Resource-combining costs of being a diet generalist in the super-generalist protist predator *Dictyostelium discoideum*

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Abstract

Consumers lie on a continuum between diet specialization on few resources to being generalist feeders on many resources. Generalism has the clear advantage of having more resources to exploit, but the costs that limit generalism are less clear. We explore two understudied costs of generalism in a super-generalist amoeba predator, *Dictyostelium discoideum*, feeding on naturally co-occurring bacterial prey. Both involve costs of combining different prey. First, amoebas exhibit a reduction in growth rate when they switch from one species of prey bacteria to another, something we call resource-switching costs. These switching costs typically disappear within a day, indicating adjustment to new prey bacteria. Second, amoebas usually divide more slowly on mixtures of bacteria compared to on single bacteria, something we call resource-mixing costs. Both results support that idea that, although amoebas can consume a huge variety of prey, they must use partially different methods and thus must pay costs to handle multiple prey, either sequentially or simultaneously.

Significance Statement

Perhaps the most fundamental conflict in nature occurs when one organism consumes another. Diet generalists benefit from the advantage of eating many prey but then must deal with many prey defences. We explore costs associated with a broad diet in a protist microbial predator, *Dictyostelium discoideum*. These predators of bacteria show a delay in growth when switched from one bacteria to another, supporting the hypothesis that they must deploy different strategies. They also experience costs when grown on many bacteria at once, suggesting that the alternative strategies for consuming different prey are partly incompatible with each other. Our findings shed light on the nature of diet generalism and highlight the complexity of predation in the microbial world.

Main Text

Introduction

Consumers vary widely in their diet breadth. Some are diet specialists that eat one or few resources, such as koalas that feed only on eucalyptus leaves (1) or snail kites that exclusively hunt apple snails (2). Some are diet generalists that consume many different resources, such as coyotes that feed on many small mammals (3) or spiders that feed on many species of arthropods (4, 5). This variation in diet breadth has important ecological and evolutionary consequences that impact the structure and stability of food-webs (6), the stability of communities to perturbations (7), within- and between-species competition (8), the strength of coevolutionary dynamics (9) and speciation (10).

The obvious benefit to being a diet generalist is the ability to exploit diverse resources, especially when resources are scarce and fluctuate in their availability (11). But there must be associated costs with diet generalism or else all consumers would be generalists. One classic explanation for diet specialization is that “the jack of all-trades is the master of none.” We expect generalists to suffer from trade-offs in resource utilization because of antagonistic pleiotropy, where mutations that are good for exploiting one resource maybe bad on another (12–14). Such trade-offs can also occur via mutation accumulation, where mutations that are neutral on one resource may be detrimental on new resources (15). Generally, there has been surprisingly little support for trade-offs in performance on different resources (12, 16, 17). Other, less studied, costs include that generalists may also be slower to adapt to a given resource because they spend less time on a given resource compared to specialists (18), and that generalists can suffer from information costs from having to track more information about their resource environment (19).

Another kind of cost that seems to be rarely considered is the cost of combining resources. We distinguish two types of combining costs. First, generalists occur in environments with shifting resource abundances and may need to switch among different resources. If different resources require different methods of exploitation, then changing among these methods can result in costs. These methods may be related to resource recognition, handling time, processing or detoxification (20, 21). We refer to these as resource-switching costs. Such costs have been observed in arctic charr, where the fish experience lower metabolic rates when fed a diet different from the one they were raised on compared to fish that did not have a dietary change (22).

A second kind of combining cost is when generalists try to feed on multiple resources at once and the specific techniques that work best for different resources are partly incompatible. It may be impossible to effectively deploy multiple optimal strategies at the same time. For example, synergistic interactions between defensive chemicals of different resources could occur such that the combined toxins are more detrimental (23). We refer to the reduction in foraging efficiency in the presence of multiple resources as resource-mixing costs. However, generalists could alternatively benefit from resource-mixing because of improved nutritional balance or through reduction in the amount of toxins ingested from any one resource or prey (24). These two kinds of combining costs aren't the same as standard evolutionary trade-offs because a generalist may do just as well on every individual resource as a specialist but do more poorly when it tries to mix or switch among resources.

The costs and benefits associated with diet breadth evolution have been extensively studied in herbivorous insects. They are one of the most abundant and diverse eukaryotic life-forms and often possess highly specialized diets (25, 26). But the study of consumers with highly

88 generalized diets provides an equally important perspective. Predators are often among the
89 most general of consumers. By definition, predators are consumers that kill more than one
90 victim in their lifetime (27), usually many more. They also tend to be much larger than their
91 victims. Thus, to avoid starvation and minimize variance in energy intake, predators may need to
92 consume many sub-optimal prey types when the most profitable prey is not abundant enough
93 (28). Many macroscopic predators consume a formidable number of prey species, but few can
94 match the diversity of prey consumed by some microbial protist predators. We call species with
95 extreme diet breadth "super-generalists". Predation by protists is a major factor accounting for
96 bacterial mortality in the environment and as a consequence plays an important role in nutrient
97 cycling (29–31). Predation by protists can determine the composition and properties of bacterial
98 communities (32) and can be an important selective pressure for bacterial defenses such as
99 biofilm formation, antibiotic production, and secretion systems (33, 34).

100 *Dictyostelium discoideum* is one such super-generalist protist predator. It is a social
101 amoeba that lives in forest soils. It is a unicellular amoeba when bacterial prey are abundant and
102 transitions to a non-feeding multicellular dispersal stage upon starvation. *D. discoideum* has
103 been the subject of extensive research because of this fascinating multicellular stage (35, 36). Its
104 feeding behavior has been less extensively studied, but it appears to qualify as a super-
105 generalist predator. It can eat the majority of bacteria it is presented with (37–39). For example,
106 one study tested 159 bacterial strains found in close association with fruiting bodies of *D.*
107 *discoideum* from forest soil habitats and found that the amoebas were able to consume 77% of
108 them (39).

109

The prey bacteria of *D. discoideum* are very diverse, ranging across at least four highly divergent bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (38). These shared a common ancestor about three billion years ago, far older than the divergence time of diverse insect prey (~400 mya) that generalist invertebrates feed on (40). Bacteria also possess highly varied defensive mechanisms against microbial predators (33, 34). They can produce many kinds of secondary metabolite toxins to repel, disable, or kill their enemies. They can use secretion systems and effectors that can kill or allow for intracellular survival within protists. Some bacteria can swim away at high speeds to escape predators. Some can group together to form biofilms to prevent ingestion by predators.

Here we investigate the two kinds of costs of combining resources in *D. discoideum*. Amoebas occur in spatially and temporally variable communities of soil bacteria (41). Amoebas can encounter patchy bacterial distributions such that they switch from hunting one species of bacteria to another. We therefore tested for resource-switching costs by seeing if amoebas perform worse when switched to a new species of bacteria compared to controls that continued to grow on the same bacterium. Amoebas will often encounter mixed communities of prey, so we also looked at resource-mixing costs, testing if amoebas performed worse than expected in multi-species bacterial communities compared to their growth in single-species communities.

Results

Growth rate of *D. discoideum* amoebas varies on different species of bacteria.

As a preliminary step we confirmed that *D. discoideum* is a generalist. We measured the growth rate of three *D. discoideum* clones on the commonly used lab food bacterium, *Klebsiella pneumoniae* and on 22 species of bacteria that had been collected as transient associates on *D.*

133 *discoideum* fruiting bodies. These fruiting bodies emerged from soil and deer feces collected in
134 the field, so the 22 bacterial species represent biologically relevant prey species for the
135 amoebas. *D. discoideum* amoebas showed wide variation in their doubling times on different
136 soil bacteria (Test bacterium: $F_{22,45}$ = 23.88, p-value < 2.2×10^{-16}). On each of the 23 bacteria, all
137 three *D. discoideum* clones grew similarly (Figure 1), ruling out the possibility that the
138 generalism of the species might be due to a mixture of individually specialized clones (42). The
139 results also confirm that the amoebas are generalist feeders on prey bacteria that are likely to
140 be encountered in nature.

141

142 ***D. discoideum* amoebas experience resource-switching costs**

143 We conducted resource-switching cost experiments to investigate how *D. discoideum* amoebas
144 proliferate on a given bacterium when previously conditioned to a different bacterium (Figure
145 2). We found that there were fewer amoebas at 3 hours in switched treatments compared to
146 controls that did not experience a prey switch (Figure 3, Treatment $F_{1,106}$ = 27.621, p -value =
147 7.69×10^{-7}; Effect size = - 0.96, 95% CI = [-1.34, -0.575], df = 106; all effect sizes in this paper are
148 the standardized measure Cohen's d). Although only some bacterial species showed individually
149 significant switching costs (Figure S1A), there was no interaction of treatment with prey species
150 (Treatment x Test bacterium: $F_{1,106}$= 0.838, p-value = 0.526) and the overall effect size was
151 strong. Possible percentage change in amoeba numbers ranges from -24.04% to -8.03% (95%
152 CI). We also found that conditioning time of amoebas (2 days or 5 days) had a similar effect on
153 switching costs (Figure S1B, Conditioning time of 2 days: Treatment p-value < 0.001; Effect size =
154 -1.094, 95% CI = [-1.63, -0.561], df =106, Conditioning time of 5 days: Treatment p-value =

155 0.0018; Effect size = -0.825, 95% CI = [-1.35, -0.301], df =106). This is inconsistent with switching
156 costs being due to evolutionary changes during conditioning since evolutionary changes should
157 be greater over 5 days than 2. The trend in the magnitude of effect sizes is also inconsistent with
158 the evolutionary hypothesis because we would expect larger effect with longer conditioning
159 time.

160

161 **No evidence for resource-switching costs if amoebas undergo spore formation before the prey**
162 **switch**

163 If resource-switching costs are due to an evolved response in amoebas, then these genetic
164 changes should be passed on through the spores. But if the costs are physiological, then
165 undergoing the social cycle would be likely to erase the effects of food conditioning and
166 therefore eliminate switching costs, because spores have very different gene expression profiles
167 compared to amoebas (43, 44). We therefore tested if *D. discoideum* still show switching costs
168 when they undergo the social cycle and spore formation before changing between prey
169 bacteria. The switched and control spores resulted in a similar number of amoebas (Figure 4,
170 Treatment: $F_{1,48} = 0.263$, p-value = 0.61; Effect size = -0.133, 95% CI = [-0.653,0.387], df = 48).
171 Possible percentage change in amoeba numbers ranges from -25.43% to 22.58%. Thus, we find
172 no support for the hypothesis that switching costs are due to an evolved response, though the
173 lower end of confidence interval of effect size does not rule out some cost.

174

175 **No evidence that resource-switching cost persists over the long term**

176 If switching costs are not due to an evolved response, as supported by our previous results, then
177 the switched amoebas should eventually recover their growth rates to the level of the controls
178 that did not switch to new food bacteria. We conducted a time-course experiment to test this
179 for all three pairs of bacteria and compared early (zero to six hours) and late (24 to 27 hours)
180 growth rates of the switched and control treatments. As before (though tested here at 6 hours
181 rather than 3 hours), the switched treatments have significantly lower early growth rates
182 compared to controls (Figure 5A, Treatment $F_{1,53} = 12.98$, p-value = 6.9×10^{-4}; Effect size =-0.931,
183 95% CI = (-1.48, -0.382), df= 53). Possible percentage change in growth rates ranges from -
184 27.47% to -3.75%. But there was no difference in late growth rate of switches and controls
185 (Figure 5B, Treatment $F_{1,53} = 0.014$, p-value = 0.90; Effect size = -0.0311 , 95% CI = (-0.549,
186 0.487), df= 53). Possible percentage change in growth rates ranges from -23.72% to 28.31%.
187 Thus, though switched amoebas proliferate slower than controls during early growth, they
188 appear to eventually catch up and perform similarly to controls, consistent with physiological
189 adjustment to the new prey. Again, the confidence interval of effect size cannot rule out some
190 cost at the late stage.

191

192 ***D. discoideum* amoebas experience resource-mixing costs in some multi-prey communities**

193 We tested if *D. discoideum* experiences costs when grown in multi-species prey communities
194 compared to expectations from their growth in single-species communities. Consistent with
195 resource-mixing costs, we observed significantly fewer amoebas than expected after growing in
196 multi-species communities (Figure 6, Treatment $F_{1,59} = 11.84$, p-value = 0.001; Effect size = -
197 0.811, 95% CI = [-1.31, -0.316], df = 59). Possible percentage change in observed and expected

amoeba numbers ranges from -25.19% to -2.79%. But there seems to be variation in these mixing costs between the different bacterial communities (Treatment x Bacterial Community: $F_{5,59}$ = 2.557 p-value = 0.036). Three of the prey communities cause significant costs as judged by non-overlap of effect size confidence intervals with zero, and none show a significant mixing benefit (Figure S2).

Discussion

Consumers lie on a spectrum between specialization on a few resources or generalism on a wide variety of resources. The balance between costs and benefits associated with these two strategies determines where the consumer falls in that spectrum and has important ecological and evolutionary consequences. But the costs of generalism are not well understood. We examined an understudied type of cost of diet generalism in the social amoeba, *D. discoideum*. As a preliminary step, we showed that the different strains of *D. discoideum* amoebas are true generalists that grow similarly on any given species of bacterium (Figure 1). Some have hypothesized that a generalist species could be a collection of individual specialists (23, 42), but our results show that this is not the case in *D. discoideum*.

Amoebas experience early resource-switching costs when moved from one prey bacterium to another, even after controlling for variation in bacterial edibility (Figures 3, 5A, S1A). The smallest costs consistent with the 95% confidence intervals were 8% and 4% and even these smallest effects seem biologically significant. We also did three experiments to test if these costs were physiological in nature or due to an evolved response. First, we found no evidence that the conditioning time (duration spent by amoebas on a given bacterium before

220 the resource switch) affected the magnitude of switching costs. If the costs were due to
221 evolution during the conditioning period, they should become stronger with longer conditioning
222 time. Second, we found no evidence that amoebas experience switching costs if they undergo
223 spore formation before prey switch (Figure 4). If this was due to an evolved response, then
224 these effects would be genetically passed on through the spores. Third, we found that switching
225 costs occur during early growth but are no longer apparent after a handful of additional
226 generations (Figure 5), faster than they would change by selection. Our conclusions from these
227 three experiments must be drawn from their failure to find significant costs, and the confidence
228 intervals of each cannot exclude some costs. However, we have three different kinds of
229 experiment, each one failing to support the hypothesis of evolutionary change and we have two
230 separate experiments showing significant costs during the early stages where physiological
231 effects were expected (Figures 3, 5A). Taken together these five experiments make a strong case
232 that these costs are physiological in nature and not due to evolution.

233 Resource-switching costs have not been extensively studied and rarely enter into
234 broader discussions of generalism, but there are other examples. Arctic charrs show reduced
235 metabolic rate when switched among amphipods, bloodworms, and *Daphnia* (22). These costs
236 might be related to irreversible changes in their digestive tracts during development which
237 increase efficiency on the specific prey they were reared, but seem to be maladaptive on new
238 prey. Some songbirds, such as American Robins and European Starlings, exhibit costs when
239 switched between very different categories of foods in their natural diets, such as insects and
240 fruit (45, 46). These switching costs can be reflected in utilization efficiencies, gut retention
241 time, and body mass. Cabbage butterflies, *Pieris rapae*, take longer to extract nectar from a
242 flower species if they are moved to it from a different flower (47). Switching between different

243 flower species has a learning cost and may be related formation of a search-image for a specific
244 flower. *Escherichia coli*, the model bacterium shows a distinct lag phase when shifting from one
245 carbon resource to another (48). The lag phase may be a result of time required to switch on
246 relevant metabolic genes or related to the ability of the cells to accurately detect the depletion
247 of the primary carbon resource and presence of the secondary resource (49).

248 We also found that *D. discoideum* amoebas experience resource-mixing costs, where
249 they proliferate less than expected in some communities with multiple species of prey bacteria.
250 Our findings are in the opposite direction from the trend in other studies that have looked at the
251 effect of mixing resources in generalists. Some studies on other protists that have looked at
252 growth rate in multi-prey communities have found an increase in growth rates (50, 51).
253 Similarly, a study on marine amphipods found that fitness on mixed diets of algal species and
254 animal matter was either improved or at least comparable to fitness on the best monospecific
255 diet (52). A meta-analysis on the effect of diet mixing that included diverse consumers found
256 that consumers grew better on mixed diets compared to the averages from single-species diet
257 (53). However, if they considered only defended prey, then mixed diet was not different from
258 the average of single-species diets. This might help explain our results. There could be negative
259 synergistic interactions between defensive chemicals of different prey bacteria such that
260 combined toxins are more detrimental to the amoebas. Another possible explanation of lower
261 success on mixtures is that the different bacterial species compete and lower their overall
262 numbers. However, we minimized this effect by not providing nutrients for bacterial growth. It is
263 not clear why *D. discoideum* shows mixing costs when other taxa do not, but it is consistent with
264 their switching costs.

265 It is interesting that we observe resource-mixing costs despite another factor that may
266 obscure them. That factor is that amoebas may choose to eat the most profitable bacteria first.
267 All but late measurements would then reflect the growth rate on the best bacterium and any
268 mixing costs might therefore be obscured. Amoebas do show preferential attraction towards
269 Gram-negative bacteria compared to Gram-positive bacteria (54). Thus, it would be interesting
270 to test if *D. discoideum* amoebas can avoid some mixing costs by preferentially eating the most
271 profitable prey bacteria first, but if they do, our data show that it is apparently not enough to
272 fully overcome such costs.

273 These costs of combining resources are consistent with the idea that *D. discoideum*
274 amoebas use partially different methods to hunt and process different prey. The costs probably
275 involve changing gene expression. *D. discoideum* amoebas transcribe partially distinct sets of
276 genes on Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria (*K.*
277 *pneumoniae*, *Pseudomonas aeruginosa*) (55). The transcriptome of *D. discoideum* was also
278 found to be highly species-specific when tested on 3 species of bacteria: *K. pneumoniae*, *B.*
279 *subtlis*, and *Mycobacterium marinum* (56). Another study found that mutations that affect the
280 ability of *D. discoideum* to grow on different bacteria were highly prey-specific (57). This
281 suggests that there are different mechanisms for hunting or processing these distantly related
282 bacteria.

283 Predation by amoebas is a complex process that can be divided into 4 broad steps:
284 search, encounter, attack, and digestion (33). The costs of combining resources could arise in
285 any of these steps. Eukaryotic phagocytes use G-protein coupled receptors (GPCRs) to detect
286 and chase bacteria and use pattern recognition receptors to recognize and eliminate bacteria
287 (58, 59). *D. discoideum* is equipped with 61 GPCRs (60). Some GPCRs are involved in the

288 amoebas' social cycle, but at least one GPCR, the folate receptor fAR1, has been implicated in
289 both chasing and engulfing *Klebsiella* bacteria (61). Other GPCRs may play a role in other aspects
290 of prey capture including chase and recognition. *D. discoideum* is also equipped with 22 genes
291 that encode as many as 4 different types of lysozymes (62). Lysozyme genes play an important
292 role in digesting bacteria. Knocking out some lysozyme genes generally reduces the ability of
293 amoebas to feed on all tested bacteria, while deletion of other lysozyme genes reduces growth
294 only on specific gram-negative bacteria (63). Future research on the mechanisms underlying
295 these costs would be valuable.

296 Why can't amoebas evolve one predation technique that works optimally on all prey
297 bacteria to eliminate the costs of diet generalism? After all, amoebas are a few hundred times
298 larger than the average bacterium by mass (64). They should be able to overwhelm most
299 bacteria with their size advantage. But bacteria possess many mechanisms to resist their
300 eukaryotic predators (33, 34, 65, 66). Morphological adaptations such as formation of
301 microcolonies, biofilms, and filamentation can prevent attack or ingestion by predators. Bacteria
302 can modify their membrane properties by changing the lipopolysaccharides on the outer
303 membrane, secreting an S-layer and sporulating among others that can help them avoid
304 recognition, ingestion, and digestion by predators. They can also produce a huge array of
305 secondary metabolites that can deter predation by protists (67, 68). Thus, each bacterial species
306 may possess a unique combination of defenses that is unlikely to yield to a common predation
307 strategy. It would be interesting to test how many different predation strategies *D. discoideum*
308 can employ. *D. discoideum* may treat some groups of bacteria similarly and some as different.

309 We suspect that environmental heterogeneity plays a large role in the maintenance of a
310 generalist strategy in *D. discoideum*. Environmental heterogeneity, especially temporal variation

311 favors generalists (69). The scale of variation can also influence the nature of generalism (70).
312 Coarse-grained environments may select for early developmental plasticity, but for a fixed
313 phenotype later in life. Fine-grained environments select for versatile generalists that are
314 capable of reversing their phenotypic response.

315 How much environmental heterogeneity amoebas experience in nature is not fully
316 known. Soil bacterial communities are certainly spatially and temporally variable, such that no
317 one bacterium may be consistently sufficiently abundant (41). But the scale is important. On a
318 microscale, soil is made up of small aggregated particles that are connected via a network of air-
319 and water-filled pores (71). The three-dimensional nature of these particles increases soil
320 surface area such that as little of 10^{-6} percent of soil surfaces may contain bacteria (72). These
321 particles generally contain bacterial patches of a limited number of clonal cells and these
322 patches can be separated by distances that are large on a microbial scale (73). Thus, it is possible
323 that amoebas are likely to experience switching costs as they move between bacterial patches.
324 Amoebas can also travel considerable distances during their social cycle through slug migration
325 and especially through the dispersal of spores by animal vectors (74). This can introduce them to
326 new soil environments with different bacterial communities. Thus, amoebas are in a selective
327 environment that probably favors generalists.

328 Resource-combining costs may be less relevant to consumers that can find one big
329 resource unit to spend their whole life on, such as phytophagous insects or parasitoid wasps.
330 However, the generalist parasitoid wasp, *Venturia canescens* shows a cost of resource switching
331 across generations, perhaps due to epigenetic or maternal effects (75). Consumers that
332 encounter diverse and defended prey species in their lifetimes are more likely to be plagued by

these costs. One example is wolf spiders that experience costs when fed a mixed diet of toxic prey (76).

Resource-combining costs are different from standard trade-offs because here generalists can handle each resource well, but they experience costs when combining different resources. These costs, like trade-offs, could eventually lead to resource specialization. We have shown that resource-combining costs are prominent in a resource generalist but it would be interesting to also study resource specialists. Are they sometimes constrained in their diets less by being unable to feed on a more general diet, but by the inability to readily switch to another suitable resource? Are they kept from having a broader diet by resource-mixing costs? We suggest that resource-combining costs deserve greater consideration than they have received in the debate on the evolution of generalism and specialism.

Materials and Methods

Growth rate of *D. discoideum* on different species of bacteria isolated from forest soil environments

We first measured the growth rate of wild clones of *D. discoideum* on *Klebsiella pneumoniae,* the lab food bacterium, and on 22 species of bacteria found in close-association with *D. discoideum* isolated by Brock et al. 2018 from forest soil environments (Table 1). These bacteria were isolated from fruiting bodies of *D. discoideum* that developed from field-collected samples of soil and deer feces from Mountain Lake Biological Station, Virginia in 2014. All *D. discoideum* strains used in this paper were also isolated from Mountain Lake Biological Station, Virginia. The bacterial species identification is based on closest partial 16S BLAST hit by Brock et al. 2018. In

355 this paper, we refer to the bacteria using names from these closest 16s BLAST hits. We
356 performed this experiment on 3 *D. discoideum* strains: QS1, QS6, and QS9. We plated 100,000
357 amoebas with 200μl of 10 OD_{600} bacterial suspension on starving agar plates. We estimated
358 amoeba numbers at 20 hours and calculated doubling times as:

359 $$Doubling\ time\ (T_D)\ = ln(2)\ \times \frac{20}{ln(N_{t=20}) -\ ln(N_{t=0})}$$

360 This assay also helped us identify reasonably edible bacteria for other experiments.

361

362 **Resource-switching cost assay in *D. discoideum* amoebas**

363 To test if resource-switching costs occur when *D. discoideum* amoebas are switched from
364 Bacterium A to Bacterium B, we first conditioned separate populations of the amoebas to each
365 species of bacterium (Figure 2, details below for three separate experiments). The switched
366 treatment for these experiments used amoebas conditioned to Bacterium A and replated with
367 Bacterium B. We plated 100,000 amoebas conditioned to Bacterium A with 200μl 20 OD_{600}
368 suspension of Bacterium B on SM/20 plates and then measured amoeba numbers after 3 hours.
369 The controls for this experiment were identical except that amoebas conditioned to Bacterium B
370 were replated with Bacterium B. If we observe fewer amoebas in switched plates compared to
371 control plates, then *D. discoideum* amoebas experience resource-switching costs. We measured
372 the costs of switching between 3 pairs of bacteria with 2 reciprocal switches for each pair. We
373 replicated this experiment with 5 *D. discoideum* strains: QS6, QS9, QS14, QS17, QS160.

374 We did three sets of experiments to rule out the possibility that these costs are instead
375 due to an evolved response in the amoebas during the conditioning period to either bacterium.

376 Switched amoebas could experience poor growth on a Bacterium B because of trade-offs
377 associated with new evolutionary adaptation to the Bacterium A during the conditioning period.
378 Control amoebas may also experience better growth rates if they have evolved and adapted to
379 Bacterium B during the conditioning period. We ruled out this complication in three ways.

380 **Effect of conditioning length of amoebas on resource-switching costs**

381 First, we tested if the length of the conditioning period of amoebas on the bacterium affected
382 resource-switching costs. If costs are due to either adaptation in switched amoebas that results
383 in evolutionary trade-offs, or to direct improvement in growth rate because of adaptation in
384 control amoebas, we expect stronger costs with longer conditioning (i.e. evolving) time. Thus,
385 we conditioned amoebas on a given bacterium for a) 2 days and b) 5 days before measuring
386 switching costs. In the 2-day treatment, we conditioned the amoebas for 40 hours by plating
387 200,000 spores of *D. discoideum* with 200ul of 1.5 OD_{600} bacterial suspension on SM/5 plates.
388 After ~40 hours, we washed off the plates with 10ml of ice cold KK2 to collect the amoebae-
389 bacteria suspension. Next, we centrifuged the suspension for 3 minutes at 300g at 10°C to spin
390 down the amoebas and discarded the supernatant containing the bacteria. We resuspended the
391 amoeba pellet in ice-cold KK2 and washed it off two more times until all the bacteria were
392 discarded. Finally, we suspended the amoeba pellet in 500μl – 1000μl of ice-cold KK2. We
393 conditioned the amoebas for the 5-day experiment similarly, but collected amoebas from the
394 resulting vegetative front after 5 days with a sterile loop and resuspending in 600ul ice-cold KK2.
395 We performed washing centrifuging steps as described above to thoroughly wash the amoebas
396 off the conditioning bacterium. A conditioning time of 2 days translates to 10-14 amoeba
397 divisions and 5 days to 25-35 amoeba divisions on good prey. If resource-switching costs are

398 because of an evolutionary response, then we expect costs to be stronger for the 5-day
399 conditioning period than the 2-day period.

400

401 **Resource-switching costs assay with *D. discoideum* spores**

402 To further distinguish between a temporary switching cost and an evolved response, we tested
403 whether allowing amoebas to go through the social cycle and subsequent sporulation before
404 prey bacteria switches erases switching costs (Figure 2). If costs are because of an evolved
405 response in amoebas, then these changes should still be evident after the social stage. But if the
406 costs are because of conditioning of the amoebas that results in temporary mismatch in
407 transcriptional tools on the new bacterium, then undergoing the social cycle should erase most
408 switching costs. This is because *D. discoideum* experiences a huge turn-over in gene expression
409 when transitioning from a unicellular lifestyle into the multicellular cycle (Parikh et al. 2010,
410 Rosengarten et al. 2015). The abundance of almost every transcribed mRNA changes at least 2-
411 fold throughout development starting from vegetative amoebas to multicellular fruiting bodies
412 (43). Therefore, any transcriptional conditioning of amoebas towards a bacterium should be
413 largely erased by development. The switched and control spores are at the same transcriptional
414 start line of dormancy.

415 We used the same design as the switching experiment for amoebas described above but
416 with sporulation at the end of the conditioning phase (~7 days). *D. discoideum* amoebas feed on
417 bacteria for the first 2-4 days of the conditioning phase, and then transition to the social cycle
418 once the bacteria are depleted. We then plated 100,000 spores from the resulting fruiting
419 bodies with bacterial suspensions on SM/20 (Figure 2). Since the spores require some time to

germinate into amoebas and start feeding on the bacteria, we counted the total number of amoebas on these plates after 36 hours.

Time course data of resource-switching costs in *D. discoideum* amoebas

As a third check on whether what we see is an evolved or a conditioned response, we conducted a time series study of the amoebas on all three pairs of bacteria to check if the growth rates of the switched treatments quickly catch up with control growth rates, which would indicate a physiological lag rather than an evolutionary change. We tracked the number of amoebas in switched and control plates after 6, 24 and 27 hours from plating. We calculated early growth rate between 0h and 6h time points and calculated late growth rate between 24h and 27h time points. We assumed exponential growth and used this formula to calculate growth rate between time points t1 and t2, where N_t stands for number of amoebas at time t:

$$Growth\ rate\ (r) = \frac{ln(N_{t2}) - ln\ (N_{t1})}{(t2 - t1)}$$

If switching costs are not an evolved response, then switches and controls should not differ in their late growth rates. We performed this on SM/5 plates with a starting number of 100,000 amoebas with 200µl of 1.5 OD_{600} suspension of the test bacterium on the *D. discoideum* clone QS9. We replicated this experiment five times.

Resource-mixing costs in *D. discoideum* amoebas

439 Next, we determined whether *D. discoideum* amoebas experience resource-mixing costs when
440 feeding on multiple species of bacteria. For this experiment, we chose 5 prey bacteria that were
441 generally highly edible from our growth rate assay of amoebas on 23 species of bacteria. We
442 generated 5 single-species communities, 6 three-species communities and 1 five-species
443 community from the 5 prey bacteria. We made 10 OD_{600} suspensions of each bacterial species
444 and mixed these suspensions in equal proportions to make a given multi-species suspension. We
445 used AX4 amoebas that were grown axenically in bacteria-free cultures to preclude any effect of
446 prior conditioning of amoebas to a given species of bacterium on the assay. We plated 100,000
447 amoebas on starving agar plates with 200µl of bacterial suspensions and measured amoeba
448 numbers in all the different treatments after 20 hours. We replicated this experiment 6 times.
449 We expect bacterial growth and consequently competition to be minimal because we
450 performed this experiment on starving agar. To test if amoebas experience resource-mixing
451 costs, we first calculated the expected number of amoebas in multi-prey treatments with data
452 from our single-prey treatments. For example, the expected number of amoebas in multi-prey
453 treatment containing *A. tumefaciens*, *S. maltophilia* and *C. testosteroni* is the average of
454 observed numbers of amoebas in single-prey treatment of those bacteria. We then compared
455 these expected numbers to our observed number of amoebas in these multi-species treatments
456 to infer costs.

457

458 **Statistics:**

459 We performed all statistical analysis in R (version 4.2.1) (77). We used general linear models
460 (with log link functions for count data) to analyze our data after testing for normality of residuals

461 using the Shapiro-Wilk's test and examining Q-Q plots. We used the "*emmeans*" package to
462 calculate estimated effect sizes (Cohen's d, a standardized measure) and 95% confidence
463 intervals for relevant contrasts (78). We calculated the largest and the smallest percentage
464 difference between treatments from the 95% C.I range of the estimated marginal means. To test
465 if *D. discoideum* amoebas experience varying doubling times on different species of bacteria, we
466 built a linear model with amoeba doubling times as the response variable and the Test
467 bacterium (23 species of bacteria listed in Table 1) as the fixed factor. We define test bacterium
468 as the bacterium on which we measured amoeba growth rates. To test if *D. discoideum*
469 amoebas experience a resource-switching cost on a given bacterium when previously grown on
470 a different bacterium, we built a linear model with log-transformed amoeba numbers after 3
471 hours of growth as the response variable and Treatment (Control, Switch), Test bacterium (At,
472 Pv, Fg, Sm, Sz, Ct), and Conditioning length (2days, 5days) as fixed factors. We also included
473 interaction effects for Treatment x Test bacterium, Treatment x Conditioning length. For the
474 similar experiment that includes a spore stage after conditioning, we used a similar linear model
475 with log transformed amoeba numbers after 36 hours as the response variable and Treatment
476 and Test bacterium as the fixed factors. We included interaction effects for Treatment x Test
477 bacterium in this model.

478 We performed the following statistical tests on time-course data collected on switches.
479 To test if there are switching costs during early proliferation we used a linear model with early
480 growth rate calculated between 0 and 6 hours as the response variable and Treatment (Control,
481 Switch) and Test bacterium (At, Pv, Fg, Sm, Sz, Ct) as fixed factor. To test if resource-switching
482 costs persist during late proliferation, we used a similar linear model with late growth rate
483 calculated between 24 and 27 hours as the response variable.

To test if *D. discoideum* amoebas experience resource-mixing costs, we tested if amoebas performed worse than expected in multi-species prey communities. We built a linear model with log transformed amoeba numbers at 20 hours as the response variable, Treatment (categorical variable for whether an observation is expected or observed) and Bacterial Community (6 Communities) and Experimenter (2 Experimenters) as fixed factors. We included an interaction effect between Treatment x Bacterial Community. ANOVA tables for all models are included in the supplement.

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673

Figures and Tables

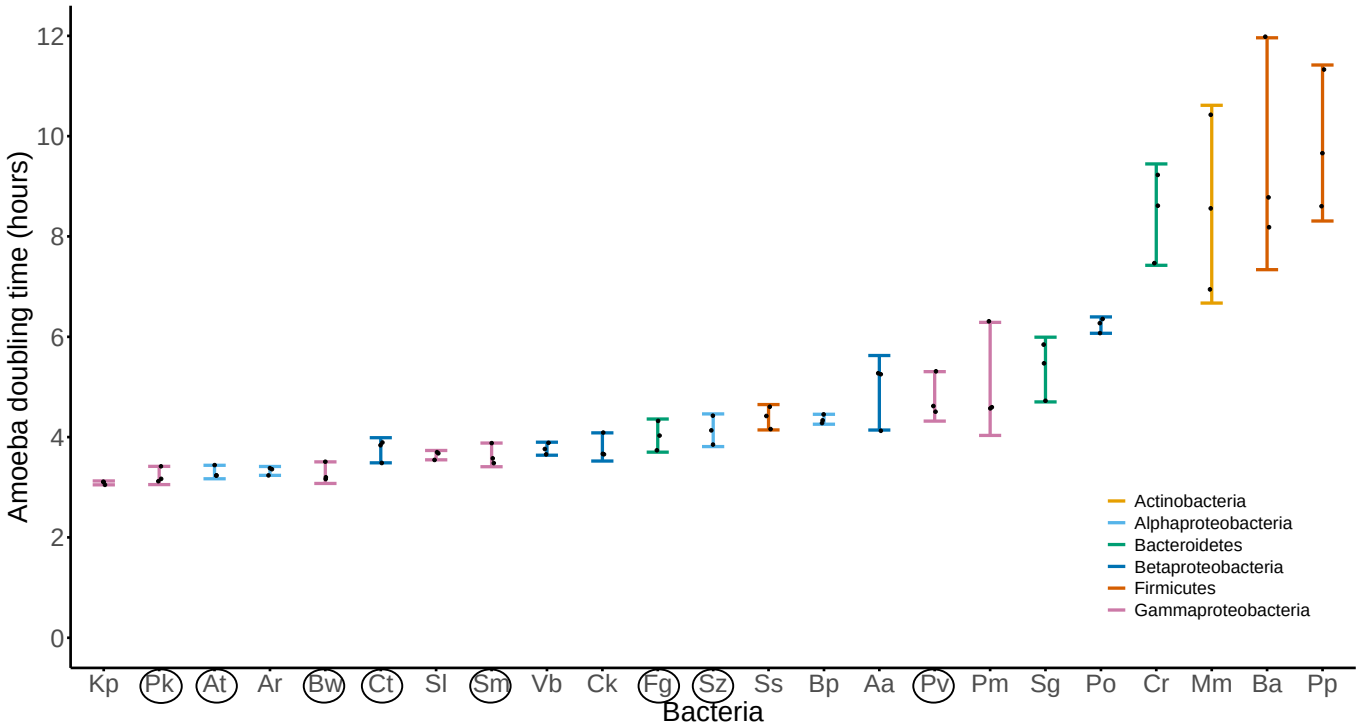

Figure 1. Doubling time of *D. discoideum* on *K. pneumoniae* and 22 species of soil bacteria closely associated with *D. discoideum*. Error bars are 95% C.I. The points represent the 3 *D. discoideum* strains. The circles represent the 8 species of bacteria used in other parts of this study. Bacteria species identities by Brock et al. 2018 based on closest partial 16S BLAST hit are listed in Table 1.

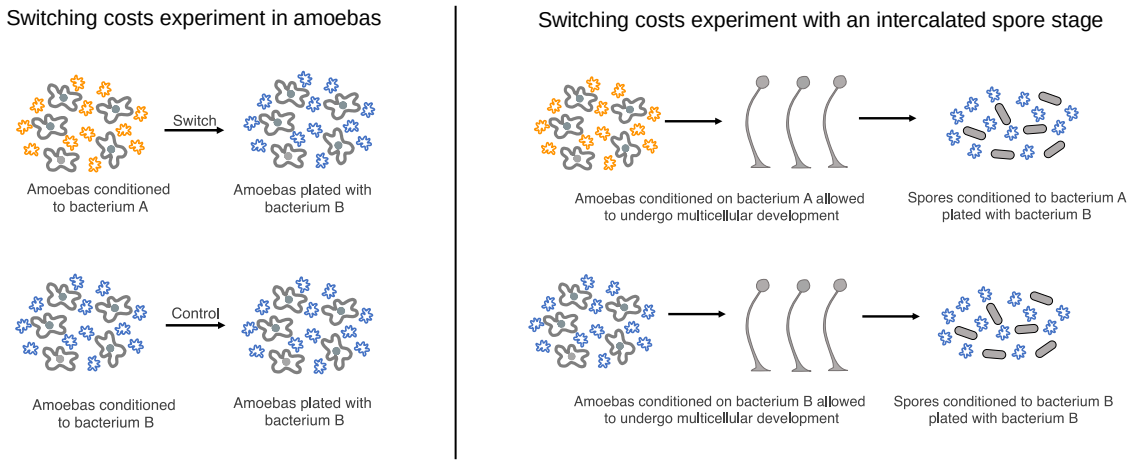

Figure 2. Schematic on the left shows resource-switching costs experiment in amoebas. Amoebas in switched treatment are conditioned on bacterium A and are switched to bacterium B for the experiment. The controls for this experiment are amoebas conditioned to bacterium B plated with a fresh culture of the same. We also varied conditioning time of amoebas on a given bacterium (2 days, 5 days). Schematic on the right outlines switching costs experiment on spores. We used the same basic switching design as described for the amoebas, except we let the amoebas undergo multicellular development on the conditioning bacterium. We then collected spores from these fruiting bodies and performed the experiment.

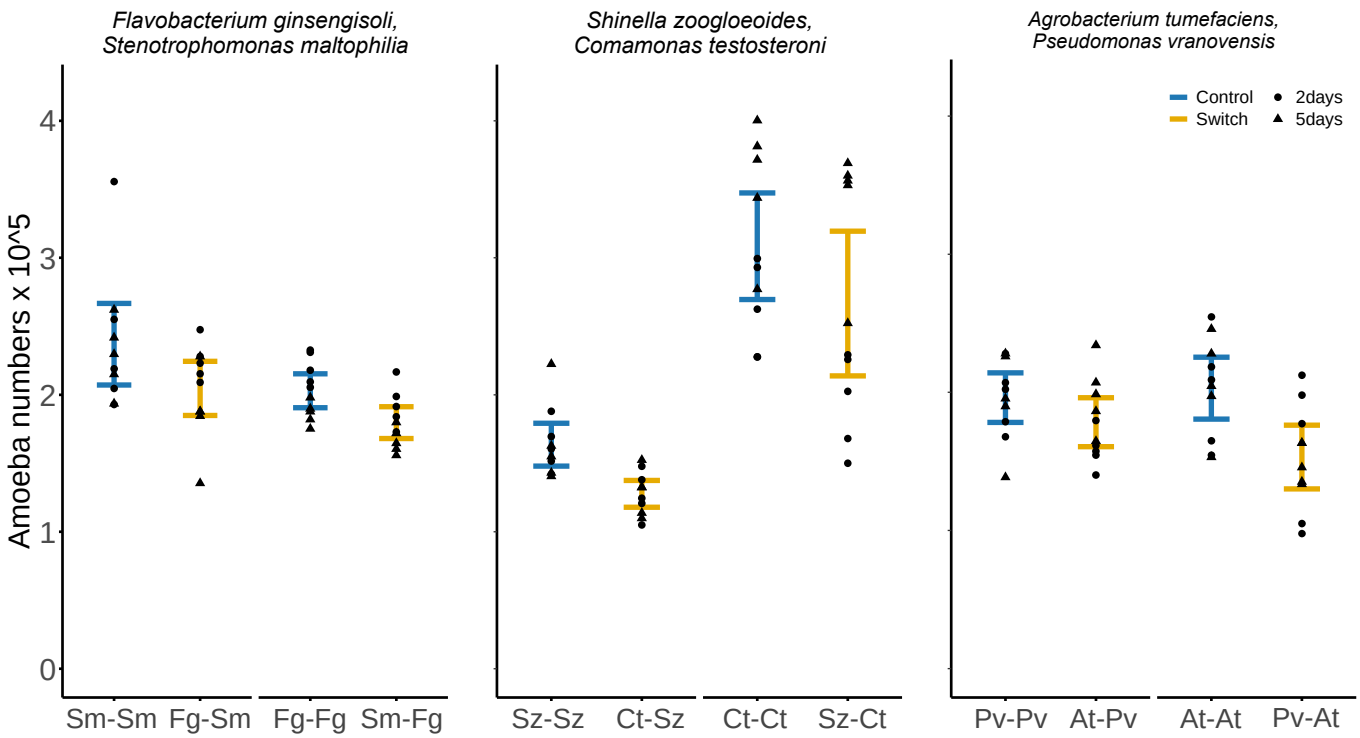

Figure 3: *D. discoideum* amoebas experience resource-switching costs: Mean amoeba numbers ± 95% C. I. of controls (blue) and switches (orange) after 3 hours of growth. The shapes represent the different conditioning times of amoebas (● - 2 days, ▲- 5 days). The points represent the five *D. discoideum* strains. There are fewer amoebas after three hours in switched treatments compared to controls. The two conditioning times are similar in the magnitude of switching costs. Interpreting x-axis labels: For example - Sm-Sm refers to amoebas grown on *S. maltophilia* moved to *S. maltophilia*, while Fg-Sm refers to amoebas grown on *F. ginsengisoli* moved to *S. maltophilia*.

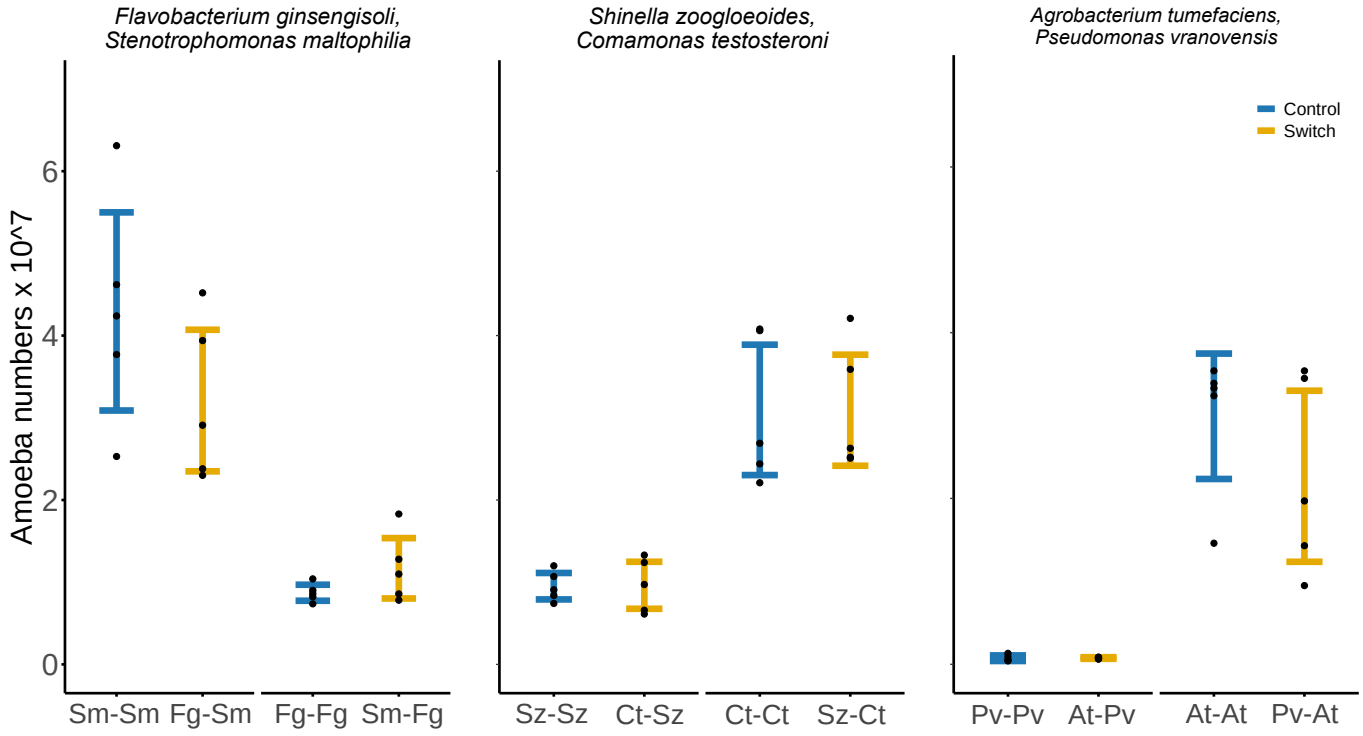

Figure 4: Switching costs disappear when spore formation precedes prey switch: the switched and control treatment have similar number of amoebas. Graph shows mean amoeba numbers ± 95% C.I. of controls and switches at 36 hours from plating. The points represent the five *D. discoideum* strains.

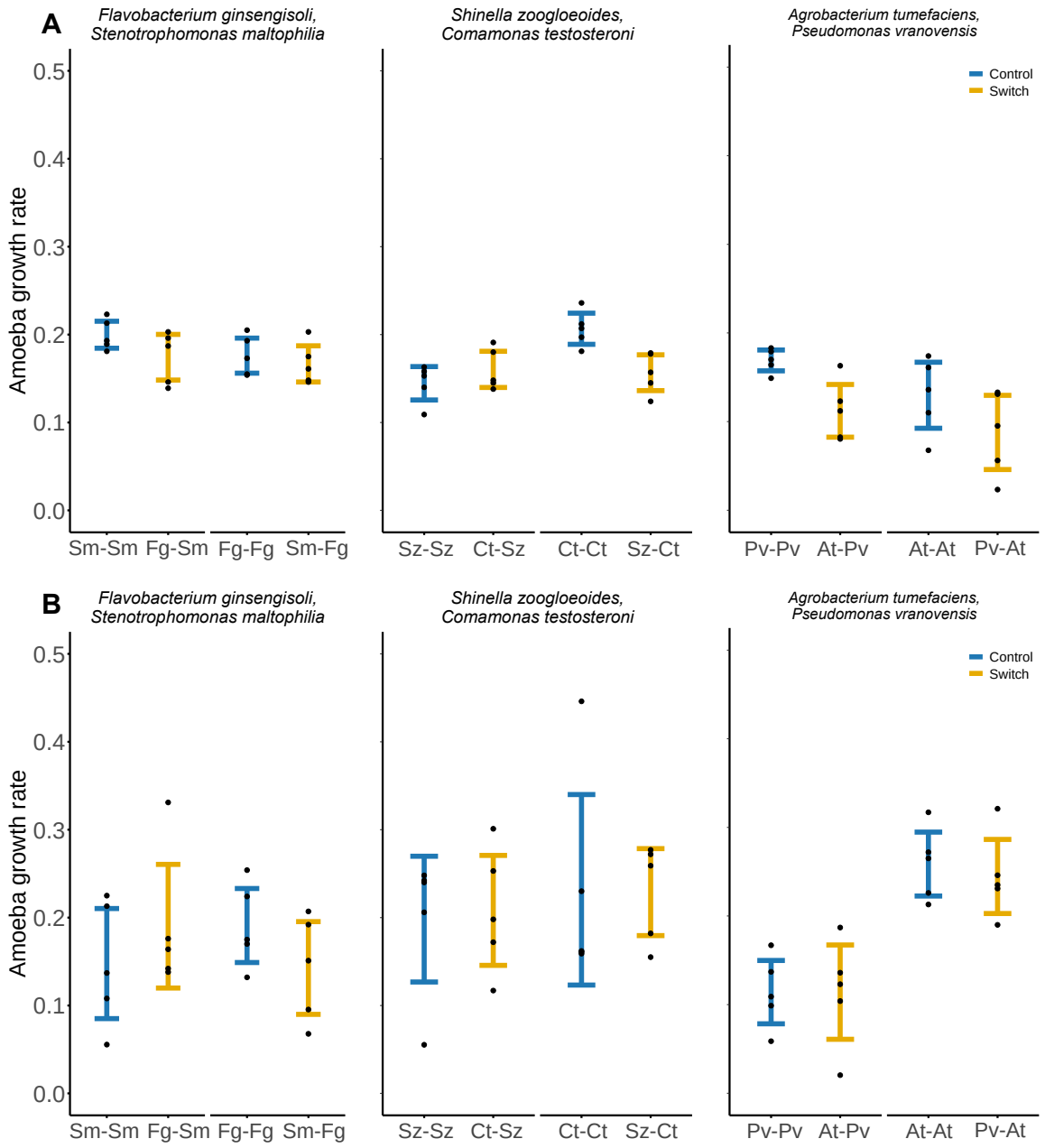

Figure 5: Switching costs occur during early growth period rather than later: **A)** Early growth rate estimates of controls and switches between 0 and 6 hours from set-up. Switched treatments have lower early growth rates compared to controls. **B)** Late growth rate estimates of controls and switches between 24 to 27 hours from set-up for. Graph shows mean growth rates ± 95%

714 C.I. We found no difference in growth rates switches and controls at the later time points. The

715 points represent 5 technical replicates of *D. discoideum* clone QS9.

716

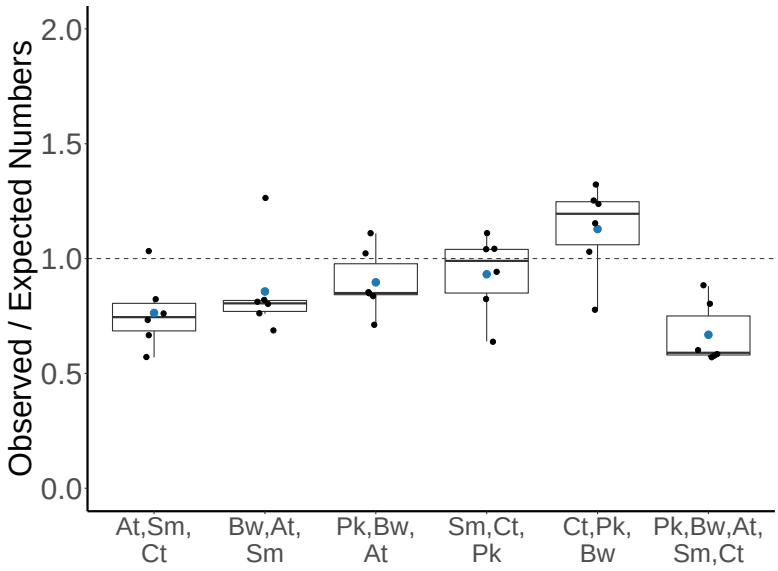

Figure 6: *D. discoideum* amoebas experience resource-mixing costs in some multi-species bacterial communites: Ratios of observed vs. expected amoeba numbers in different bacterial communities. A ratio lower than one indicates costs of resource mixing. The blue dot marks the mean. At- *Agrobacterium tumefaciens*, Bw- *Buttiauxella warmboldiae*, Ct - *Comamonas testosteroni*, Sm - *Stenotrophomonas maltophilia*, Pk - *Pseudomonas kuykendallii*.

724 **Table 1: List of 23 species of bacteria used in *D. discoideum* growth rate experiments. ***
725 **Species identification of bacterial isolates by Brock et al. 2018 based on closest partial 16S**
726 **BLAST hit.**

Strain number	Closest 16S sequence	Phylum/Class	Abbreviation
Lab clone	*Klebsiella pneumoniae*	Gammaproteobacteria	Kp
14P 8.1.1	*Pseudomonas kuykendallii*	Gammaproteobacteria	Pk
18P 8.2.2	*Buttiauxella warmboldiae*	Gammaproteobacteria	Bw
18P 2.2.1	*Agrobacterium tumefaciens*	Alphaproteobacteria	At
20P 9.1.2	*Agrobacterium rubi*	Alphaproteobacteria	Ar
14P 4.3.1	*Serratia liquefaciens*	Gammaproteobacteria	Sl
14P 6.2.3	*Stenotrophomonas maltophilia*	Gammaproteobacteria	Sm
18P 6.2.3	*Comamonas testosteroni*	Betaproteobacteria	Ct
20P 10.2.4	*Variovorax boronicumulans*	Betaproteobacteria	Vb
14P 8.1.4	*Comamonas kerstersii*	Betaproteobacteria	Ck
20P 3.2.2	*Flavobacterium ginsengisoli*	Bacteroidetes	Fg
20P 9.1.1	*Shinella zoogloeoides*	Alphaproteobacteria	Sz
20P 6.1.2	*Brucella papionis*	Alphaproteobacteria	Bp
5S 2.1.1	*Staphlycoccus saprophyticus*	Firmicutes	Ss
5P 5.1.1	*Pseudomonas vranovensis*	Gammaproteobacteria	Pv
18P 8.1.1	*Achromobacter aegrifaciens*	Betaproteobacteria	Aa
14P 5.3.2	*Pseudomonas migulae*	Gammaproteobacteria	Pm
20P 10.2.1	*Sphingobacterium ginsensidimutans*	Bacteroidetes	Sg
20P 2.1.2	*Pandoraea oxalativorans*	Betaproteobacteria	Po
14P 4.3.2	*Chryseobacterium rhizosphaerae*	Bacteroidetes	Cr
14P 6.2.1	*Microbacterium maritypicum*	Actinobacteria	Mm
20P 7.2.1	*Bacillus aryabhattai*	Firmicutes	Ba
20P 2.1.1	*Paenibacillus pabuli*	Firmicutes	Pp

Supplemental information:

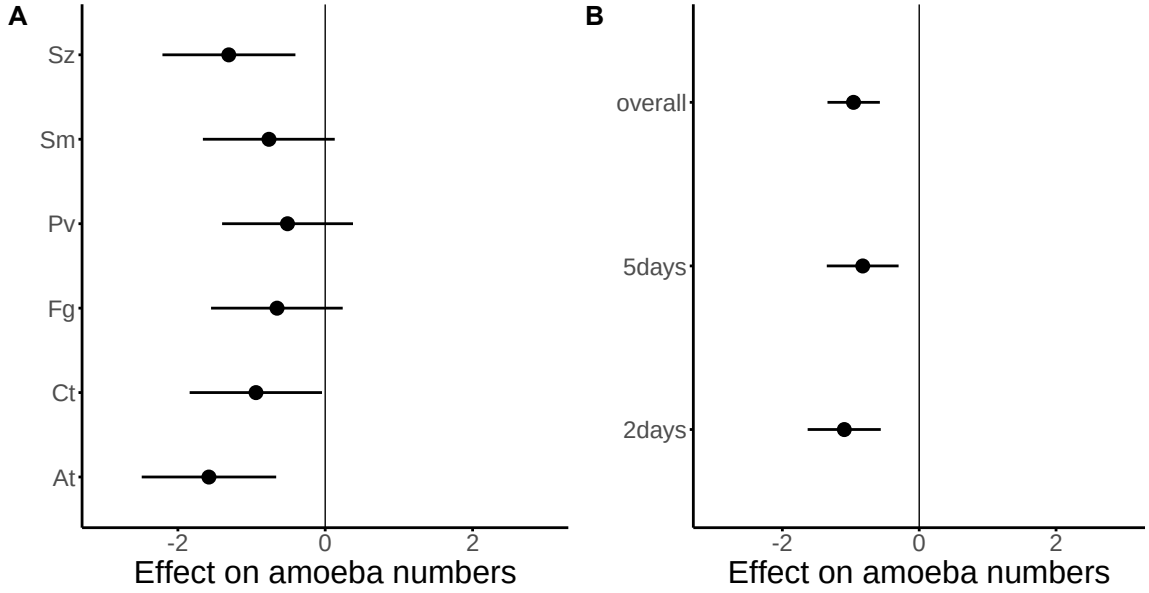

Figure S1: Estimated effect size (Cohen's d) ± 95% C.I. of amoeba numbers of the switched treatment minus the control treatment. **A)** shows that switched treatment in all test bacterium generally had a roughly similar negative effects on amoeba numbers. Plot **B)** shows that switched treatments of the different conditioning time had a similar negative effect on amoeba number compared to controls.

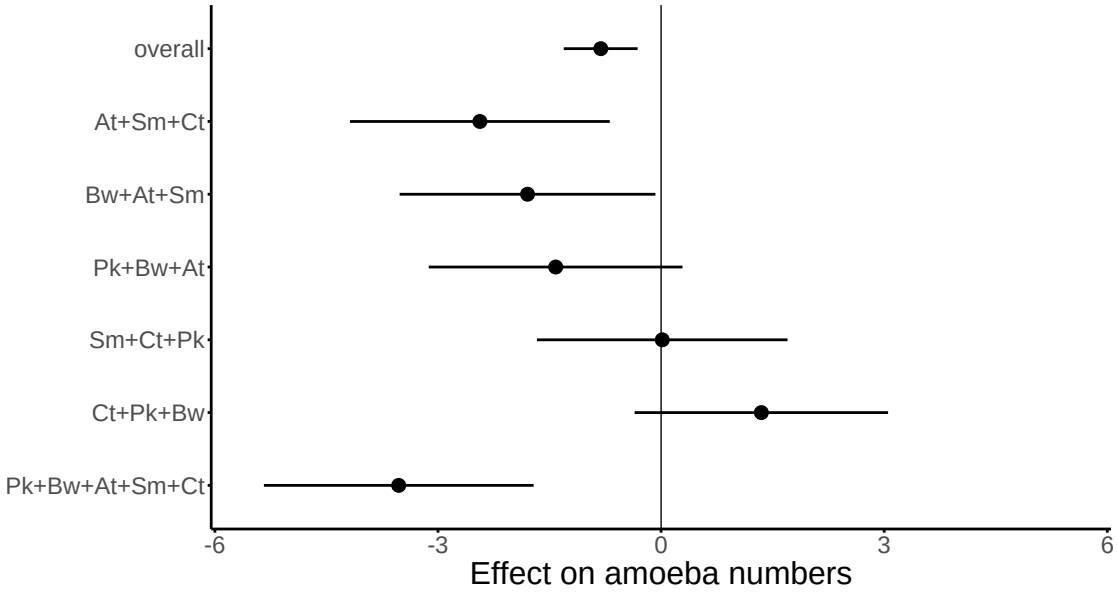

Figure S2: Estimated effect size (Cohen's d) ± 95% C.I. of observed – expected amoeba numbers in resource-mixing experiment averaged across observers. We see support for mixing costs overall and expected trend in four out of the six bacterial communities.

Table S1: ANOVA of amoeba growth rate assay

Formula: anova(lm (log(amoeba_number) ~ Test_bacterium))

	Df	Sum sq	Mean Sq	F_value	Pr(>F)
Test_bacterium	22	299.346	13.3794	24.949	2.20E-16
Residuals	46	24.668	0.5363		

Table S2: ANOVA of amoeba switching assay

Formula: anova(lm(log(amoeba_number) ~ Treatment x Test_bacterium + Treatment x Conditioning_length)

	Df	Sum sq	Mean Sq	F_value	Pr(>F)
Treatment	1	0.965	0.9648	27.621	7.69E-07
Test_bacterium	5	4.958	0.9917	28.392	2.00E-16
Conditioning_length	1	0.057	0.0567	1.623	0.205
Treatment x Test_bacterium	5	0.146	0.0293	0.838	0.526
Treatment x Conditioning_length	1	0.019	0.019	0.543	0.463
Residuals	106	3.702	0.0349		

Table S3: ANOVA of spore switching assay

Formula: anova(lm(log(amoeba_number) ~ Treatment x Test_bacterium + Treatment x Conditioning_length))

	Df	Sum sq	Mean Sq	F_value	Pr(>F)
Treatment	1	0.03	0.03	0.264	0.61
Test_bacterium	5	106.78	21.355	186.402	<2e-16
Treatment x Test_bacterium	5	0.68	0.136	1.191	0.328
Residuals	48	5.5	0.115		

Table S4: ANOVA of early growth rate data

Formula: anova(lm(log(amoeba_number) ~ Treatment + Test_bacterium))

	Df	Sum sq	Mean Sq	F_value	Pr(>F)
Treatment	1	0.012	0.012	12.988	0.0006923
Test_bacterium	5	0.043	0.009	9.561	1.52E-06
Residuals	53	0.048	0.001		

Table S5: ANOVA of late growth rate data

Formula: anova(lm(log(amoeba_number) ~ Treatment + Test_bacterium))

	Df	Sum sq	Mean Sq	F_value	Pr(>F)
Treatment	1	0.000	0.000	0.015	0.9045156
Test_bacterium	5	0.123	0.025	5.469	0.0003944
Residuals	53	0.239	0.005		

Table S6: ANOVA of resource-mixing data

Formula: anova(lm(log(amoeba_number)~Treatment *Community + Experimenter))

	Df	SumSq	MeanSq	F-value	Pr(>F)
Treatment	1	0.4564	0.4564	11.8394	0.001071
Community	5	1.6847	0.3369	8.74	3.01E-06
Experimenter	1	24.3675	24.3675	632.0609	2.20E-16
Treatment x Community	5	0.4929	0.0986	2.5571	0.036767
Residuals	59	2.2746	0.0386		