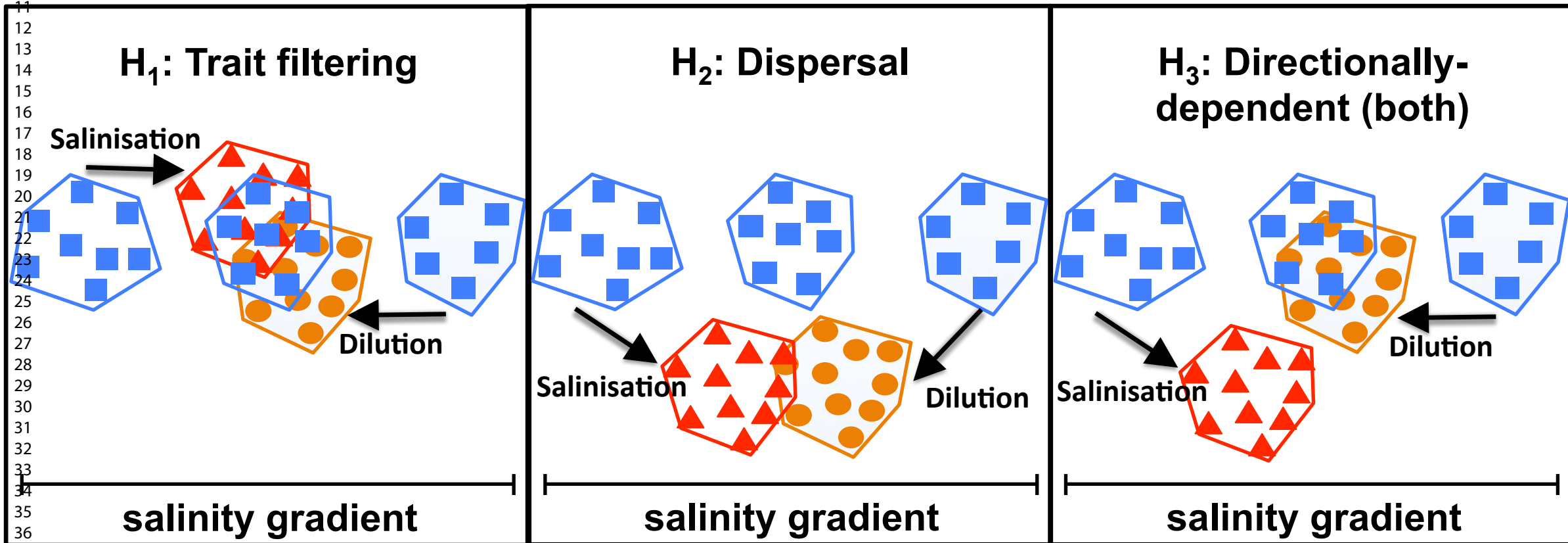
Supplementary Information S1. Identification of rivers affected by salinisation through isotopic analysis

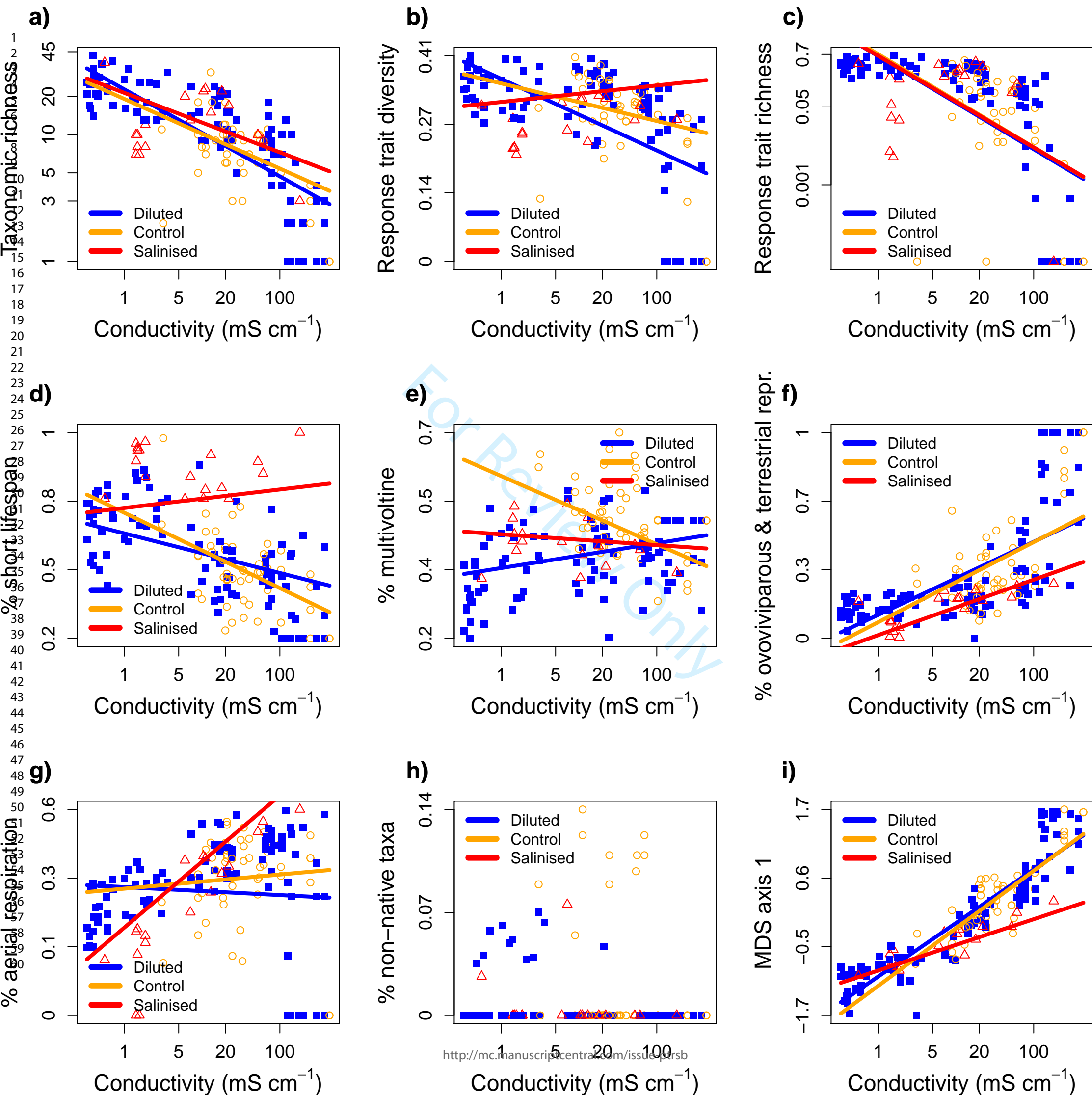
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Supplementary Information S2. R code and data to reproduce the analysis presented in this study.

Supplementary Information S3. Additional model details and results.

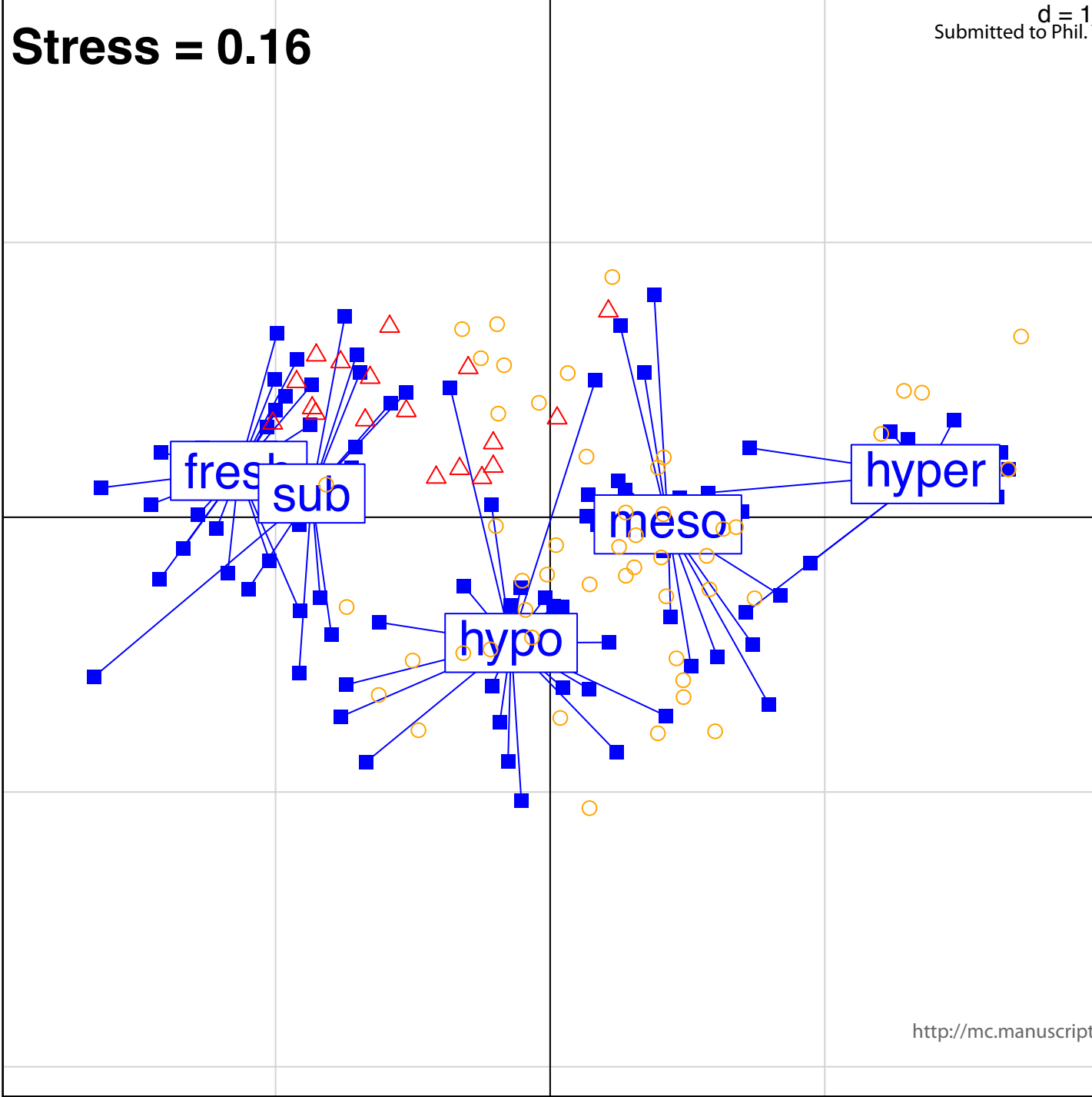
For Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41



a) Stress = 0.16

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41



b)

