Minimal requirements for membrane adaptation of the bacterial model organism *M. extorquens*

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Abstract

Cells, from microbes to man, adapt their membranes in response to the environment to maintain functionality. How cells sense environmental change/stimuli and adapt their membrane accordingly is unclear. In particular, how lipid composition changes and what lipid structural features are necessary for homeostatic adaptation remains relatively undefined. Here, we examine the simple yet adaptive lipidome of the plant-associated Gram-negative bacterium *Methylobacterium extorquens* over a range of chemical and physical conditions. Using shotgun lipidomics, we explored adaptivity over varying temperature, hyperosmotic and detergent stress, carbon sources, and cell density. Globally, we observed that as few as 10 lipids, representing ca. 30% of the lipidome, characterized by 9 structural features account for 90% of the total changes. We revealed that variations in lipid structural features are not monotonic over a given range of conditions (e.g. temperature) and are not evenly distributed across lipid classes. Thus, despite the compositional simplicity of this lipidome, the patterns in lipidomic remodeling suggest a highly adaptive mechanism with many degrees of freedom. Our observations reveal constraints on the minimal lipidomic requirements for an adaptive membrane and provide a resource for unraveling the design principles of living membranes.

33 1. **Introduction**

34 All organisms have at least one membrane that is crucial for compartmentalizing and coordinating
35 biochemical processes within the cell. And yet, nearly a century since the discovery that membranes
36 are made of a lipid bilayer (Gorter and Grendel, 1925) and half a century since integral membrane
37 proteins were proposed in the Fluid Mosaic model (Singer and Nicolson, 1972) we still lack
38 fundamental insight into the design principles required to engineer a functional cell membrane. Much
39 of the membrane's functionality is associated with the activity of membrane proteins, which perform
40 a diverse range of tasks from signaling to transport. The activity of such proteins is in turn crucially
41 dependent on the biophysical properties of the membrane such as viscosity, thickness, curvature and
42 bilayer asymmetry (Hunte and Richers, 2008; Klose et al., 2013; Pomorski and Menon, 2006; Sanders
43 and Mittendorf, 2011). These properties are largely dictated by membrane lipids (Ernst et al., 2016;
44 van Meer et al., 2008).

45

46 Cells produce many lipids with different structures that, in their unique combinations, determine the
47 membrane's biophysical state. Moreover, cellular lipidomes can vary from tens, for example in the
48 bacterial lipidome reported here, to hundreds of individual lipid species in mammalian cells (Sampaio
49 et al., 2011; van Meer et al., 2008). A major challenge in membrane research is, therefore, to
50 understand how cells regulate individual lipid abundances to maintain an adaptive, functional
51 membrane. Recent progress reconstituting synthetic membranes from the bottom up is paving the
52 way towards the goal of building a self-sustaining adaptive membrane and presently one of the
53 challenges is to implement adaptive lipid remodeling (Brea et al., 2016; Buddingh and van Hest,
54 2017; Hardy et al., 2015; Tang et al., 2014). Complex lipidomes can be remodeled to modulate
55 collective membrane properties such as viscosity, lipid packing, thickness and phase properties
56 (Klose et al., 2013; Levental et al., 2016). For instance, increasing the proportion of lipids containing
57 double bonds (acyl chain saturation) or changing the number or position of double bonds along the
58 phospholipid acyl chains, can alter the overall membrane viscosity (Quinn, 1981).

59

60 Singular observations of lipidome composition do not inform which properties the cell membrane
61 senses and adapts to, nor which minimal suite of lipid structure are required for swift adaptation.
62 Lipidomics has provided global insights into the complexity of cellular lipidomes from yeast to man
63 and can provide insight into how cells remodel lipid composition during adaptation (Ejsing et al.,
64 2009). To date, however, there still are only few studies systematically characterizing lipidomic
65 remodeling across a range of conditions and stressors. The most comprehensive study of this kind

66 was performed on yeast (Klose et al., 2012), whose lipidome contains over 150 unique lipids,
67 providing unprecedented insight into the adaptation of a complex eukaryotic lipidome.
68
69 Complex organisms possess membranes with diverse and niche-specific functional requirements.
70 While understanding complex lipidomes is important, such systems are not ideal for exploring the
71 fundamental principles underlying lipidome adaptation. Since all membranes require means to
72 homeostatically adapt to perturbations (Kaiser et al., 2011), a simpler organism that is capable of
73 adapting to a broad range of environmental conditions would be a more suitable model system to
74 elucidate the minimal lipidomic requirements for adaptation. Bacteria are an attractive target for this
75 endeavor since some are among the simplest organisms, capable of surviving over a broad range of
76 environments, and many species have been well-characterized and established as model organisms
77 for cell biology.
78
79 Here, we characterized the lipidomic adaptivity of the Gram-negative bacterium *Methylobacterium*
80 *extorquens*. *M. extorquens* has a small lipidome, with 25 phospholipid species as compared with over
81 100 in E. Coli (Jeucken et al., 2019). Despite its relatively small lipidome, as a plant- and soil-
82 associated bacterium, *M. extorquens* must adapt to a broad range of chemical and physical conditions
83 (Vorholt, 2012). We explored the lipidome variation over varying temperature, osmotic and detergent
84 stress, carbon source, and cell density. Surprisingly, we find that most lipidome features remain
85 stable, with only minimal modulations even under significant perturbations. However, other lipidome
86 features are highly plastic and contribute to membrane adaptation. Analysis of structural features such
87 as saturation, acyl-chain length or head-group revealed signatures of adaptive lipidomes. Globally,
88 we observed that as few as 10 lipids, < 30% of all available species, account for the majority of
89 lipidomic adaptation across all perturbations. The patterns of lipidome remodeling that we report here
90 provide a resource for exploring the minimal design principles of living membranes and for designing
91 an adaptive synthetic membrane.
92
93 **2. Results and Discussion**
94
95 **2.1 Experimental Design**
96 To determine lipidomic remodeling induced by environmental perturbations, we measured the
97 lipidomes of *M. extorquens* under physical and chemical challenges to cellular membrane
98 homeostasis (Figure 1a; Figure 1-Figure supplement 1a). To this end, we varied temperature, osmotic
99 conditions, detergent stresses, carbon sources, and cell densities (Figure 1-Figure supplement 2a).

100 Cells were harvested during early exponential growth to minimize their impact on media chemical
101 composition. Temperature was varied from 6 to 30 ˚C to challenge the viscosity of the membranes.
102 Salt (NaCl) and detergent (Triton X-100) concentration were varied to produce osmotic and detergent
103 stresses, respectively. Cells were grown on two concentrations of methanol (0.1 and 1% v/v) as the
104 sole carbon source to evaluate the effect of metabolism on lipidomic remodeling. Finally, to
105 characterize lipidomic adaptation at varying cell densities, cells were harvested at early, mid and late
106 exponential, as well as stationary growth stages (Figure 1b). Cellular lipid content was analyzed by
107 high resolution shotgun mass spectrometry (Ejsing et al., 2009) to determine absolute abundances of
108 25 phospholipids and two hopanoid species, distributed between 5 lipid classes and representing over
109 90 mol% of the lipidome of *M. extorquens* (Sáenz et al., 2015)(Figure 2).
110
111 It has been previously shown that *M. extorquens* produces lipids with 16 and 18 carbon atoms-long
112 acyl chains, and with up to 2 double bonds per acyl chain (Bradley et al., 2017; Wallace et al., 1990).
113 Consistently, the major PG, PE, and PC species have cumulative chain length of 36 or 34 carbons
114 with two double bonds per lipid (e.g. 18:1/18:1 and 18:1/16:1) and minor species with up to 4 double
115 bonds per lipid. We also observed methylation of diplopterol (2Me-DIP) which has previously been
116 reported in cyanobacteria and methylotrophic bacteria including *Methylobacterium* (Bradley et al.,
117 2017; Rashby et al., 2007; Stampf et al., 1991; Summons et al., 1999). Recent work suggests that 2-
118 methylation may be particularly prevalent among plant-associated bacteria (Ricci et al., 2014). In the
119 following sections, we dissect the data in Figure 2 in order to reveal patterns in lipidomic variation
120 across experimental conditions.
121
122 2.2 **Lipidomic variation across experimental conditions**
123 To broadly characterize the effect of environmental perturbations on membrane remodeling, we
124 evaluated the cumulative lipid variability for each growth condition. Presently there is no gold
125 standard for evaluating lipidomic variability. Lipid variability has previously been reported as the
126 variance over mean of lipid abundance (Klose et al., 2012). This analysis reveals how much a lipid
127 varies relative to its abundance, such that even a very low abundance lipid that has a small change in
128 relation to a total lipidome could have a high variance over mean. However, in this study we aimed
129 to characterize variations that would have an impact on the bulk adaptive properties of the membrane.
130 Since small changes in abundance do not have a considerable effect on the bulk adaptive properties
131 of the membrane, we focused on lipids with high absolute changes in abundance. Therefore, we
132 calculated lipid variability as the standard deviation of abundances over a range of conditions. The
133 total lipidomic variability was then calculated as the aggregate standard deviation of relative

134 abundance for all individual lipid species over the range of the various conditions (e.g. temperature,
135 growth stage, etc.). (Figure 3a). This analysis revealed that temperature has by far the largest effect
136 on lipidomic remodeling, with >2-fold more variability than any other condition. Surprisingly, the
137 smallest effects were observed with detergent challenge.
138
139 To compare general lipidomic features for each individual experimental condition, we performed a
140 principle component analysis (PCA) on lipid species abundances (Figure 3b). PCA is a method that
141 allows for the comparison of multidimensional data by reducing variations down to two-dimensions
142 called principle components. Distance between each point on the PCA plot is proportional to variation
143 in lipid composition between conditions. Most conditions clustered closely with the standard growth
144 condition, shown in red. Thus, lipid composition was not strongly affected by detergent, methanol,
145 low salt concentrations, or during the progression through early, mid, and late growth stage. In stark
146 contrast, bacteria grown at low temperatures, in stationary growth phase, or at high salt conditions
147 (0.2 M NaCl) did not cluster with the standard growth condition, indicating a high extent of lipidome
148 remodeling. Furthermore, temperature had an orthogonal effect compared to stationary growth and
149 high salt conditions, indicating that a different set of lipidomic features is involved in responses to
150 those conditions.
151
152 It is surprising that a membrane destabilizing detergent (Helenius, 1975) did not result in lipidomic
153 remodeling. This could possibly be attributed to the ability of Gram-negative bacteria to resist
154 chemicals through the robustness imparted by barrier function of lipopolysaccharide at the cell
155 surface and active removal of toxins by multidrug transporters (Nikaido, 1996; 2003; Nikaido and
156 Vaara, 1985; Piddock, 2006). The insensitivity of the lipidome to methanol was also unexpected.
157 Klose et al (2012) showed that the yeast lipidome varied greatly with differing carbon substrates
158 based on a similar PCA analysis. Our result suggests that methanol and succinate metabolism do not
159 require different membrane properties in *M. extorquens*. Nonetheless, the dominant effect of
160 temperature is consistent with the large diurnal temperature variations that *M. extorquens* must adjust
161 to in its native habitat on plant leaf surfaces and in soils. It is possible that the mechanisms of lipidome
162 adaptation and the lipidomic composition in *M. extorquens* have evolved to be particularly well-
163 suited for temperature changes.
164
165 2.3 **Variation in individual lipid species**
166 We next examined the individual lipid species involved in membrane adaptation in *M. extorquens*.
167 The highest abundance species generally show the largest degree of variability (Figure 4a and 4b).

168 However, there are some low abundance species that exhibit large variations, notably lipids with 3
169 and 4 double bonds. An important point of emphasis is that low abundance or undetectable species at
170 standard growth conditions can become major components under certain conditions, playing a crucial
171 role in adaptation. The most striking result is that the majority of lipid species do not vary
172 substantially under any conditions, suggesting that only a small fraction of the lipidome is required
173 for adaptive membranes.
174
175 We then asked how many and which lipid species are highly variable for each set of conditions. We
176 defined the highly variable lipid species as those that account for 90% of the total lipidomic variability
177 within any given condition. By this measure we observed that 10-13 lipids are highly variable over
178 any given condition (Figure 4c), meaning that only around 30% of the lipidome is involved in
179 remodeling during adaptation. Of these 10-13 highly variable lipids, 8 are highly variable over all of
180 the conditions tested. These results revealed that only a fraction of the lipidome is involved in adaptive
181 remodeling and point towards the minimum complexity of lipid species required for membrane
182 adaptation. In principle, it is possible that as few as 8 lipid species could support adaptation over the
183 range of experimental conditions examined in this study.
184
185 2.4 **Variation of lipid structural features**
186 Membrane biophysical properties are modulated by changes in the abundance of lipid structural
187 features. For instance, increasing the number of double bonds per lipid reduces membrane viscosity
188 during adaptation to decreasing temperature, whereas various lipid headgroups can impart curvature
189 or surface charge. We analyzed how the various structural features of *M. extorquens* lipids are
190 modulated to glean insight into their sense and response mechanisms of membrane adaptation.
191
192 Headgroup features were differently regulated, as PC, PE and PG were relatively plastic, whereas CL
193 and diplopterols are relatively invariant (Figure 5a). For acyl chains, most of the variability is
194 observed for lipids with 2 and 3 double bonds and with total chain length of 34-36 carbons (Figure
195 5b). When we group acyl chain features separately for each headgroup (e.g. PC-specific unsaturation)
196 PC shows the largest variability for both unsaturation and chain length, whereas PG acyl chain
197 features are nearly invariant. This shows that acyl chain features are regulated independently within
198 each headgroup, and provides a starting recipe for designing a responsive synthetic lipidome.
199
200 To assess how much different features varied for each experimental condition we calculated the
201 variance of lipid features for each range of conditions as depicted by the heat map in Figure 5c. The

highest variation in lipid classes is observed over temperature and growth stage. Interestingly, total lipid unsaturation varies over temperature but is relatively invariant over all other conditions. The most variation in total lipid chain length varies with temperature and salt concentration. These results reveal that membrane adaptation of total lipid features exhibit different patterns in response to different experimental conditions. This shows that the lipidomic adaptation mechanism is capable of unique, orthogonal responses to different perturbations.

2.5 **Progressive remodeling of lipids across experimental conditions**

Assessing lipidomic data for each step within each condition allows us to compare lipids as we go stepwise through the changes. For instance, how does the lipidome compare when the growth temperature is lowered from 30 to 20 to 13 and to 4 degrees, or as cells progress from early to mid, to late and finally to stationary growth phase? We again consider lipids grouped by their features as this allowed us to monitor structural variations that directly impact the membrane's biophysical properties (Figure 6; Figure 6-Figure supplement 1-4).

Class

Lipid class abundances are relatively invariant over most conditions (Figure 6). PC abundance increases with decreasing temperature, with a pronounced change at 6 ˚C. PE abundance increases considerably when cells are in stationary growth stage. These results suggest that lipid class distribution is only adjusted under extreme conditions.

Double Bonds

The total number of double bonds per lipid varies substantially with temperature and is nearly invariant for other conditions. As temperature decreases the abundances of DB3 and DB4 lipids increase, while abundances of DB1 and DB2 decrease. This contrasts with other organisms such as yeast where primarily DB1 and DB2 are adjusted with temperature change (Klose et al., 2012). It is possible that having additional double bonds enhances Methylobacterium's ability to adapt to broad temperatures that they experience on plant surfaces and in soils.

The variation of double bonds per lipid within each headgroup class is not evenly distributed across classes. For instance, for varying temperatures PC shows the highest remodeling whereas PG is nearly invariant. Additionally, for varying salt and growth stages where total unsaturation has little or negligible variability, class-specific PC-DB and CL-DB vary considerably. These variations in PC-DB and CL-DB are nearly equal and opposite resulting in no apparent change in total DB content.

236

237 *Chain length*

238 Total chain length is highly conserved across all conditions, and only varies substantially at 6 ˚C.
239 Since total chain length has a large effect on membrane thickness, which in turn must be matched
240 with membrane protein transmembrane domain length, it is not surprising that this feature is so
241 invariant. The large change in total CL at extreme low temperature suggests that the membrane must
242 take extreme measures to adapt as it approaches the end of the range of viable growth temperature.

243

244 Similar to unsaturation, chain length within each headgroup class shows much higher variability than
245 total chain length. For instance, from 30 to 20 ˚C total chain length remains constant while PC chain
246 length decreases and is compensated for by increased chain length in PG, PE and CL. Similar
247 remodeling is also observed for growth stage and salt concentration. Additionally, PC exhibits the
248 highest chain length variation.

249

250 *Diplopterol 2-methylation*

251 A particularly striking variation was observed for the sterol analogue diplopterol and its 2-methylated
252 derivative (Figure 7). Diplopterol is the major hopanoid in *M. extorquens* and is localized in the outer
253 membrane (Hancock and Williams, 1986; Sáenz et al., 2015). We previously demonstrated that
254 diplopterol has a sterol-like ability to modulate the order of saturated lipids such as
255 lipopolysaccharides in the bacterial outer membrane (Sáenz et al., 2012). Subsequent work
256 demonstrated that 2-methylation of hopanoids increases their membrane rigidifying effect (Sáenz et
257 al., 2015; Wu et al., 2015). Therefore, variations in diplopterol 2-methylation could play a role in the
258 adaptation of the outer membrane. While total diplopterol abundances (diplopterol + 2-methyl-
259 diplopterol) did not vary substantially under any conditions, 2-methylation of diplopterol is highly
260 variable in all conditions except methanol and detergent (Figure 7a and b). There is a progressive
261 increase in 2-methyl-diplopterol abundance with decreasing temperature and increasing salt
262 concentration (Figure 7c), consistent with transcriptional regulation of this process by global stress
263 response pathways (Kulkarni et al., 2013). These results show for the first time that 2-methylation is
264 dynamically regulated in response to varying growth conditions and is important for outer membrane
265 adaptation.

266

267 Our observations highlight three characteristics of structural lipid adaptation. First, we observed that
268 adaptivity of features over a particular range of conditions (e.g. temperature) does not involve a
269 monotonic pattern of feature remodeling, but rather can involve different features over intervals of a

270 given condition. Second, certain features such as acyl-chain unsaturation and diplopterol 2-
271 methylation are highly variable and are remodeled over a broad range of conditions. However, other
272 features such as lipid class and acyl-chain length are relatively invariant except under extreme
273 conditions of low temperature and stationary growth stage, suggesting that chain length and
274 headgroup distribution have multiple constraints (e.g. maintaining membrane thickness or surface
275 charge). Notably, even the tightly constrained features are adjusted under extreme perturbations.
276 Third, acyl chain features are regulated independently within each headgroup class. Thus, even when
277 total acyl-chain unsaturation and length appears invariant, there can be extensive acyl chain
278 remodeling within specific headgroup classes. Such class-specific remodeling of unsaturation may
279 result in adaptation that is localized to the inner or outer membrane, or to specific regions within each
280 membrane, such as highly curved regions at the poles, one particular membrane leaflet, lipids that are
281 closely associated with protein transmembrane domains, or potentially lateral membrane domains.
282 On the other hand, constraining total unsaturation while internally shifting double bonds from PC to
283 CL might be a means of varying intrinsic curvature while maintaining total membrane viscosity.
284
285 **Summary and Outlook**
286 In this study, we systematically characterized the lipidome of *M. extorquens* over a range of growth
287 conditions to gain insights into membrane homeostatic adaptation. We demonstrated that membranes
288 composed of only a few lipid species are capable of adapting specifically to a broad range of
289 conditions, in particular temperature. Lastly, we showed that lipidomes must be assessed by lipid
290 species composition, but also by the composite structural features conferred by binned combinations
291 of lipids, such as the total number of double bonds or the global sum of acyl chain lengths, since
292 combination of these structural features ultimately dictates the physical state and functionality of
293 membranes.
294
295 We probed the lipidome composition across a broad range of viable conditions spanning temperature,
296 hyperosmotic and detergent stress, carbon sources, and cell density. While individual lipid species do
297 adapt to changing conditions, eight lipid species are variable in all experimental regimes (Figure 4)
298 and the majority of lipids remained surprisingly stable. PCA analysis reveals that most lipidomes are
299 highly similar across conditions (Figure 3). This demonstrates that the lipidome is robust and that
300 there are strong constraints on lipidome composition and remodelling. One of the outstanding
301 conundrums in membrane biology is to understand why there are so many lipids (Dowhan, 1997;
302 2017), even a relatively simple organism like *E. Coli* has over 100 phospholipid species (Jeucken et
303 al., 2019). Recapitulating such a complex system from first principles seems daunting. However, the

304 lipidome of *M. extorquens* demonstrates that a synthetic membrane capable of supporting life over a
305 broad range of environmental conditions could be constructed with a relatively small number of lipid
306 species.
307
308 The lipidome adaptability is also constrained by structural elements of individual lipids. Instead of
309 analysing lipidomes purely by lipid species, it is therefore critical to assess the global structural
310 composition. For example, total phospholipid chain length, that influences membrane thickness, is a
311 highly conserved feature that only varies under extreme conditions such as low temperature (Jensen
312 and Mouritsen, 2004). Interestingly, phospholipid acyl chain length was highly conserved globally,
313 but variable within certain headgroup classes. Other constraints do not have such clear implications.
314 For instance, phospholipid headgroup composition only varies at low temperature or in stationary
315 growth phase, possibly suggesting conservation of membrane surface charge as a key constraint.
316 Also, the acyl chain composition among phosophilpids with PG headgroup is nearly invariant across
317 all conditions, suggesting that there could be biochemical constraints on PG remodelling. Low PG
318 remodelling has also been observed in cyanobacteria (Pittera et al., 2018), showing that this constraint
319 is not limited to *M. extorquens*. In contrast, we learned that PC acyl chain composition is highly
320 variable and appears to take on the brunt of adaptive remodelling. While the physicochemical and
321 physiological basis for these observations remains to be fully explored, the patterns and constraints
322 in lipidome remodelling that we report here serve as a resource for designing a minimal adaptive
323 membrane and highlight the need for multi-level analysis of lipidome data.
324
325 Understanding how *M. extorquens* adapts with so few lipids, and even fewer that are responsive,
326 remains an open challenge. In particular, given that lipid remodelling did not involve a monotonic
327 change, but rather seemed to involve different features varying over different intervals of a given
328 range of conditions, such as temperature. One possibility is that the presence of isoprenoids, sterol-
329 like hopanoids, buffer changes in membrane properties and thereby minimizes the need for
330 phospholipid remodelling. Our previous data, however, shows that hopanoids primarily interact with
331 lipopolysaccharides in the outer leaflet of the outer membrane, and thus would not be relevant to
332 variations of the phospholipidome within the inner membrane or inner leaflet of the outer membrane
333 (Sáenz et al., 2015; 2012). Alternatively it has also been proposed that in bacterial membranes lacking
334 sterol analogues or complex lipidomes, integral membrane proteins may serve a role in adaptation
335 (Kaiser et al., 2011). Similarly unexplored are the sense and response pathways that underlie such
336 complex patterns of lipidome adaptation. It has emerged in recent years that there can be dedicated
337 membrane sensors, which are involved in regulating lipid biosynthesis pathways in response to

perturbed membrane properties (Ernst et al., 2016; Puth et al., 2015; Saita and de Mendoza, 2015). It is presently unknown how many different sensors are required for adaptation, and how many orthogonal parameters can be distinguished by such sensors. Building on the lipidomic resource presented here, *M. extorquens* is a promising model organism to explore minimal mechanisms for cell membrane adaptation. The adaptive lipidome of *M. extorquens* provides a critical resource as a starting point to elucidate the regulatory systems underlying membrane adaptation.

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Author contributions

Sandra Rizk and Daniel Grosser performed bacterial growth experiments. Andrej Schevchenko and Oksana Lavrynenko performed MS analysis. Grzegorz Chwastek contributed to writing, editing and data analysis. Michał Surma performed MS data analysis and editing. Magda Rucińska performed bioinformatics analysis. Helena Jambor conceived and designed the figures. James Sáenz conceived the project, obtained funding, supervised the project, and wrote the manuscript. All authors read, edited and approved the final manuscript.

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364 **Methods**

365

366 **Media, growth conditions**

367 For all cultivations a defined media, here referred to as Hypho-TMM, was used (Delaney et al., 2013).
368 All liquid cultures were grown at 30°C and pH 7 with shaking at 150 rpm unless stated otherwise.
369 For all experiments we used a strain of *M. extorquens PA1* with the cellulose synthase genes knocked
370 out to minimize clumping and improve cell density measurements (Delaney et al., 2013). Cultures
371 were started from a glycerol stock stored at -80°C were plated on Hypho-TMM agarose plate and
372 grown at 30°C. A liquid pre-culture was set up by sterile picking a single colony and transferring it
373 to 5 ml of Hypho-TMM. This pre-culture was grown over night to near stationary phase. The
374 experimental culture was started by inoculation to OD_{600nm} 0,02 with pre-culture and grown until a
375 final OD_{600nm} of 0,2 - 0,3 (early log-phase) was reached. Several conditions were tested including the
376 influence of salinity, carbon source (MeOH vs. succinate), detergent (up to 5% Triton-X100) and
377 temperature (6 – 30°C). Cells were also harvested at mid-, late- and stationary growth stages. A
378 defined volume representing 6 OD units (1 OD unit = 1 mL of OD 1) was sampled and pelleted. The
379 harvested cells were washed twice with fresh media. The pellet was snap frozen in liquid nitrogen
380 and stored until further processing at -20°C.

381

382 *Hypho-TMM recipe:*

383 1,45 mM K_2HPO_4; 1,88 mM NaH_2PO_4; 45,6 µM sodium citrate; 1,2 µM $ZnSO_4$; 1 µM $MnCl_2$;
384 18 µM $FeSO_4$; 2 µM $(NH_4)_6Mo_7O_{24}$; 1 µM $CuSO_4$; 2 µM $CoCl_2$; Na_2WO_4; 0,5 mM $MgCl_2$;
385 8 mM $(NH_4)_2SO_4$; 20 µM $CaCl_2$
386 30 mM PIPES
387 15 mM succinate unless stated otherwise

388

389 **Shotgun mass spectrometry**

390 For the lipidomic analysis by mass spectrometry the lipids of our harvested cells were extracted twice
391 by the method of Blight and Dyer (Bligh and Dyer, 1959). The total lipid extracts representing 6 OD
392 units of *M. extorquens* were analyzed by quantitative shotgun lipidomics (Ejsing et al., 2009) adapted
393 for the quantification of hopanoids (diplopterol, and 2-methyl-diplopterol). Absolute concentrations
394 of diplopterol and methyl-diplopterol were determined using deuterated cholesterol as internal
395 standard.

396

397 *Measurement of phospholipids*

398 In order to determine the phospholipid classes and species present in the bacterial extracts the MS/MS
399 experiments were performed. Based on fragmentation of lipid species the total lipidome was
400 represented by 4 phospholipid classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE),
401 phosphatidylglycerol (PG) and cardiolipin (CL). For absolute quantification extracts were diluted
402 with MS mix containing the 0,5 µM PC (34:0), PE (34:0), PG (34:0) mixture and 0,05 µM CL (57:4).
403 MS spectra were acquired on Q Exactive tandem mass spectrometer (Thermo Fisher Scientific,
404 Bremen, Germany) equipped with a robotic nanoflow ion source TriVersa NanoMate (Advion
405 BioSciences, Ithaca NY). Spectra were acquired in negative MS mode with the mass resolution of
406 $R_{m/z\ 400}$ = 280000 with AGC target value of 10^6 and maximum injection time of 1 s in the range of
407 masses 400 – 1000 m/z. Raw data were processed using LipidXplorer software (Herzog et al., 2012;
408 2011).
409
410 *Measurement of diplopterol*
411 The extract was dried down and re-dissolved in MS mix (7,5 mM ammonium acetate in
412 isopropanol/methanol/chloroform 4:2:1) spiked with cholesterol D7 standard to the final
413 concentration of 0,13 mM. MS spectra were acquired on Q Exactive tandem mass spectrometer
414 (Thermo Fisher Scientific, Bremen, Germany) equipped with a robotic nanoflow ion source TriVersa
415 NanoMate (Advion BioSciences, Ithaca NY). Gas backpressure was 1.25 psi and ionization voltage
416 was 0.95 kV. Spectra were acquired in positive MS mode with the mass resolution of $R_{m/z\ 400}$ = 140000
417 with AGC target value of 10^6 and maximum injection time of 1 s in the range of masses 360 – 455
418 m/z. Raw data were processed using LipidXplorer software.
419
420 **Data analysis**
421 Lipid abundances (mol% of total) of biological triplicates for all conditions are provided in the
422 supplementary materials. One of the replicates at 13 ˚C yielded erroneous abundance data due to a
423 problem with the internal standard in that replicate and we have therefore excluded it from our
424 analyses.
425
426 Variation of lipid species across experimental conditions was calculated by taking the standard
427 deviation of lipid abundances for replicates over a given range of conditions (e.g. temperature, salt,
428 etc.). Total lipidomic variation was calculated as the cumulative variation of individual lipid species
429 for a given range of conditions.
430

Visualization of lipidome variation for all conditions was investigated with principle component analysis (PCA). PCA was performed by using the R prcomp function in RStudio with default parameters, where the calculation is carried out by a singular value decomposition of the (centered and scaled) data matrix. Visualization in single plots performed using the ggbiplot function. PCA allows the identification of latent variables (principal components) in the data based on 14 observed variables. The plot is based on principal components 1 and 2, which explain 50.1% and 16.5% of the total variance of the data, respectively.

Variations in abundance of lipid features were analyzed by binning lipid species according to structural features including total chain length, saturation, class (e.g. phospholipid head group and diplopterols), and 2-methylation of diplopterol. Acyl chain length and saturation were binned for all phospholipids (global acyl chain features), and separately within each head group (head group specific acyl chain features).

Figure Captions

Figure 1. Overview of experimental outline and lipids analyzed in this study. (A) Overview of experimental design and exemplary data obtained. **(B)** Schematic of lipid structural features measured in this study: headgroups of phospholipids and isoprenoids, acyl chain length and acyl chain saturation (number of double bonds).

Figure 1-figure supplement 1. Bacterial growth rates (Herbert et al., 1956) for all experimental conditions.

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Figure 6. Step-wise adaptation of lipids by feature at changing environmental growth conditions. (A) Abundances of lipids grouped by class, acyl chain length and acyl chain saturation when changing growth temperature, probing at different growth stages, and varying salt (NaCl) concentration of the growth media. **(B)** Abundances of phospholipids grouped by acyl chain features, with headgroups indicated by color (blue: PG, green: PE, olive: PC, magenta: CL). This reveals compensatory effects in lipid adaptability: while abundance of PC with 2 double bonds increases with

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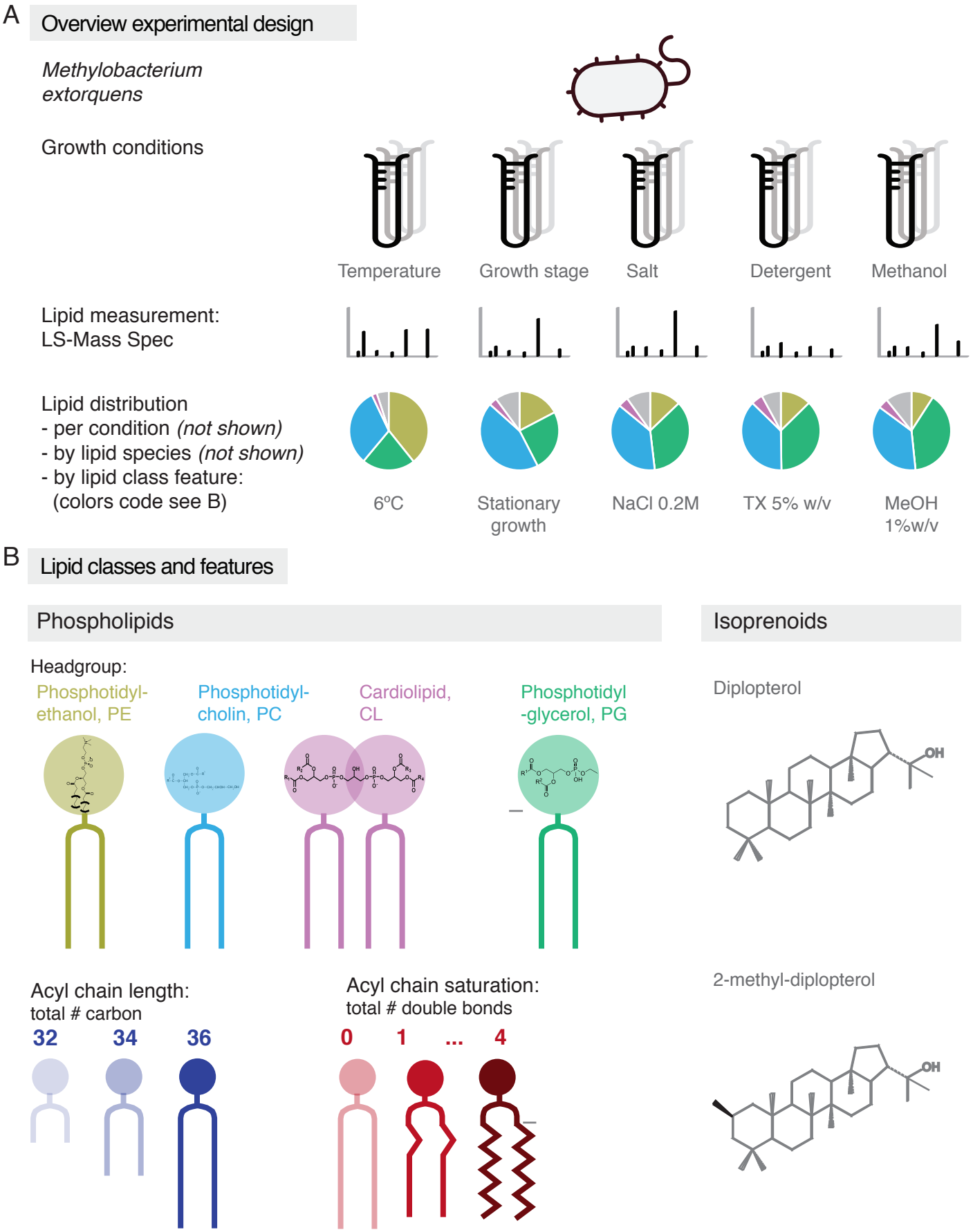

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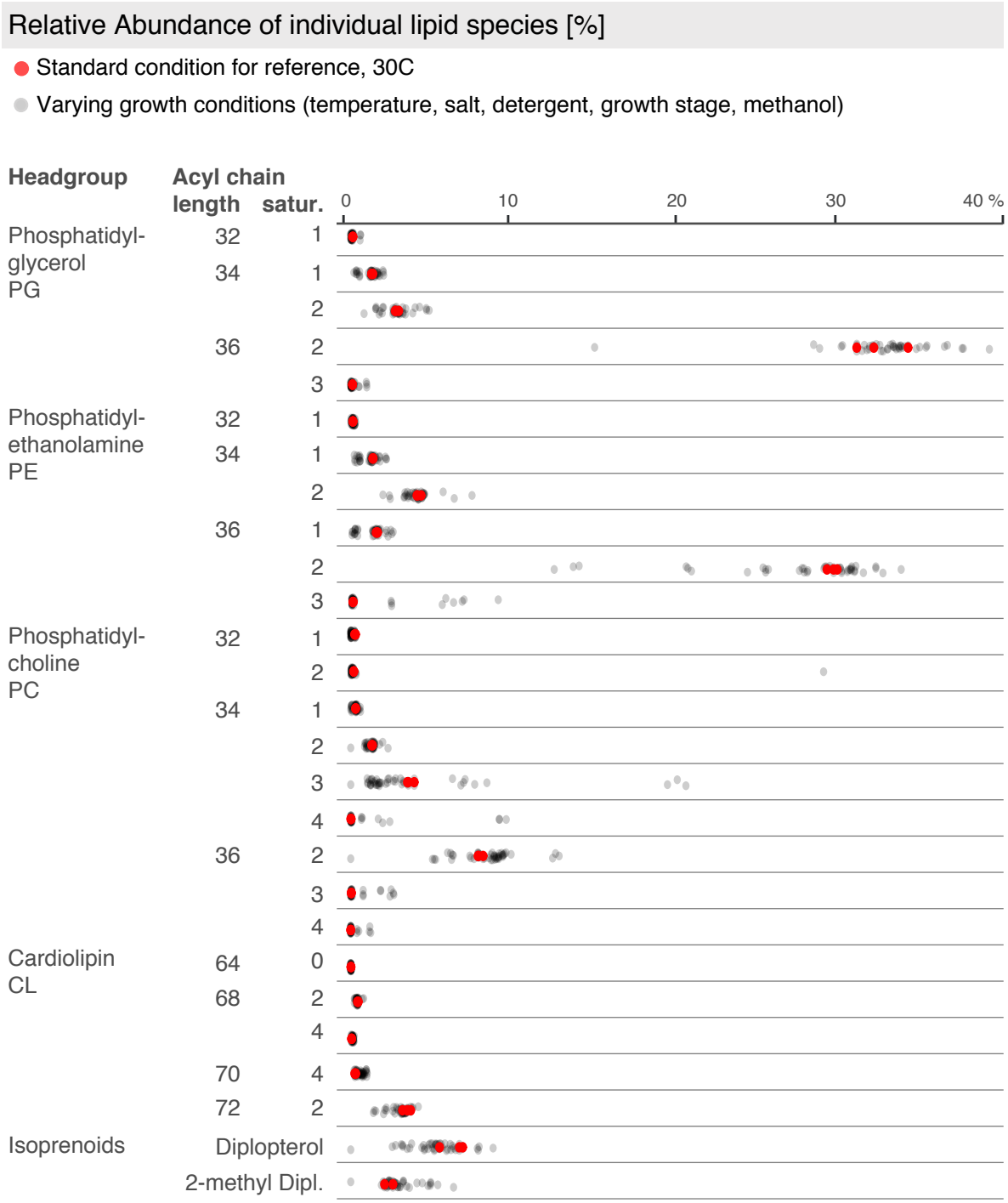

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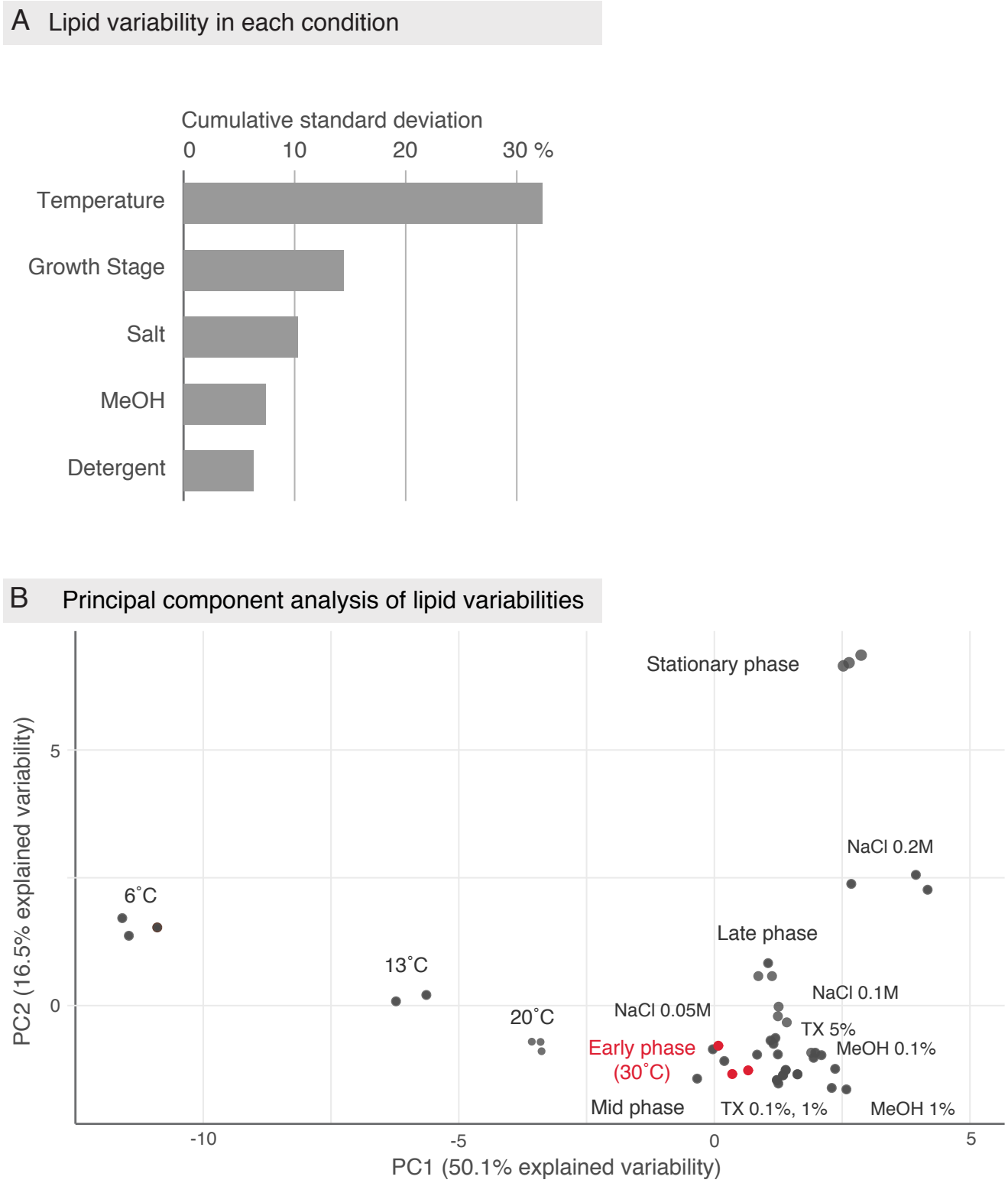

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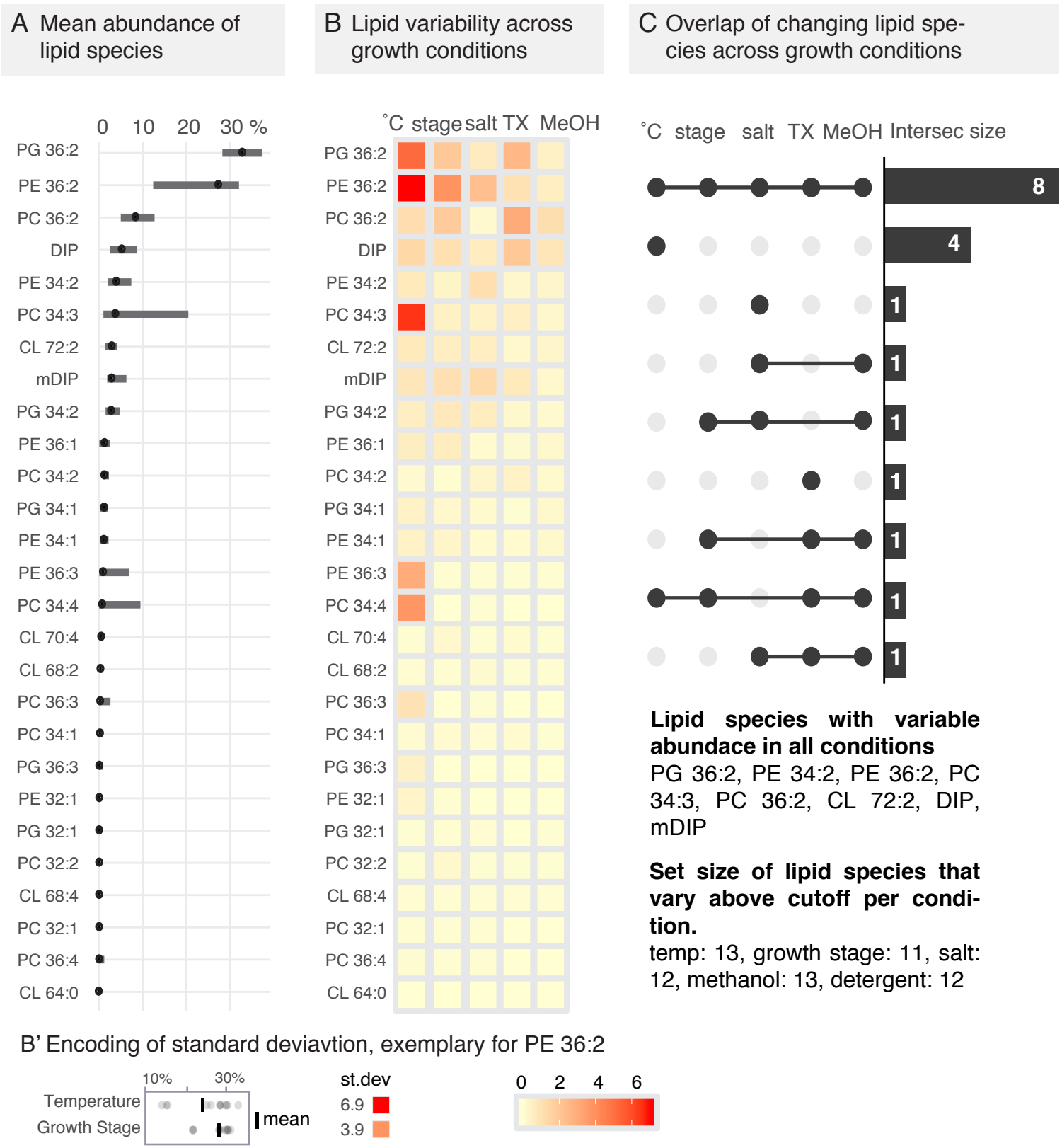

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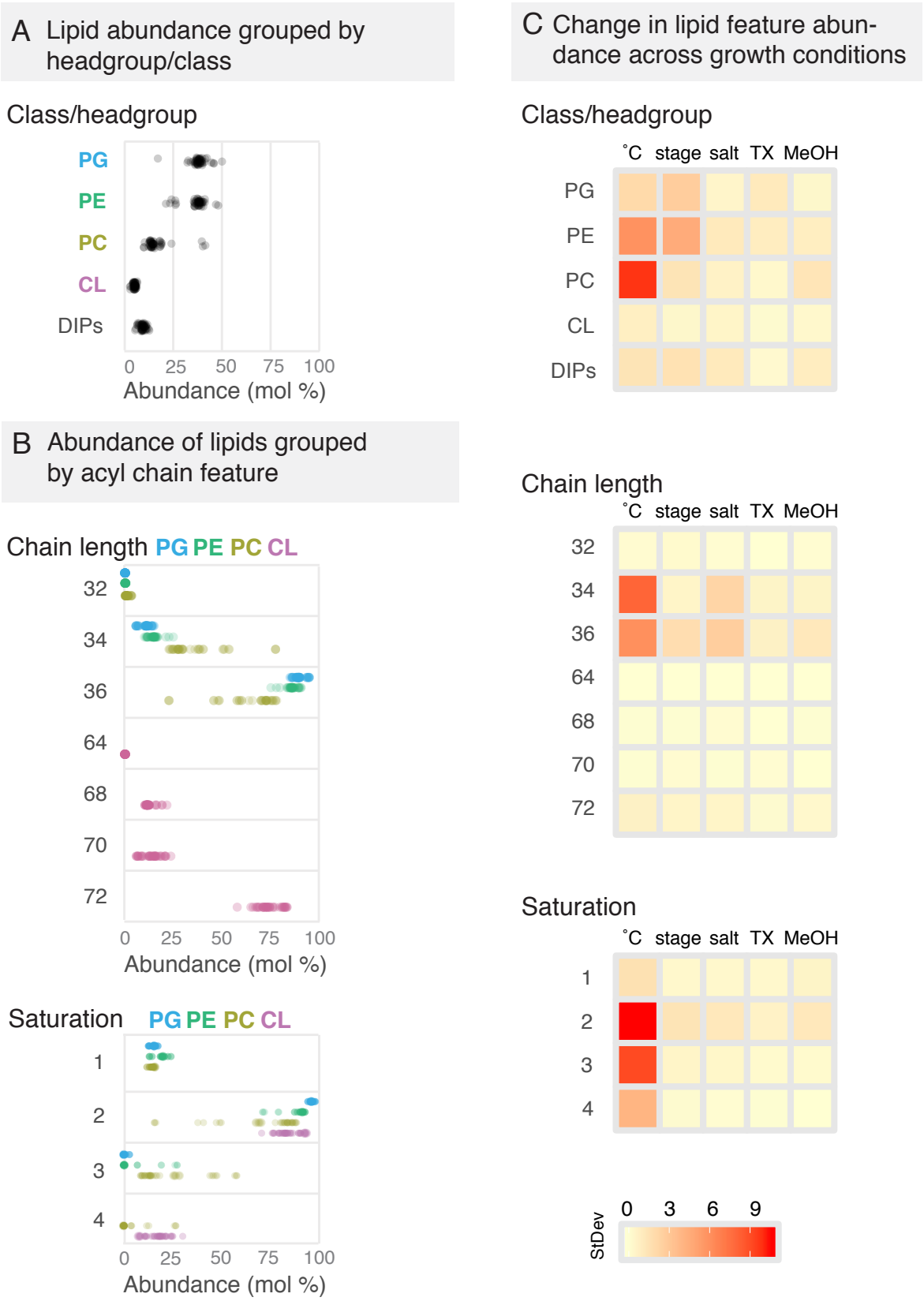

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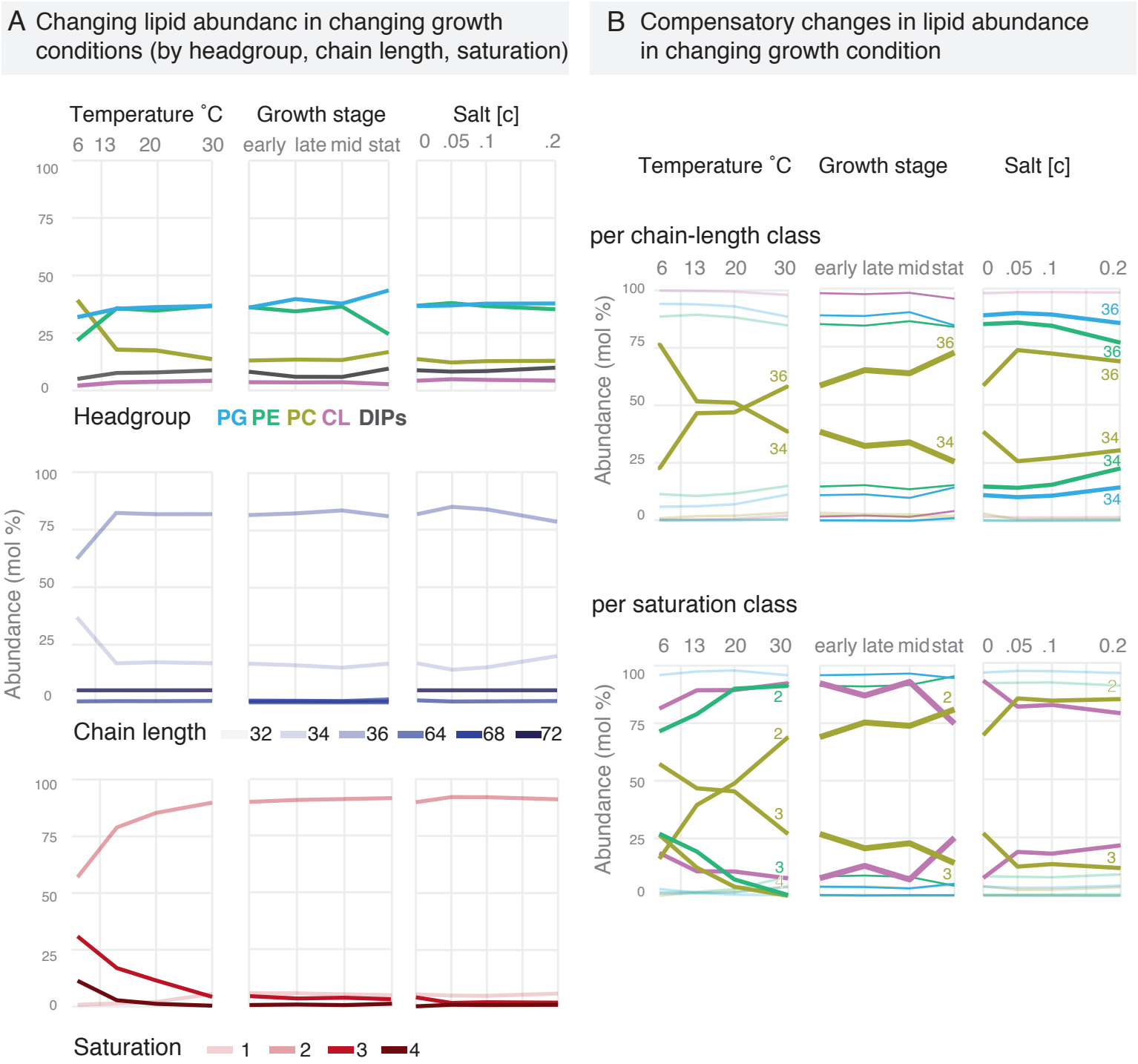

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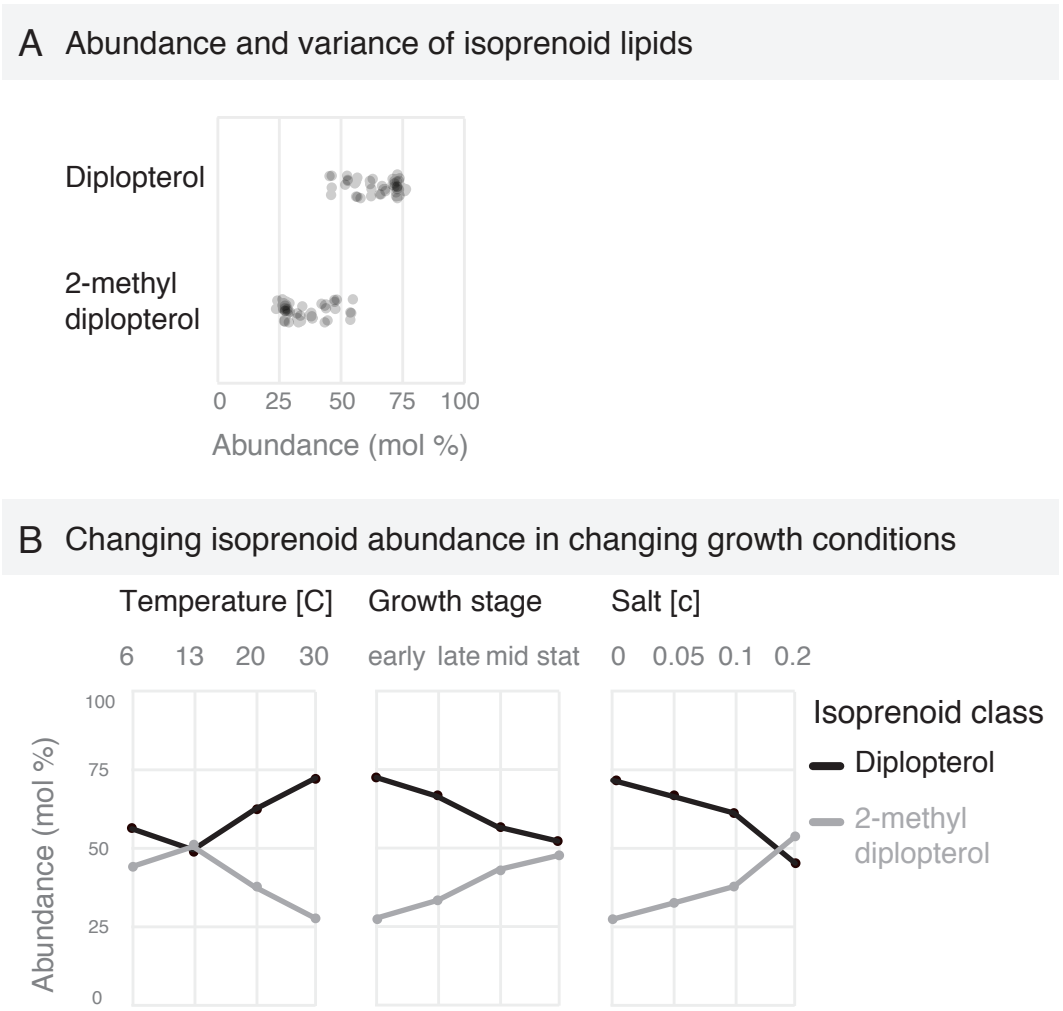

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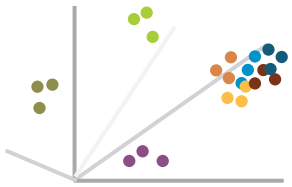
Methylobacterium extorquens
Gram-negative plant-based model to explore membrane adaptability

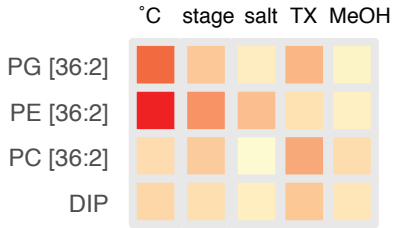
Systematic exploration of diverse enviornmental conditions
The standard growth condition (30°C, early phase) was adapted to new regimes.

Quantitative Mass-spec
Lipids were quantitatively measured by LS-Mass Spec

PCA showes robust lipidome across conditions, remodeling is notable in variable temperature con-



Variability across conditions: 8 lipid species were adapted in all conditions.



Lipidome composition by lipid feature remodelling e.g. of headgroup/class shows robustness also at the lipid structure level.

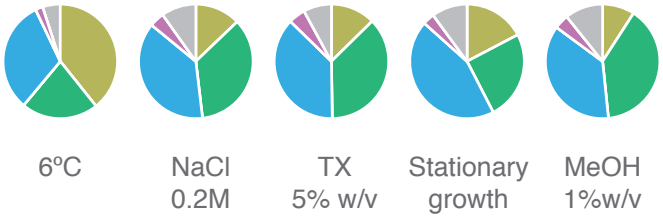
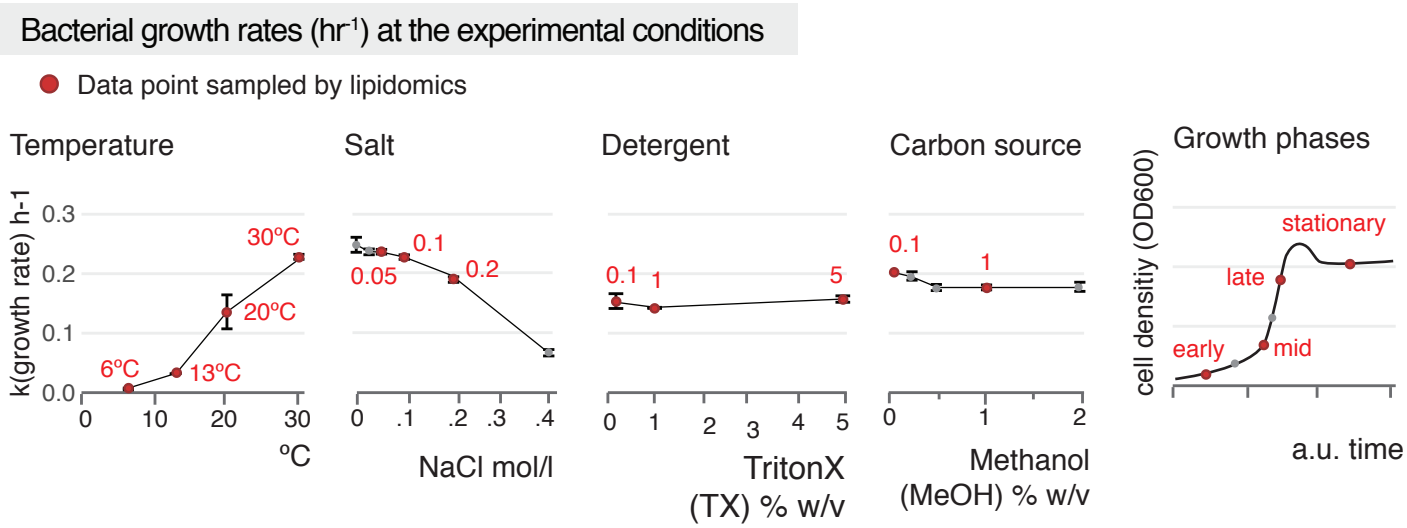


Figure 1 - supplement 1 Graphical abstract



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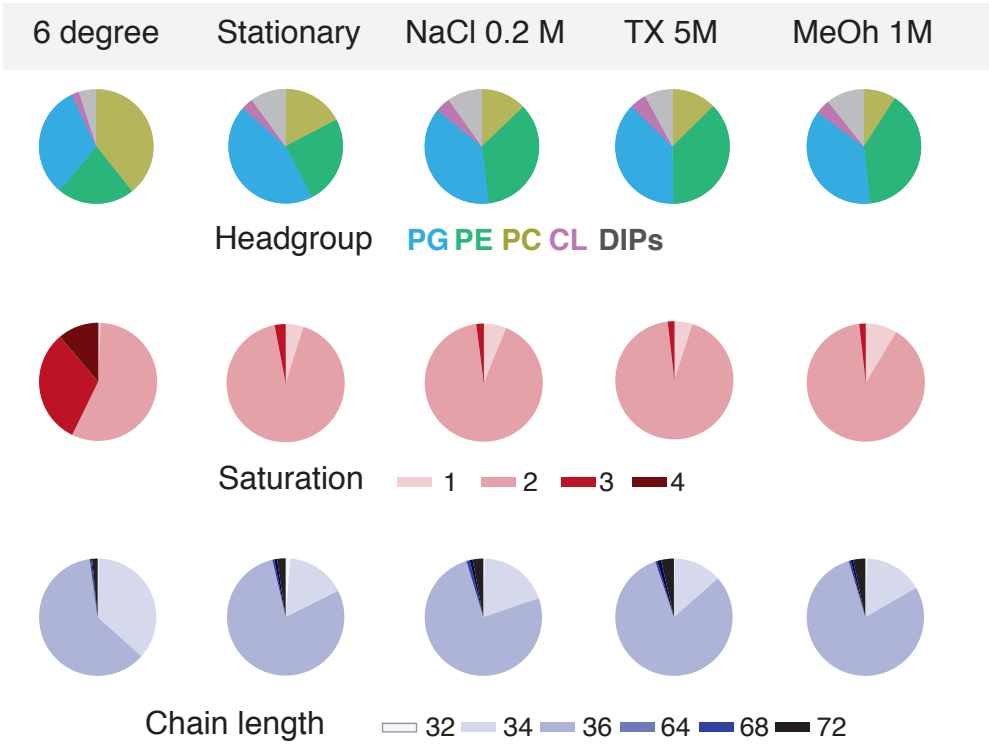

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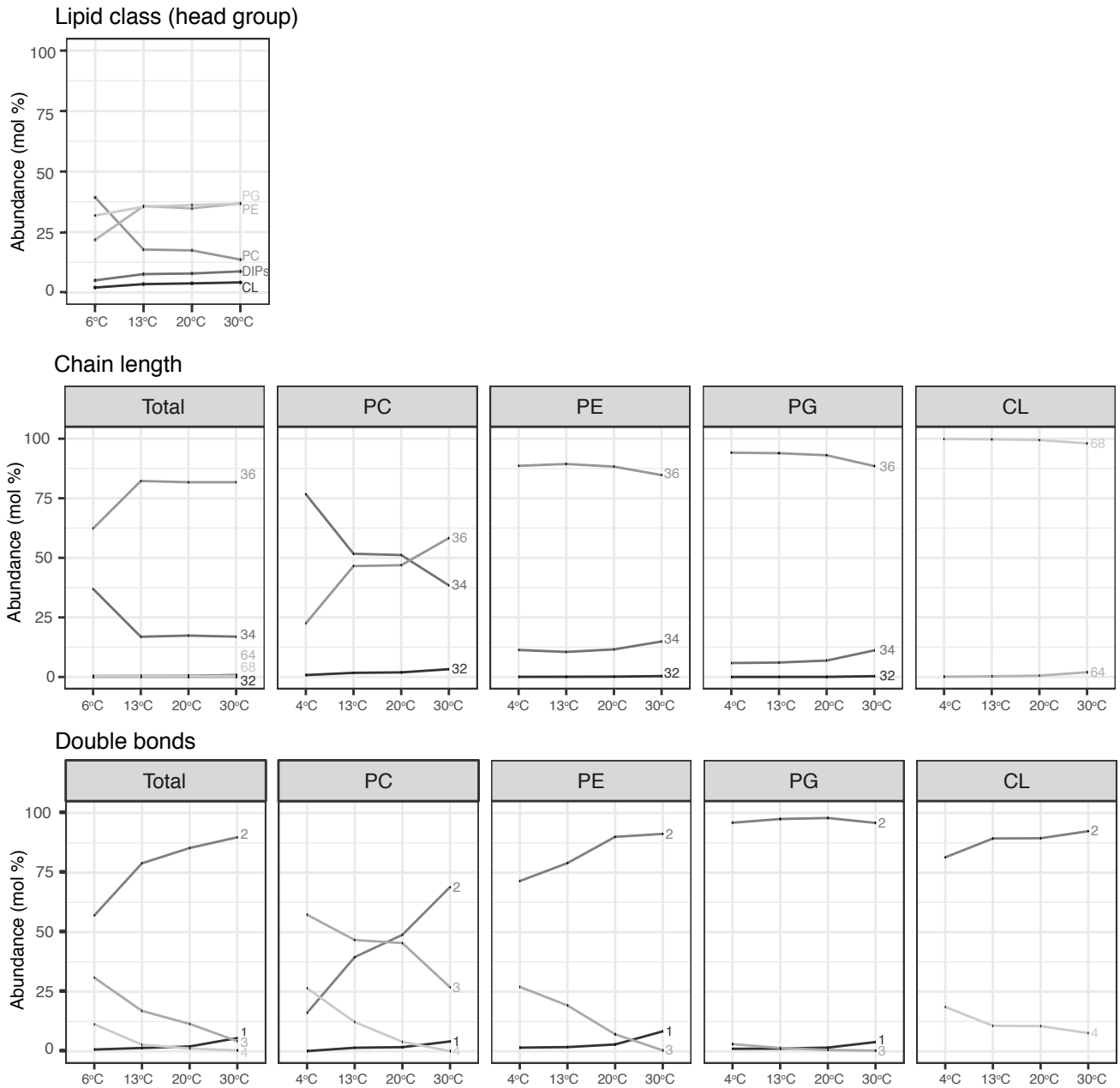

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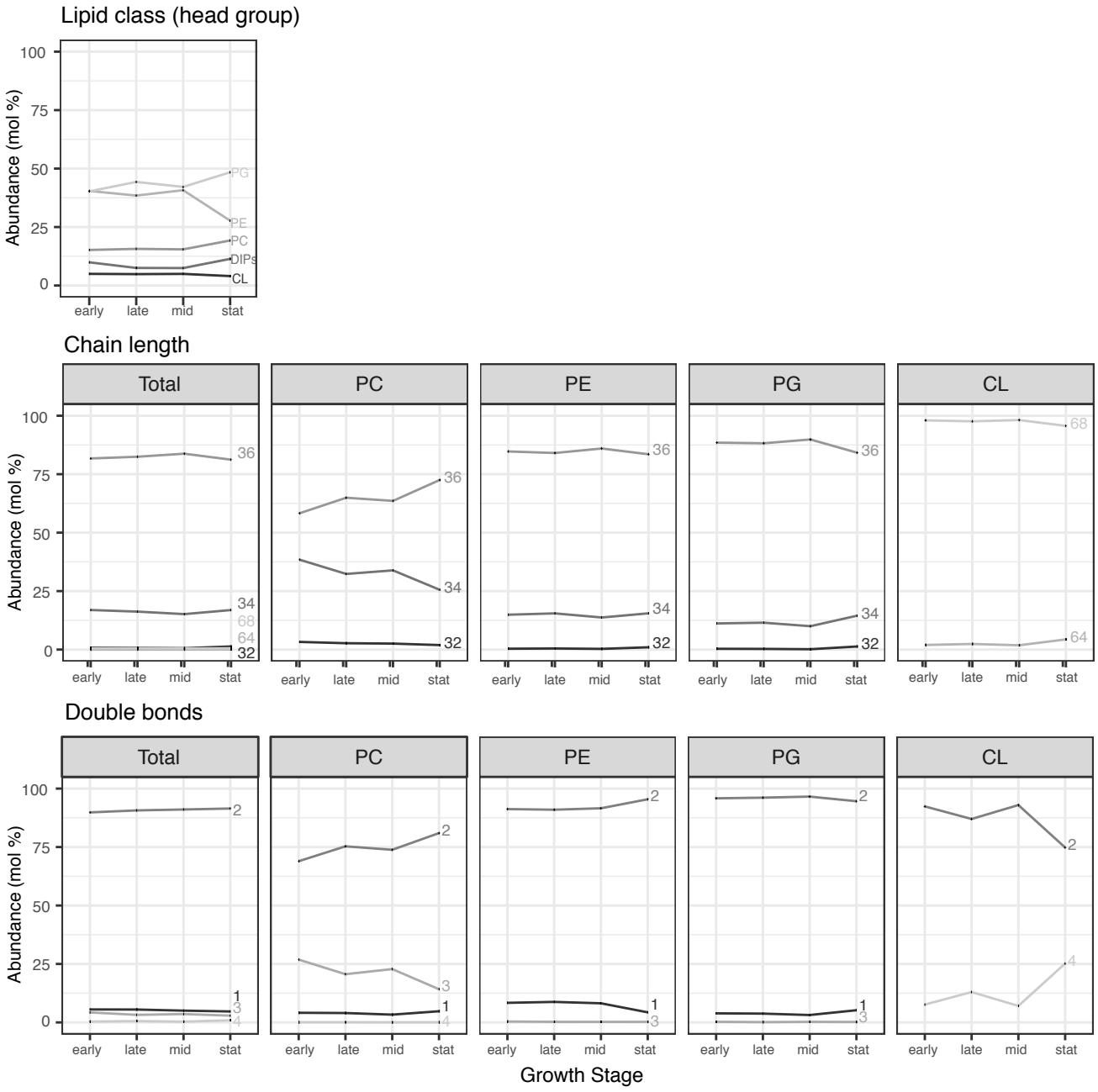

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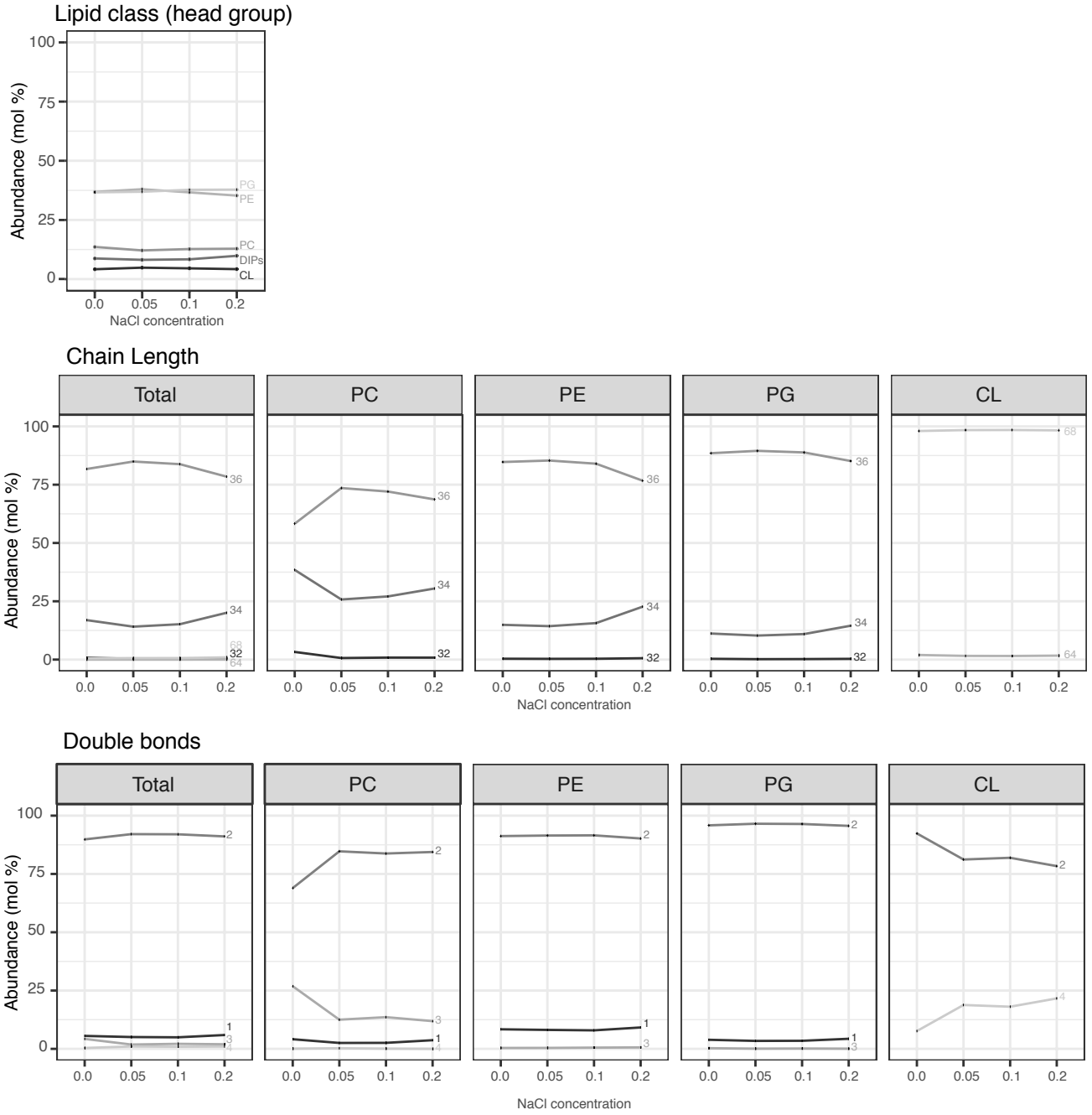

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