

# The Gut Microbiota and Host Metabolic Physiology / Growth



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# Defining nutritional status according to the World Health Organization



Multi-center reference growth study (1997 – 2003)

WHO Multi-Center Growth Reference Study from approximately 8500 children from widely differing ethnic backgrounds and cultural settings (Brazil, Ghana, India, Norway, Oman and the USA) assessing the growth and development of infants and young children around the world.

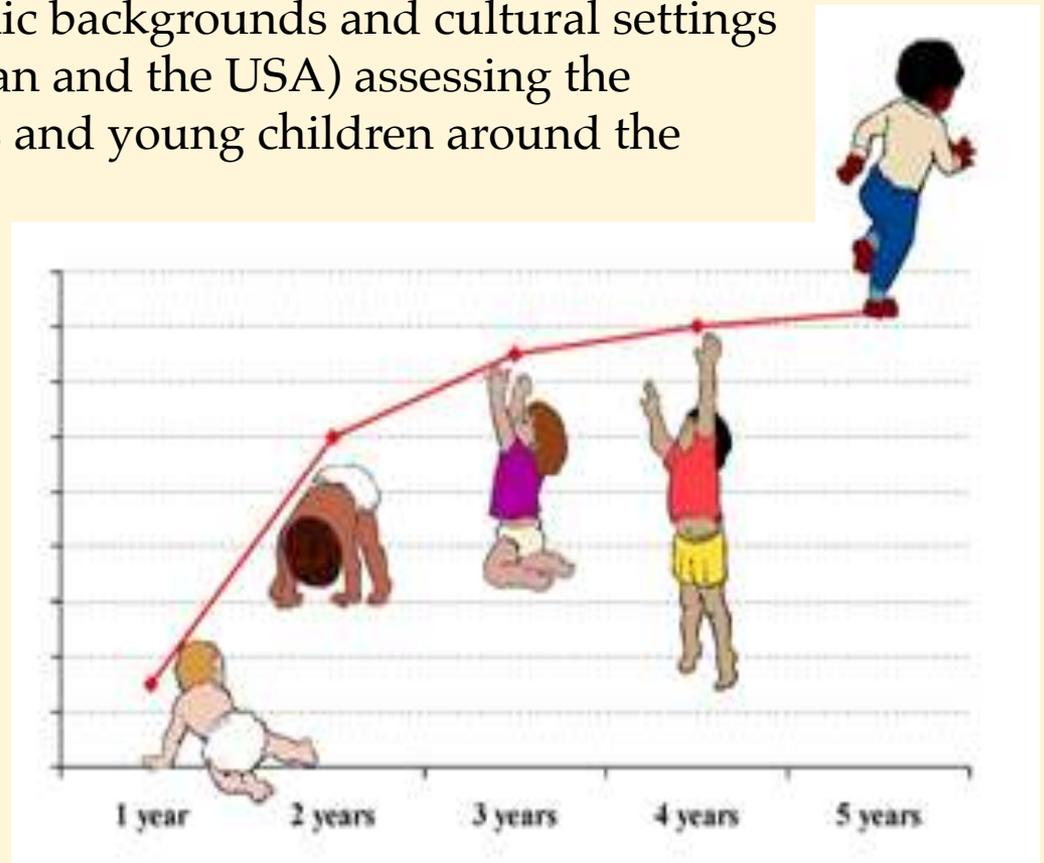
Z-scores:

Height-for-Age

Weight-for-Age

Weight-for-Height

**A single international standard that is the best description of growth for all children from birth to five years of age.**



# Defining nutritional status according to the World Health Organization



## Diagnostic criterion and presentation of Severe Acute Malnutrition:

WLZ score < -3



Bipedal edema



MUAC < 11.5 cm



## Therapeutic foods to treat malnutrition

Plumpy-Nut RUTF



Khichuri



Halwa

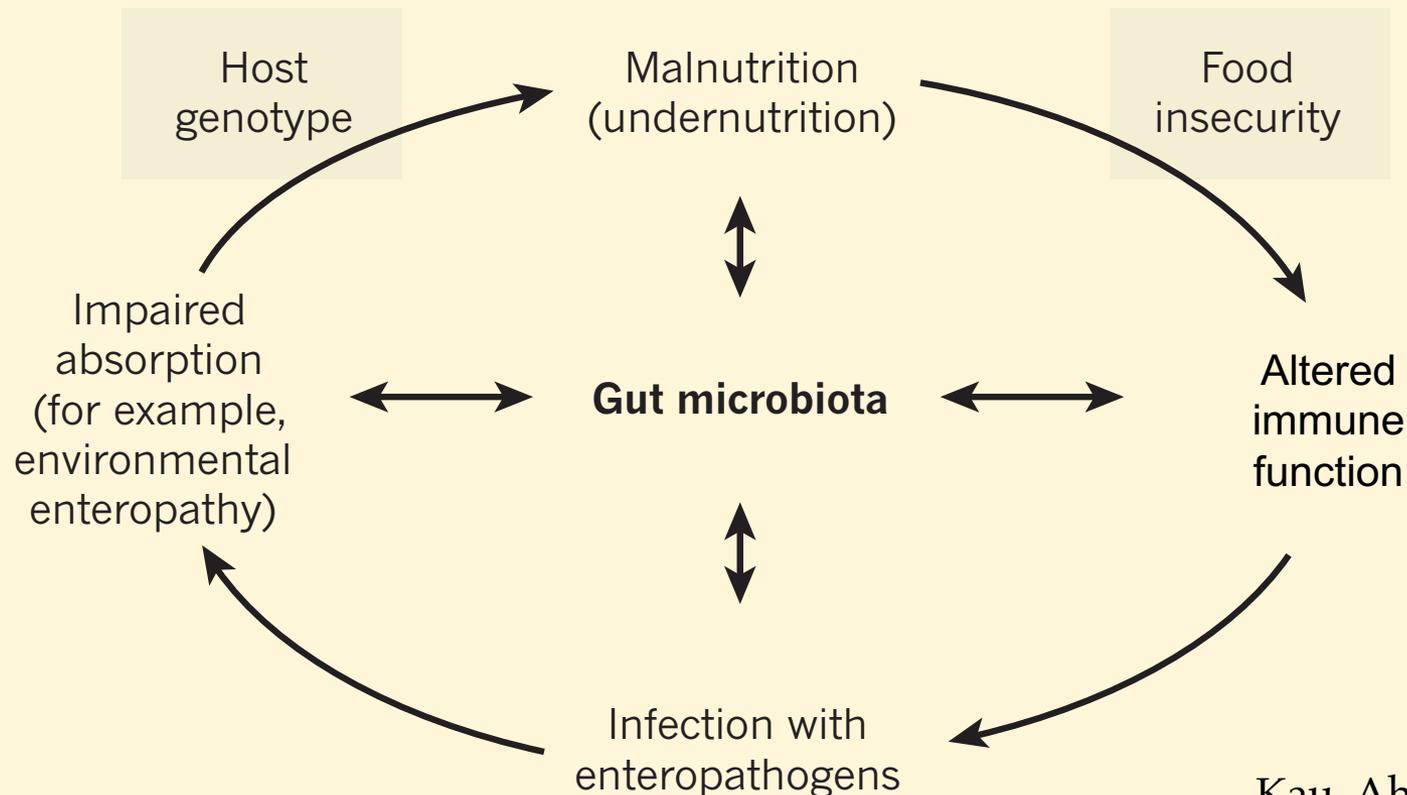


	RUTF	Khichuri	Halwa
Energy (Kcal)	530-545	145	240

# The gut microbiota as part of the vicious cycle of malnutrition



Undernutrition (malnutrition) is not caused by food insecurity alone, but by a variety of intra- and inter-generational factors, all of which could be influenced by the gut microbiota.



# The gut microbiota can be thought of as another organ in the human body



The human gut microbiota: **community of microbes** that live along the human gut.

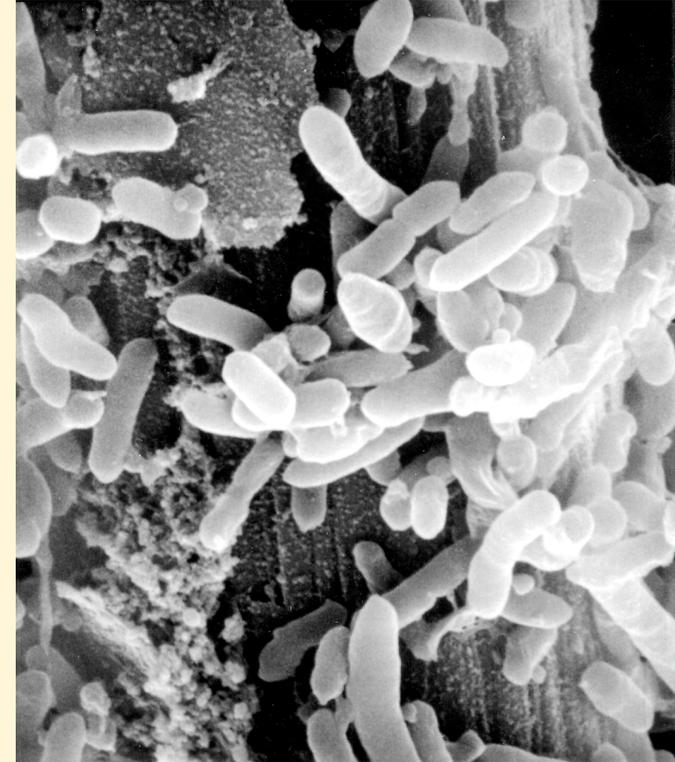
Microbiome refers to the genetic sequence of these microbes.

**Food** shapes the balance of power in this community and can in turn affect:

- Nutritional absorption
- Metabolism
- Immunity and Vaccination
- Susceptibility to Infections

## **Central Hypothesis:**

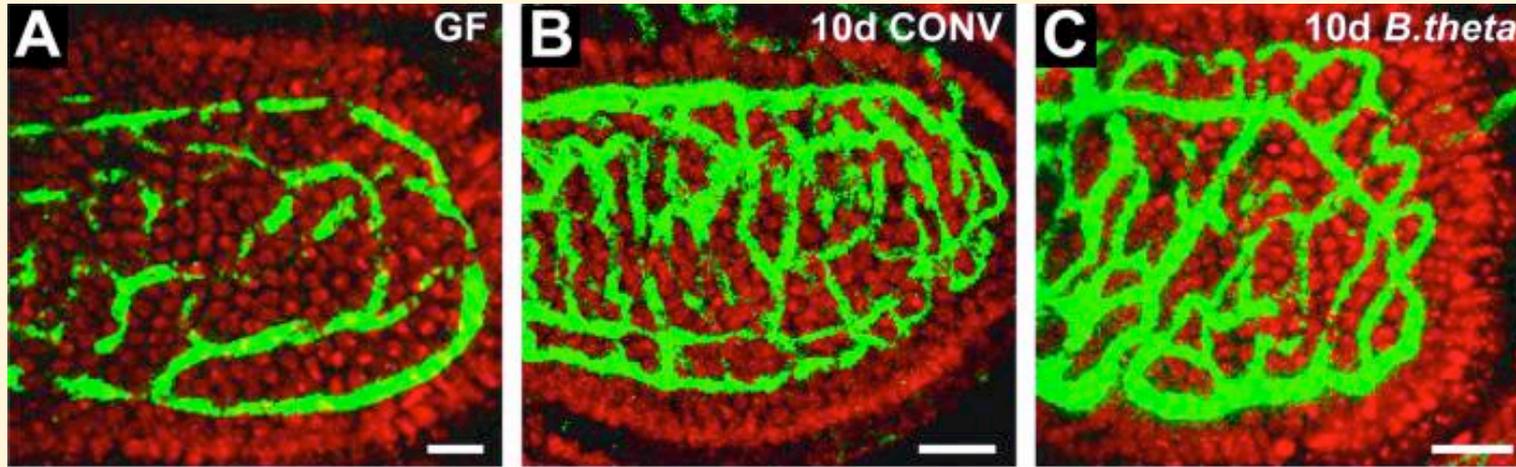
Human gut microbes develop like the rest of our organs, and this development is affected in undernutrition



# Microbes in turn influence host physiology and development



Villi microvascular network regulated by microbes via Paneth cells



Stappenbeck, Hooper and Grodon, PNAS 2002

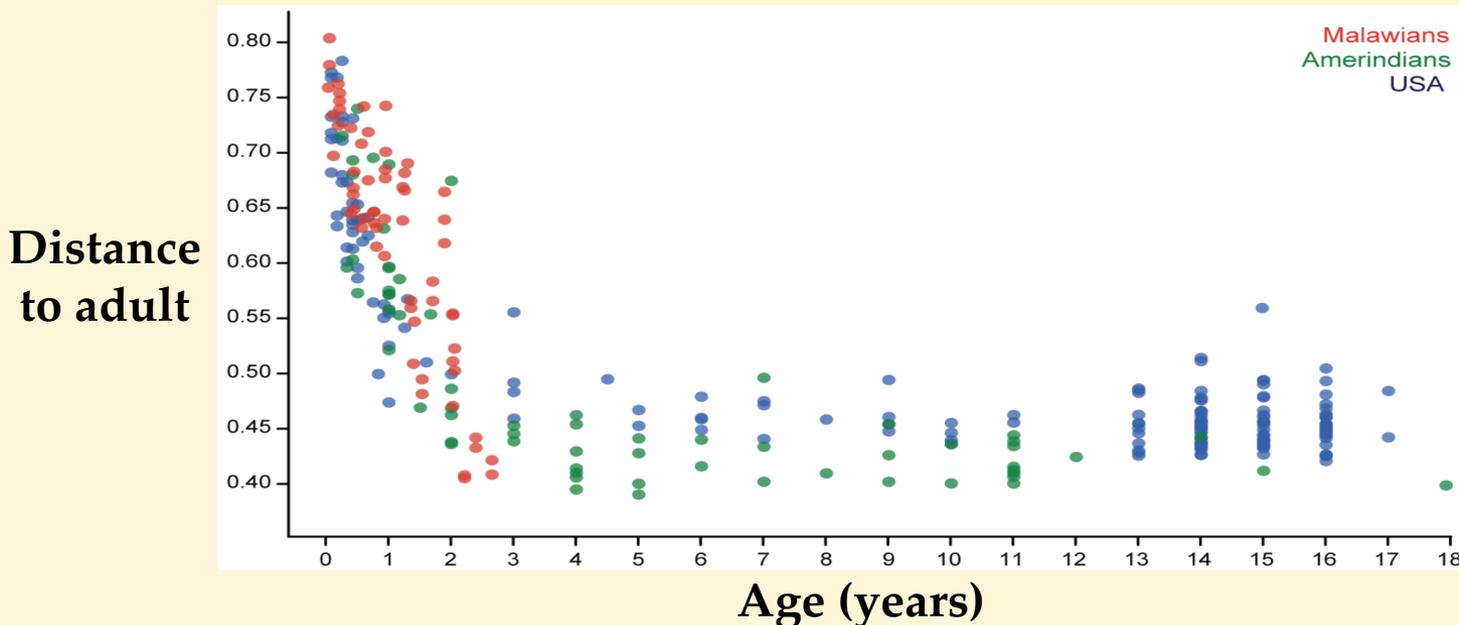
- Gut barrier function
- Modulation of regulatory T cells and other immune cell populations
- Intestinal motility
- SCFA production
- TMAO production, tryptophan, bile salt metabolism

# Gut microbial communities change dramatically in early life



Age is a major factor in shaping what bacterial communities look like in the gut

Based on a pair-wise phylogenetic distance based approach, completely **unsupervised**, children approximate their parents over the time course of two years



Yatsunenکو  
et al., 2012



# Collaboration to study undernutrition in Mirpur, Bangladesh



## Collaboration with ICDDR,B

Oral Rehydration therapy was discovered here, now implemented worldwide as life-saving therapy for cholera.

Study participants are inhabitants of an urban slum area in Mirpur, Dhaka

Birth cohort and nutritional status data were collected along with biospecimens.



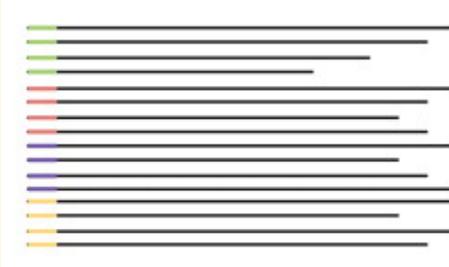
Photos reproduced with consent courtesy of icddr,b Bangladesh

# Methods to characterize the bacterial component of the gut microbiota



Pulverize bio-specimens  
& extract genomic DNA

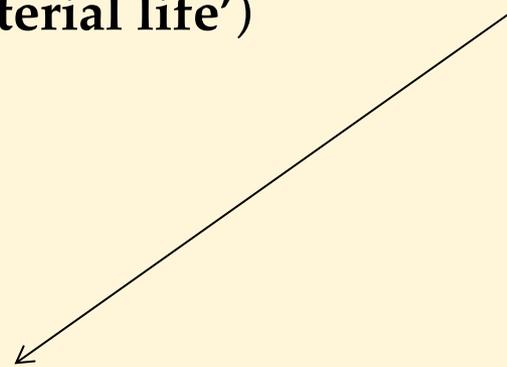
Biospecimens provide a  
non-invasive measure of  
bacterial diversity in the  
gastrointestinal tract



PCR amplify sample DNA  
using a 16S rRNA gene  
(phylogenetic 'barcode of  
bacterial life')



Multiplex high  
throughput  
sequencing



Group related sequences into Operational Taxonomic Units (OTU) based  
on alignment with 97% sequence identity.

**Bacterial species/strains/taxa are defined based on 97% identity**

# Application of a machine learning approach to developing microbiota maturation metrics



**Question: Given a healthy child's diaper biospecimen, can we accurately predict the age of a child? Can we use this as a measurement of a child's gut development?**

Random Forests: decision-tree based machine learning method applied to **identify bacterial strains that can serve as markers of the aging process.**

Advantages:

1. Non-parametric assumptions
2. Ability to deal with large number of features relative to data-points (predictors >> number of data points)
  1. Provides a ranking of features that assist with feature selection
  2. Suited for sparse dataset types (tables with lots of 0's)

Disadvantages:

black-box nature of algorithm, possibility of over-fitting, limited by the data provided for training

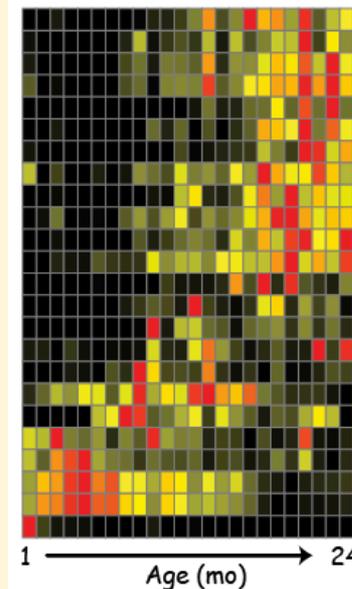
# Defining the stages of gut microbiota development using RF regression

Healthy infants/  
children from  
Bangladeshi  
study population;  
sampled monthly  
from birth to two  
years

Use Random Forests (machine  
learning) to identify bacterial  
taxa that are most  
discriminatory for different  
stages in assembly of the  
microbiota

$Y$  is age of child ~  $X_i$  are the abundances of  
each type of gut bacteria,  $i$

Create sparse model based on  
most age-discriminatory taxa



Each row is a  
different age-  
discriminatory  
strain

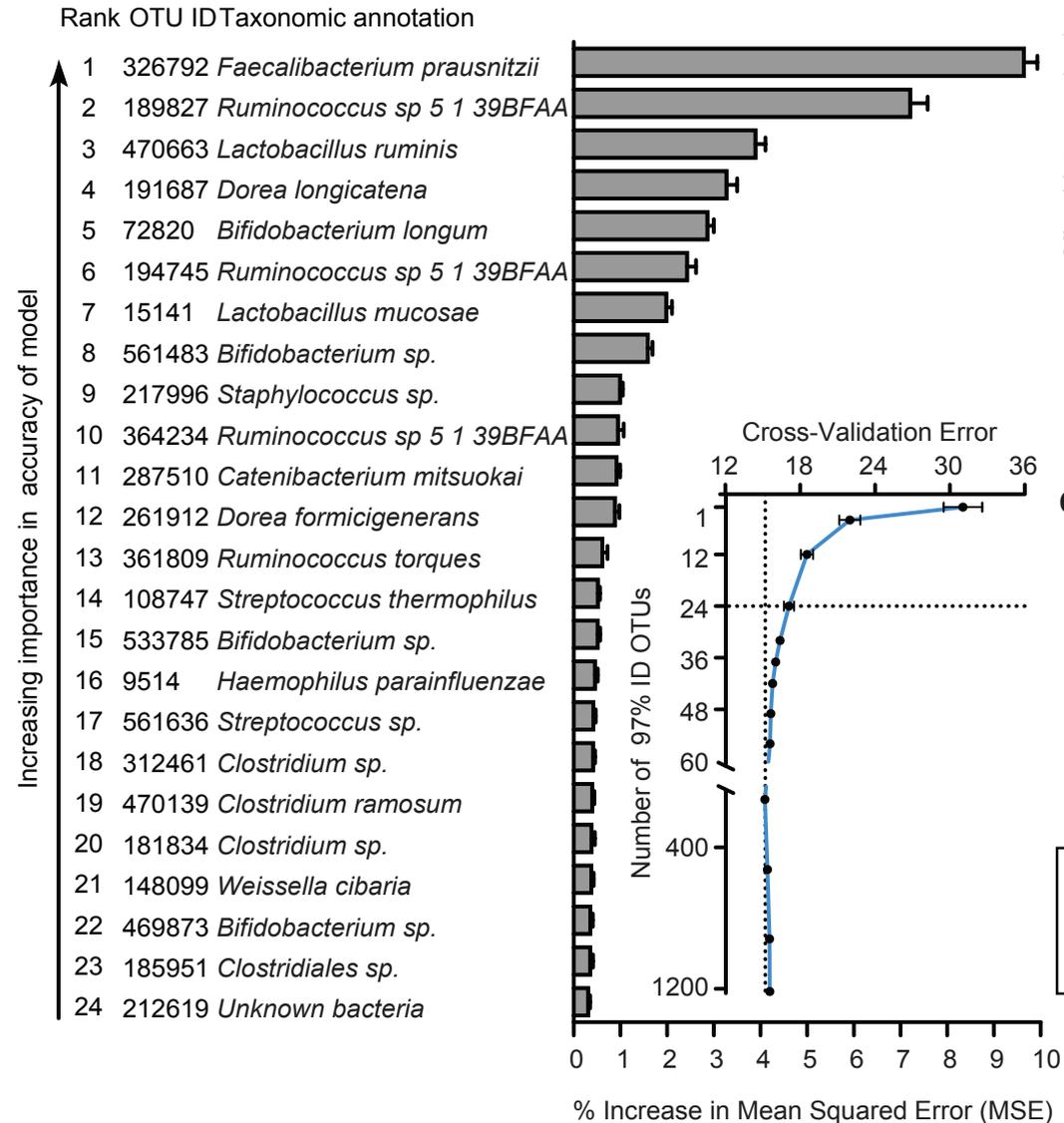
Relative abundance  
of a bacterial taxon  
Min Max

# Identification of bacterial taxa as biomarkers of healthy gut microbiota maturation



Steps to identification of age-discriminatory taxa:

1. Rank bacterial taxa in order of mean-squared error, permutation-based importance score
2. Cross-validate within training set to estimate number of taxa needed for accurate prediction
3. Generate sparse model and validate model in samples from a different set of unrelated children



# Validation of a sparse Bangladeshi model consisting of age-discriminatory taxa



Training set

Validation sets

**B**

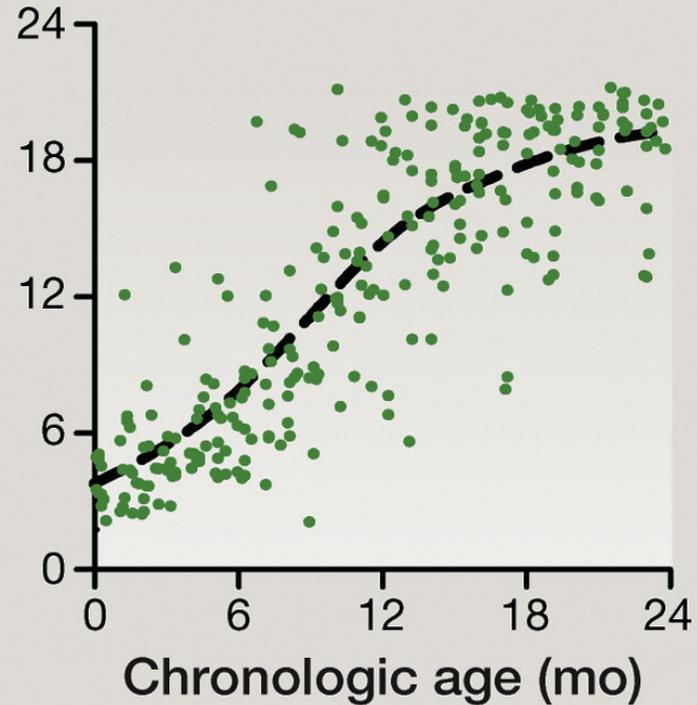
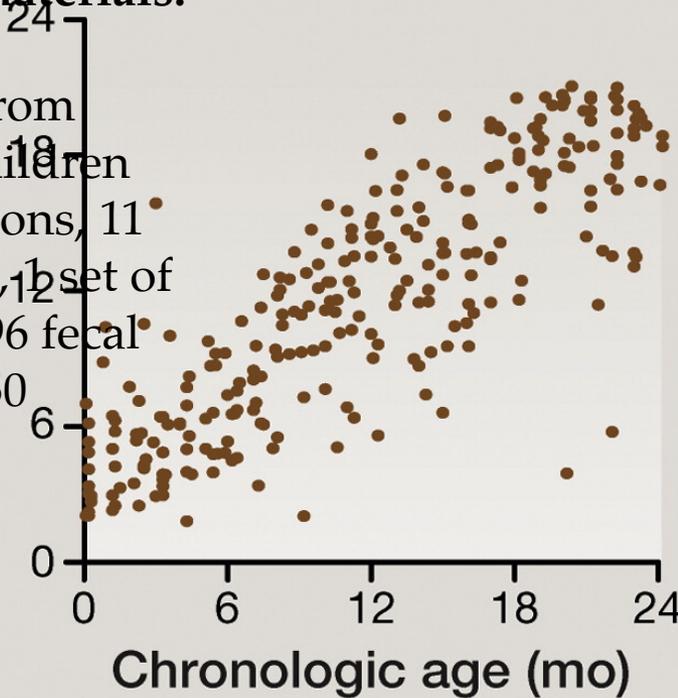
Age-discriminatory model training

Model applied to other children

Starting materials:

Samples from healthy children (25 singletons, 11 twin pairs, 12 set of triplets; 96 fecal samples from 50 children)

Microbiota age (mo)



Discriminatory taxa

Applied to singletons

Applied to twins

Applied to triplets

Relative abundance of bacterial taxon

Max

100747 *Streptococcus thermophilus*

561636 *Streptococcus* sp.

561483 *Bifidobacterium* sp.

72820 *Bifidobacterium longum*

217996 *Staphylococcus* sp.

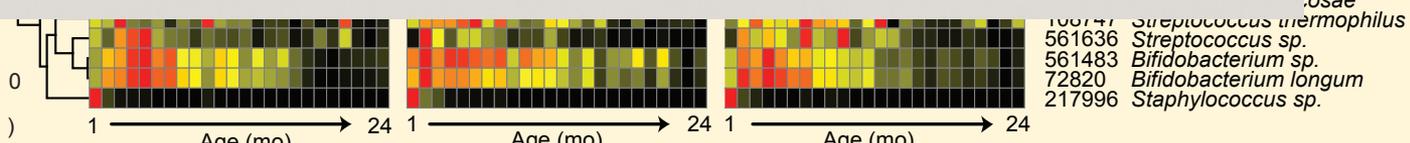
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Subramanian et al., 2014

# Definition of two microbiota metrics based on the Bangladeshi model

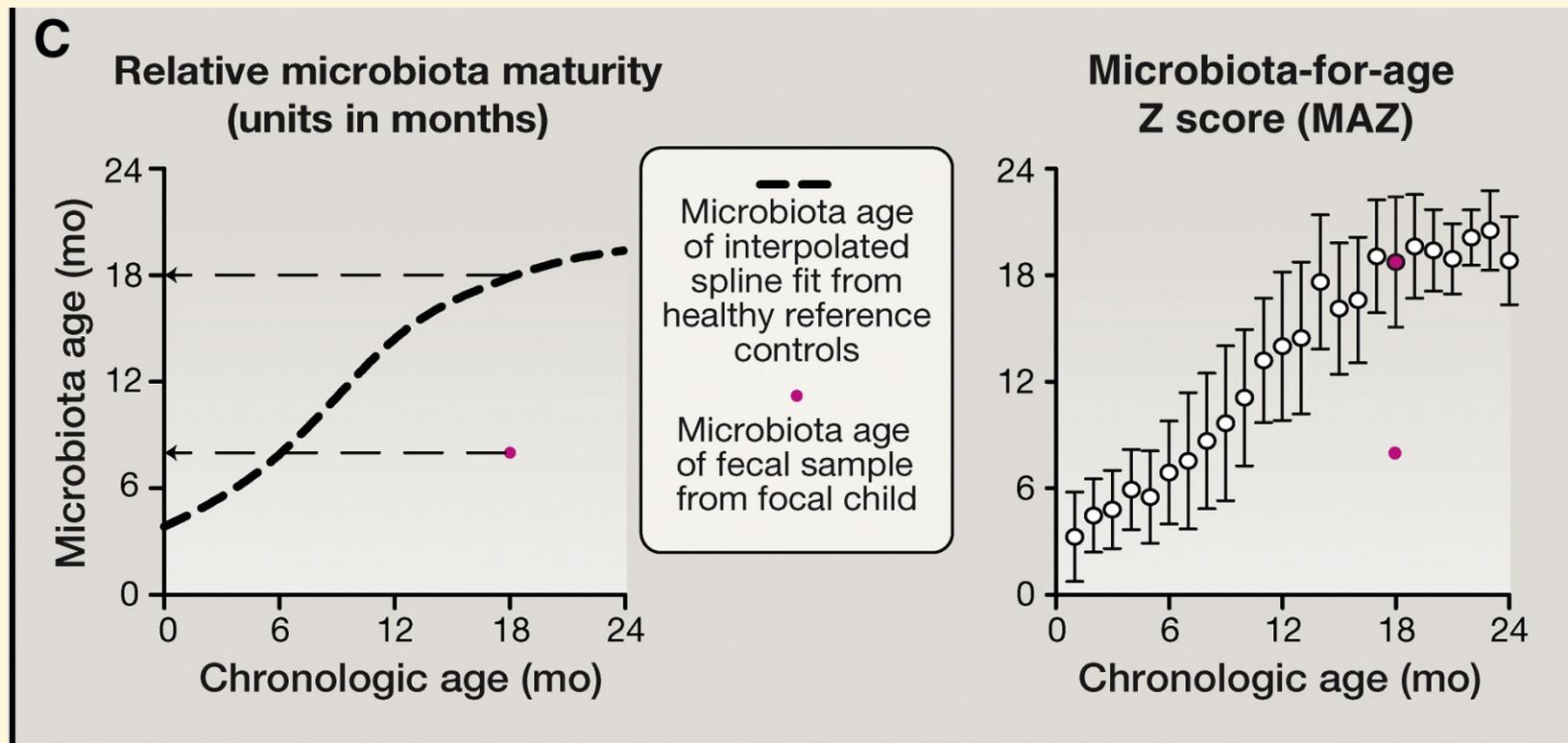


(1) Relative microbiota maturity =

microbiota age of a given child –  
microbiota age for healthy children  
of similar chronological age

(2) Microbiota-for-age Z score (MAZ)

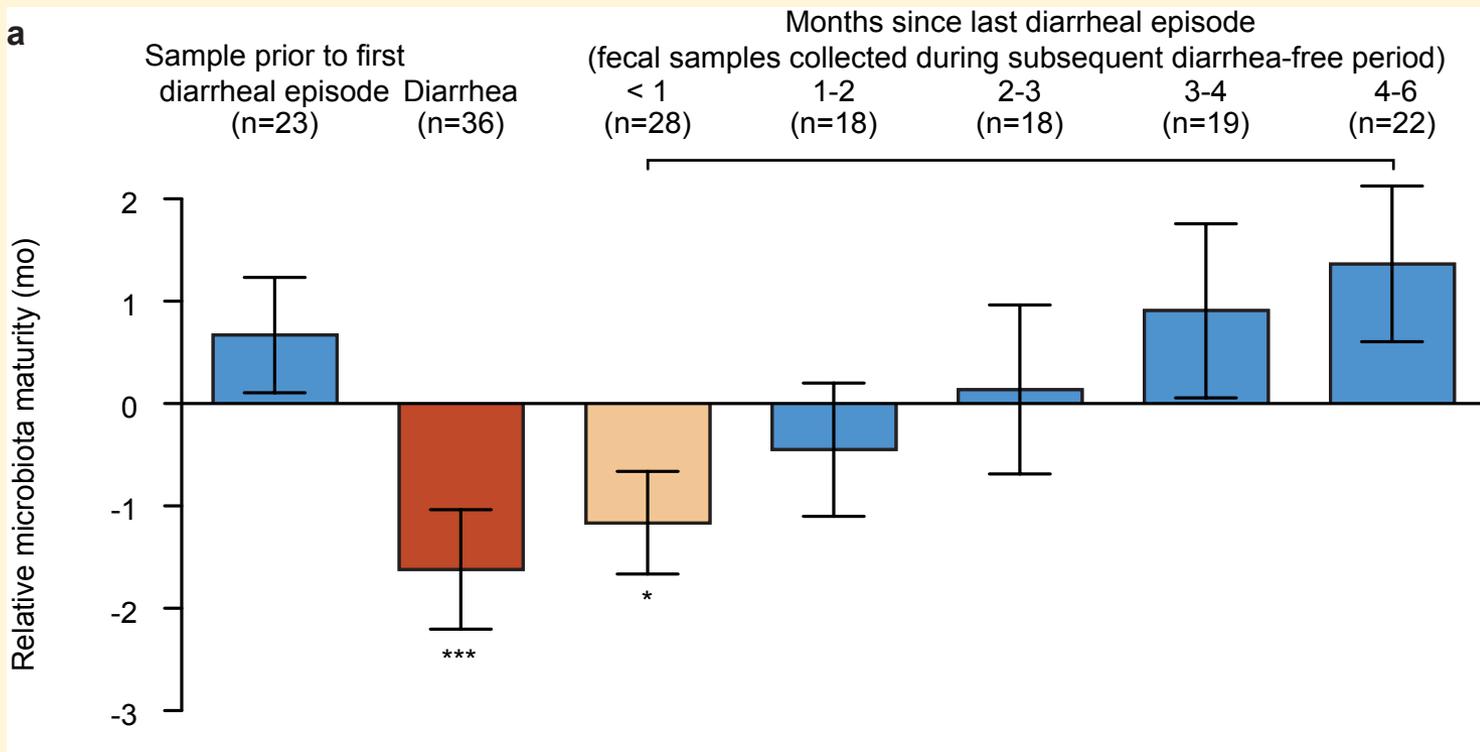
$$\frac{(\text{predicted microbiota age} - \text{median})}{\text{standard deviation of microbiota age}}$$



# Application of metrics to a cohort of Bangladeshi twins and triplets



Diarrheal episodes in anthropometrically healthy children are associated with a transient reduction in maturity levels that recovers.



ANOVA of linear mixed models:  
'diarrhoea', 'month following diarrhoea' and 'presence of formula in diet' have significant effects, \* $P < 0.05$ , \*\* $P < 0.01$



# Presentation of severe acute malnutrition (SAM)



Weight-for-Length Z score  $< -3$  SD  
Bipedal edema, MUAC  $< 11.5$  cm

Stabilization with Milk Suji (porridge)

Oral Rehydration

Antibiotics

Administration of therapeutic foods

Plumpy-  
Nut  
RUTF



Khichuri



Halwa



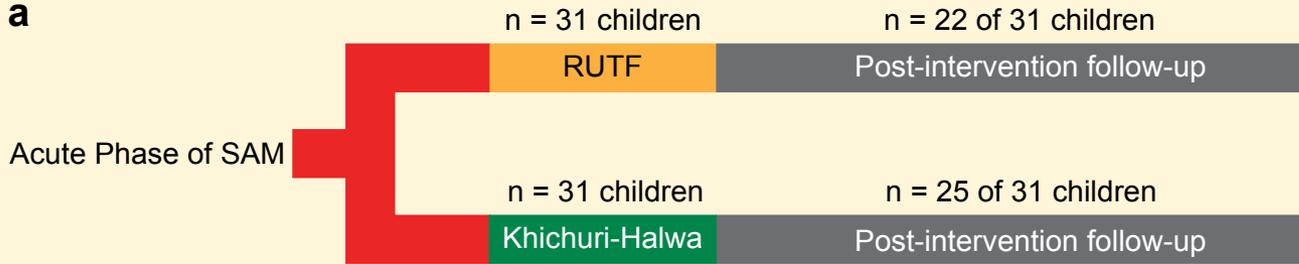
# Inpatient treatment of children with severe undernutrition



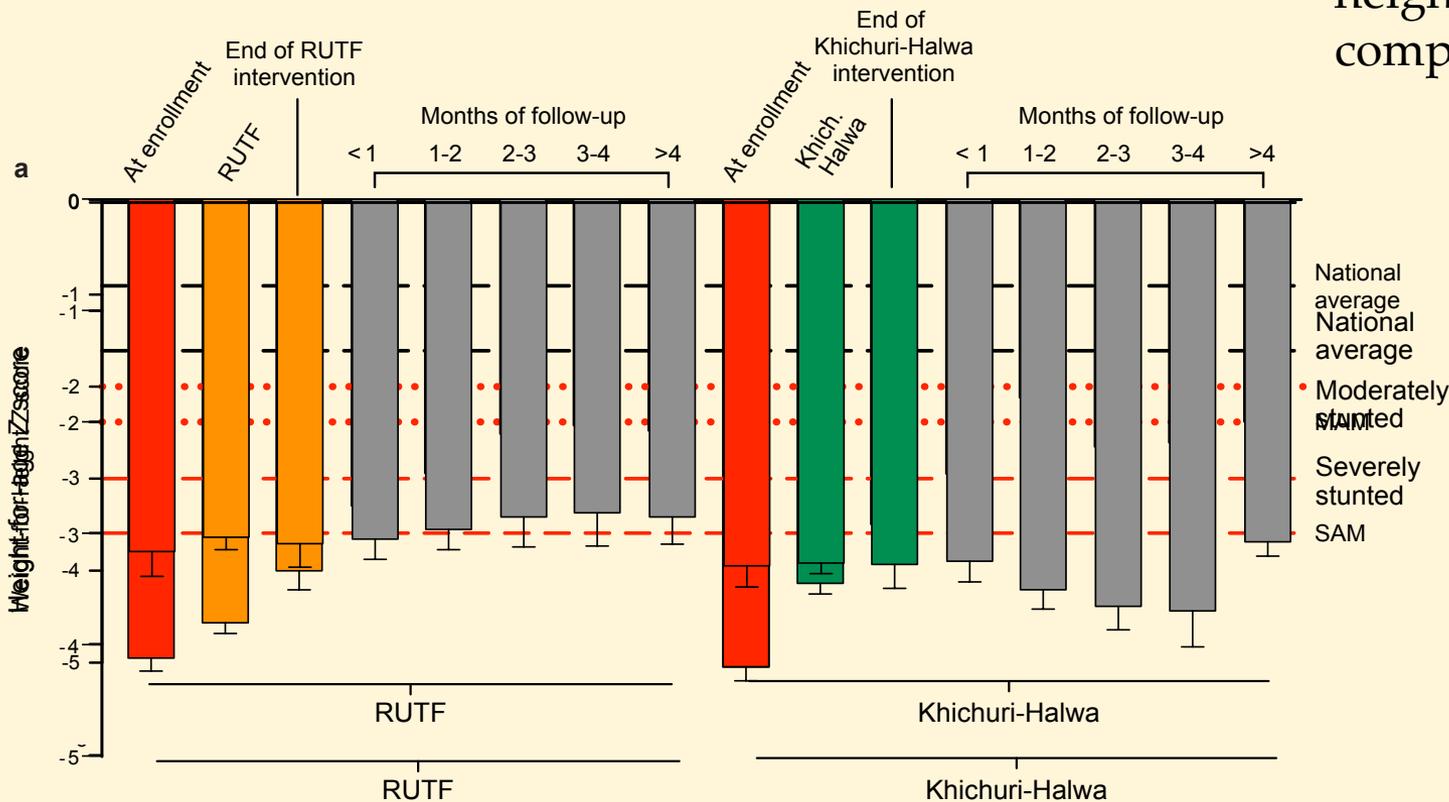
# Randomized clinical trial comparing two food interventions to treat SAM



a

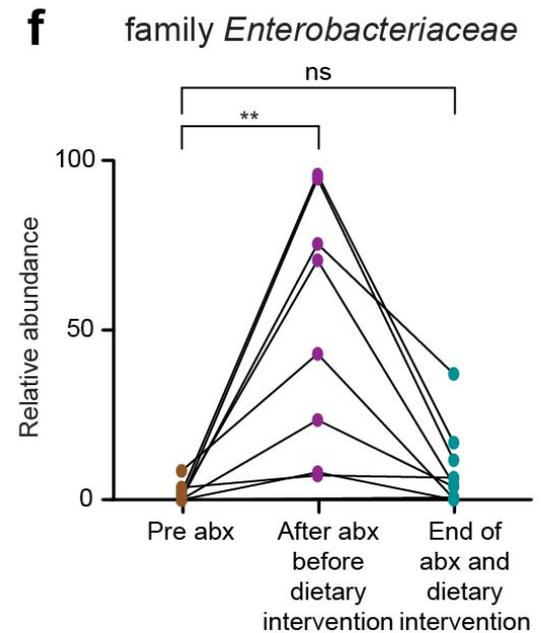
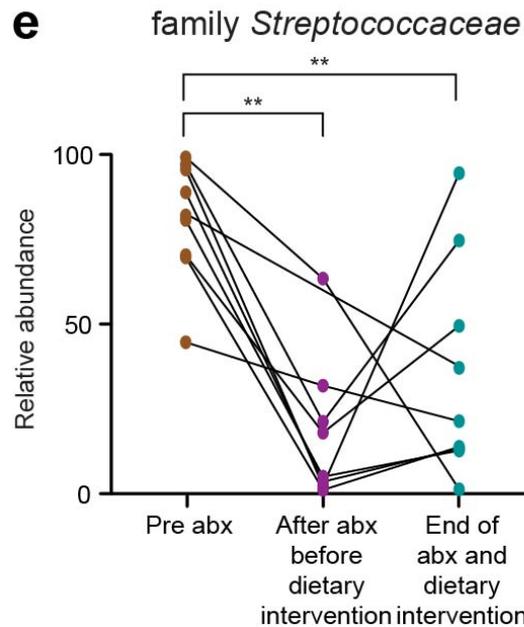
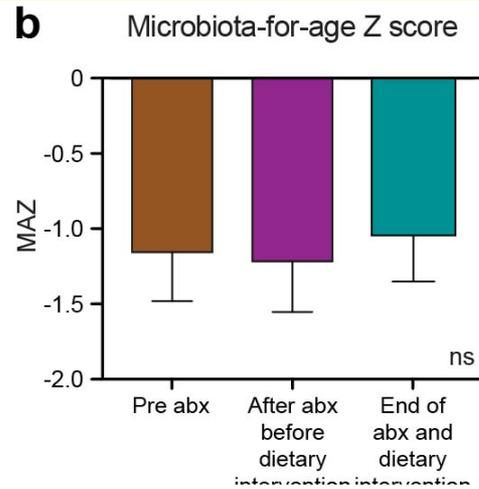
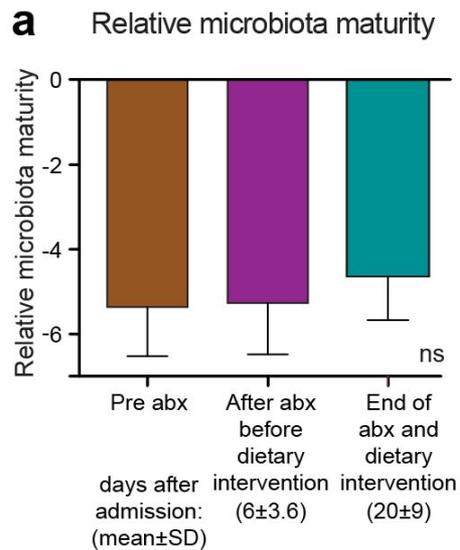


Despite treatment, children don't improve in terms of age-adjusted height and not completely with weight.





# Microbiota immaturity evident prior to administration of antibiotics



# Conclusions from Bangladesh study



Two types of therapeutic foods produce an improvement in microbiota maturity indices that was not sustained

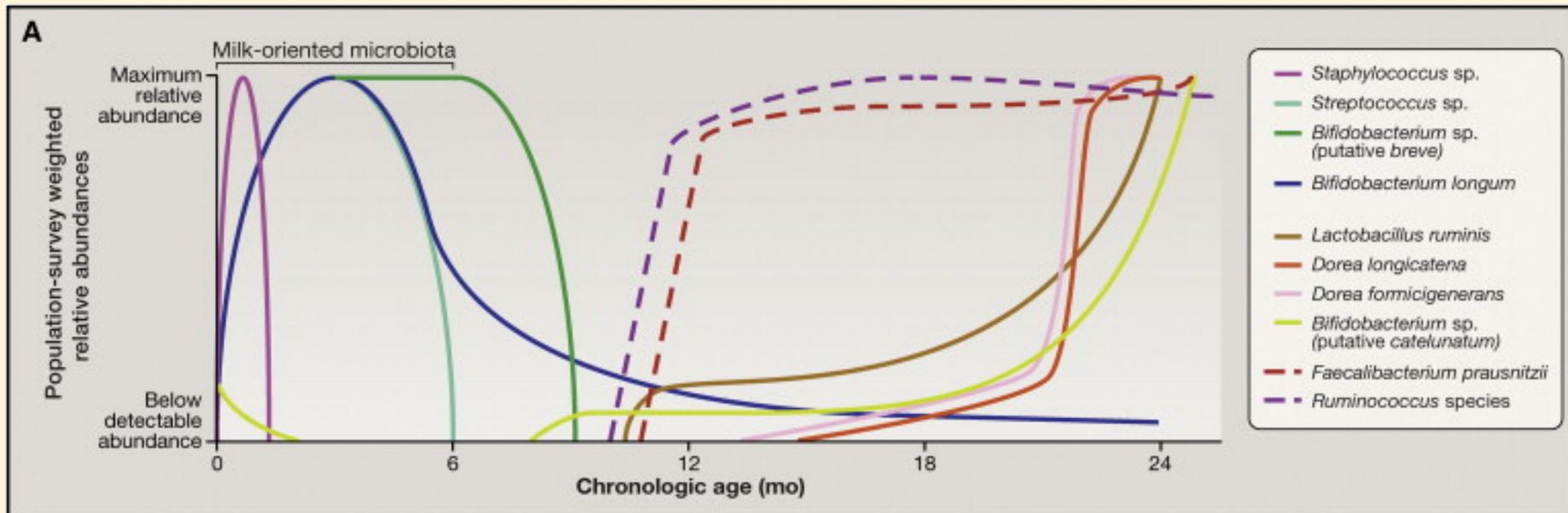
More prolonged food-based interventions of varying composition and / or addition of gut microbes may be beneficial

Age-discriminatory taxa may themselves serve as therapeutic agents and / or targets of next generation microbiota-directed therapeutic foods

# Gut microbial milestones as metrics to monitor therapeutic interventions



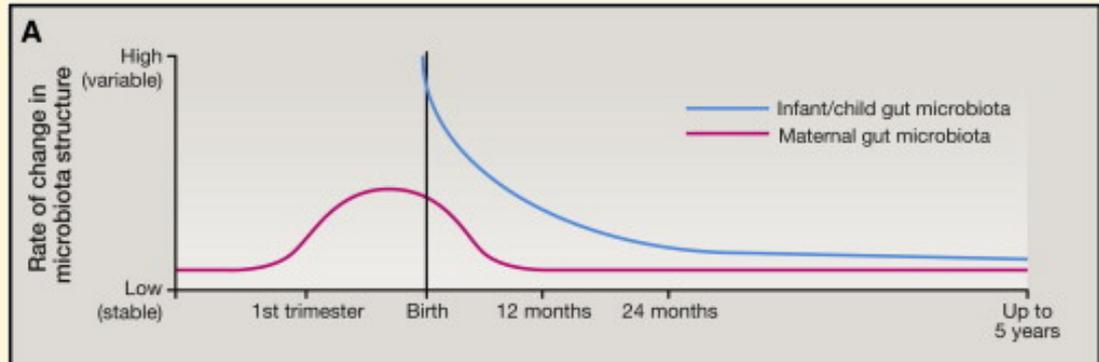
Bacterial strains discriminatory for age in healthy children provide a way to characterize malnourished states and human postnatal development



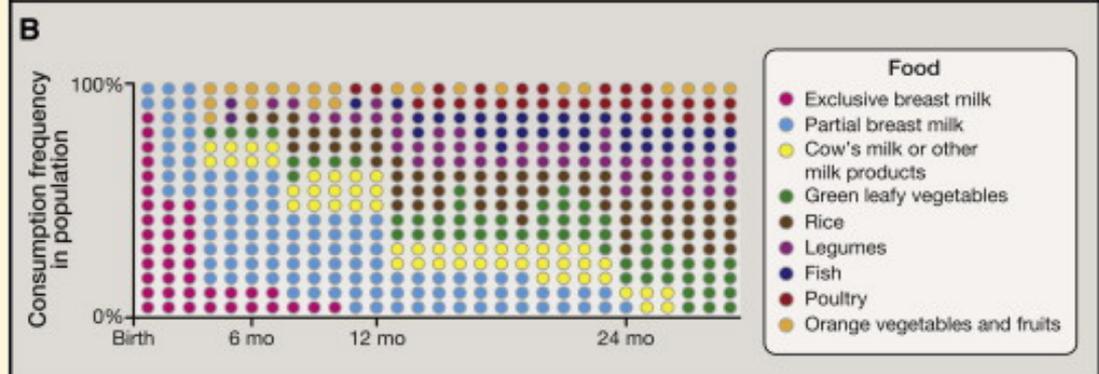
Beyond their diagnostic value these features can be now investigated in terms of their **functional roles** in cell-based, animal and human studies

# Further questions?

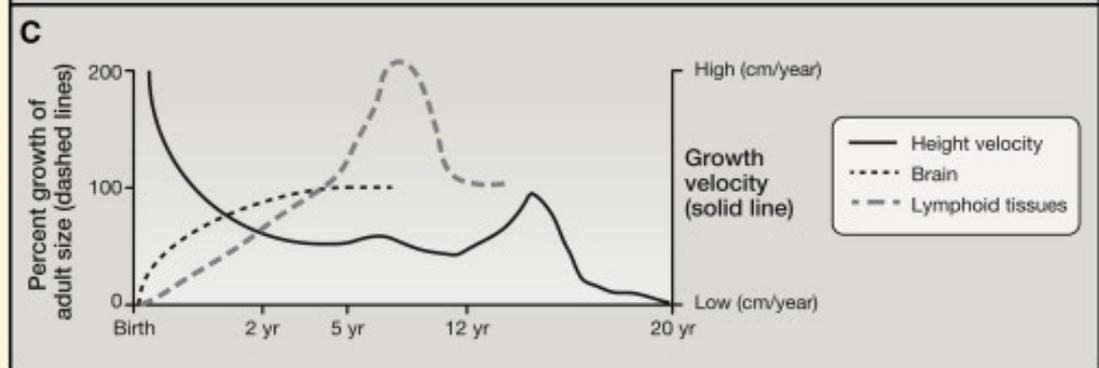
Relationship between **maternal microbes and infant microbes?**



Relationship between **complex food consumption patterns and gut bacteria?**



Relationship between gut microbial organ development and development of other organs?  
**Brain, Bone, Immune System Development**

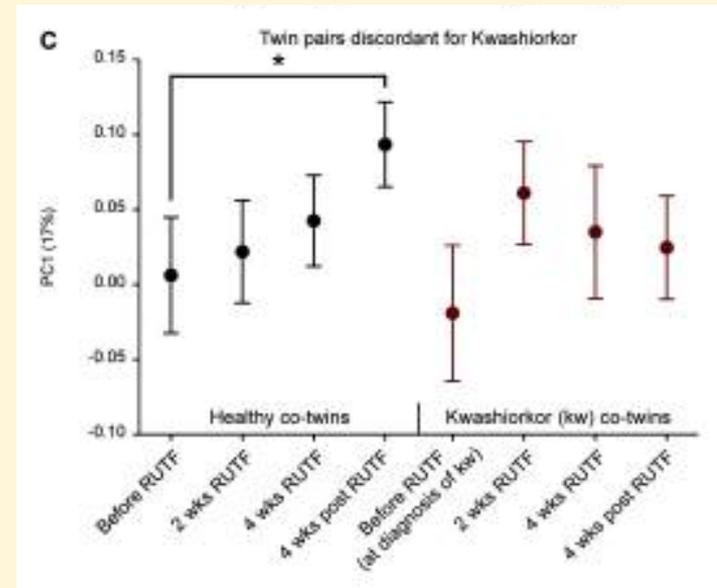
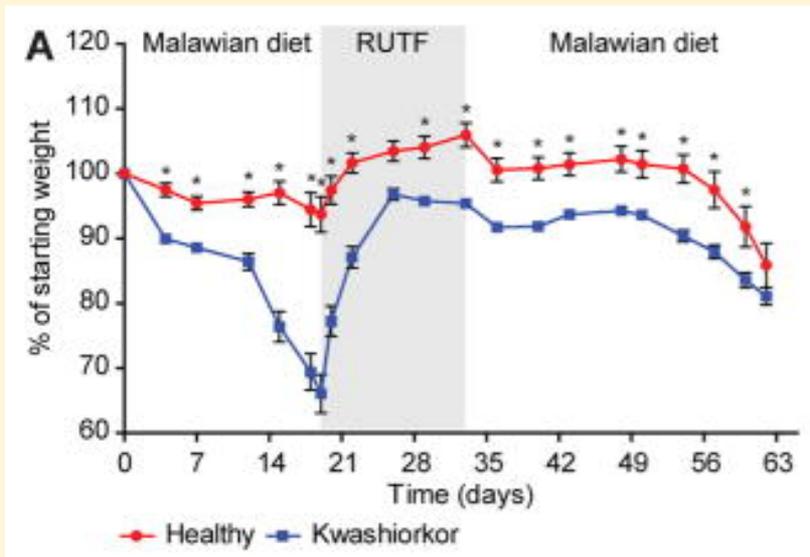




# Testing causality in a humanized gnotobiotic model of kwashiorkor



## Malawian twin pair discordant for kwashiorkor

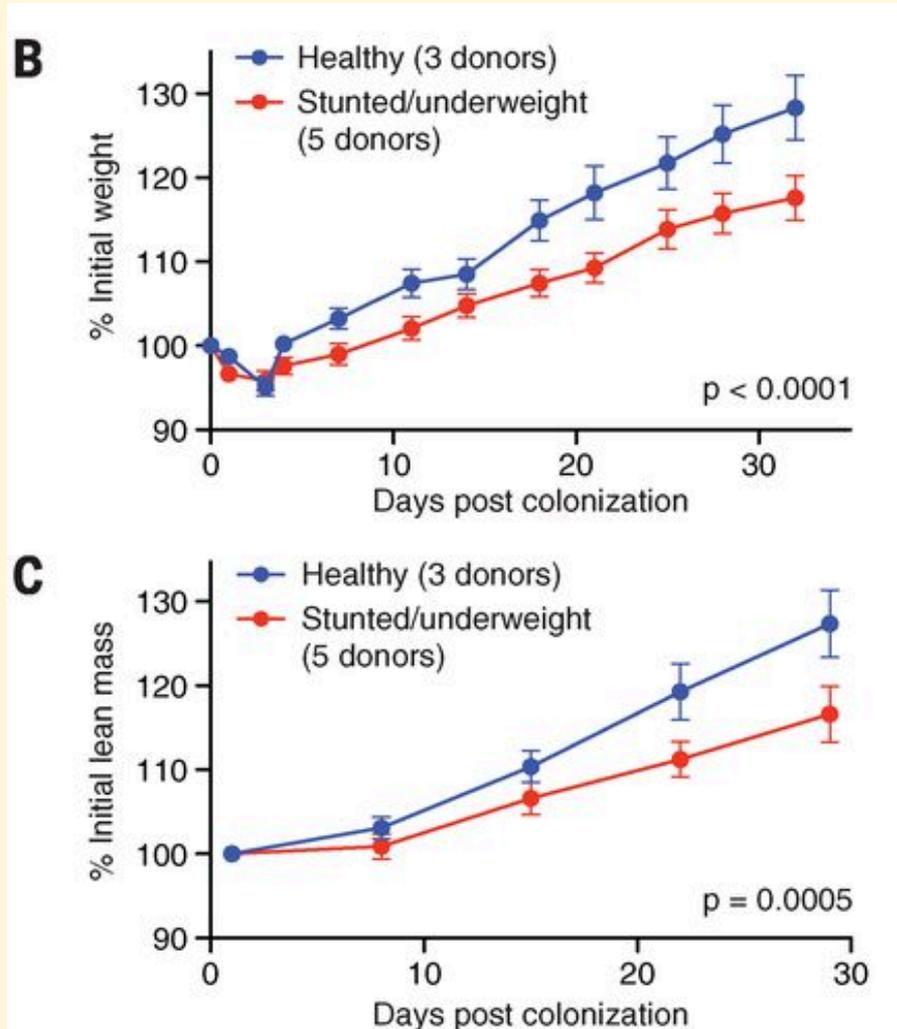


Smith, Yatsunenکو et al., Science 2013

# Testing causality in a humanized gnotobiotic model of stunting

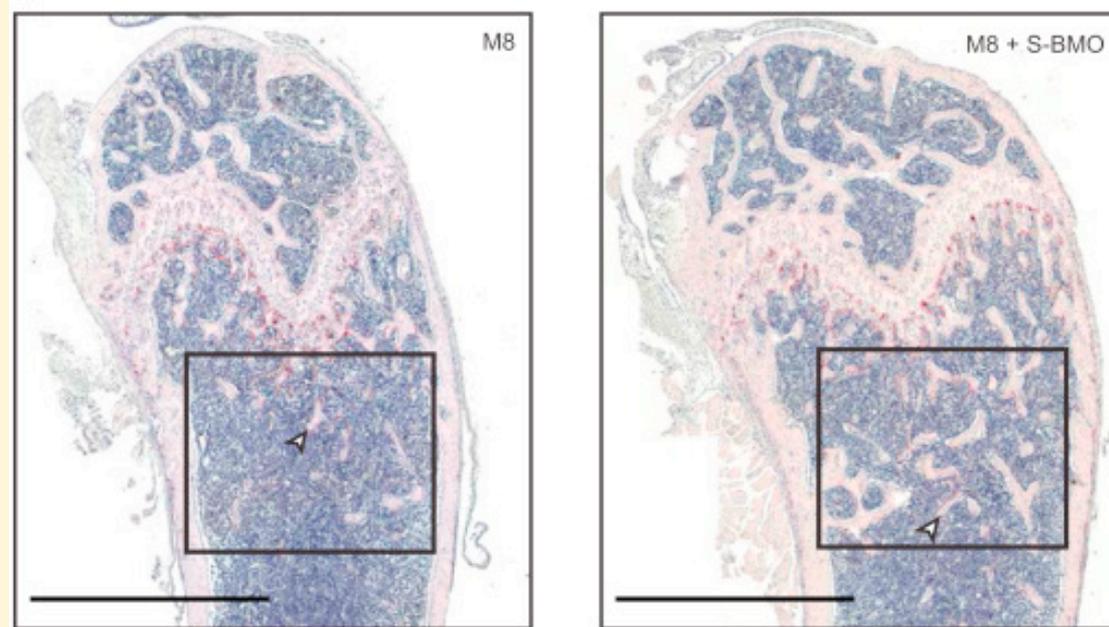
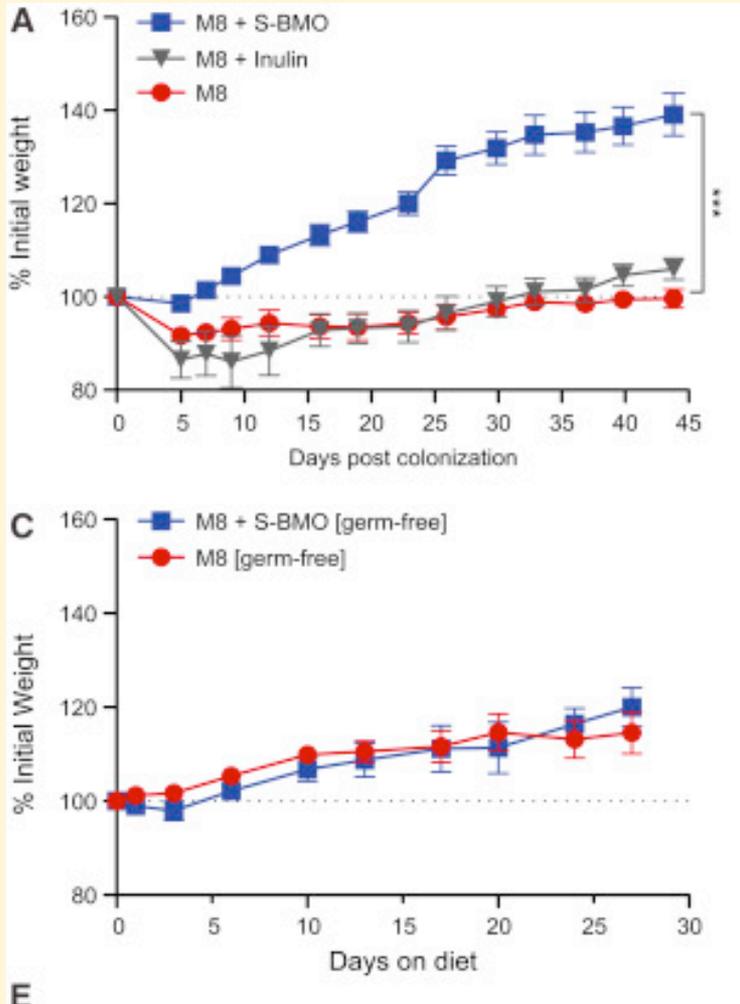


Malawian singletons with chronic forms of undernutrition



Blanton et al.,  
Science 2016

# Pleiotropic effects in humanized mouse models of undernutrition



Charbonneau et al., Cell 2016

# Neonatal sepsis and an early synbiotic interventions



## A randomized synbiotic trial to prevent sepsis among infants in rural India

Pinaki Panigrahi<sup>1,2</sup>, Sailajanandan Parida<sup>3</sup>, Nimai C. Nanda<sup>4</sup>, Radhanath Satpathy<sup>5</sup>, Lingaraj Pradhan<sup>6</sup>, Dinesh S. Chandel<sup>7</sup>, Lorena Baccaglioni<sup>1</sup>, Arijit Mohapatra<sup>5</sup>, Subhranshu S. Mohapatra<sup>5</sup>, Pravas R. Misra<sup>5</sup>, Rama Chaudhry<sup>8</sup>, Hegang H. Chen<sup>9</sup>, Judith A. Johnson<sup>10</sup>, J. Glenn Morris Jr<sup>10</sup>, Nigel Paneth<sup>11</sup> & Ira H. Gewolb<sup>12</sup>

**Table 2 | Effect of synbiotic treatment on sepsis and other morbidities in the first 60 days of life**

Outcome variables	Control <i>n</i> =2,278 (%)	Synbiotic <i>n</i> =2,278 (%)	RR (95% CI)	NNT (95% CI)	<i>P</i> value
Death and sepsis (primary outcome)	206 (9.0)	123 (5.4)	0.60 (0.48, 0.74)	27 (19, 47)	<0.001
Deaths	4 (0.2)	6 (0.3)	1.50 (0.42, 5.31)	NA*	0.526†
Sepsis (A + B + C)	202 (8.9)	117 (5.1)	0.58 (0.46, 0.72)	27 (19, 44)	<0.001
A. Sepsis/pSBI—culture-positive septicaemia	27 (1.2)	6 (0.3)	0.22 (0.09, 0.53)	108 (71, 232)	<0.001
Gram-negative sepsis	16 (0.7)	4 (0.2)	0.25 (0.08, 0.75)	190 (110, 699)	0.007
Gram-positive sepsis	11 (0.5)	2 (0.1)	0.18 (0.04, 0.82)	253 (142, 1160)	0.012

Community-based, double-blind, placebo-controlled randomized trial in 149 randomly chosen villages in Odisha state

# Microbiota directed complementary foods for undernutrition



Tilapia



Egg



Sweet pumpkin



Whole wheat flour



Milk Powder



Peanuts



Potato



Spinach



Red lentils



Banana



Rice



Chickpeas

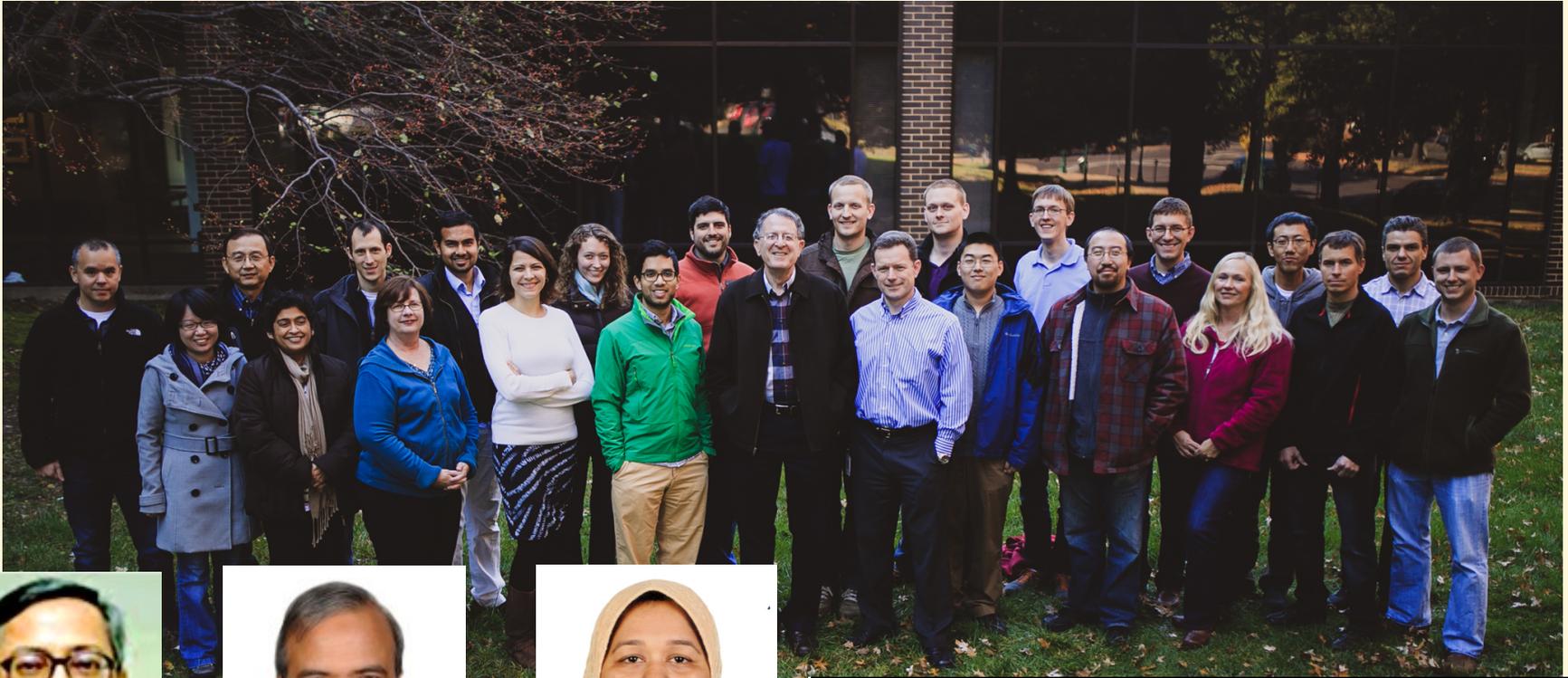
# Preserving and nurturing our resources for a sustainable future



# Acknowledgements



## Gordon Laboratory



Tahmeed  
Ahmed



Rashidul  
Haque



Sayeeda  
Huq

