

# Models of microbial communities: diversity & functioning



Microbial communities provide many ecosystem services:

Decomposition

Carbon and nitrogen cycling

Waste water treatment

Bioremediation: pollution, oil spills

(Rivers et al. 2013 ISME Journal. 11:789)

Nutrients for plant roots, exclude pathogens

(Philippot et al. 2013 Nat. Rev. Microb. 7:2315)

and disservices:

Disease

metabolic function

## Human gut:

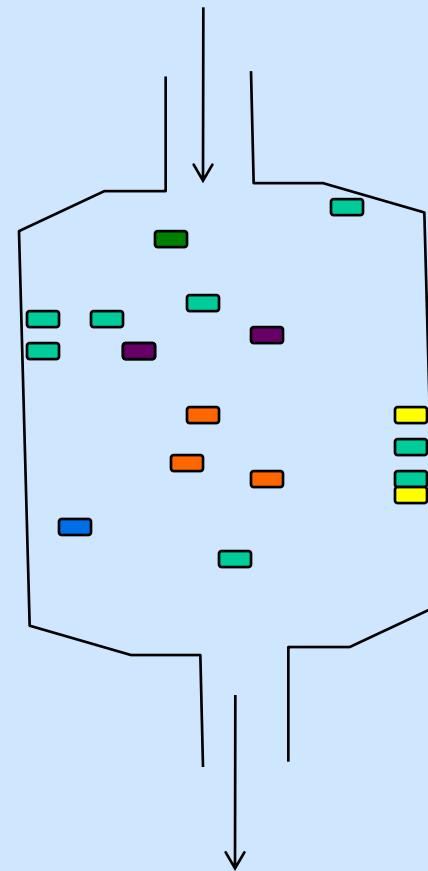
$10^{15}$  bacteria, thousand species, many unculturable  
(highest density in colon - anaerobes)

Invasion: pathogens or probiotics?

Homeostasis to maintain function

Fitness and inheritance

A separate organ



## Human gut:

- Diversity (next generation sequencing)
- Function: nutrition; protection
- Dynamics: effects of antibiotics
- Management: pre- and pro-biotics
- Evolution? Models versus experiments

Robinson et al. 2010. From structure to function: the ecology of host-associated microbial communities.  
*Microb. Mol. Biol. Rev.* 74: 453-476

## Human gut: diversity

Most bacteria are unculturable

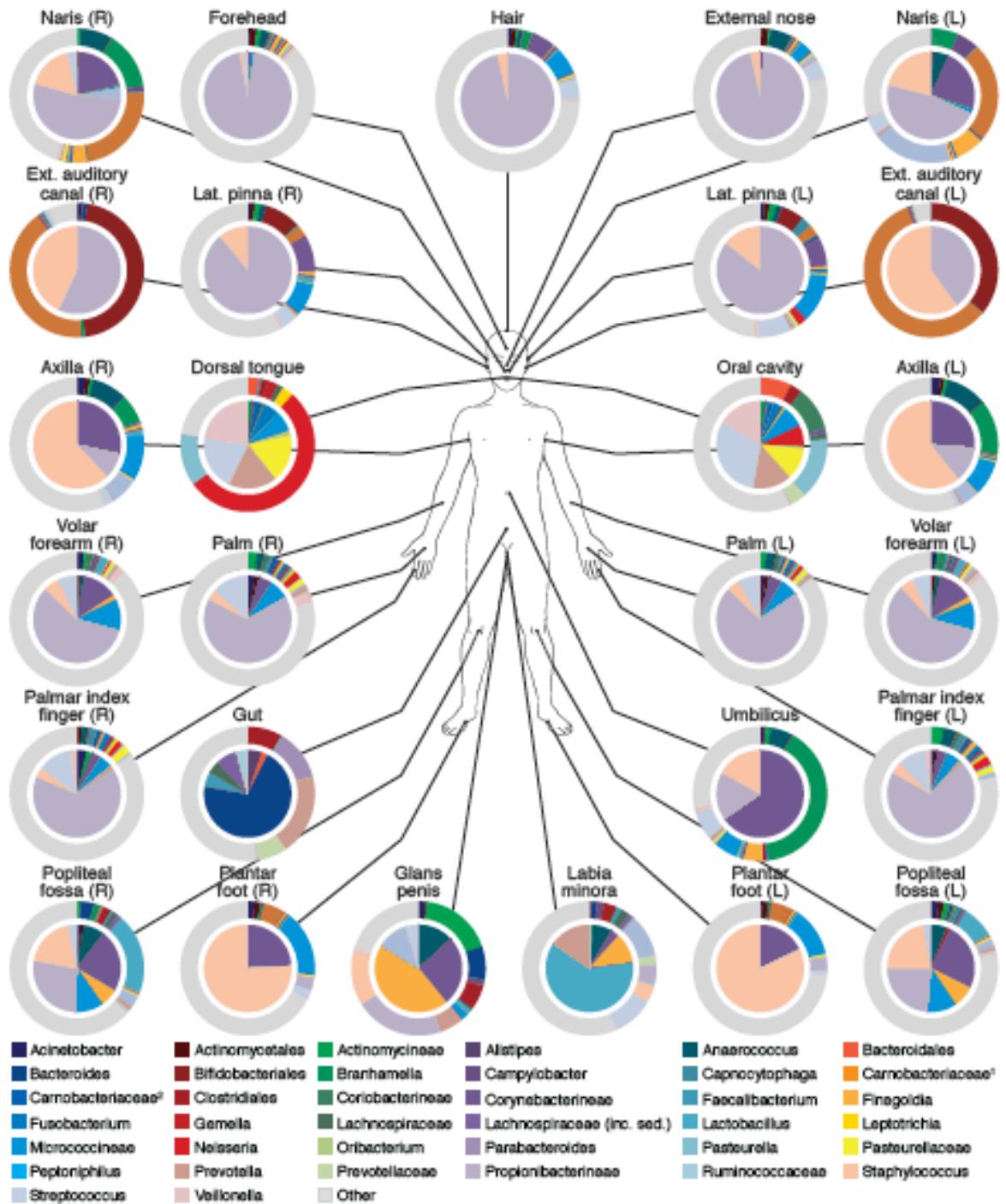
Only discovered existence using 16S rRNA sequences

Recent studies can sample millions of sequences

Costello et al. 2009  
Bacterial community  
variation in human  
body habitats.  
Science 326:1694

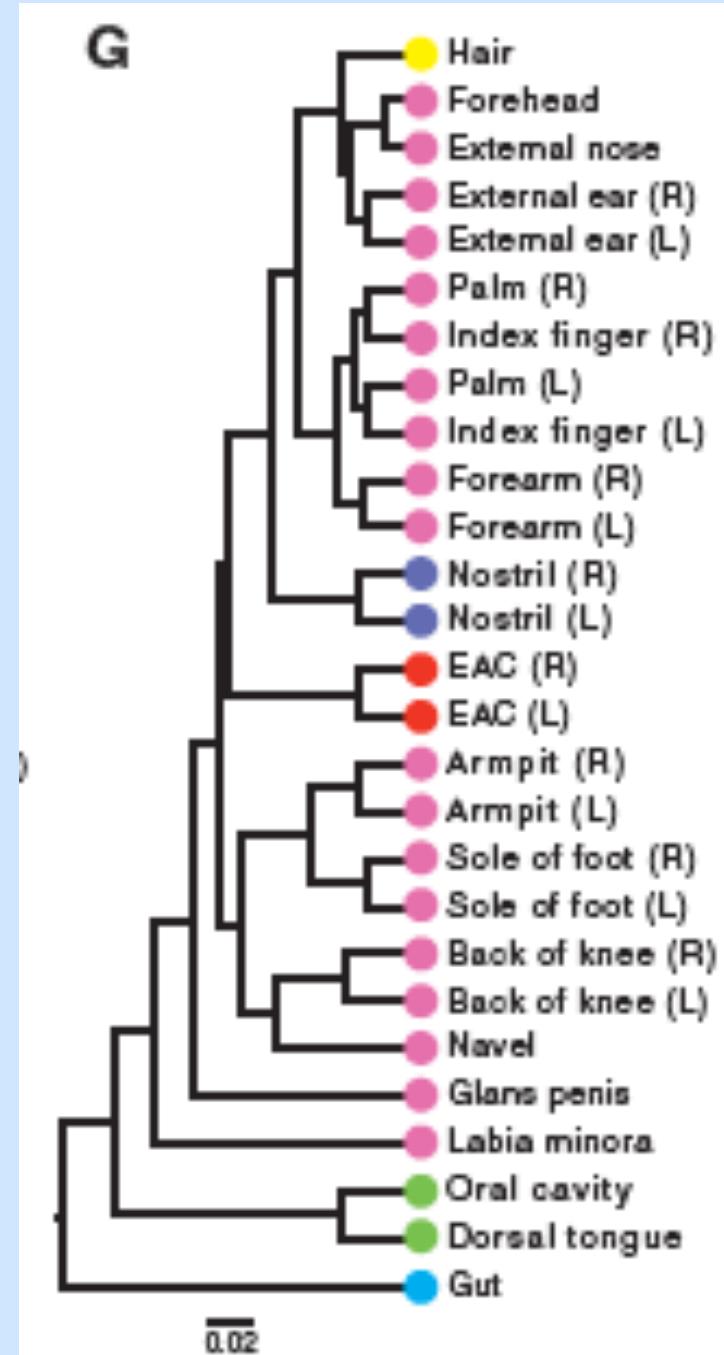
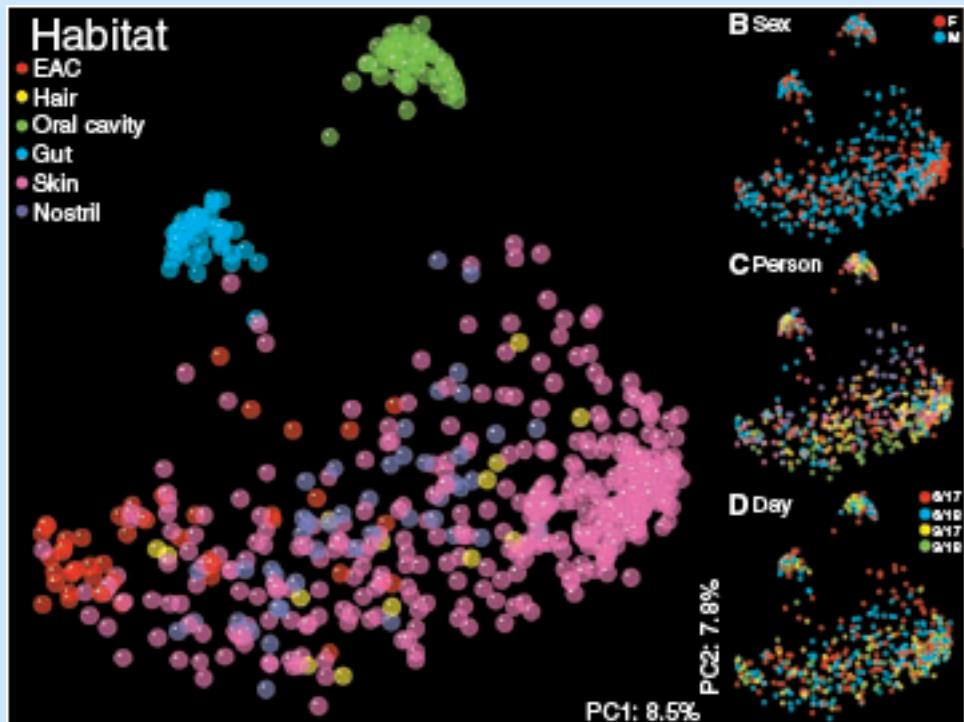
454 sequencing

>1,070,000  
sequences of V2  
region of 16S



Costello et al. 2009

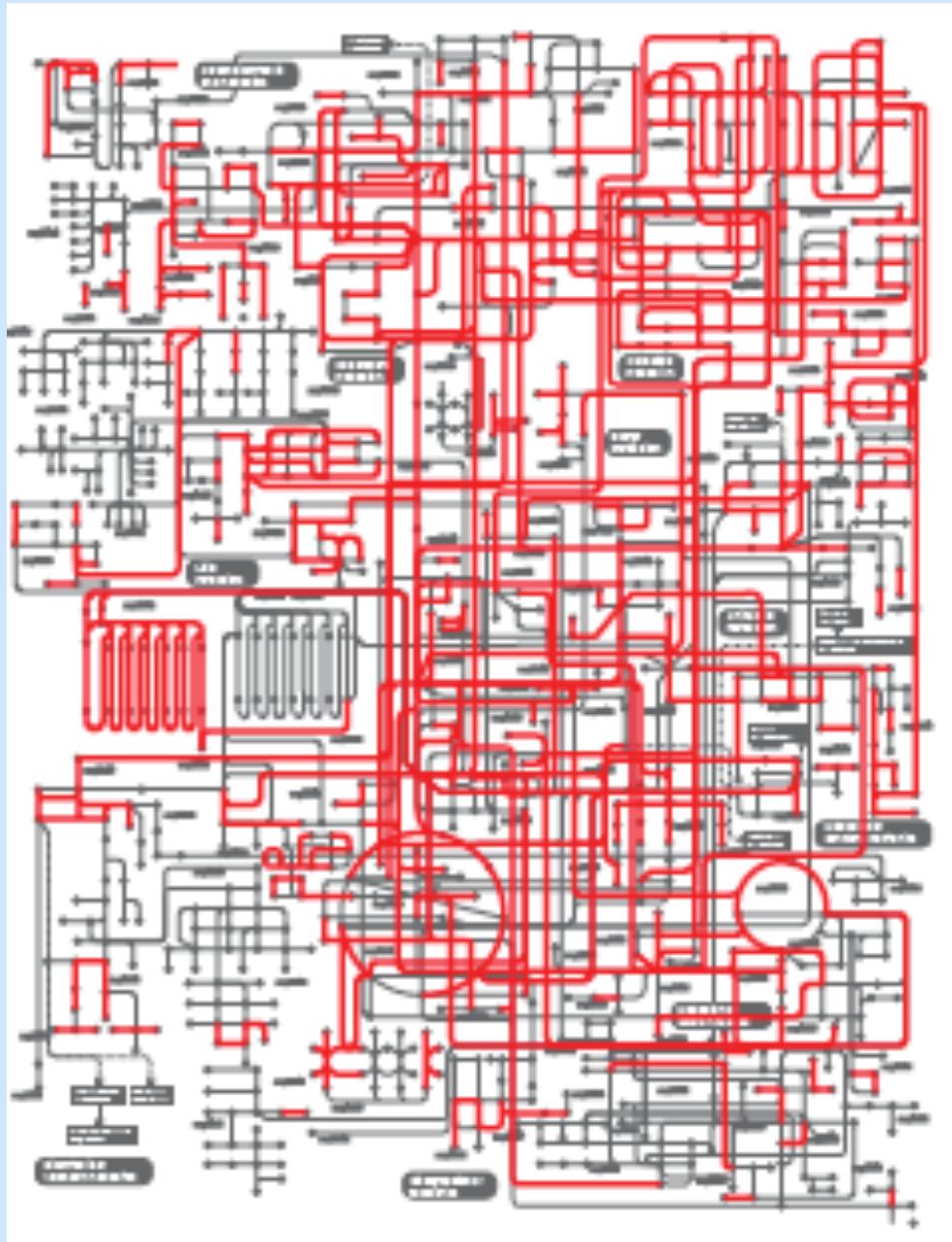
## Description of diversity and patterns



Qin et al. 2010  
A human gut microbial  
gene catalogue.  
*Nature* 464:59

576.7 Gb sequence  
from 124 individuals

Inferred 3.3. million  
unique genes



# A human gut microbial gene catalogue.

General house-keeping genes

Genes for binding to host proteins (e.g. fibrinogen)

Biodegradation of complex sugars, e.g. pectin

Bacteria ferment sugars to short chain fatty acids  
=> host uses them in muscle, heart, brain...

Function of different species?

## Sampling?

576.6 Gb from 124 individuals

= 4.65 Gb bases per community

If there were 1000 species, equal abundance, average of 4.65 Mb per species; ~ 1X coverage of genome

In fact, abundances are not even

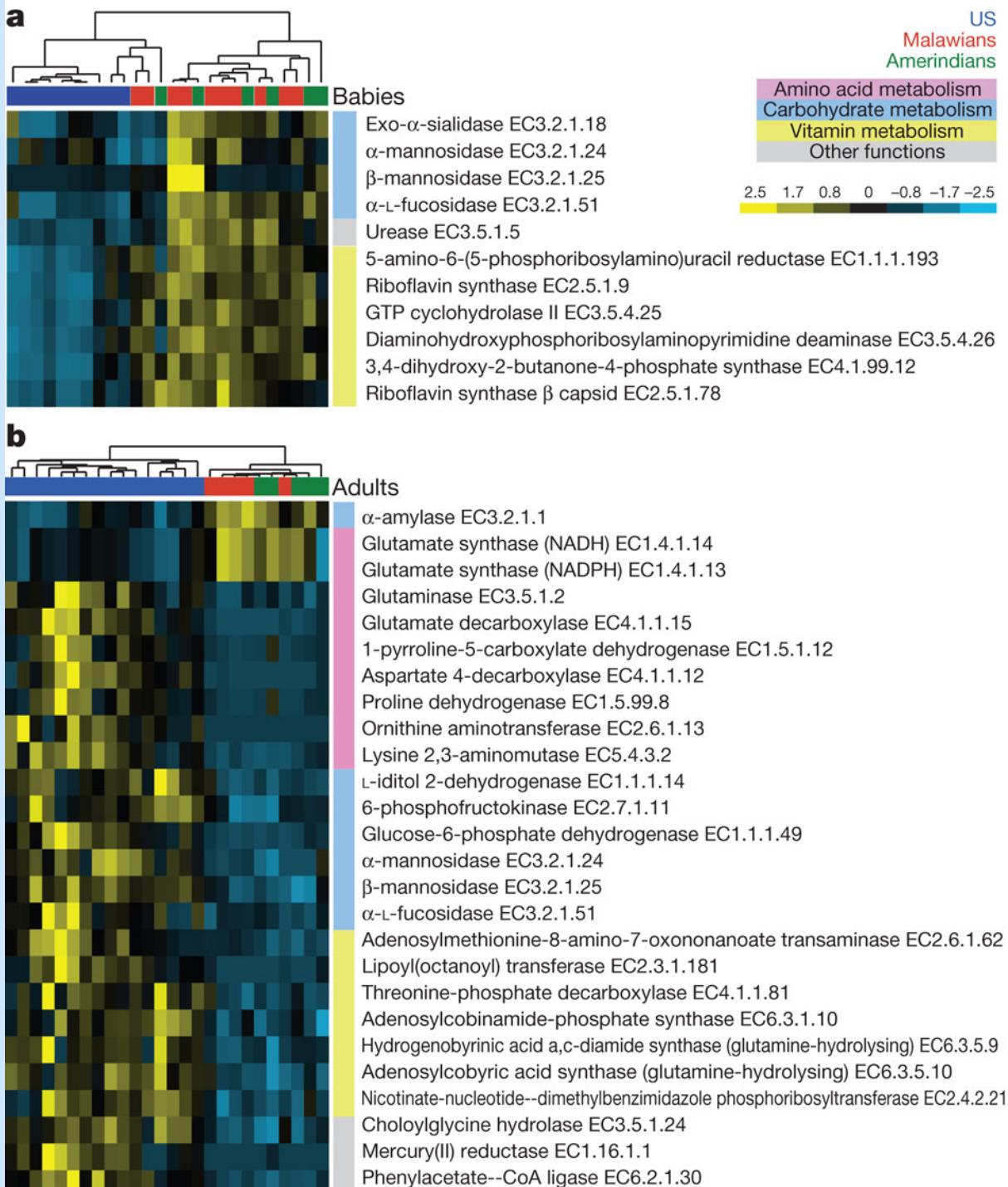
Good for metabolic overview, poor for species sampling

# Case study: human gut:

Differences in abundance of particular genes

e.g. more riboflavin synthesis in Malawi & Amerindian babies

Yatsunenko et al. 2012.  
Human gut microbiome viewed across age and geography. Nature  
486:222-227



## Human gut: function

Need ecology of component species or functional groups  
to model dynamics etc.

Bioinformatics to predict function from genomes

Direct evidence?

# Function: gnotobiotic mice



Sterile mice born by cesarean section and maintained in controlled environments.

## Function: gnotobiotic mice

Protects against injury to gut wall; improves healing after injury to gut wall

Protects against invasion by pathogens

Aids energy extraction and storage

Important for development of healthy immune system

=> tends not to separate bacterial species, but could do so. Some differences to human system.

## Function: chemical methods

Stable isotope probing (e.g. labeled starch or glucose)

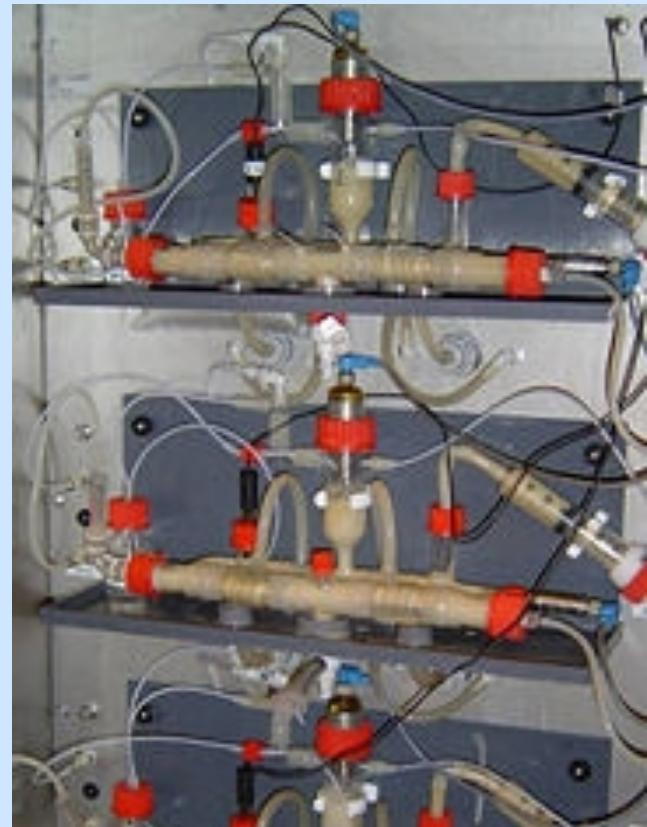
Look for enrichment of heavy isotopes in 16S sequences  
to identify taxa using particular nutrients

e.g. *Ruminococcus bromii*  
ferments starch to acetate

*Eubacterium rectale*  
converts acetate to butyrate

Hard *in vivo*, use *in vitro* simulators

TIM-2 human gut simulator



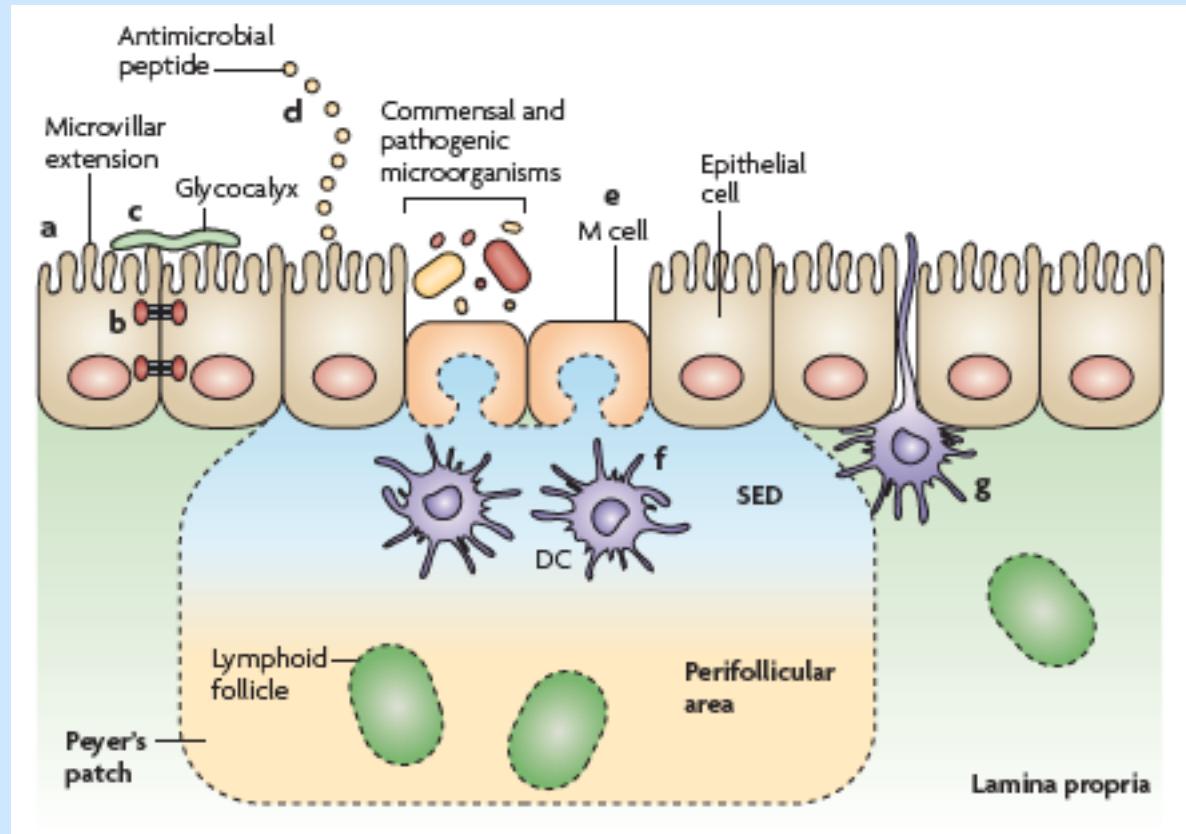
# Function: immune system

Gut walls rich in immune cells

Special cells for sampling intestine

Inflammation; secrete broad-range antimicrobial peptides

Feedback loop with Short-chain fatty acids (inhibit inflammatory response)



Commensals stimulate proper development of immune system  
Immune system can influence community of commensals

## Human gut: dynamics

Community composition variable through time and among individuals.

### Succession in juveniles

Antibiotics reduce total bacterial counts and especially some taxa (depending on antibiotic)

Diet affects composition:

< Bacteroidetes; > Firmicutes in obese patients, which reverses if switched to low fat diet

high fibre/olive oil alter community in mice

# Human gut: management

Gastric health – inflammation, damage to gut, nutritional or dietary problems, protection from pathogens

Depends on interaction between host and microbial community

**Prebiotics:** indigestible food additives that stimulate growth of beneficial bacteria

e.g. inulin, fructo-oligosaccharides increase *Bifidobacterium* populations (more generally “fibre”)



# Human gut: management

Probiotics: living organisms taken to alter host community with intended health benefit

*Lactobacillus* and *Bifidobacterium* treatments ameliorated diarrhea and irritable bowel syndrome

Could modulate host immune system, compete with detrimental species (for resources or exclude from epithelium walls), produce toxins

- ⇒ How can they invade complex community?
- ⇒ How predict consequences?

## Human gut: evolution

Potential for evolution and co-evolutionary interactions:  
productive environment with high turnover

But also for colonisation of pre-adapted bacteria

How important is evolution for dynamics and function of  
gut communities?

Longer-term coevolution with host: gut biota is inherited  
from mother, potential for selection at higher levels

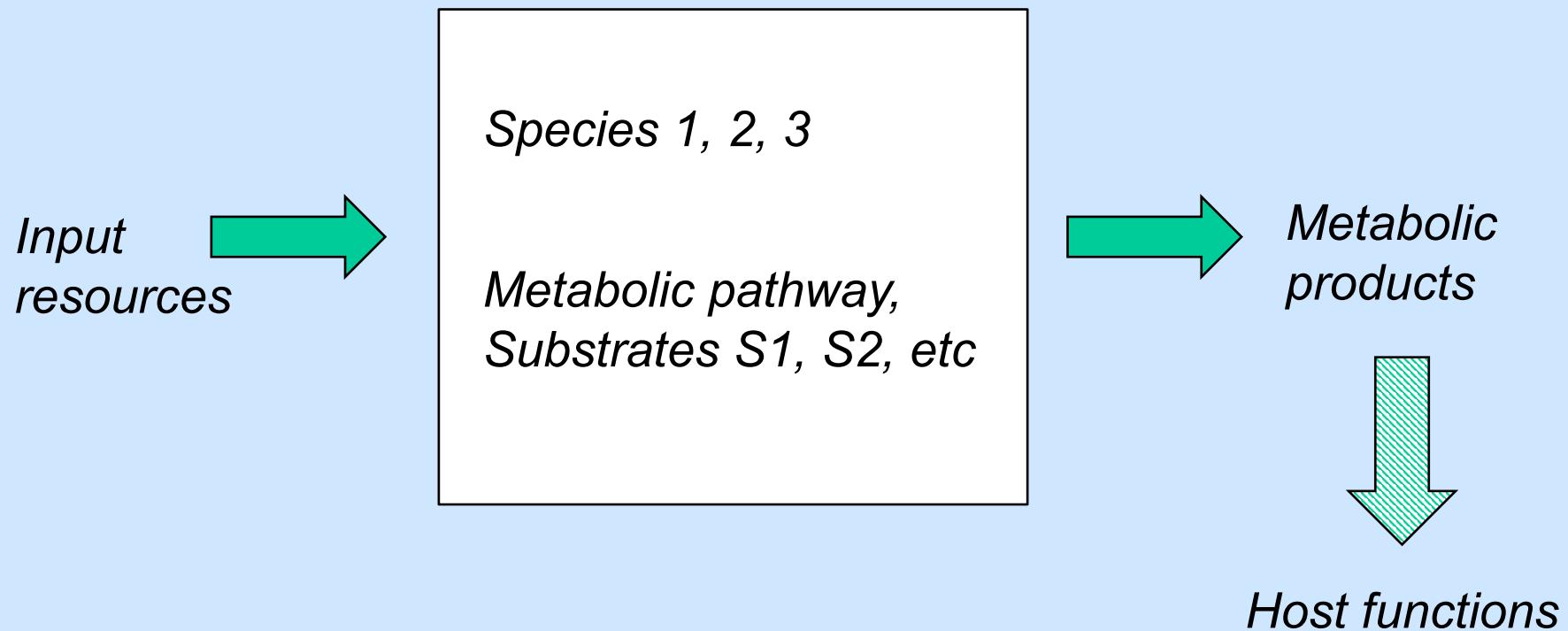
With a change in diet, how does the gut microbiota respond?

Ecological and evolutionary responses

How predict and interpret dynamics?

# Mathematical model to predict species interactions, evolution and functioning in microbial decomposer communities

Bacterial species metabolizing C-sources



Simplify: no phage, feedbacks, space...

# Model growth of species on multiple substrates

Substrate 1

$$\frac{dS_1}{dt} = D(Q_1 - S_1) - \frac{k_{cat_1} E_1 S_1 N_1}{(Km_1 + S_1)}$$

Substrate 2

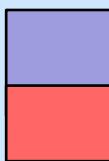
$$\frac{dS_2}{dt} = \frac{k_{cat_1} E_1 S_1 N_1}{(Km_1 + S_1)} - \frac{k_{cat_2} E_2 S_2 N_1}{(Km_2 + S_2)} - DS_2$$

Species 1

$$\frac{dN_1}{dt} = \frac{c_1 k_{cat_1} E_1 S_1 N_1}{(Km_1 + S_1)} + \frac{c_2 k_{cat_2} E_2 S_2 N_1}{(Km_2 + S_2)} - DN_1$$

E1

E2



# 1) Resource use specified by enzyme allocation

Substrate 1

$$\frac{dS_1}{dt} = D(Q_1 - S_1) - \frac{kcat_1 E_1 S_1 N_1}{(Km_1 + S_1)}$$

Substrate 2

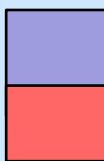
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Species 1

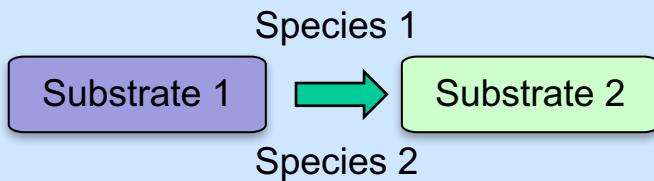
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E1

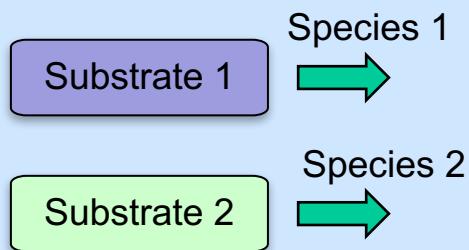
E2



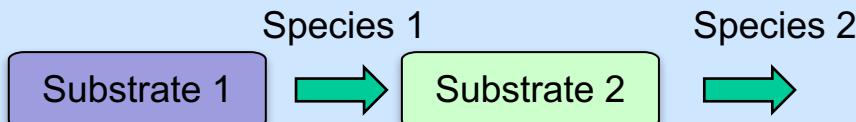
## 2) Species interactions determined by pattern of resource use



Competition



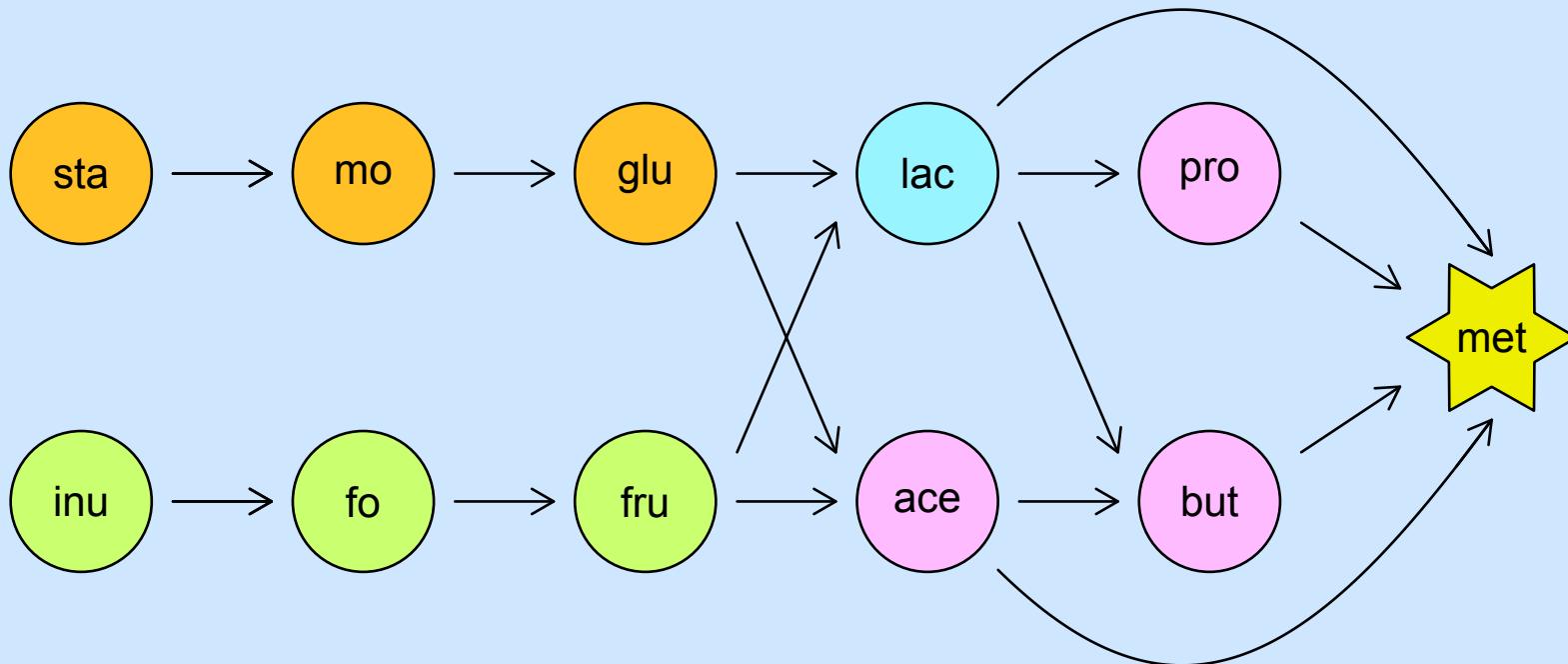
No interaction



Facilitation (cross-feeding)

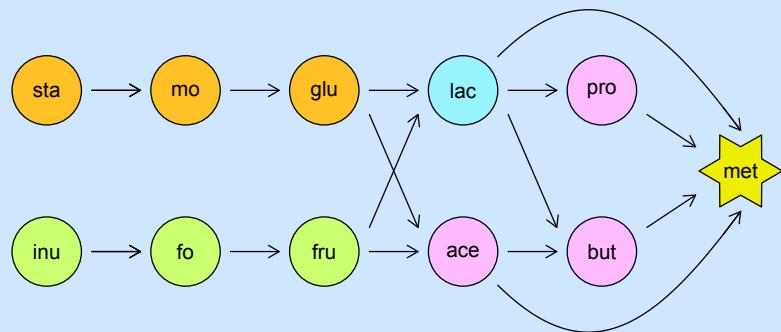
# Case study: human gut:

Anaerobic fermentation of indigestible macromolecules into short-chain fatty acids



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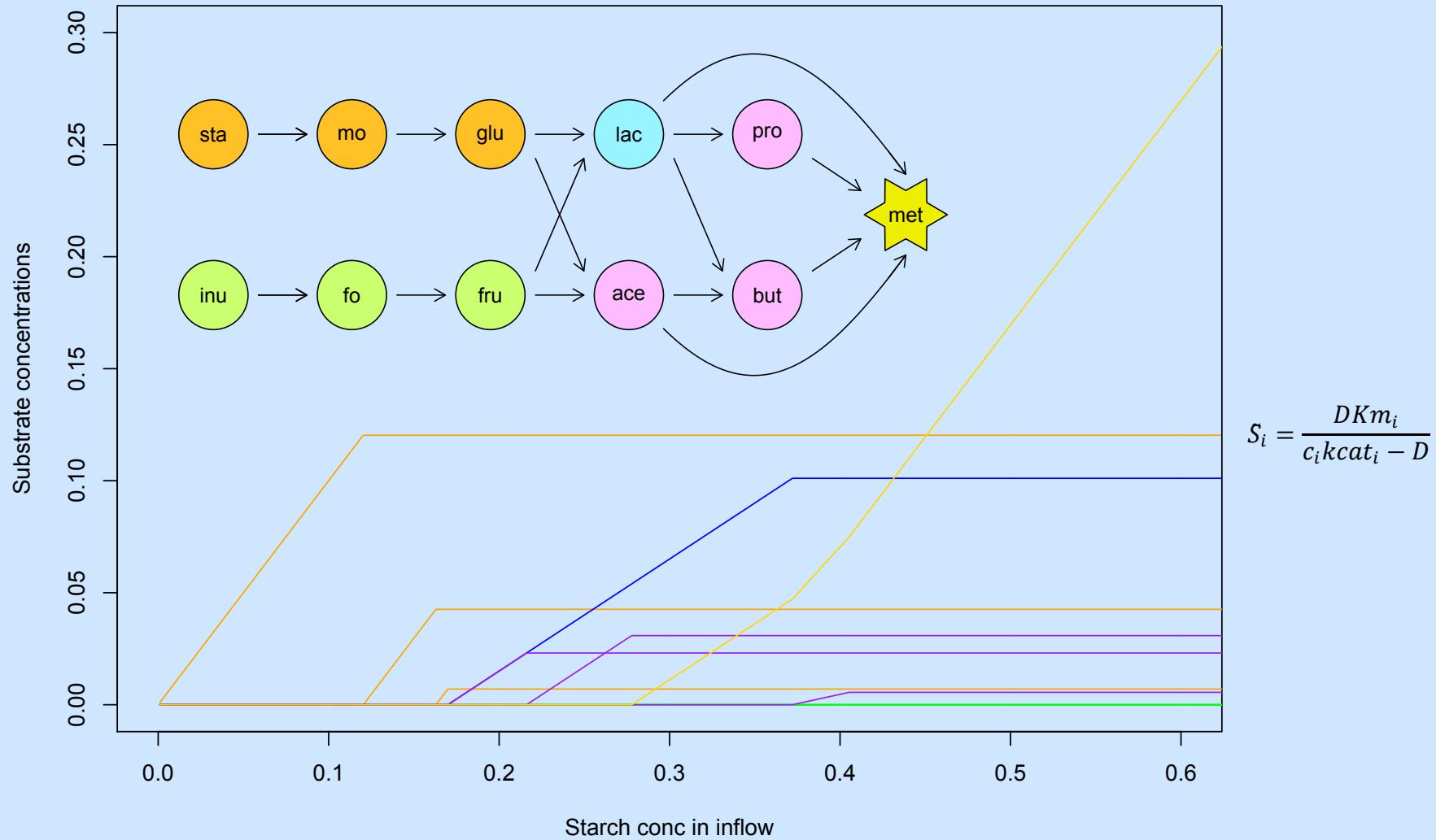


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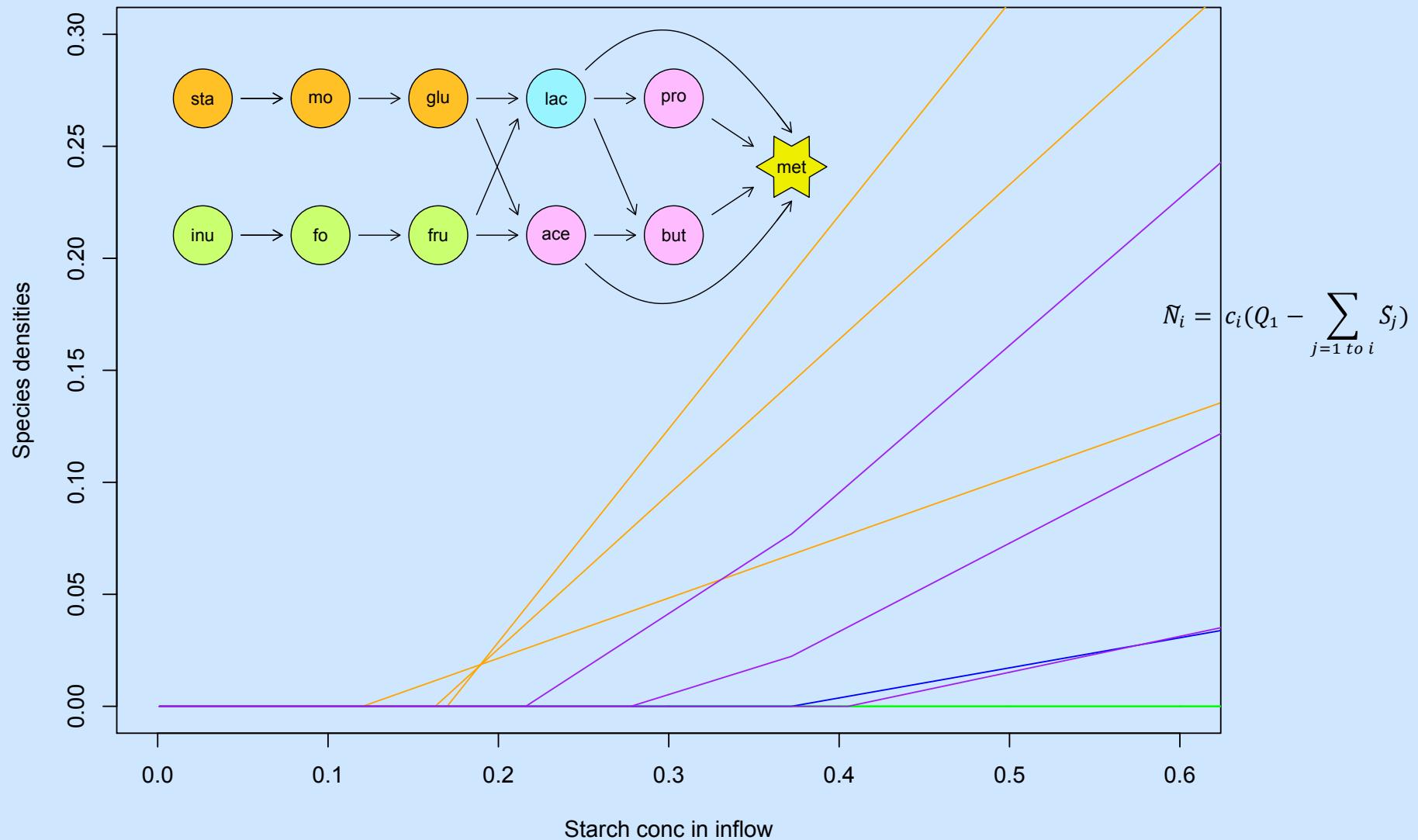


= Short Chain  
Fatty Acids (SFCA)

# Can solve steady-state for specialists



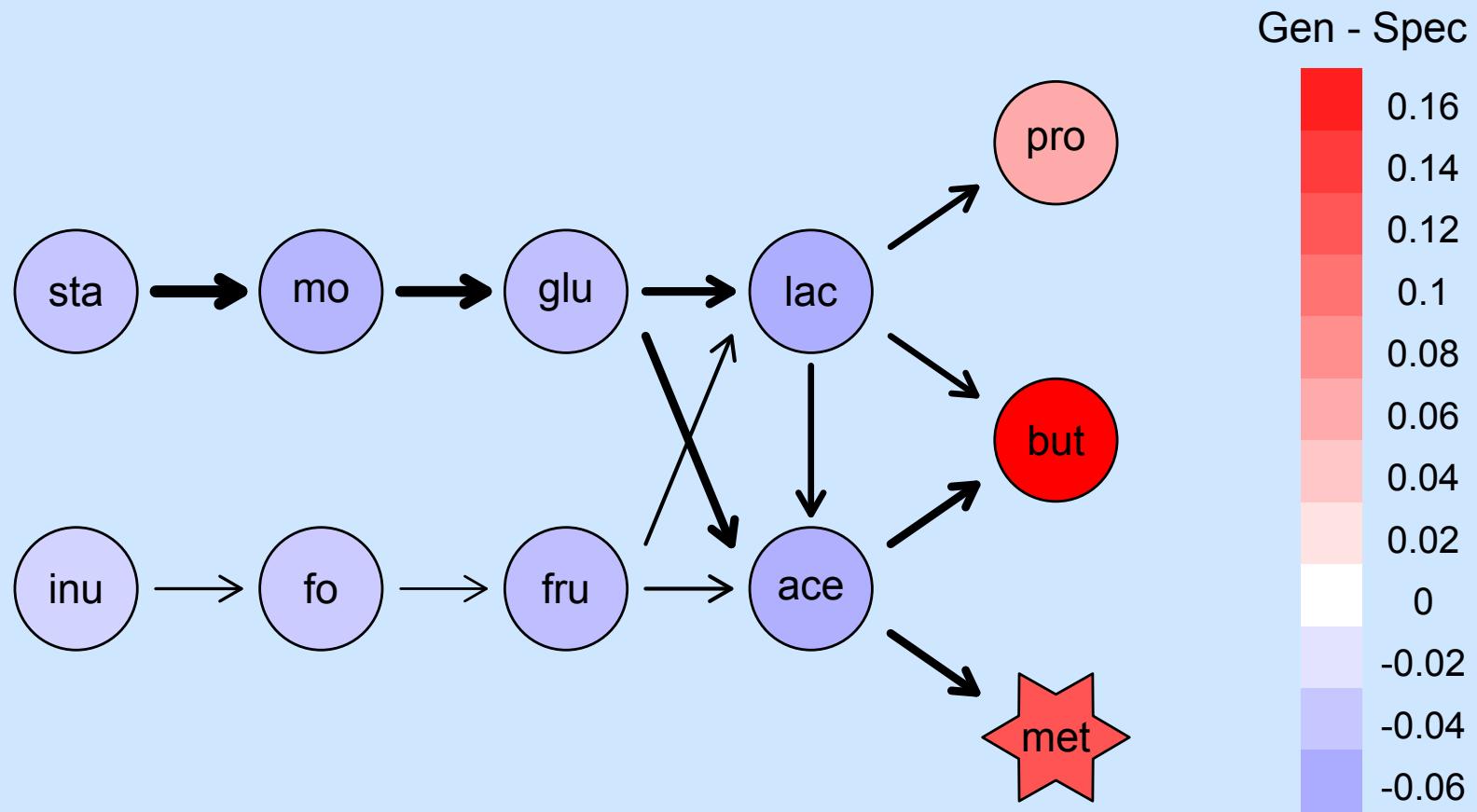
# Can solve steady-state for specialists



## Metrics of functioning:

- 1) Low concentration of input S (e.g. decomposition/detoxification)
- 2) High biomass (e.g. biofuels)
- 3) High conc of intermediate substrate (e.g. SCFA in gut)
- 4) High conc of end product (e.g. gas biofuel)
- 5) Particular target ratio of substrates (e.g. balanced SCFA ratios)
- 6) Total of a set of substances (e.g. total SCFA production)

Can simulate for generalists, serial transfer



Add in evolution: allocation E1:E2 can evolve (sums to 1)

Substrate 1

$$\frac{dS_1}{dt} = D(Q_1 - S_1) - \frac{kcat_1 E_1 S_1 N_1}{(Km_1 + S_1)}$$

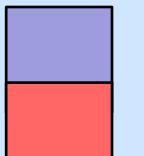
Substrate 2

$$\frac{dS_2}{dt} = \frac{kcat_1 E_1 S_1 N_1}{(Km_1 + S_1)} - \frac{kcat_2 E_2 S_2 N_1}{(Km_2 + S_2)} - DS_2$$

Species 1

$$\frac{dN_1}{dt} = \frac{c_1 kcat_1 E_1 S_1 N_1}{(Km_1 + S_1)} + \frac{c_2 kcat_2 E_2 S_2 N_1}{(Km_2 + S_2)} - DN_1$$

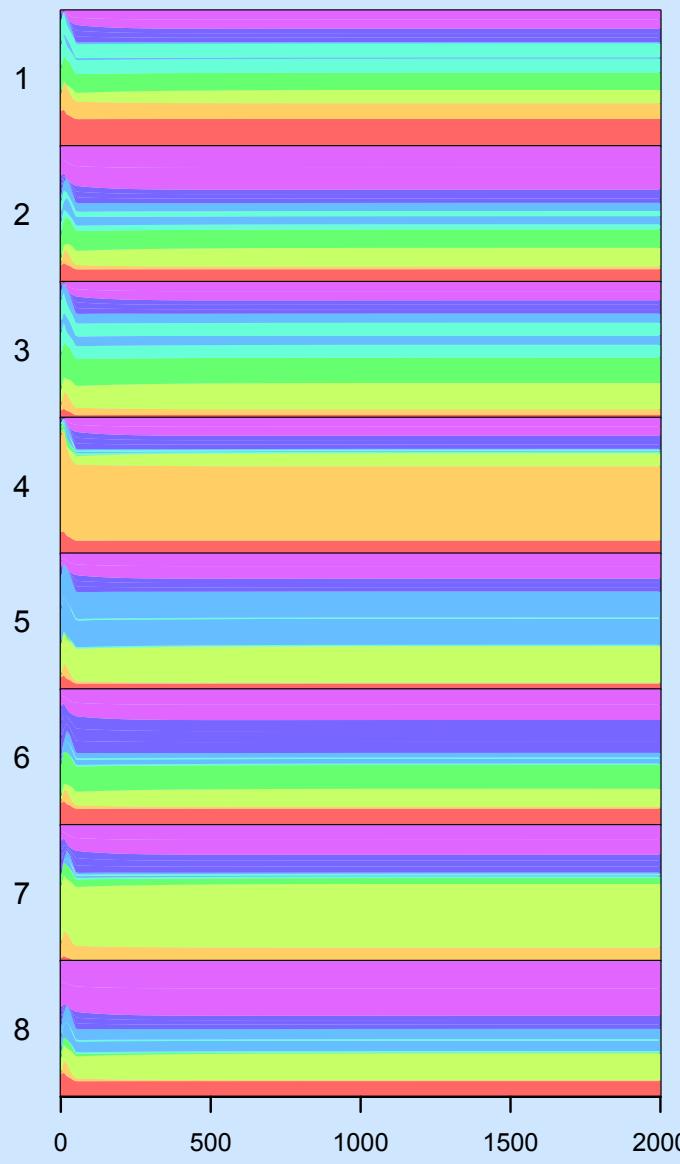
E1  
E2



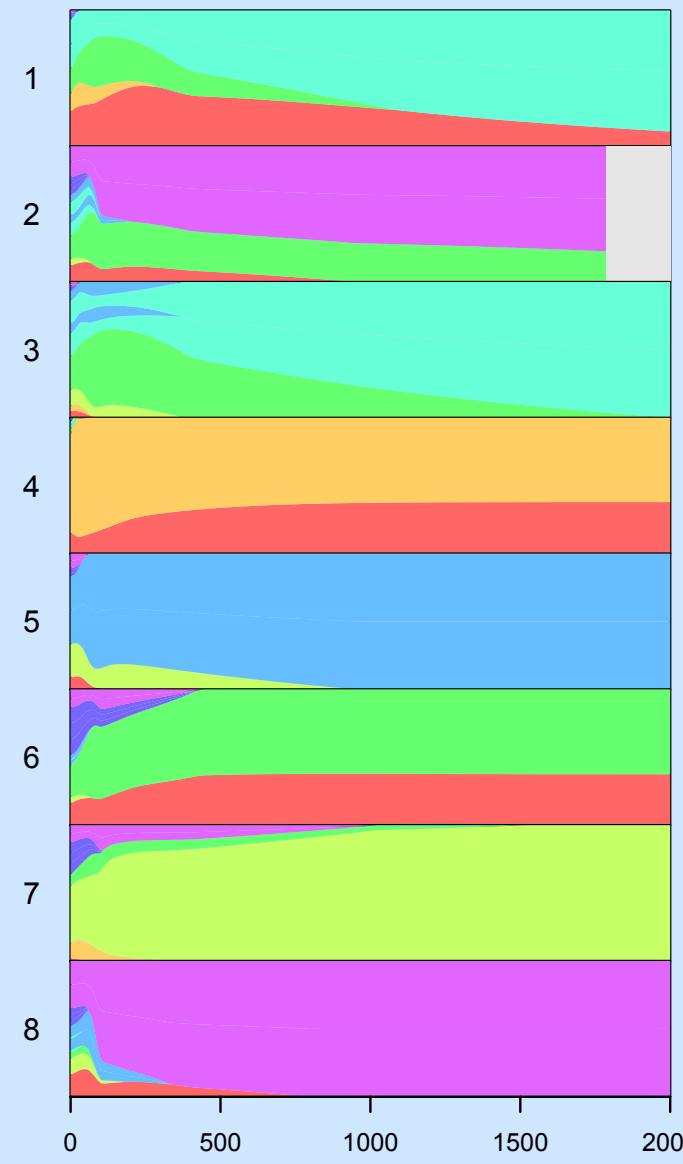
$$\frac{dE_1}{dt} = \mu \frac{\partial \left( \frac{1}{N} \frac{dN}{dt} \right)}{\partial E_1}$$

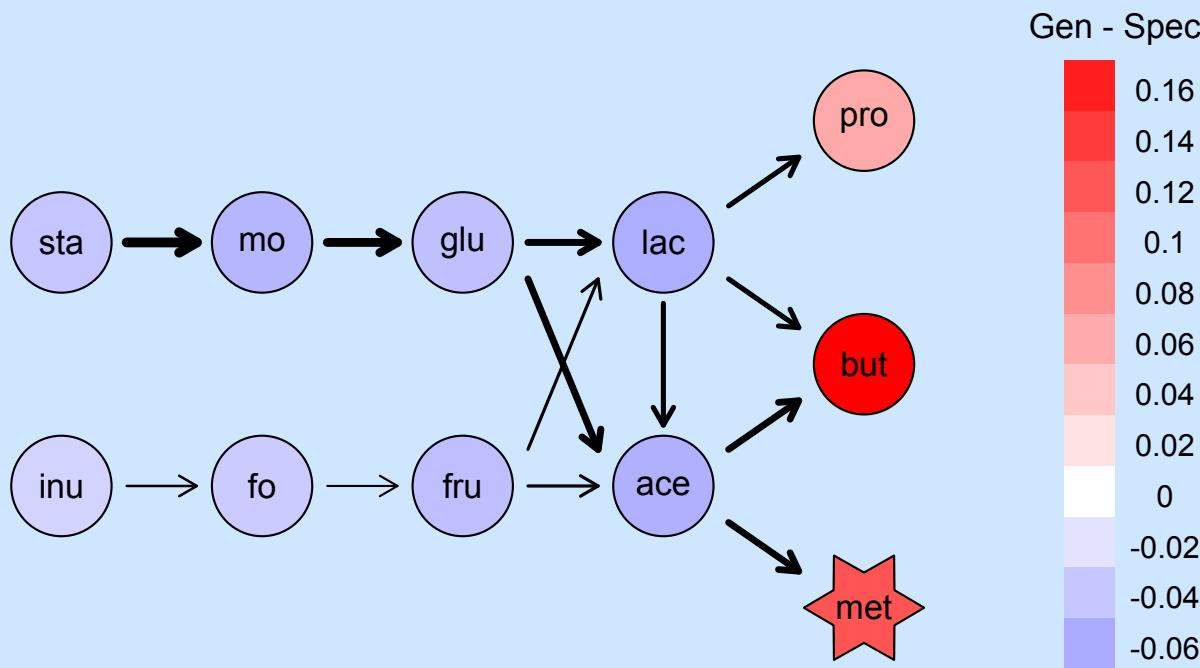
# Consequences of evolution depend on trade-off

a=1

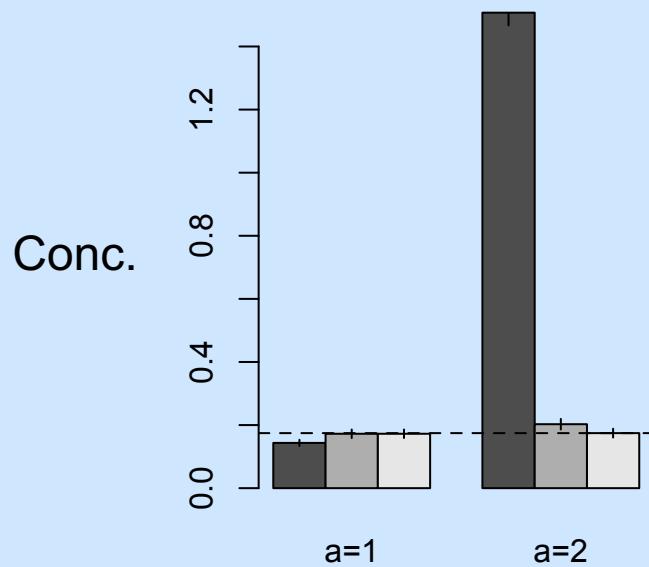


a=2

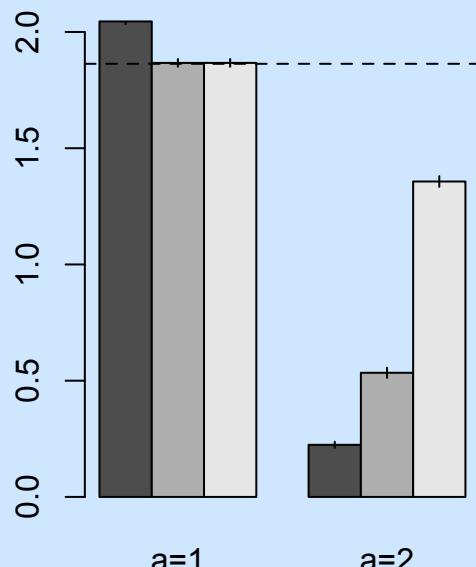




**A) Mean conc S1**



**B) Mean conc S10**



Eco-generalist  
Evo-generalist  
Evo-specialist

# Top down: perturbing human gut microbiota

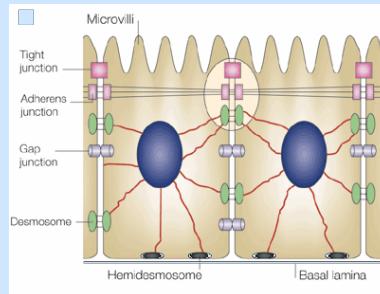


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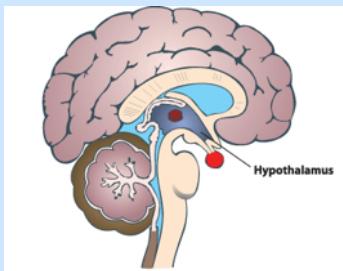
= Short Chain  
Fatty Acids (SCFA)

SCFA +



= PPY + GLP1  
(anorectic gut hormones)

PPY +



= reduced appetite

Gemma Walton, Jonathan Swann, Glenn Gibson, Univ. Reading  
Laura Johnson, Gary Frost, Arianna Psichas, Imperial College London



3 healthy volunteers (no recent history of antibiotic/probiotic use)



3 stool samples collected. Stools were weighed and diluted roughly 1:5 with PBS



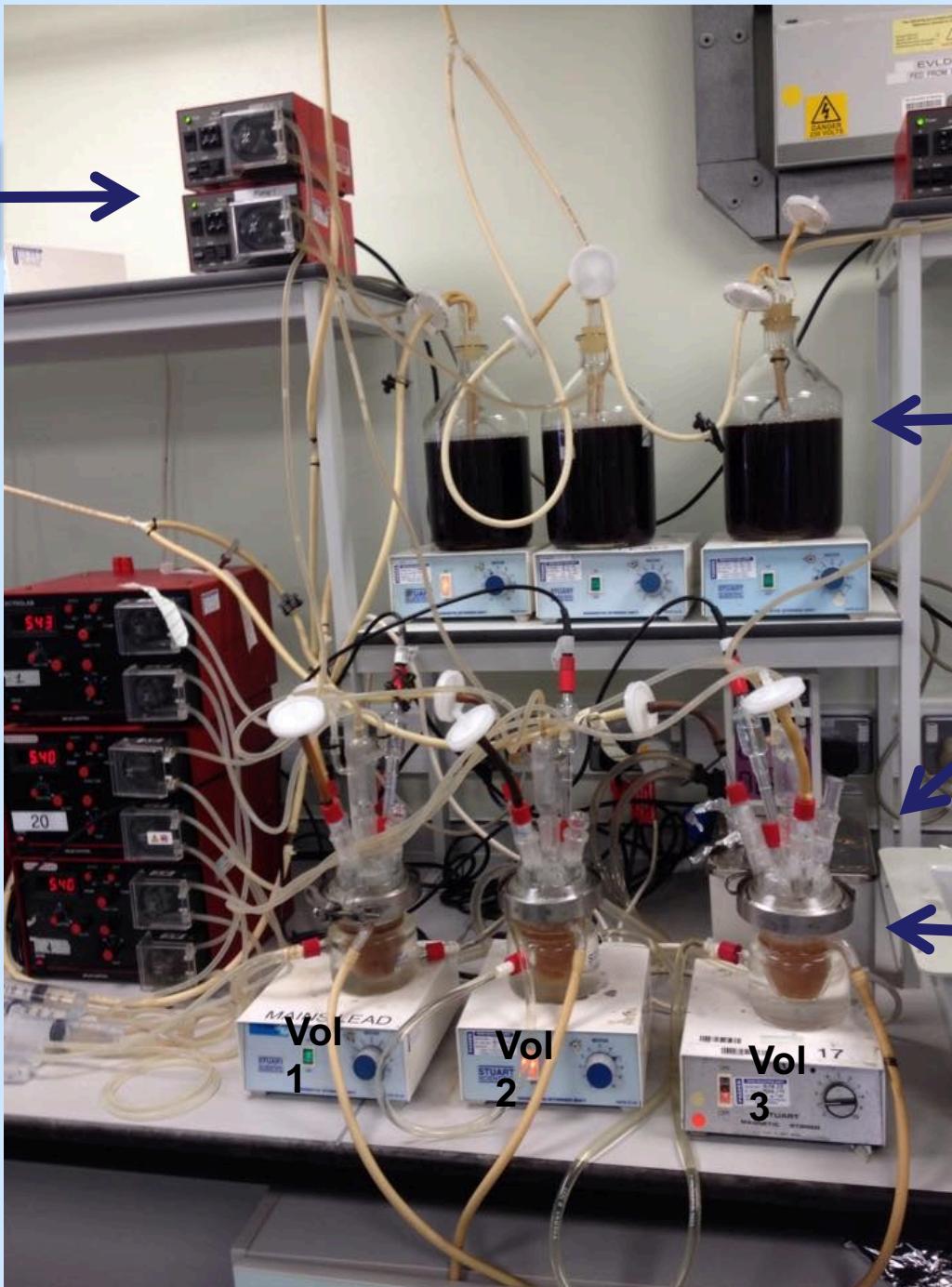
Diluted stools were homogenised using a Stomacher 400



Faecal slurries were added to vessels containing 100 ml nutrient rich gut model media. This was left for two weeks for bacteria to acclimatise and chemostats to reach a steady state.



Flow rate – 6.25  
ml/h



pH controllers –  
pH 5.4 – 5.6

Basal nutrient media  
supplemented with  
inulin

Water bath – 37.  
C

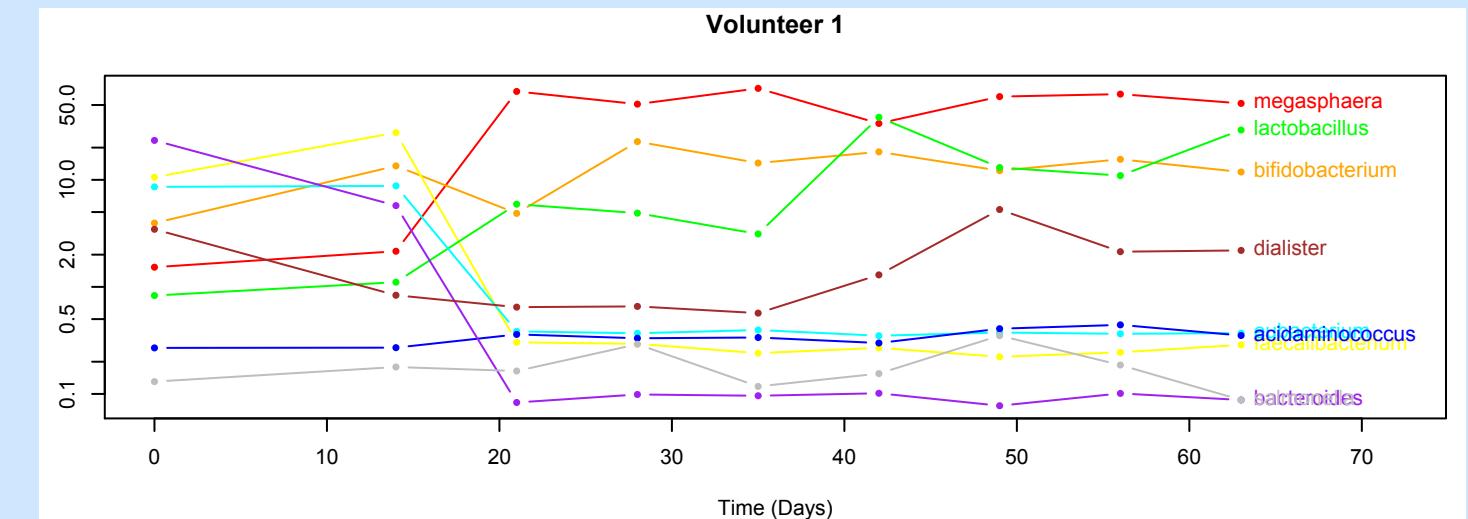
Chemostats

mixed

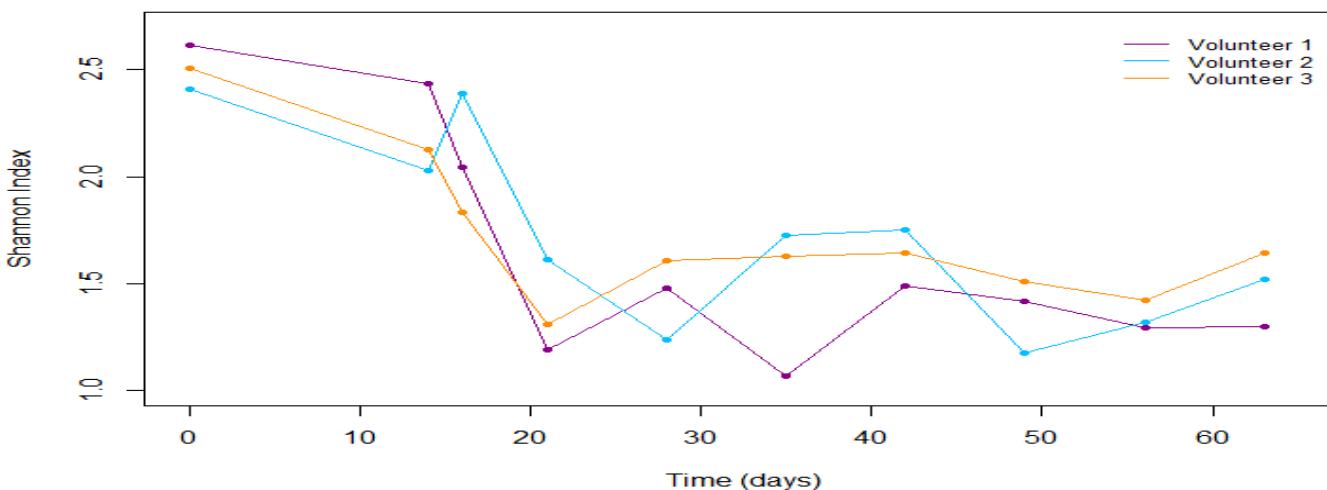
inulin



Taxa  
Log(f)



Diversity



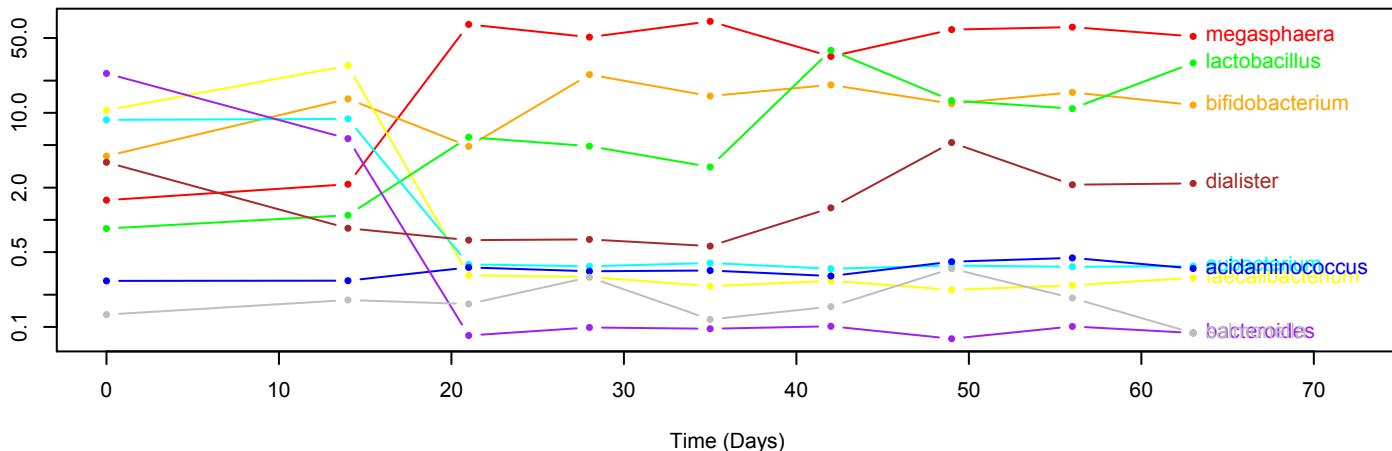
mixed

inulin

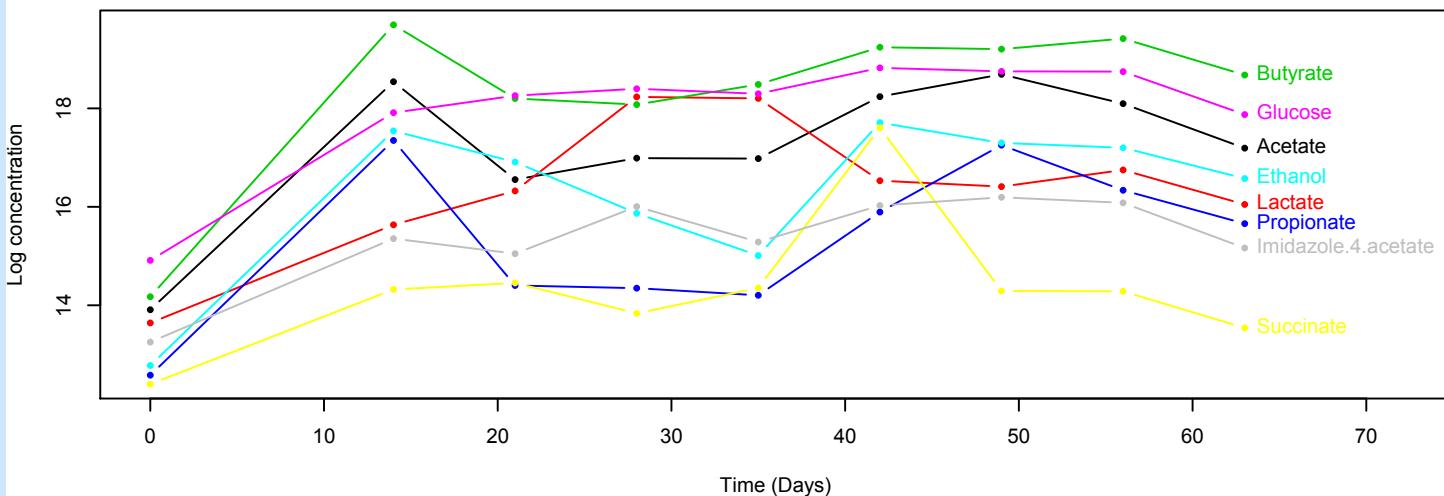


Taxa  
Log(f)

Volunteer 1

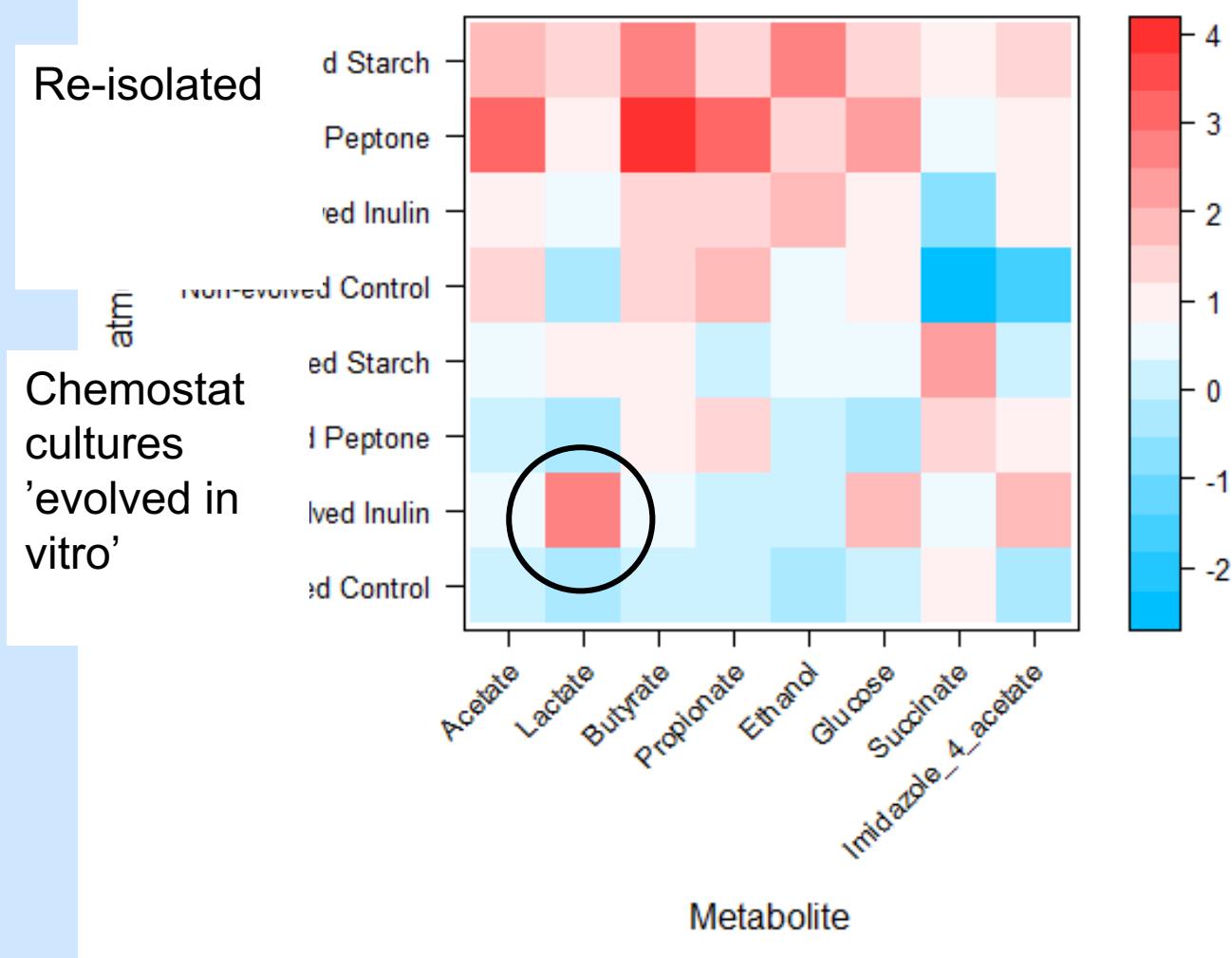


metabolites



Communities re-isolated from volunteers produce higher acetate, butyrate, propionate on all diets.

## Evolved inulin community produces more lactate, glucose, i4a.



# Evolution or just ecological sorting?

Contig	Gene	Product	Location (interval)	Depth	Allele Frequency	Polymorphism Type	Reference Nucleotide	Variant Nucleotide	Base Change	Nonsynonymous?	AA Change
MELS00001	<i>icaB</i>	Poly-beta-1,6-N-acetyl-D-glucosamine N-deacetylase	192, 302	105	1	Substitution	G	A	G -> A	No	
MELS00001	<i>mprA</i>	Transcriptional repressor MprA	133, 764	59	1	Substitution	G	A	G -> A	Yes	A -> V
MELS00001	<i>mprA</i>	Transcriptional repressor MprA	133, 902	60	1	Substitution	G	A	G -> A	Yes	A -> V
MELS00002	-	-	98532 - 98533	371	1	Insertion	-	A	+A	-	-
MELS00004	<i>por3</i> *	Pyruvate synthase	115, 713	624	1	Substitution	A	G	A -> G	Yes	V -> A
MELS00008	<i>yidA</i> 1 *	Sugar phosphatase YidA	32, 707	111	1	Substitution	A	G	A -> G	No	-
MELS00009	<i>gdpP</i>	Cyclic-di-AMP phosphodiesterase GdpP	85, 014	116	1	Substitution	C	T	C -> T	Yes	F -> L
MELS00009	<i>gdpP</i>	Cyclic-di-AMP phosphodiesterase GdpP	85, 731	119	1	Substitution	A	G	A -> G	Yes	T -> A
MELS00012	hypothetical protein	Hypothetical protein	64, 186	86	1	Deletion	C	-	-C	Yes	Frameshift
MELS00014	<i>yadA</i> 2	Adhesin YadA	52,855	217	1	Substitution	G	T	G -> T	Yes	N -> K
MELS00018	<i>rpoB</i>	DNA-directed RNA polymerase subunit beta	38,424	90	1	Substitution	A	G	A -> G	Yes	I -> V
MELS00020	hypothetical protein	Hypothetical protein	21082 - 21083	80	1	Insertion	-	T	+T	Yes	Frameshift
MELS00020	<i>fdhF</i> *	Formate dehydrogenase H	23,319 - 23, 321	79	1	Deletion	GCA	-	-GCA	No	-
MELS00025	- (repeat region, CRISPR)	-	10,136	148	1	Substitution	C	T	C -> T	No	-

# Approaches:

## a) Bottom-up

Construct simplified communities, manipulate conditions, track evolution: **species interactions & functioning evolves**

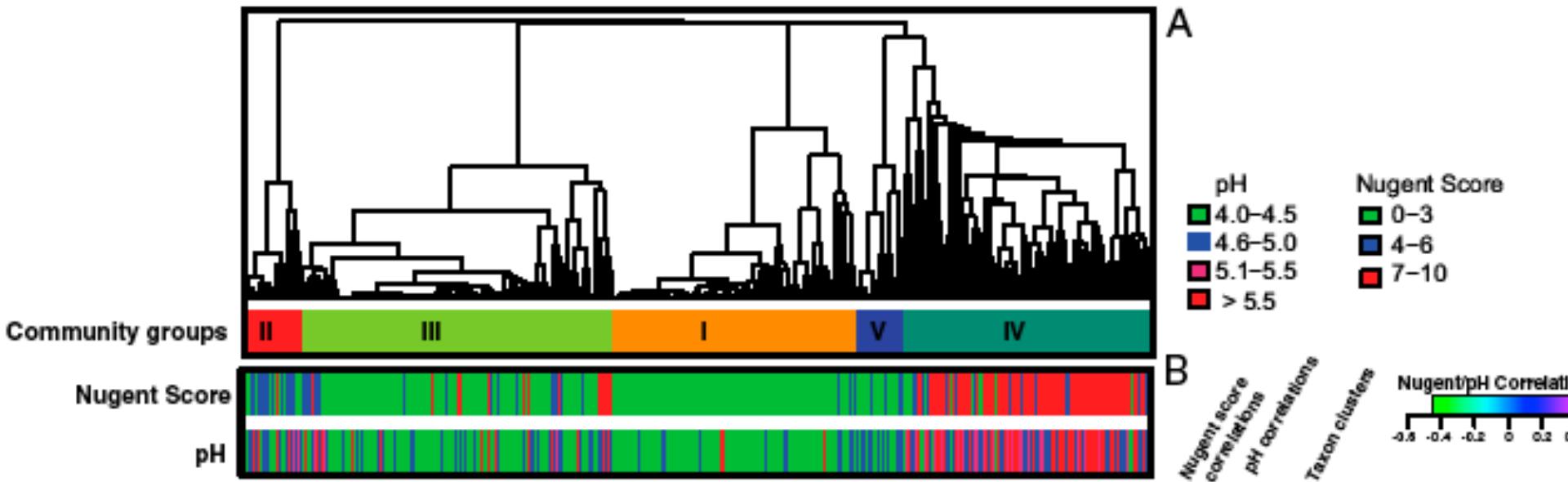
## b) Top down

Culture whole gut communities in vitro, manipulate conditions, track responses: **new methods to track mechanisms**

## c) Mathematical models

Generate predictions for expected evolution of resource use, species interactions and functioning: bacterial metabolism

# Many other similar systems of interest



5 classes of community in vaginal microbiome

4 dominated by different *Lactobacillus* species (keystone species)

Major difference in pH (much higher) in fifth class => consequences for fertility, protection from pathogens

Significant differences among racial groups

# Insect gut microbiota

...protection from disease

Koch H. & Schmid-Hempel 2011. PNAS

Honeybees and  
*Crithidia bombi*

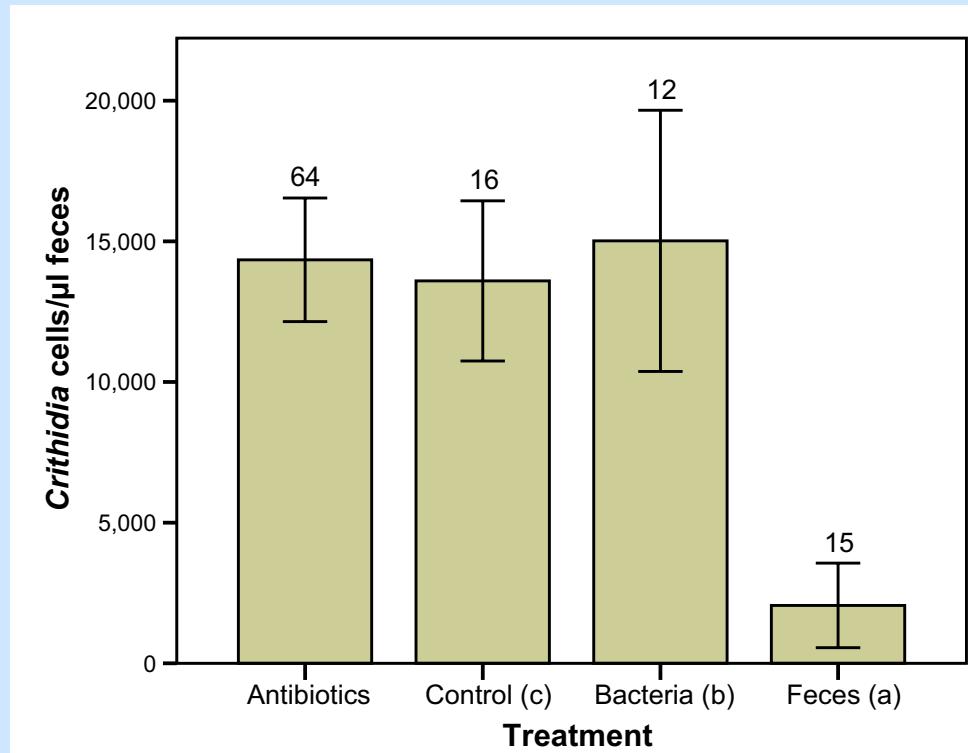
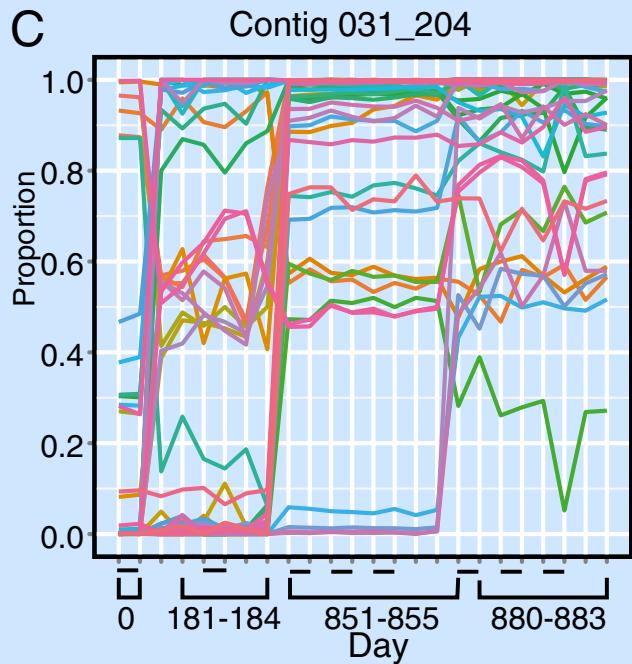


Fig. 1. Comparison of the number of *C. bombi* cells per  $\mu\text{L}$  of feces at 7 d postinfection for the three treatment groups and the antibiotics-fed individuals. The sample size of each group is listed above the bars. (Error bars:  $\pm 1$  SE.)

# Evolution in gut virome

## Track changes in SNPs over time



And use CRISPR sequence  
To infer host and coevolution

Minot et al. 2013.  
PNAS. 110:12450

Table 1. CRISPR arrays from bacterial metagenomic sequence targeting viral contigs detected in this study

CRISPR	Organism hosting CRISPR	No. of spacers associated with repeat	Median spacer length (bp)	Viral contig targeted
CRISPR-2	<i>Ruminococcus bromii</i> L2-63 (temperate phage)	64	30 (29–31)	232_308
CRISPR-3	Unknown	38	30 (21–33)	112_6
CRISPR-7	Unknown	64	36 (22–40)	051_116 75
CRISPR-21	Unknown	59	35 (30–38)	111_52
CRISPR-31	<i>Eubacterium siraeum</i> V10Sc8a	110	37 (25–40)	132_57
CRISPR-32	<i>Eubacterium siraeum</i> V10Sc8a	230	37 (22–46)	132_57
CRISPR-37	<i>Bacteroides fragilis</i> NCTC 9343	32	30 (29–30)	111_52