1 **Disturbance During Biofilm Community Succession Promotes Cooperation and**

2 **Diversity**

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16 **Running title:** Disturbance in biofilm communities

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Abstract

26   Diversity-disturbance relationships have found widespread application in ecology,

27   conservation and biodiversity management. In spite of their explanatory power, these

28   conceptual frameworks have yet to be systematically applied to understanding succession in

29   diverse microbial biofilms. Here we investigate community assembly in biofilms formed in

30   replicate microbial bioelectrochemical systems using time-course sequencing of community

31   16S rRNA genes, corresponding to hundreds of operational taxonomic units (OTUs). For the

32   first time we present a statistical model showing that a simple diversity-disturbance

33   relationship can be used to explain dynamic changes in high diversity biofilm communities.

34   This simple model reveals that succession in these systems is guided towards either a low

35   diversity, generalist-dominated biofilm or a high diversity, cooperative-specialist biofilm,

36   depending on the level of endogenous disturbance measured within the community. The

37   pattern observed shows remarkable symmetry with findings from macro-scale communities

38   such as grasslands, forests and coral reefs.

Introduction

41   Mixed species biofilms can be comprised of thousands of bacterial taxa with a vast network

42   of potential trophic interactions. Predicting the behaviour of these systems poses a similar

43   challenge to that faced by ecologists studying macro-scale communities. Two of the most

44   widely investigated ecological relationships are those between productivity and diversity as

45   well as between disturbance and diversity. While some promising results have previously

46   been obtained using simplified microcosms, thus far the operation of these relationships

47   during succession within high diversity biofilm communities have not been detected

48   (Konopka *et al*, 2015). The discovery of simple, familiar ecological patterns within diverse

49   biofilms could provide a unique insight into the seemingly intractable complexity of mixed

50   microbial community dynamics (Dini-Andreote *et al*, 2015).

52   A range of previous studies have shown clear relationships between community diversity and

53   productivity (Grime, 1973a; Grime, 1973b; Gurevitch, 1986; Tilman *et al*, 1996; Grime, 1997;

54   Buckling *et al*, 2000; Kassen *et al*, 2000; Gravel *et al*, 2011). The best known of these

55   productivity diversity relationships (PDRs) is the unimodal or "hump backed" distribution

56   (Grime, 1973). This predicts an increase in diversity with productivity which peaks at

57   intermediate levels of productivity and then declines at high levels due to competitive

58   exclusion. Disturbance diversity relationships (DDRs) are also mainstay conceptual

59   frameworks in ecology (Connell, 1978; Wilkinson, 1999; Molino and Sabatier, 2001; Svensson

60   *et al*, 2012). Disturbance has been shown to mitigate competitive exclusion, increasing

61   diversity (Connell, 1978; Huston 1979) and cooperative interactions (Brockhurst *et al*, 2007).

62   This is because competitive exclusion relies on the long-term exploitation of a small

63   competitive advantage within a crowded system (Hautier et al, 2009). Disturbance damages

64   established organisms, creating gaps which allow substitution with alternative community

65   members. These gaps can also be exploited by less competitive organisms which may have

66   greater rates of growth, dispersal or persistence (Tilman, 1994; Schnitzer and Carson, 2001).

67   Disturbance can be exogenous or endogenous. Exogenous disturbance is caused by unique or

68   external factors; in macro scale systems this includes human activity or natural disasters. On

69   the other hand, endogenous disturbance arises from many smaller routine events within the

70   community (Peh *et al*, 2011). In macro-scale communities, sources of endogenous

71   disturbance include forest tree-falls (Attiwill, 1993), herbivory and pathogens (Ayres and

72   Lombardero, 2000), and may be induced by exogenous events such as forest clearing or

73   climate change (Overpeck *et al*, 1990). Analogous processes in microbial communities are

74   predation, phage infection, production of bioactive molecules (e.g. antibiotics) or biofilm

75   disaggregation.

76

77   Advances in sequencing methods now permit characterisation of communities comprised of

78   thousands of distinct bacterial taxa within a single analysis run (Caporaso *et al*, 2011). This is

79   especially useful for microbial ecology, as large numbers of individual microbial taxa can be

80   simultaneously detected based on their 16S rRNA gene. Therefore, in a time-course

81   experiment, an entire community can be resolved into a comprehensive breakdown of

82   operational taxonomic units and their relative abundances, as well as how these change with

83   time. As a consequence, the fundamental rules governing community assembly should be

84   detectable so long as other factors including inoculum, medium feed rate and biofilm

85   substrate are kept constant. For comparison, the analysis for succession sequences in macro-

86   scale communities requires labour spanning entire careers (Silvertown, 2006; Enquist *et al*,

87   2009).

88 Microbial fuel cells (MFCs) are bioelectrochemical systems (BES) that utilise the metabolic
89 properties of bacterial biofilms to convert an electron donor, usually a carbon source, into
90 carbon dioxide and hydrogen ions while transferring electrons to the anode on which they
91 are growing, which acts as the final electron acceptor. Hydrogen ions migrate through a cation
92 selective membrane towards the cathodic chamber, while electrons travel the external circuit
93 to reduce an electron acceptor (e.g. molecular oxygen in air breathing systems) at the cathode
94 (Figure 1). MFCs can be used as continuous biofilm culture systems by feeding the electron
95 donor, usually a sugar, over extended periods of time (Logan *et al*, 2006; Beecroft *et al*, 2012;
96 Stratford *et al*, 2014). In those systems, the electron transfer rate has been found to correlate
97 strongly with CO_2 production and with the decrease in chemical oxygen demand in the anode
98 chamber (Thurston *et al*, 1984). Therefore, the electrical output of the system can be used as
99 a convenient indicator of community productivity. The stoichiometry of the reactions
100 required within the biofilm have been characterised in detail and assigned to distinct
101 metabolic types (Freguia *et al*, 2008, Hodgson *et al*, 2016). In a carbohydrate-fed MFC (such
102 as our system), the fuel is converted into fermentation products which are then consumed in
103 electrogenic reactions mediated by anodophiles (Freguia *et al*, 2008; Kiely *et al*, 2011). This
104 results in two broad types of community members, fermentative cells and anodophilic
105 respirators, which correspond to the biochemical processes taking place within the biofilm.
106 Organisms carrying out fermentation obtain metabolic energy by conversion of substrates
107 into e.g. volatile fatty acids such as acetate, while anodophiles obtain their energy through
108 the respiration of a carbon source, such as those fermentation products, using an anode as
109 the final electron acceptor (Figure 1). Anodophiles must possess a respiratory electron
110 transport chain in order to be electrogenic, and respiration is therefore a physiological
111 necessity. Many bacterial species are generalists, capable of performing both respiratory and
112 fermentative metabolism to obtain energy. These generalist species possess an electron
113 transport chain in addition to the ability to ferment sugars. Bacteria can be identified and
114 categorised as one or the other of these types: fermenter only (F), non-fermentative
115 respirator (R), or respiratory fermenter (RF). Each of the alternative specialist types (R and F)
116 requires the other to efficiently utilise the available resources, while the generalist species
117 (RF) are potentially capable of powering the fuel cell without the need for cooperative
118 syntrophy.

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119   We show here that microbial electrochemical systems can be used as a model for the balance

120   between cooperative and competitive interactions within rich bacterial communities during

121   community succession. Using microbial fuel cells as an experimental platform, we

122   demonstrate that endogenous disturbance is associated with increased cooperation and

123   diversity within multispecies bacterial biofilms. This relationship can also explain why

124   diversity is greatest at intermediate levels of biofilm productivity.

125

126   **Results**

127   Bacteria colonise the electrode rapidly and biofilms were established after 15 days. In spite

128   of identical medium composition and reactor dilution rate, communities are highly dynamic

129   and show substantial changes in composition with time. These changes occur not only

130   through increases in sequence abundance but also sequence losses for many taxa. Some

131   communities are more dynamic and experience larger losses whilst others are relatively

132   stable. Community dynamics are most apparent from measurements of DNA content per area

133   unit, with detectable DNA per cm^2 increasing from a range of 48.1 - 99.7 ng μl^{-1} at 15 days to

134   97.1 – 154 ng μl^{-1} at 91 days.

135

136   Diversity values varied widely among reactors at a given time point and between different

137   time points (Figure 2). At 15 days, diversity averaged 9.82 species equivalents while at 40 days

138   this value was 8.08, and 9.66 at 90 days; overall, there was no significant trend across time,

139   r= 0.09, p = 0.69. It is noteworthy that all communities had similar diversity at 15 days (8.12 -

140   12.14 species equivalents). Productivity increased with time, from a value of 456 mW m^{-3} at

141   15 days rising to 884 mW m^{-3} at 40 days and 1340 mW m^{-3} at 90 days. The relationship

142   between productivity and diversity revealed a probability distribution in which all of the 5

143   most diverse communities were found to occur in the centre of the productivity range around

144   925 mW m^{-3} (Figure 3A).

145

146   The proportion of RF bacteria increased with time, r= 0.43, p = 0.049 (Figure 2A), and RF

147   abundance positively correlated with MFC absolute power density, r = 0.55, p = 0.01

148   (regression not shown). This is clearly observed in the data presented in Figure 2: At later time

149   points, higher abundance of RF is associated to a more productive community. RF abundance

150   negatively correlated with community Shannon diversity, r = - 0.75, p < 0.001 (Figure 4). This

5

151   mutually exclusive relationship appears as a bifurcation in the pattern of succession for both

152   diversity and RF abundance, first appearing between 20 and 40 days (Figure 2). Combining

153   data for generalist (RF) sequence abundance with Shannon diversity measurements (units =

154   Shannon Index) allows the construction of a multiple linear model, R = 0.75, p = 0.003 (Table

155   1). The multiple linear model revealed positive relationships between the abundance of

156   generalist (RF) bacteria and productivity as well as a positive relationship between diversity

157   and productivity. This model explains 56% of all variance in power output between MFC

158   communities. These relationships are integrated into a conceptual framework, summarised

159   in Figure 6. Candidates for explaining this residual variation include fouling of the cathode or

160   the ion exchange membrane (Zhuang *et al*, 2012), variation in the performance of different

161   taxa, and stochastic variation. Most strikingly, the measured historical disturbance for a

162   community predicts its succession towards either a high diversity community of specialists (R

163   or F) or alternatively a low diversity generalist community. Disturbance (cumulative historical

164   sequence loss) was strongly associated with community diversity, r = 0.81, p < 0.001 (Figure

165   5B). Partial regression between Shannon diversity and disturbance, controlling for community

166   age, reveals an even stronger relationship, R = 0.89, p < 0.001 (regression not shown). This

167   relationship demonstrates that cumulative disturbance with time (rather than community

168   age) is more strongly associated with the observed trend. Disturbance also predicts generalist

169   (RF) relative abundance, r = - 0.49, p = 0.037 (Figure 5A).

170

171   **Discussion**

172   In our model system, communities contained bacteria that exclusively utilised fermentative

173   or respiratory metabolism, as well as generalist organisms capable of both metabolic

174   processes (Figure 1) (Freguia *et al*, 2008, Hodgson *et al*, 2016). We have shown that both

175   greater diversity within specialist communities and a high relative abundance of generalists

176   increases electrogenic activity. As our purpose in this work was to detect simple patterns

177   corresponding to those observed in macro-scale systems, we used the least complex

178   statistical models which were sufficient to reveal these relationships. A multiple linear model

179   (MLM) using both metrics as independent variables, while controlling for the amount of cells

180   present (extractable DNA), explained a majority of the variation in MFC power density (Table

181   1). Where a generalist can function equally well within two niches, it is expected that it will

182   have an advantage over specialists and be competitively dominant (Wilson and Yoshimura,

183   1994). This results in a diversity productivity relationship in which diversity is greatest at

184   intermediate values of productivity. The operation of this principle in our system is strongly

185   supported by the observation that all 5 of the highest diversity communities occur in the

186   centre of the productivity range (Figure 3). This relationship is reminiscent of the hump-

187   backed distribution discovered in other ecosystems (Grime, 1973; Huston, 1979). In line with

188   the predictions of diversity disturbance relationships, disturbance was associated with

189   reduction of the advantage possessed by generalists; and as a consequence, was associated

190   with increased syntrophy between fermentative and respiratory bacteria. This diversity-

191   disturbance relationship (DDR) coincided with community succession toward either a low

192   diversity generalist or high diversity specialist community. We found in our system that the

193   DDR has a monotonic pattern of increase in diversity with disturbance. This monotonic

194   relationship is expected when the reproductive rates of all species exceed the maximum rate

195   of mortality due to disturbance (Svensson *et al*, 2012). Under these conditions disturbance

196   will never be high enough to reduce diversity, truncating the unimodal distribution. As most

197   culturable bacteria have very short division times compared with our measurement intervals,

198   the monotonic relationship would reasonably be expected for our system.

199

200   Synthetic systems have been suggested as experimental tools to control environmental

201   constraints and reduce variability in microbial ecology studies (Konopka *et al*, 2015). Our

202   results clearly demonstrate the potential for microbial electrochemical systems to be used as

203   microcosms in which to model fundamental ecological relationships existing within microbial

204   ecosystems, as it has been previously proposed (Dolfing 2014). Thus far, the bulk of microbial

205   electrochemistry research is directed towards extending the range of feed-stocks, improving

206   power output, optimising cell design and finding novel electrogenic bacteria (Jia et al, 2003;

207   Logan *et al*, 2006; Chaudhury and Lovley, 2003; Yong *et al*, 2012; Zhuang *et al*, 2012).

208   Microbial fuel cells generate a direct current which gives an instantaneous and easily

209   measured proxy for community productivity (Thurston *et al*, 1984). The advent of next

210   generation sequencing methods also allows the collection of detailed community abundance

211   data for hundreds or thousands of species across replicate systems at multiple time points.

212   This can be combined with the ability of MFCs to support high diversity biofilm cultures across

213   extended time horizons, rendering anode associated electrogenic biofilms a tractable model

214 for microbial community succession. This approach has allowed us to demonstrate the
215 operation of a diversity disturbance relationship within electrode associated biofilms.
216

Conclusions

218 A large amount of evidence has previously shown that diversity improves productivity in
219 ecosystems (Tilman *et al*, 1996; Loreau *et al*, 2001). This corresponds to one possible
220 trajectory within our Multiple Linear Model. Dominant organisms have also been shown to
221 contribute more to system productivity than species with subordinate rankings (Smith and
222 Knapp, 2003). This corresponds to the second possible trajectory within our 3D MLM. Our
223 findings suggest that both of these relationships can function in MFC biofilm communities.
224 Increased biodiversity improves community productivity for mixtures of specialists. However,
225 a higher abundance of competitive dominants will also increase productivity. During
226 succession, generalists (RF) will tend to prevail over specialist organisms and disturbance will
227 tend to mitigate their advantage. Succession can follow either path from the same starting
228 point and the trajectory taken depends on the level of disturbance measured. The alternative
229 trajectories of succession we have observed, when examined together, explain the
230 approximate unimodal distribution of diversity along the dimension of biofilm productivity.
231 This pattern contributes to explaining variation in community structure where starting abiotic
232 conditions are otherwise identical.
233

Materials and Methods
235 **Microorganisms and media.** MFCs were inoculated with anaerobic digester sludge
236 originated in a biosolids mesophilic digester (Cog Moors Sewage Treatment Works, Cardiff,
237 UK). Solids were removed by sieving through a 0.6-mm mesh (Endecotts Ltd., UK), and the
238 collected material was stored at 4°C. The culture medium contained (g l^{-1}): NH$_4$Cl, 0.31;
239 NaH$_2$PO$_4$·H$_2$O, 5.38; Na$_2$HPO$_4$, 8.66; KCl, 0.13. The pH was adjusted to 7.0, and the medium
240 was supplemented with 12.5 ml l^{-1} of a trace mineral solution and 12.5 ml l^{-1} of a vitamin
241 solution (Lovley et al. 1984). The medium used for the batch operation contained 5 g l^{-1}
242 sucrose, while the medium for the continuously operated MFCs contained 0.1 g l^{-1} sucrose.
243 All media were autoclaved at 121°C for 15 min, except for the vitamins, mineral and sucrose
244 solutions, which were sterilised by filtration through a 0.2-μm pore size membrane
245 (Nalgene, USA).

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247 **MFC set up.** MFCs consisted of 9 cm^3 Perspex anode chambers and cover plates, with

248 stainless steel metal plates serving as a contact between the air-breathing cathode and the

249 electrical circuit. The anode (geometric area: 32 cm^2) was made of carbon fibre veil (PRF

250 Composite Materials, UK) with polyvinyl alcohol binder to improve anodic capacity. The

251 anode was connected to the electrical circuit with an insulated Ni/Cr wire weaved across the

252 anode. The air-breathing cathode was made of type A carbon cloth (geometric area 9 cm^2;

253 E-TEK, USA) coated with 4 mg cm^{-2} of Pt black catalyst and polytetrafluoroethylene binder.

254 The Pt side of the cathode was painted with 0.5– 1.0 mg cm^{-2} of Nafion perfluorinated ion-

255 exchange ionomer (5% w/v in lower aliphatic alcohols and H_2O). Anode and cathode

256 chambers were separated by a Nafion-115 proton-exchange membrane (20 cm^2, DuPont,

257 USA). The membrane was pre-treated by boiling sequentially for 1 h in 6% w/v H_2O_2, H_2O,

258 0.5 M H_2SO_4 and H_2O. The pre-treated PEM was stored in deionised water sheltered from

259 light until assembling the MFC.

260

261 **MFC operation.** Replicate MFCs were inoculated with a 10% v/v suspension of anaerobic

262 digester sludge in sucrose-containing medium. MFCs were operated for approximately 2

263 weeks to ensure establishment of the anodic biofilm. During that time, the anodic

264 suspension was replaced with fresh N_2-purged fresh medium until a stable biofilm was

265 present, as assessed by constant output and repeatable cycles of voltage generation. The

266 MFCs were then operated in continuous mode, supplying fresh medium at a flow rate of

267 0.18 mL min^{-1}. The MFCs were operated at room temperature (21–22°C), and anaerobic

268 conditions were kept by maintaining a continuous flow of oxygen-free N_2. Samples were

269 taken for chemical and microbial community analysis.

270

271 **Chemical analyses.** Total carbohydrate consumption was calculated as the difference

272 between the concentration of carbohydrates in the influent and the effluent medium,

273 determined by a colorimetric method (Dubois et al. 1956). Chemical oxygen demand (COD$_{Cr}$)

274 was analysed according to the standard method (SFS 5504 1988). Coulombic efficiency was

275 calculated as previously described (Logan et al. 2006). The pH of the effluent medium was

276 monitored using a pH meter (Mettler Toledo MP220, Switzerland).

277

278   **Electrochemical measurements.** The electrical output was monitored using a battery tester

279   (Arbin BT2000, Arbin Instruments, USA) controlled by dedicated software (MITS Pro, Arbin

280   Instruments, USA) across a fixed external resistance of 40 kΩ. Polarisation curves were

281   recorded by measuring the decrease in voltage when external resistances were varied over

282   the range 700 kΩ to 500 Ω, for 5 min for each resistance. The volumetric power density was

283   calculated as $P=UI/V$, where U is the measured voltage, I is the current and V is the volume

284   of the anodic suspension. The ohmic internal resistance of the MFCs was measured by

285   electrochemical impedance spectroscopy. Impedance spectra were recorded between

286   anode and cathode in the frequency range of 0.1 Hz– 1 MHz and with a sinusoidal

287   perturbation of 10 mV amplitude under open circuit voltage using a frequency response

288   analyser (Solartron Analytical 1260) and a potentiostat/galvanostat (Solartron Analytical

289   1287, Solartron Analytical, UK) (Zhao et al. 2008).

290

291   **Microbial community analysis.** Total DNA samples were obtained from the anodic biofilm

292   and from the anodic suspension using FastDNA Spin Kit for Soil (MP Biomedicals, UK). MFCs

293   were temporarily disassembled in an aseptic environment, and a 1 cm^2 fragment of the

294   anode was cut out with a sterile scalpel. The MFCs were immediately reassembled and

295   operation was restarted. For the planktonic DNA samples, anode suspension samples were

296   centrifuged (10,000×g, 5 min), washed three times with 1 ml phosphate-buffered saline

297   (NaCl, 8.0 g.l-1; KCl, 0.2 g.l-1; Na2HPO4, 1.15 g.l-1 ; KH2PO4, 0.2 g.l-1; pH 7.3) and

298   resuspended in 100 µl of nuclease-free water (Promega, UK).

299

300   **Sequence analysis.** Fifty-four samples were analysed in Mothur using the 454 SOP Pipeline

301   (Langille et al 2013). The forward primer used was 28F-GAGTTTGATCNTGGCTCAG. To reduce

302   sequence error, trim.seqs() was implemented, with the parameter minlength set to 250. The

303   kmer searching method was employed to align sequences by using a Silva bacterial database

304   (www.arb-silva.de/), with the flip parameter set to true, allowing for the reverse complement

305   of the sequence to be aligned for better results. The screen.seqs command was implemented

306   to remove any sequences outside the 2.5%-tile to 97.5%-tile range of sequences. The

307   filter.seqs was used to remove empty columns from the alignment, which gives the length of

308   filtered alignment to 897. To classify the sequences, we used a RDP database/reference

309   sequence files and the Wang method (Wang et al 2007).

310   To minimise artefacts and maintain the same number of reads in each sample, singleton OTUs
311   and OTUs < 10 reads in any sample were collated into OTU_singletons and OTU_rare
312   phylotypes, respectively. The taxonomic affiliation of the partial 16S rRNA gene sequences
313   (from Phylum to Genus) were determined using the RDP MultiClassifier script (Wang et al
314   2007) to generate the RDP taxonomy while species level taxonomies of the OTUs were
315   determined using the USEARCH algorithm (Edgar 2010) combined with the cultured
316   representatives from the RDP database. Alpha and beta indices were calculated from these
317   datasets with Mothur and R using the Vegan package (Oksanen et al 2013).
318   PICRUSt analysis allows for phylogenetic prediction of organismal traits using the 54 samples
319   from the Mothur analysis. The shared file (representing the number of times that an OTU is
320   observed in multiple samples) generated by Mothur was converted into a biom file
321   (make.biom), from where it is possible to follow the PICRUSt metagenomics pipeline (Langille
322   et al 2013). The first step was the normalisation of the OTU table (biom), enabling the creation
323   of a metagenomics functional prediction table (predict_metagenomes.py). Using --
324   type_of_prediction parameter, allows for KEGG Orthologs predictions and COGS analysis on
325   the samples. We used the collapse predictions into pathways function
326   (categorize_by_function.py) to examine KEGG results from a higher level within the pathway
327   hierarchy.
328
329   **Statistical analysis and model construction.** Sequencing data was used to calculate the
330   Shannon index, a measure of the diversity of the biofilm microbial communities. This can also
331   be expressed as a true diversity value with units of equivalent species (ES) (Hill, 1973;
332   Stratford *et al*, 2014). We classified the OTUs identified in the anodic biofilms into metabolic
333   types based on their known metabolism or that of a closely related species. The metabolic
334   types R (non-fermentative respirator), F (fermenter) and RF (respiratory fermenter) were
335   assigned to taxa comprising 99% of total community sequences. Abundance values used in
336   statistical analysis represent relative abundance of sequences attributed to organisms
337   presenting the particular metabolic type in a survey of the literature. Raw data and details of
338   the references used for classification are provided in the supplementary material. The effect
339   of endogenous disturbance in bacterial biofilms was estimated from changes in the biofilm
340   population. While it is currently impossible to detect individual disturbance events or
341   mortality on an individual cellular level in microbial communities, an estimate can be

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342 calculated by measuring total sequences lost between measurement intervals and
343 normalising these losses to the change in total sequences detected. Sequence losses
344 measured in this way provide an estimate for disturbance in the time interval between
345 measurements. The models built here are robust to the use of either the raw loss in
346 sequences or the normalised value. Disturbance may also be interpreted as an indication of
347 other dynamic processes within the system, e.g. cell removal from the biofilm by localised
348 disaggregation or similar mechanisms not constituting mortality. These definitions of
349 disturbance have previously been used interchangeably (Brockhurst *et al*, 2007) and are used
350 in this context for our analysis. Here we define cumulative disturbance as the sum of all losses
351 in the abundance of individual taxa between sampling points measured up to a given time
352 point. In our system, all MFCs were inoculated with the same starting community and treated
353 consistently thereafter, minimising externally induced variation in biofilm disturbance
354 between communities. This means that losses within the microbial community should be
355 predominantly due to endogenous disturbance rather than operator intervention. No
356 assumptions have been made regarding the source and nature of disturbance during
357 construction of our quantitative models. All correlations and regressions were carried out
358 using SigmaPlot v.12.3 (Systat Software, San Jose, CA).

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495   **Legend to Figures**

496

497   **Figure 1.** Simplified summary of current understanding of the biochemistry underlying general

498   operation and syntrophy within MFCs.

499

500   **Figure 2.** Divergent succession in electrogenic microbial communities; communities tend towards

501   either generalist (RF) dominated or diverse (syntrophic mixture of specialist R and F bacteria). (A)

502   Change in the proportion of generalist (RF) with time, r= 0.43, p = 0.049. (B) Change in Shannon

503   diversity (units = equivalent species) with time, r= 0.09, p = 0.69. Open circles identify communities

504   rich in generalists (RF) while closed grey circles identify communities with greater diversity. Symbol

505   coding identifies the same communities in both (A) and (B).

506

507   **Figure 3.** Alternative community profiles, either high diversity or generalist-dominant community

508   types. (A) 3D graph simultaneously showing power density (absolute), Shannon diversity and relative

509   abundance of RF (generalist) microbes. Generalists are defined as those organisms with the capacity

510   for both respiration and fermentation (RF). (B) Plot showing a comparison between values of power

511   density predicted by a simple linear model with the values observed for all communities. See Table 1

512   for linear model details.

513

514   **Figure 4.** Relationship between the relative abundance of generalist and Shannon diversity in MFC

515   communities, r = - 0.75, p < 0.001.

516

517   **Figure 5.** Relationship between historical disturbance in MFC communities and (A) generalist

518   abundance, r = -0.49, p = 0.037. (B) Shannon diversity, r = 0.81, p < 0.001.
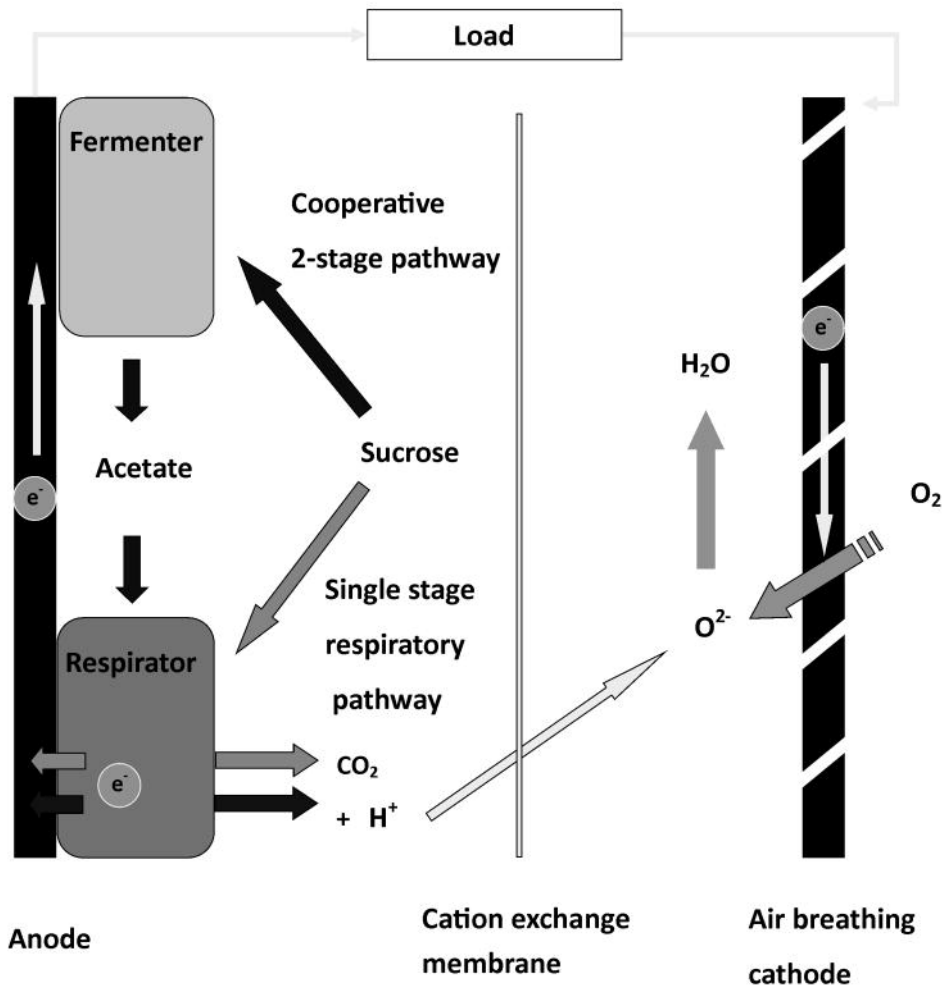
519

520   **Figure 6**. Schematic of alternative minimal community structures in MFCs under different

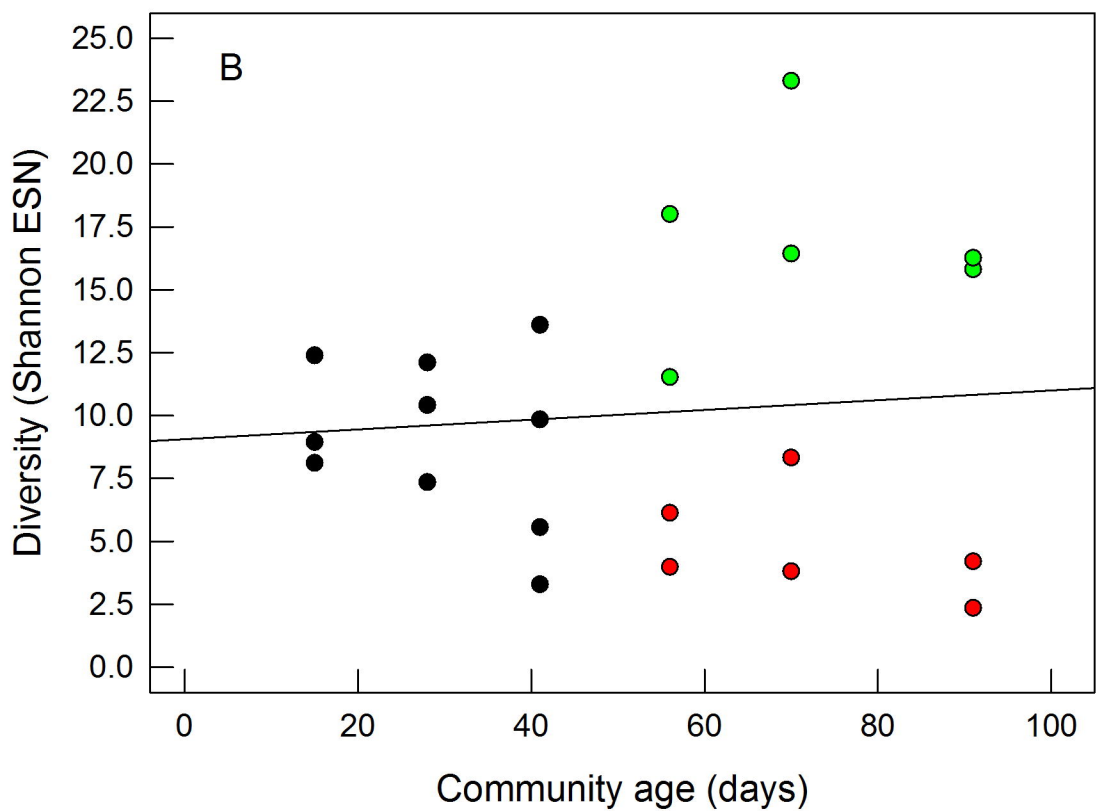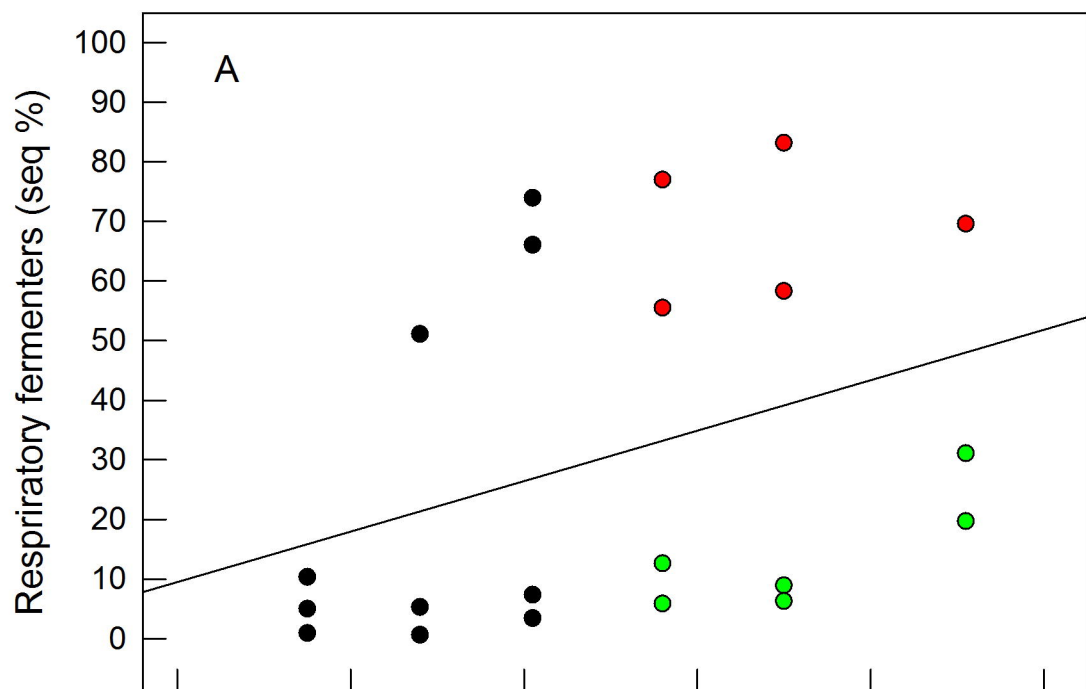521   disturbance regimes. Arrows indicate transfer of chemical species between biofilm cells.

522

523

17

524 **Table 1.** Summary for multiple linear model (MLM) predicting the productivity of MFCs.

525 Power density per unit of detected community DNA was used as the dependent variable,

526 while relative abundance of generalist taxa, DNA per cm^2 of electrode and Shannon diversity
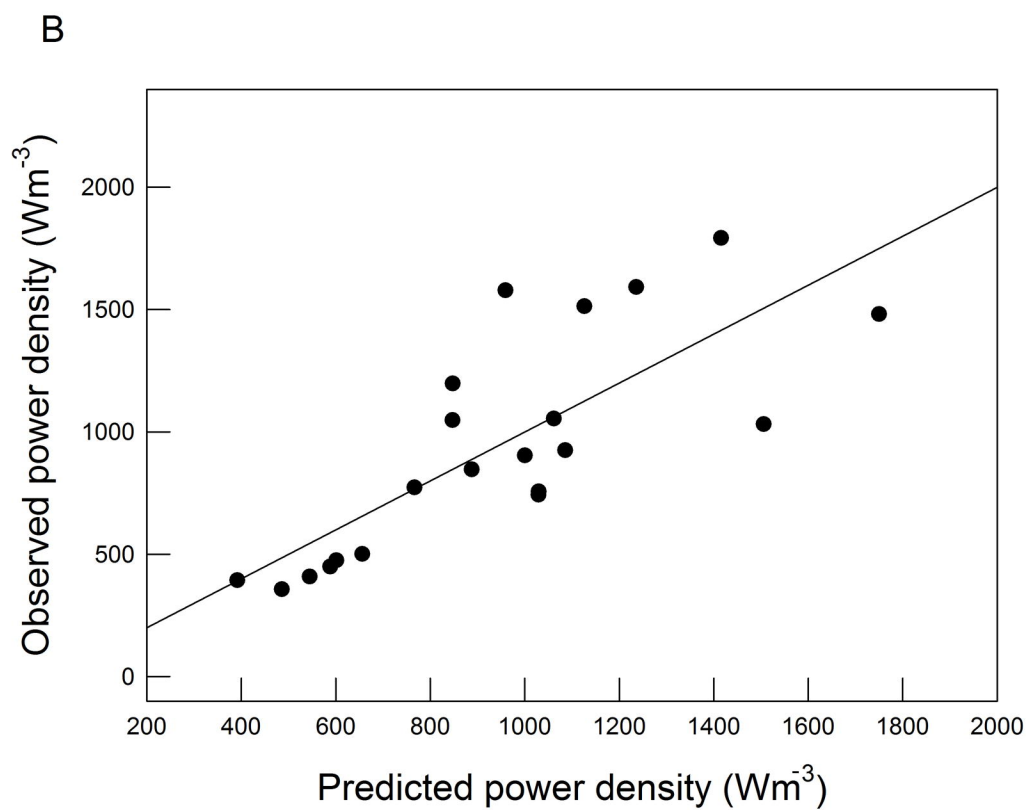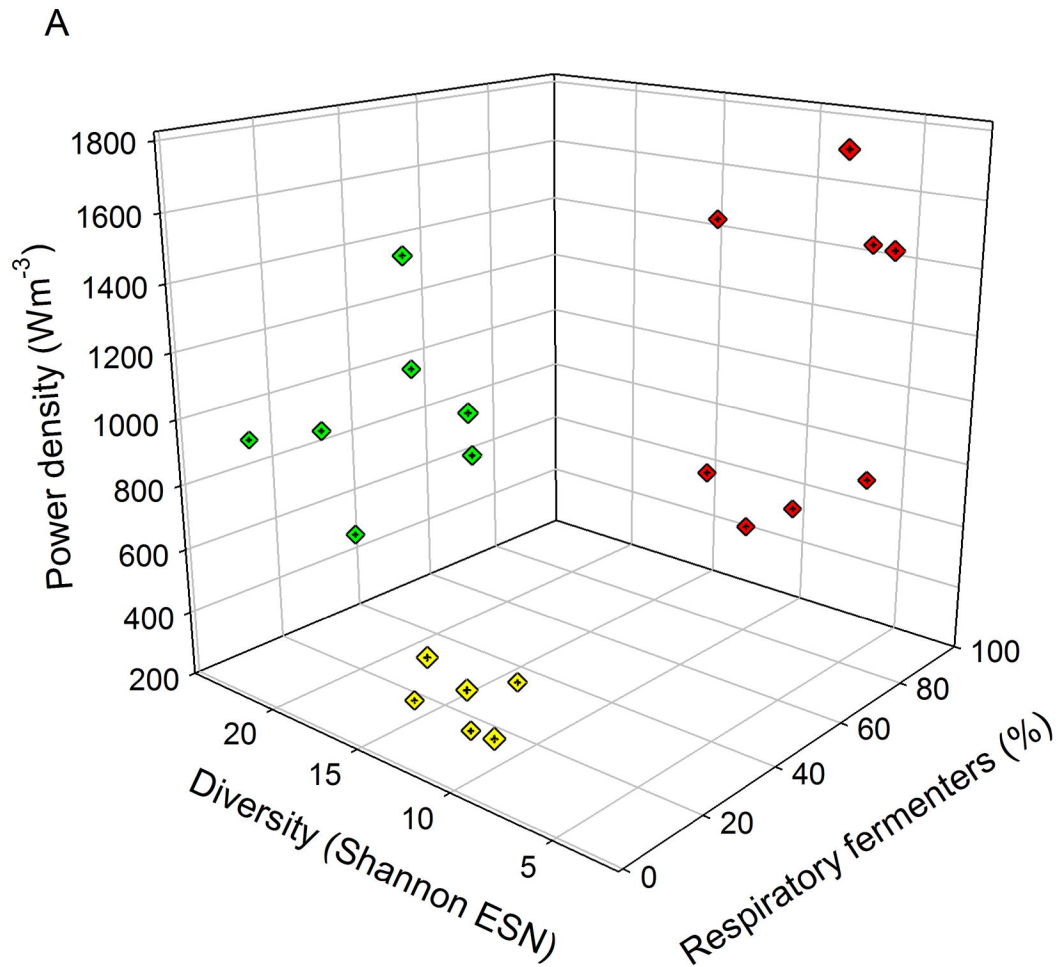
527 were used as the independent variables.
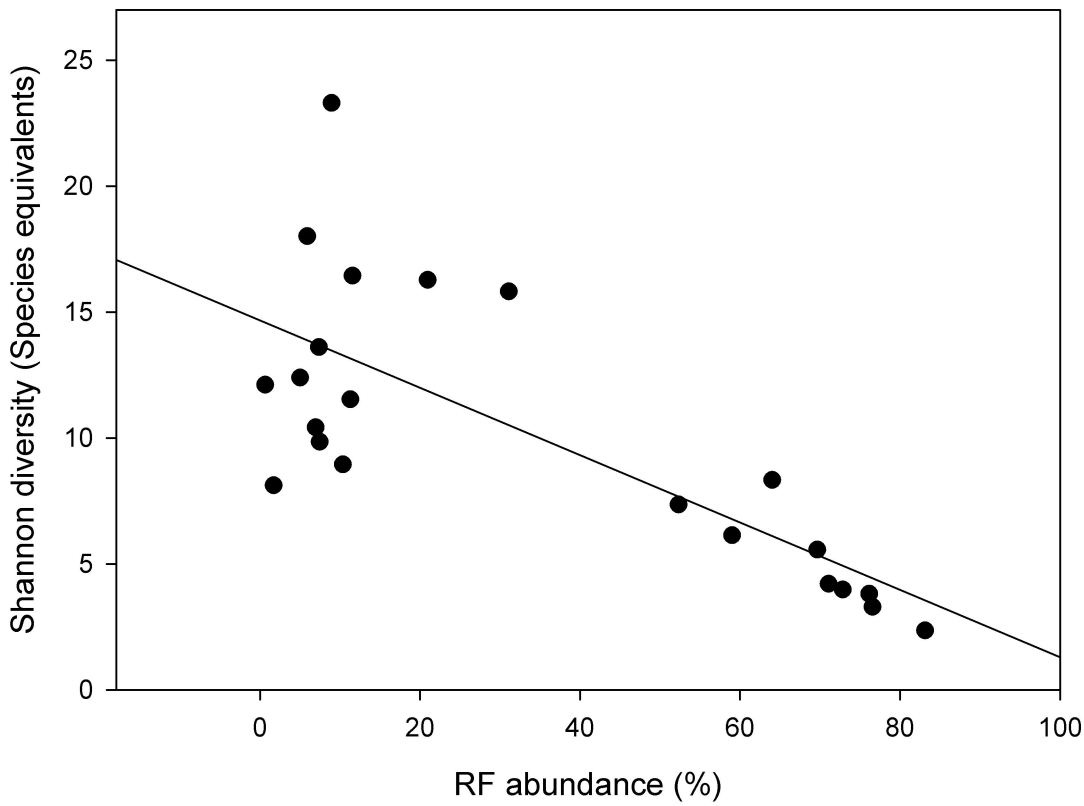
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529

Multiple linear model summary

	R	R²	R² adj	p
	0.75	0.56	0.48	0.003

530

	Coefficient	Std. Error	t	P
531 532				
533 Constant 534	- 9.422	7.814	- 1.206	0.244
535 Shannon Index 536	9.289	2.757	3.370	0.004
537 Generalist abundance (%) 538	0.223	0.0526	4.236	< 0.001
539 DNA concentration (µg/cm^2)	- 0.0688	0.0271	- 2.539	0.021

540

541

18

Load

Fermenter

Cooperative
2-stage pathway

Acetate

e^-

Sucrose

Single stage
respiratory
pathway

Respirator

e^-

CO_2

$+ \; H^+$

H_2O

O^{2-}

e^-

O_2

Anode

Cation exchange
membrane

Air breathing
cathode
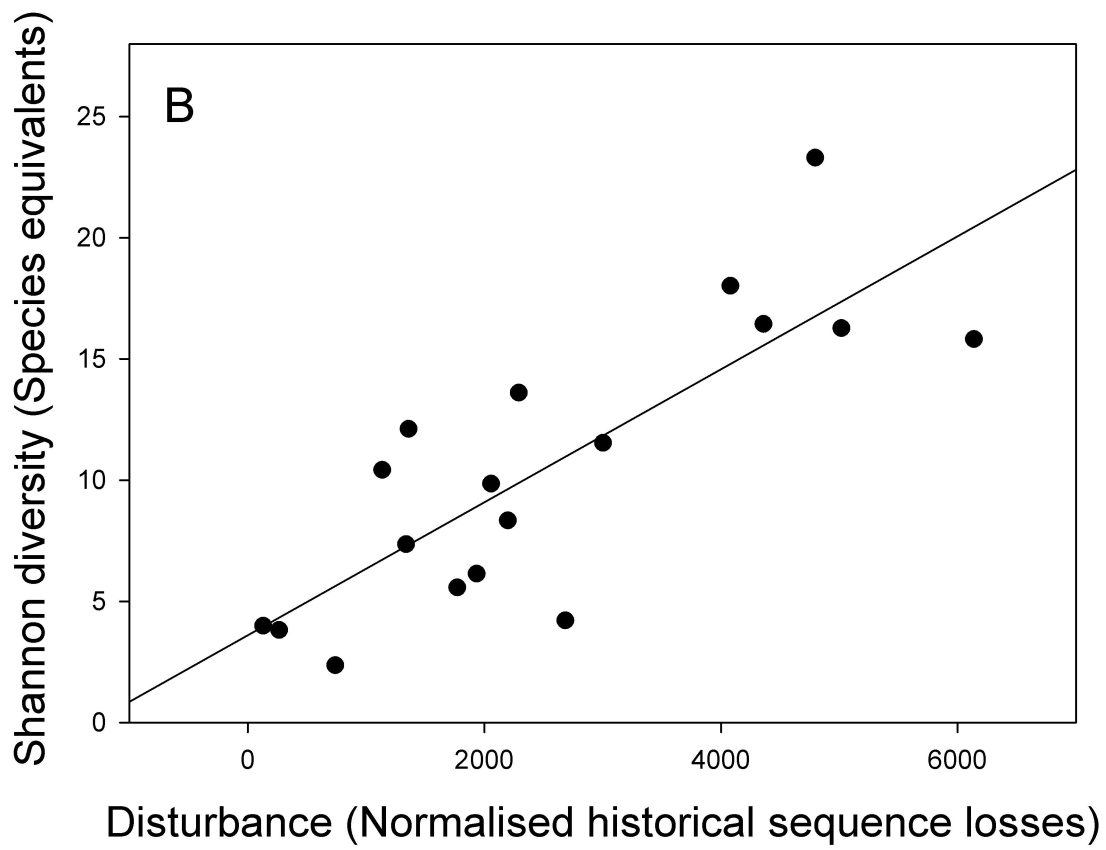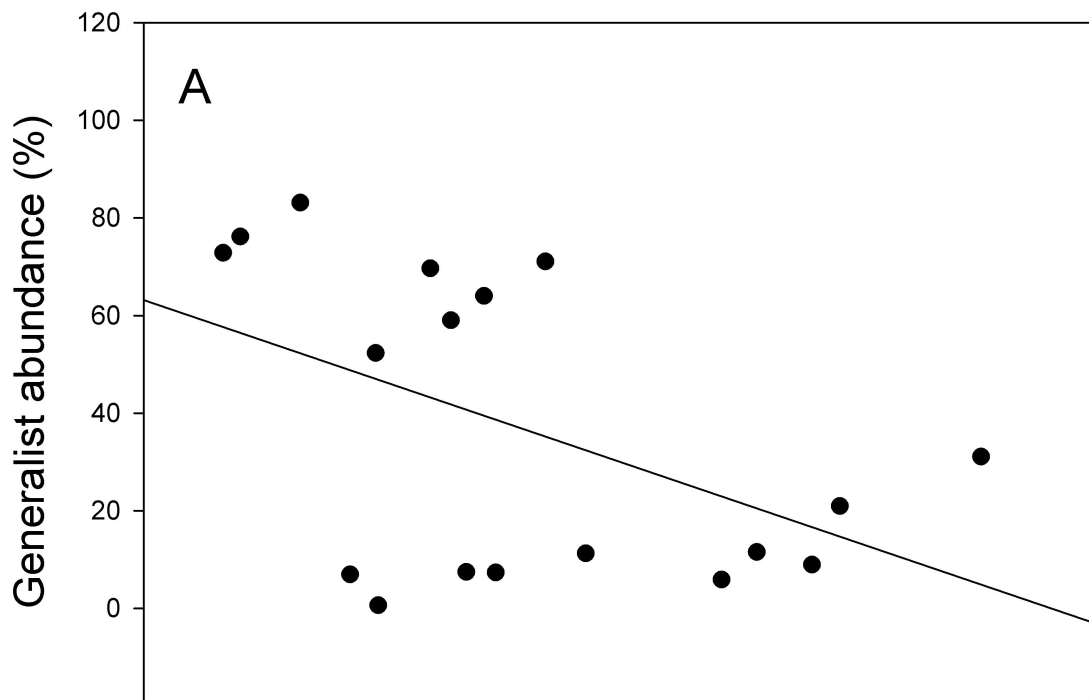
A High disturbance

Anode

Specialist Fermenter (F)

Carbohydrate

VFA

Specialist Respirator (R)

H$^+$

CO$_2$

e$^-$

e$^-$

B Low disturbance

Anode

Generalist RF

Carbohydrate

H$^+$

CO$_2$

VFA

Carbohydrate

Generalist RF

H$^+$

CO$_2$

e$^-$

e$^-$