

SUPPLEMENTARY INFORMATION

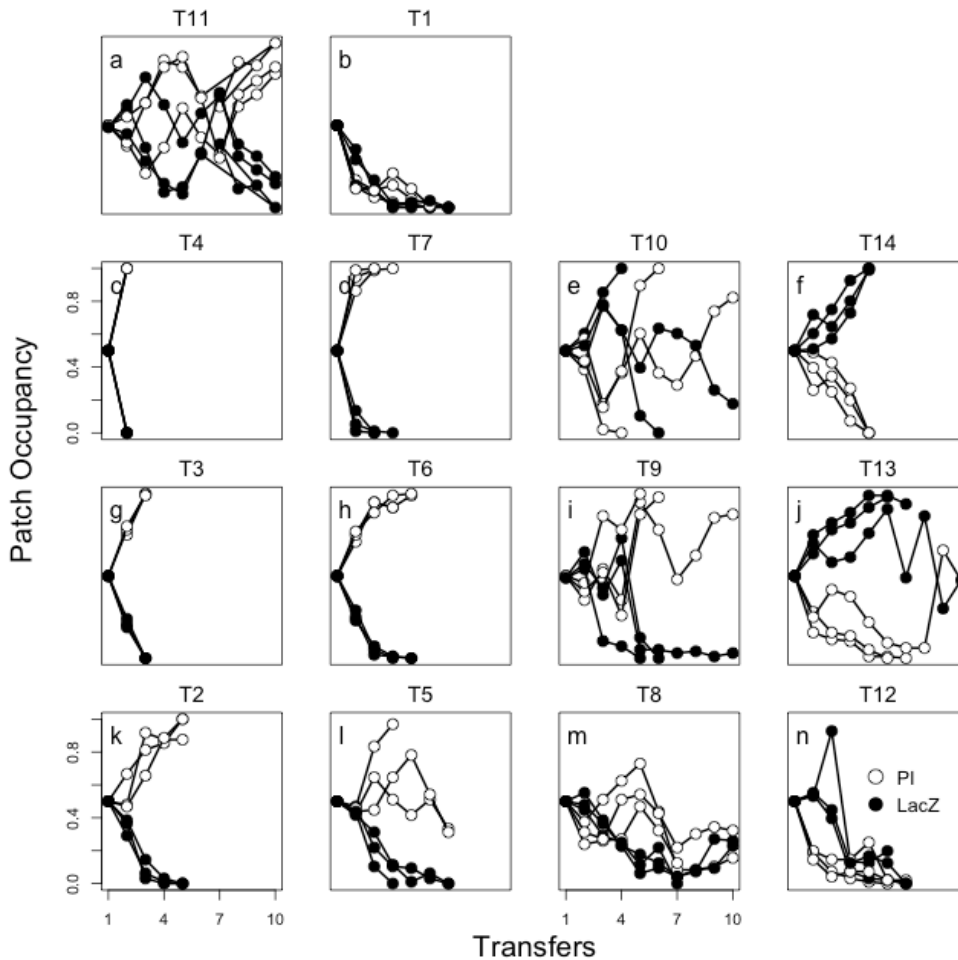
Supplementary Table S1: Treatment parameters. The “Treatment” column shows the treatment numbers (corresponding to those in Fig. 2 for treatments T1-T14). The isolation experiments are H7 and H11 and correspond to the initial parameters used in treatment T7 and T11. Metacommunity “isolation” refers to the proportion by which absolute colonization rates were reduced in the isolation experiment. “Dilutions LacZ” (the weak competitor) and “PI” (the strong competitor) are the negative exponent (base 10) of the “Concentration LacZ” and “PI” after dilution for each treatment. The “PI dilution reduction factor” represents a proportional reduction in the volume of PI before serial dilution was implemented (the strength of the trade-off).

Treatment	Isolation	Dilutions LacZ (10 ^{-x})	Dilutions PI (10 ^{-x})	Concentration LacZ	Concentration PI	PI dilution reduction factor (trade-off)
T1	-	7	7.968	1.00E-07	1.08E-08	0.892
T2	-	6.5	7.468	3.16E-07	3.40E-08	0.892
T3	-	6	6.968	1.00E-06	1.08E-07	0.892
T4	-	5.5	6.468	3.16E-06	3.40E-07	0.892
T5	-	6.5	7.55	3.16E-07	2.82E-08	0.911
T6	-	6	7.256	1.00E-06	5.54E-08	0.945
T7	-	5.5	6.962	3.16E-06	1.09E-07	0.965
T8	-	6.5	7.633	3.16E-07	2.33E-08	0.926
T9	-	6	7.545	1.00E-06	2.85E-08	0.972
T10	-	5.5	7.456	3.16E-06	3.50E-08	0.989
T11	-	5	7.368	1.00E-05	4.29E-08	0.996
T12	-	6.5	7.715	3.16E-07	1.93E-08	0.939
T13	-	6	7.833	1.00E-06	1.47E-08	0.985
T14	-	5.5	7.95	3.16E-06	1.12E-08	0.997
H7	0.7	5.5	6.962	3.16E-06	1.09E-07	0.966
H7	0	5.5	6.962	3.16E-06	1.09E-07	0.966
H7	0.9	5.5	6.962	3.16E-06	1.09E-07	0.966
H11	0.7	5	7.368	1.00E-05	4.29E-08	0.996
H11	0	5	7.368	1.00E-05	4.29E-08	0.996
H11	0.9	5	7.368	1.00E-05	4.29E-08	0.996

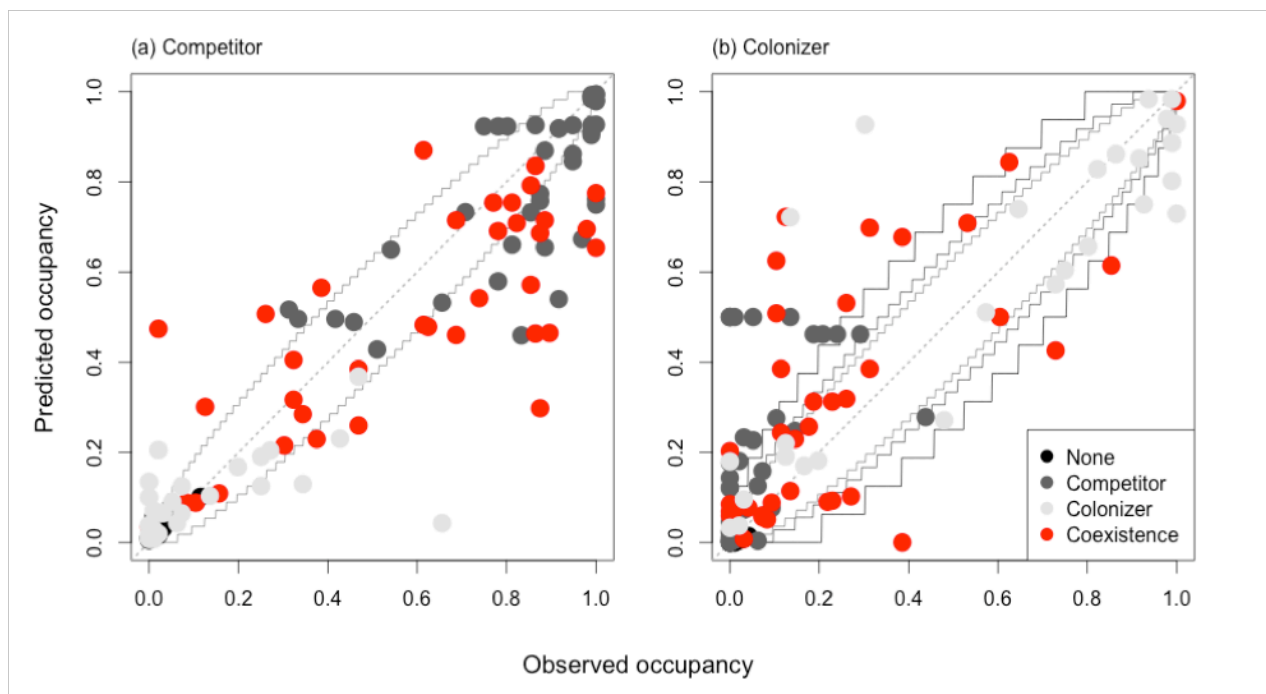
Supplementary Table S2. Generalized linear model (GLM) fitting with a binomial error distribution and a logit link function (GLM function in R ⁶¹). Levels of significance: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1. The response variable is the proportion of occupied patches by each strain in each replicate (averages of transfers 8-10). The trade-off predictor is the 'Competitor dilution reduction factor' (see Table S2) or the proportional reduction in the overnight culture volume of the competitor prior to dilution (the reduction in colonization rate). Strain is the strain identity; colonizer (LacZ) or competitor (PI). Treatment T1 was not included in this analysis because the extinction of both strains was predicted. Strain details $n = 78$.

Coefficients:	Estimate	Std. Error	Z value	Pr(> z)
(Intercept)	33.86	13.29	2.547	0.010864*
Trade-off	-34.31	13.73	2.499	0.012444*
Strain	-74.75	20.82	3.589	0.000331***
Trade-off x Strain	75.67	21.44	3.530	0.000416***

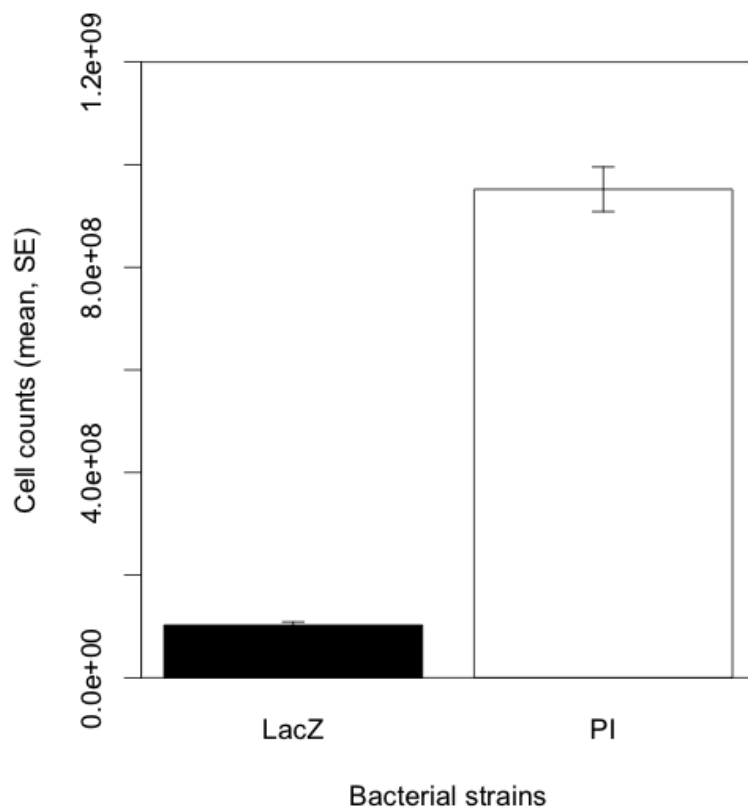
Supplementary Figure S1: **Time series for all treatments (T1-T14) from the main experiment.** Panel a shows treatments T11 and T1, which are outside the three main absolute dilution levels (see Fig. 2). Panels c-f, g-j and k-m each cross the coexistence zone along a range of absolute dilution rates (see Fig. 2). Each treatment included three replicates and was run for a maximum of 10 transfers. Replicates were stopped after the extinction of one or both strains occurred. Different circle colors indicate different strains: LacZ, black circles; PI, white circles.



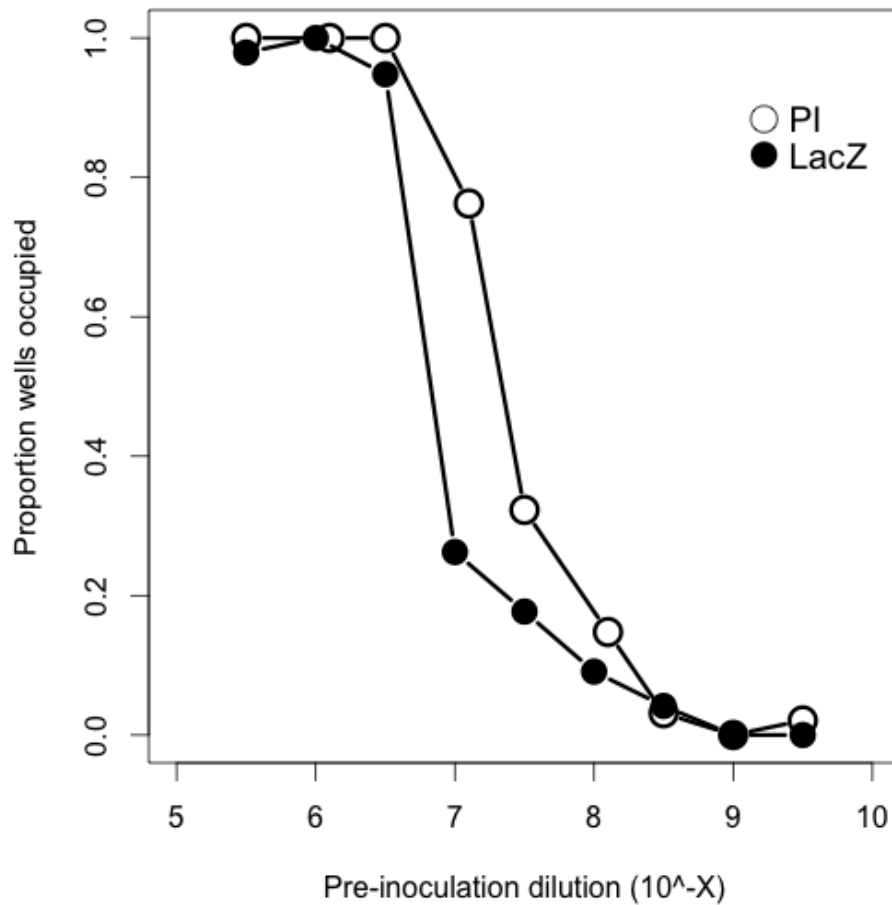
Supplementary Figure S2: **Observed and predicted occupancy for each strain after each transfer.** Colors indicate predicted persistence scenario. Four different scenarios are indicated by colored circles: the persistence of the two strains (red); the competitor persists (dark gray); the colonizer persists (light gray) and none of the strains persist (black). The dashed line indicates the 1:1 (perfect) match between predicted and observed values. Points above and below this line indicate that observed occupancies were greater or less than those predicted by the model, respectively. Full lines indicate 95% confidence envelopes from the stochastic binomial distribution of colonization events. Predicted and observed occupancy for the colonizer are scaled to available patches (i.e. those unoccupied by the competitor), and the different envelopes correspond to increasing proportions of available patches (from 1 – inner envelope to 0.2 – outer envelope).



Supplementary Figure S3: **Growth of LacZ (weak competitor) and PI (strong competitor)**. Cells were counted after 24 hours of growth in wells filled with 200 μl KB in a 96-well microplate using droplet plating (15 μl droplets diluted to a 10^{-6} concentration, replicated 3 times) on a selection of 5 wells with equal inoculation proportions of each strain. Bars represent means values for the total estimated number of cells in a single well and error bars represent standard error.



Supplementary Figure S4: **Proportion of wells containing each strain in 96-well plates as a function of the pre-inoculation dilution.** Different colors of circles indicate different strains: LacZ, black circles; PI, white circles. Overnight cultures of each strain were serially diluted equal amounts before inoculation onto separate 96-well microplates and grown for 24 h in 200 μ l of KB. Each dilution level per strain was not replicated. Notably, the initial cell density was not equalized among strains prior to this trial, generating variation among the curves for each strain.



Supplementary Methods

This methods section describes two experiments that manipulated competition-colonization trade-offs of bacterial metacommunities in experimental microcosms. In the first experiment – the main experiment – we quantitatively manipulated two key parameters: trade-off strength and absolute colonization rate. In the second experiment – the isolation experiment – we manipulated the isolation of each community from the species pool by imposing reductions in overall colonization rate. These experiments were conducted with marked *Pseudomonas fluorescens* strains of known competitive ability that can be made to approximate a two-species system. The supplementary methods detail three aspects of the design: 1) competition assays; 2) measuring abundances; and 3) the experimental protocol.

Competition assays. We assayed the competitive ability of both strains after 24 and 48 h in KB medium at 28°C in 96-well (240- μ l) microplates (Falcon USA #353072). We used the range of relative inoculation densities of the two strains that would be used in our experiment by diluting LacZ and PI from overnights (incubated together) at rates corresponding to the same 14 treatments implemented in the main experiment (Supplementary Table S1; three replicates per treatment). This method produced a range from equal initial proportions of each strain to strongly biased toward LacZ. After 24 h of growth (conditions described in Methods *Bacterial strains*), we used a 96 pin replicator (Boeckel well model 140500) to check for the presence/absence of each strain from one microplate per treatment (selected at random). One microliter of culture was filed onto petri dishes that contained a mixture of LB agar (Luria-Bertani medium with agar; 5 g.l⁻¹ yeast extract

+ 10 g.l⁻¹ tryptone + 5 g.l⁻¹ NaCl, autoclaved 20 min at 121°C), X-gal and IPTG. PI colonies (yellow) can easily be distinguished from the LacZ (blue) on this medium. We further confirmed the pin replicator results using droplet plating (15- μ l droplets diluted to a 10⁻⁶ concentration, replicated 3 times) on a selection of 5 wells from the microplate with equal inoculation proportions of each strain (Supplementary Fig. S3).

We observed complete exclusion of LacZ after 24 h in all equal proportion wells and in 99% of the 1344 wells in the 14 microplates. However, when the initial inoculation proportion was biased toward LacZ by one order of magnitude or greater, complete exclusion in the remaining 1% of wells was not observed until after 48 h with plating using a pin replicator. Importantly, PI dominated these wells so that our measurement procedure for microplates (see Methods *Basic experimental design*) recorded these wells as occupied by PI even though LacZ was not excluded until 48 h. From this, we considered the competitive exclusion of LacZ by PI in patches as effectively instantaneous after 24 h.

Measuring abundances. Preliminary trials showed that optical density (OD) measurements (650 nm) of growth were sufficiently accurate for our high cell density microplates and that a simple linear relationship exists between OD values and bacterial cell counts ($R^2 = 0.943$, $P < 0.0001$, $F = 133$, $\text{cells} = 1.247 \times 10^8 * \text{OD} + 0.971$). We developed this equation by droplet plating overnight cultures ($n = 10$, 5 per strain) with cell counts (replicated 3x at 10⁻⁶ dilution for plating) ranging from 3.33×10^5 cells/15 μ l to 7.14×10^7 cells/15 μ l and regressing the counts against OD

values ($n = 10$) for the same cultures. All OD measurements were performed with a FLUOstar Optima spectrophotometer (BMG LABTECH).

We developed a procedure to determine the presence of a strain in a well by calculating maximum growth thresholds for each strain. This calculation could be achieved because the mean OD-estimated growth at 10^{-5} initial dilution of LacZ (0.606, +/- 0.026) is substantially lower than PI (1.092, +/- 0.037). After 24 hours, these differences hold across all dilutions used in the experiment, with the maximum LacZ value of 0.666 never exceeding the minimum PI value of 0.893. In addition, the minimum growth of LacZ is always greater than OD values for wells with blank medium. Given these values, we considered that wells with OD values < 0.750 could be classified as LacZ, whereas those > 0.750 could be classified as PI. We are confident that this method is accurate; although an average of 4% of wells identified as PI at 24 h had not yet reached exclusion of LacZ in high dilution treatments, 100 % of wells classified as PI at 24 hours showed exclusion at 48 h. Wells with values less than the maximum blank value +0.10 were identified as no-growth wells. Due to the large volume of microplates, some minimal error in OD measurement is expected over the course of the experiment. To ensure the accuracy of the identification of LacZ and PI, a randomly selected replicate from each treatment was pin-replicated each transfer using a 96-pin replicator. Following 48 h incubations, the correlation between OD strain/no-growth classifications and plated values was strong and highly significant (PI: $R^2 = 0.996$, $F = 18477$; LacZ: $R^2 = 0.969$, $F = 2285$, $P < 0.0001$; $n = 75$ for both strains).

Detailed experimental protocol. Here, we provide a detailed account of our transfer procedure, which involved setting up initial conditions, determining relative abundances of each strain, and generating the colonizer pool for the subsequent transfers. This procedure was supported by an R script (see Supplementary Note) that compiles and analyzes information about observed growth and occupancy by taking into account experimental treatments (i.e. trade-off strength and colonization rate). The script outputs the inoculation volumes for the subsequent transfer independently for each replicate. This procedure allowed us to follow the dynamics of each replicate separately and control for potential daily variation in overnight growth from our frozen stocks. For clarity, we divided this protocol into six steps 1) initial conditions, 2) building pool of colonizers, 3) implementing trade-offs, 4) inoculation, 5) measuring abundances, and 6) characterizing colonizers pool.

1. **Initial conditions.** To achieve an initial dilution approaching the limit of <100% successful colony growth across the microplate, we diluted 4000 μl of LacZ and 2344 μl of PI (OD corrected, higher OD value corrected to lower) in culture volume separately for each strain 5x serially in 18 ml of M9 minimal salts medium (NH_4Cl , 1 g l^{-1} ; Na_2HPO_4 , 6 g l^{-1} ; KH_2PO_4 , 3 g l^{-1} ; NaCl , 0.5 g l^{-1} , autoclaved 20 min at 121°C). Using a multi-channel pipette, 10 μl of each strain was added with 190 μl of KB in two separate halves of a 96-well microplate as arbitrary non-equilibrium starting conditions. All microplates were placed in the incubator at the same time at 28°C for 24 hours, after which OD was measured for each microplate. All transfers included three

- microplates designated as “blanks.” Blank microplates received 10 µl of M9 minimal salts media without bacteria, but were otherwise subjected to the same procedures as the treatment microplates. A second set of overnight tubes was grown with these microplates to initiate the second transfer.
- 2. Building a pool of colonizers:** The first step to generate the colonizer pool was to determine how much of each strain is present in the metacommunity. This task was achieved by categorizing the growth value for each well as either LacZ or PI (see the *Measuring abundances* section) to calculate regional abundances of each strain per replicate. Because the dilution amount was computed using cell densities, all OD values were converted to estimated cell counts using the equations previously described in the *Measuring abundances* section.
 - 3. Implementing trade-offs:** The estimated cell counts were subsequently multiplied by the proportion of each microplate occupied by each strain and the strength of the trade-off (applied as a proportional reduction to PI only and produced using predicted dilution rates from our model; see Supplementary Table S1 for values) and corrected against the overnight OD values to determine the volume of each strain (adjusted from 200 µl) to add. The equation implemented for PI was $\text{Volume} = \text{cells} * \text{trade-off} * (\text{cells}/\text{cells_overnight}) * 200$, and for LacZ, it was $\text{Volume} = \text{cells} * (\text{cells}/\text{cells_overnight}) * 200$. Note that for “no trade-off” scenarios, the dilution rates were not equal between the two strains because the final growth of PI is always greater than LacZ. To equalize the number of cells

inoculated, the dilution rate was higher for PI (1.08^{-08} , and 1.0^{-07} for LacZ) under “no trade-off” scenarios.

4. **Inoculation:** The inoculation volume generated by the previous step was subsequently diluted serially in wells with 1600 μ l (the initial volume was adjusted up or down to accommodate the exact volumes added for each strain).
5. **Measuring abundances:** Abundances were estimated in each microplate metacommunity by estimating optical density at 650nm on a FLUOStar spectrophotometer. See details on the abundances estimates in the “Measuring abundances” above.
6. **Characterize colonizer pool:** A custom made script in R was used to estimate the relative abundances of each strain in the colonizer pool based on the data from step 5). This script takes in account the bacterial in each individual well and calculates the exact contribution of each strain for the colonizers pool of the subsequent step. These calculations were done separately for each replicate of each treatment.

Ending the experiments: We stopped transferring a replicate if one or two strains reached zero occupancy. After each transfer, all microplates were discarded, and new microplates were inoculated using data from the previous transfer. All replicates were processed independently at each step.

Supplementary Note: R script describing the calculations involved in each transfer, including serial dilution and routines for correcting variation within 96-well microplates due to edge effects.

```
#####  
#  
# This R script describes the calculations involved in the experiments described in the paper:  
#  
# Competition-colonization dynamics in experimental bacterial metacommunities  
#  
# 1. George Livingston*, 2. Miguel Matias*, 3. Vincent Calcagno, 4. Claire Barbera, 5. Marine  
# Combe, 6. Mathew A. Leibold and 7. Nicolas Mouquet  
#  
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# Austin, TX 78712, USA  
#  
# For additional information please contact the corresponding at at glivingston@utexas.edu  
#  
#####  
# libraries  
  
library(SCiAn)  
  
#####  
# set working directory  
  
path <- c("/your path/")  
  
#####  
# set parameters  
  
# upload table with parameters  
FINALtable <- read.csv(paste(path,"/treatment_tables/treatments_table.csv",sep=""))  
  
# set number of transfers transfer  
transf=10  
  
# set the overnight values measured using optical density  
piOV=c(0.9132)
```

```

IOV=c(0.6316)
blankOV=c(0.466)

# remove the background signal from the growth medium
piOV=piOV-blankOV
IOV=IOV-blankOV

# calculate cells concentrations based on previously established relationship
logCellIOVI <- 3.316*piOV+.899
logCellIOVL <- 3.316*IOV+.899

# determine the actual cell densities for each strain
CellIOVI <- 10^logCellIOVI
CellIOVL <- 10^logCellIOVL

# set treatments (this example refers to the second habitat destruction experiment)
treatments=c("L5","L8","5","8","H5","H8")

# set number of replicates
replicates=c(1:3)

#####
# Calculate mean and max blanks

# Load blank plates and using Scian functions. These functions are available at CRAN and
  facilitate the upload from Spectrophotometer's output files.

data.blank=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_B_", 1, ".csv", sep="")))
blank1 <- data.frame(position=NA,measure=NA)
for (i in 1:length(data.blank)) {blank1[i,2]<-data.blank[i]}

data.blank=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_B_", 2, ".csv", sep="")))
blank2 <- data.frame(position=NA,measure=NA)
for (i in 1:length(data.blank)) {blank2[i,2]<-data.blank[i]}

data.blank=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_B_", 3, ".csv", sep="")))
blank3 <- data.frame(position=NA,measure=NA)
for (i in 1:length(data.blank)) {blank3[i,2]<-data.blank[i]}

# determine the max blank value
max(blank1[,2])
max(blank2[,2])
max(blank3[,2])

# determine the mean blank value per rows and columns to adjust for potential edge effects
firstrowmaxblank <- max(cbind(blank1[1:12,2],blank2[1:12,2],blank3[1:12,2]))
firstrowmeanblank <- mean(cbind(blank1[1:12,2],blank2[1:12,2],blank3[1:12,2]))
siderowmaxblank <-

```

```

max(cbind(blank1[c(24,36,48,60,72,84),2],blank2[c(24,36,48,60,72,84),2],blank3[c(24,36,48,60,72,84),2]))
siderowmeanblank <-
mean(cbind(blank1[c(24,36,48,60,72,84),2],blank2[c(24,36,48,60,72,84),2],blank3[c(24,36,48,60,72,84),2]))
bottomrowmaxblank <- max(cbind(blank1[85:96,2],blank2[85:96,2],blank3[85:96,2]))
bottomrowmeanblank <- mean(cbind(blank1[85:96,2],blank2[85:96,2],blank3[85:96,2]))
interiormeanblank <- mean(cbind(blank1[-c(1:12,24,36,48,60,72,84,85:96),2],blank2[-c(1:12,24,36,48,60,72,84,85:96),2],blank3[-c(1:12,24,36,48,60,72,84,85:96),2]))

```

determine the max blank values for the entire set of plates using boxplot.stats function. These adjustments are essential to improve the accuracy of the method of determining the type of strain based on their growth

```

allblank <- rbind(as.matrix(blank1[1:12,2]),as.matrix(blank2[1:12,2]),as.matrix(blank3[1:12,2]))
blankstats <- boxplot.stats(allblank)
firstrowmaxblank <- blankstats$stats[5]

```

```

allblank <-
rbind(as.matrix(blank1[c(24,36,48,60,72,84),2]),as.matrix(blank2[c(24,36,48,60,72,84),2]),as.matrix(blank3[c(24,36,48,60,72,84),2]))
blankstats <- boxplot.stats(allblank)
siderowmaxblank <- blankstats$stats[5]

```

```

allblank <-
rbind(as.matrix(blank1[85:96,2]),as.matrix(blank2[85:96,2]),as.matrix(blank3[85:96,2]))
blankstats <- boxplot.stats(allblank)
bottomrowmaxblank <- blankstats$stats[5]

```

```

allblank <- rbind(as.matrix(blank1[-c(1:12,24,36,48,60,72,84,85:96),2]), as.matrix(blank2[-c(1:12,24,36,48,60,72,84,85:96),2]), as.matrix(blank3[-c(1:12,24,36,48,60,72,84,85:96),2]))
blankstats <- boxplot.stats(allblank)
interiormaxblank <- blankstats$stats[5]

```

```
#####
```

```
# Main loop
```

```
counter=1
```

```
for (treat in treatments) {
```

```
  for (rep in replicates) {
```

```
    #Read DO measure file
```

```
    data.plaque=formatDF(read.cinetic(paste(path,"/data/D_", transf,"_", treat,"_", rep, ".csv" , sep="")))

```

```
    mat <- data.frame(strain=NA,measure=NA)
```

```
    for (i in 1:length(data.plaque)) {mat[i,2]<-data.plaque[i]}

```

Determine the type of strain based on optical density with adjustments for edge effects. The cut-offs are adjusted based on daily overnights and blanks.

```
# Interior
for (i in 1:96) {
  if (mat[i,2] < interiormaxblank+0.010) {mat[i,1]="NG"} # No growth
  if (mat[i,2] >= interiormaxblank+0.010) {if (mat[i,2] < 0.800) {mat[i,1]="L"}} # Lac
strain
  if (mat[i,2] >= 0.800) {mat[i,1]="PI"} # Pi strain
}

# Cut-offs top row!!
for (i in 1:12) {
  if (mat[i,2] < firstrowmaxblank +0.010) {mat[i,1]="NG"} # No growth
  if (mat[i,2] > firstrowmaxblank +0.010) {if (mat[i,2] < 0.870) {mat[i,1]="L"}} # Lac
strain
  if (mat[i,2] > 0.870) {mat[i,1]="PI"} # Pi strain
}

# Cut-offs bottom row!!
for (i in 85:96) {
  if (mat[i,2] < bottomrowmaxblank +0.010) {mat[i,1]="NG"} # No growth
  if (mat[i,2] > bottomrowmaxblank +0.010) {if (mat[i,2] < 0.870) {mat[i,1]="L"}} # Lac
strain
  if (mat[i,2] > 0.870) {mat[i,1]="PI"} # Pi strain
}

# Cut-offs side row!!
for (i in c(24,36,48,60,72,84)) {
  if (mat[i,2] < siderowmaxblank+0.010) {mat[i,1]="NG"} # No growth
  if (mat[i,2] > siderowmaxblank+0.010) {if (mat[i,2] < 0.870) {mat[i,1]="L"}} # Lac
strain
  if (mat[i,2] > 0.870) {mat[i,1]="PI"} # Pi strain
}

#### Edge effect corrections ####

# Calculate means for each position
mfirstrow <- mean(mat[1:12,2])
innertop <- mat[13:48,2]
minnertop <- mean(innertop[-c(12,24,36)])
sidetop <- mean(innertop[c(12,24,36)])
mbottomrow <- mean(mat[85:96,2])
innerbottom <- mat[49:84,2]
minnerbottom <- mean(innerbottom[-c(12,24,36)])
sidebottom <- mean(innerbottom[c(12,24,36)])
```



```

# correct top
for (i in 1:12) {mat[i,2] <- mat[i,2]*(minnertop/mfirstrow)}
# correct bottom
for (i in 85:96) {mat[i,2] <- mat[i,2]*(minnerbottom/mbottomrow)}
# correct last column top
for (i in c(24,36,48)) {mat[i,2] <- mat[i,2]*(minnertop/sidetop)}
# correct last column bottom
for (i in c(60,72,84)) {mat[i,2] <- mat[i,2]*(minnerbottom/sidebottom)}

```

```

# Blank correction
mat[1:96,2]=mat[1:96,2]-interiormeanblank # remove interior blank

```

Daily transfer routine

1. Take the mean DO values and divide them by overnight DO values for each strain.
Multiply these proportions by 400ul for each strain to caculate DO corrected volumes (VL,VI).

```

#Calculate the mean measure by strain and counts
matPI <- subset(mat,mat$strain=="PI",select="measure")
countsPI <- nrow(matPI)
meanPI <- mean(matPI)
if (countsPI==0) meanPI=0

```

```

logCellI <- 3.316*meanPI+.899
CellI <- 10^logCellI # actual values 10^6 cells

```

```

matL <- subset(mat,mat$strain=="L",select="measure")
countsL <- nrow(matL)
meanL <- mean(matL)
if (countsL==0) meanL=0

```

```

logCellL <- 3.316*meanL+.899
CellL <- 10^logCellL

```

Daily overnight adjustment

```

piadj <- CellI/CellOVI
ladj <- CellL/CellOVL

```

Dilution volumes adjustment

```

VI1 <- piadj*400
VL1 <- ladj*400

```

2. Calculate from the layout the number of wells (out of 96) occupied for each strain.

Multiply VL and VI from #1 by these proportions.

```
propI <- countsPI/96  
propL <- countsL/96
```

Volume by occupancy adjustment

```
VI2 <- VI1*propI  
VL2 <- VL1*propL
```

3. Multiply volume by occupancy (VI) by the treatment specific reduction factor.

VI3 <- VI2*FINALtable[counter,"IDF"] # The table with reduction factors can be found in the supplementary material provide with the paper

```
VL3 <- VL2
```

4. Multiply VI3 by the destruction factor (This step is only for the second experiment)
This factor reduces the volume added to the dispersal pool by a proportion equivalent to the global reduction in dispersal rate

```
if (treat=="L5") {  
  VI4 <- VI3*FINALtable[counter,"Destruction"]  
  VL4 <- VL3*FINALtable[counter,"Destruction"]  
}  
if (treat=="L8") {  
  VI4 <- VI3*FINALtable[counter,"Destruction"]  
  VL4 <- VL3*FINALtable[counter,"Destruction"]  
}  
if (treat=="H5") {  
  VI4 <- VI3*FINALtable[counter,"Destruction"]  
  VL4 <- VL3*FINALtable[counter,"Destruction"]  
}  
if (treat=="H8") {  
  VI4 <- VI3*FINALtable[counter,"Destruction"]  
  VL4 <- VL3*FINALtable[counter,"Destruction"]  
}  
if (treat=="5") {  
  VI4 <- VI3  
  VL4 <- VL3  
}  
if (treat=="8") {  
  VI4 <- VI3  
  VL4 <- VL3  
}
```

Initial dilution. The corrections below are implemented to ensure all inoculation volumes are greater than 10ul.

Dilutions are implemented in 2ml x 4 x 6 microplates by changing tips after each ejection and with 200ul of transfer volume except for some final volumes which are 632ul if it is a half

dilution step

```
dill=NA
if (VI4<10) { # correction in case the volume of VI4 is <10
dill <- VI4
  VI4=VI4*10
  IniDil <- 2000-VI4-VL4
}
if (VI4<10) { # correction in case the volume of VI4 is still <10
VI4=VI4*10
IniDil <- 2000-VI4-VL4
}
if (VI4<10) { # correction in case the volume of VI4 is still <10
VI4=VI4*10
IniDil <- 2000-VI4-VL4
}
dill=NA
if (VL4<10) { # correction in case the volume of VL4 is <10
dill <-VL4
VL4=VL4*10
IniDil <- 2000-VI4-VL4
}
```

```
if (VI4>=10) {IniDil <- 2000-VI4-VL4}
if (VL4>=10) {IniDil <- 2000-VI4-VL4}
```

Output table

```
FINALtable[counter,"Transfer"] <- transf
FINALtable[counter,"Max_blank"] <- interiormaxblank
FINALtable[counter,"Mean_blank"] <- interiormeanblank
FINALtable[counter,"OV_Lac"] <- IOV
FINALtable[counter,"OV_PI"] <- piOV
FINALtable[counter,"CellIOVI"] <-CellIOVI
FINALtable[counter,"CellIOVL"] <-CellIOVL
FINALtable[counter,"CountsI"] <- countsPI
FINALtable[counter,"CountsL"] <- countsL
FINALtable[counter,"MeansI"] <- meanPI
FINALtable[counter,"MeansL"] <- meanL
FINALtable[counter,"CellI"] <-CellI
FINALtable[counter,"CellL"] <-CellL
FINALtable[counter,"piadj_"] <- piadj
FINALtable[counter,"ladj_"] <- ladj
FINALtable[counter,"VI1_"] <- VI1
FINALtable[counter,"VL1_"] <- VL1
FINALtable[counter,"propI_"] <- propI
FINALtable[counter,"propL_"] <- propL
FINALtable[counter,"VI2_"] <- VI2
```

```

FINALtable[counter,"VL2_"] <- VL2
FINALtable[counter,"VI3_"] <- VI3
FINALtable[counter,"VL3_"] <- VL3
FINALtable[counter,"VI4_"] <- VI4
FINALtable[counter,"VL4_"] <- VL4
FINALtable[counter,"Initial.dilution"] <- IniDil
FINALtable[counter,"correctionI"] <- dill
FINALtable[counter,"correctionL"] <- dilL

counter=counter+1
}# for (rep in replicates)

} #for (t in treat)

write.table(FINALtable[-c(2,7,8,9,11,14,15,16,17,18)],paste(path,
transf, ".txt", sep=""),row.names=FALSE)

#####
# Plot dynamics

graphtable <- FINALtable[,c("Treatment", "Replicate", "propL_", "propl_")]

graphL <- tapply(graphtable$propL_,graphtable$Treatment,mean)
graphI <- tapply(graphtable$propl_,graphtable$Treatment,mean)
labels <- unique(FINALtable[, "Treatment"])

graphbind <- cbind(labels, graphL, graphI, graphL+ graphI)

barplot(t(graphbind[,-1]),beside=TRUE,legend.text=c("Lac", "Pi", "Total"))

#####

```

Supplementary References

61. R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: <http://www.R-project.org/>.