

OSLO AND AKERSHUS  
UNIVERSITY COLLEGE  
OF APPLIED SCIENCES

# DEEx

Disease and Environmental Exposures

## Mikroorganismer i norsk barnehagestøv: Venn eller fiende?

**Anders Benteson Nygaard**

PhD Candidate

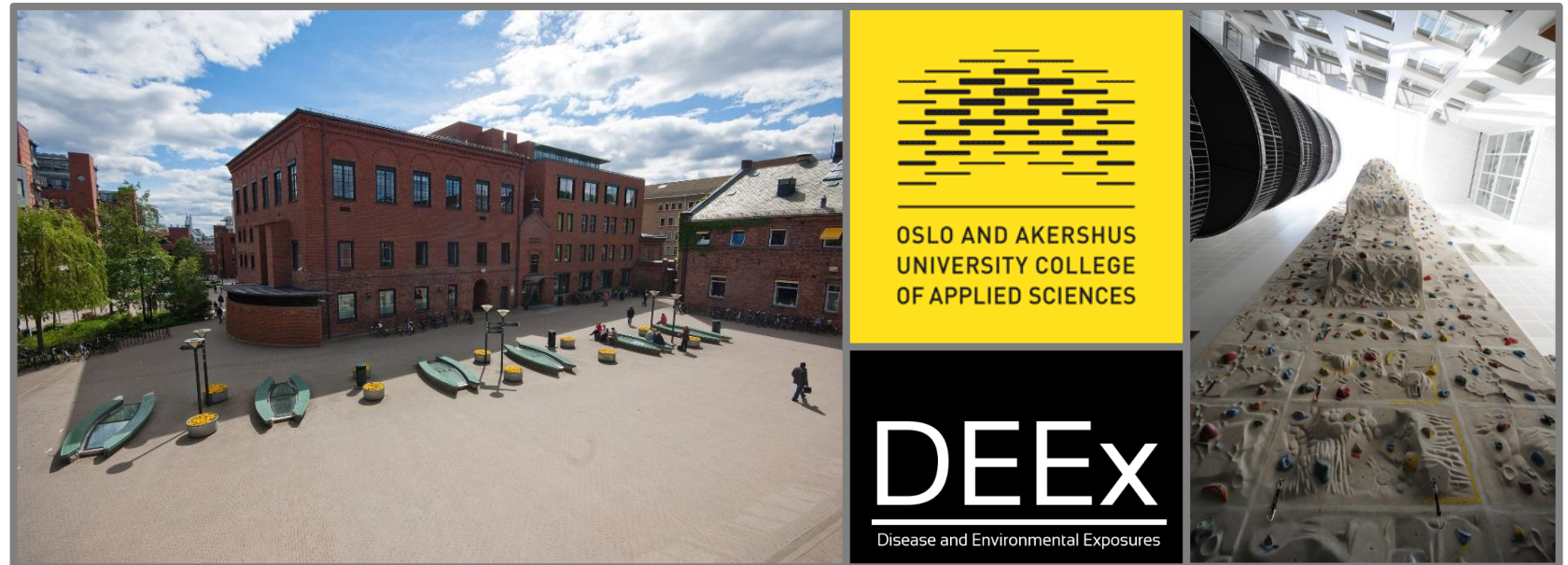
Oslo and Akershus University College (HiOA)



Nasjonal  
inneklimakonferanse

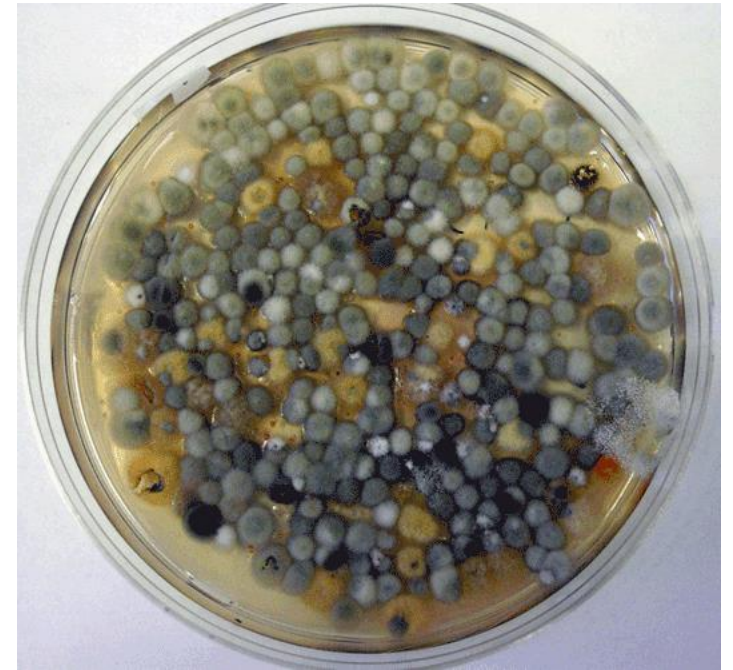
# Hvem er jeg

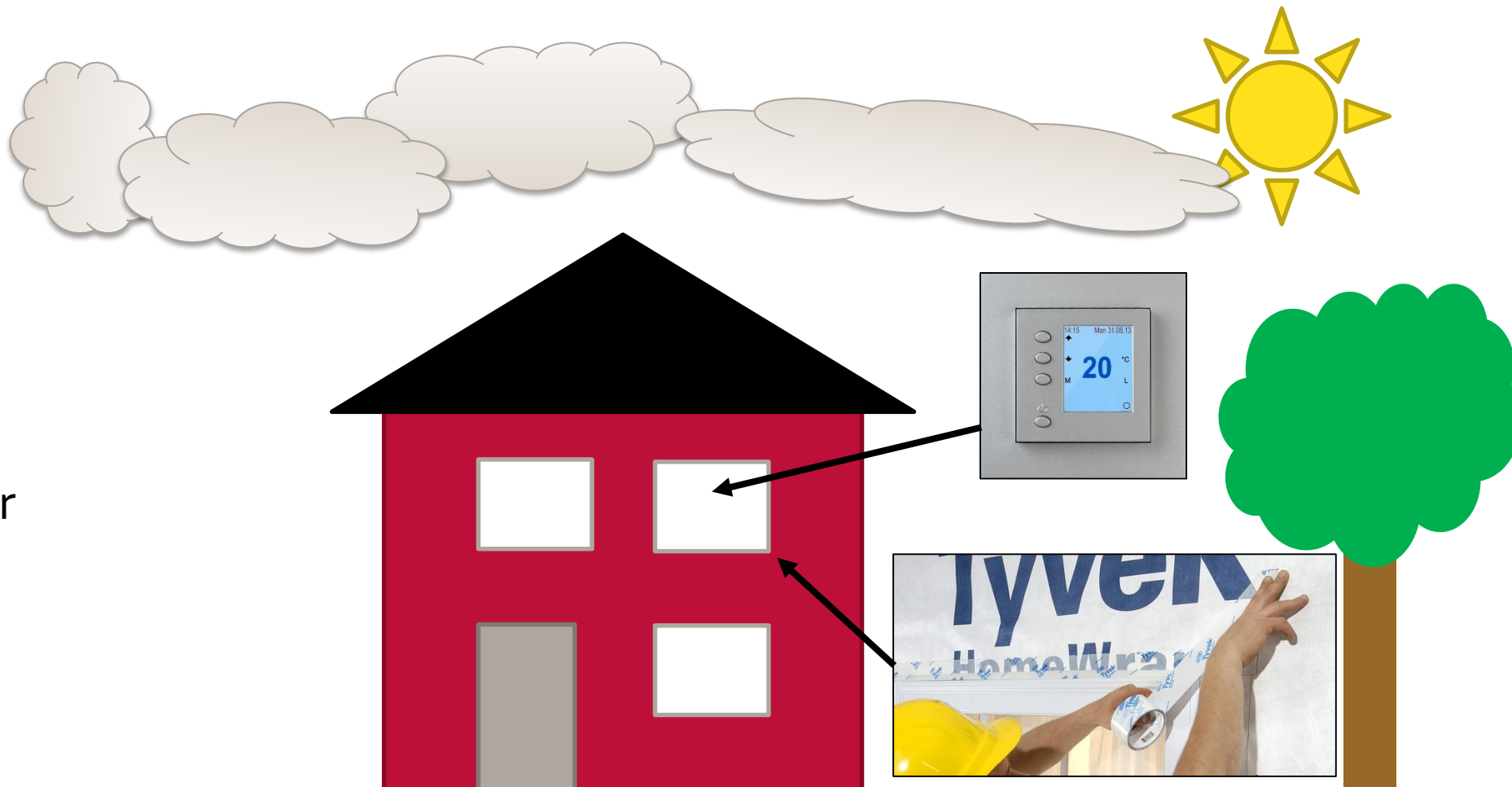
- Doktorgradsstipendiat ved Høgskolen i Oslo og Akershus
  - Fakultet for teknologi, kunst og design, Institutt for bygg- og energiteknikk
  - Fakultet for helsefag, Institutt for naturvitenskapelige helsefag
- Bakgrunn
  - B.Sc. Biologi
  - M. Sc Biomedisin
  - ... og tømrer



# Mikroorganismer og inneklima

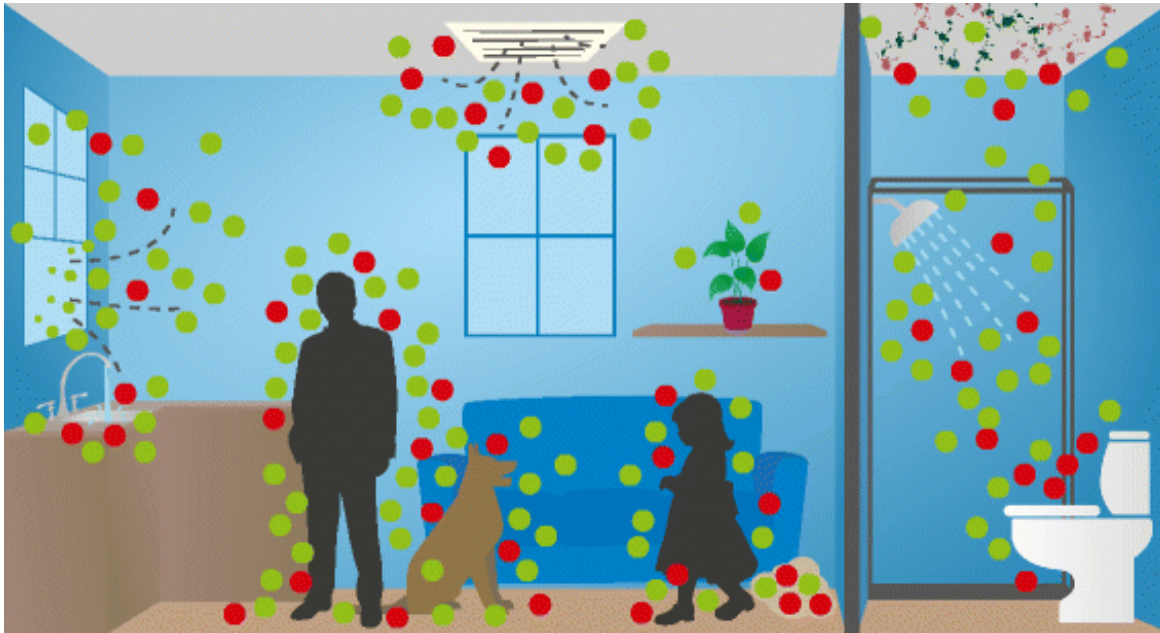
- Mikroorganismer
  - Bakterier
  - Muggsopp
- Kartlegging
  - Prøvetakning
  - Dyrkning
  - Mikroskopi





- Avskjermet
- Selektive kilder
- Statisk

# Bakterielle kilder



Prussin, A. J. and L. C. Marr (2015). "Sources of airborne microorganisms in the built environment." *Microbiome* 3(1): 1-10.

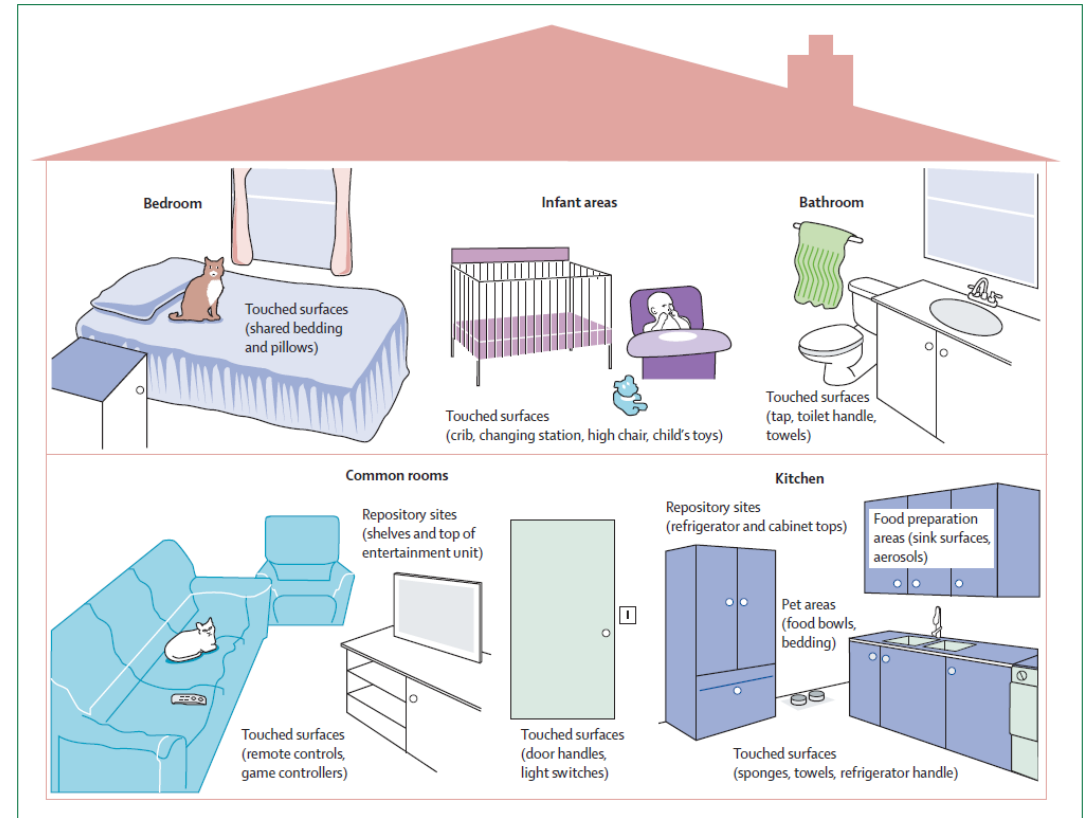


Figure 2: Potential points of transmission of staphylococci within households

Davis MF, Iverson SA, Baron P, Vasse A, Silbergeld EK, Lautenbach E, et al. Household transmission of methicillin-resistant *Staphylococcus aureus* and other staphylococci. *The Lancet Infectious Diseases*. 2012;12(9):703-16.

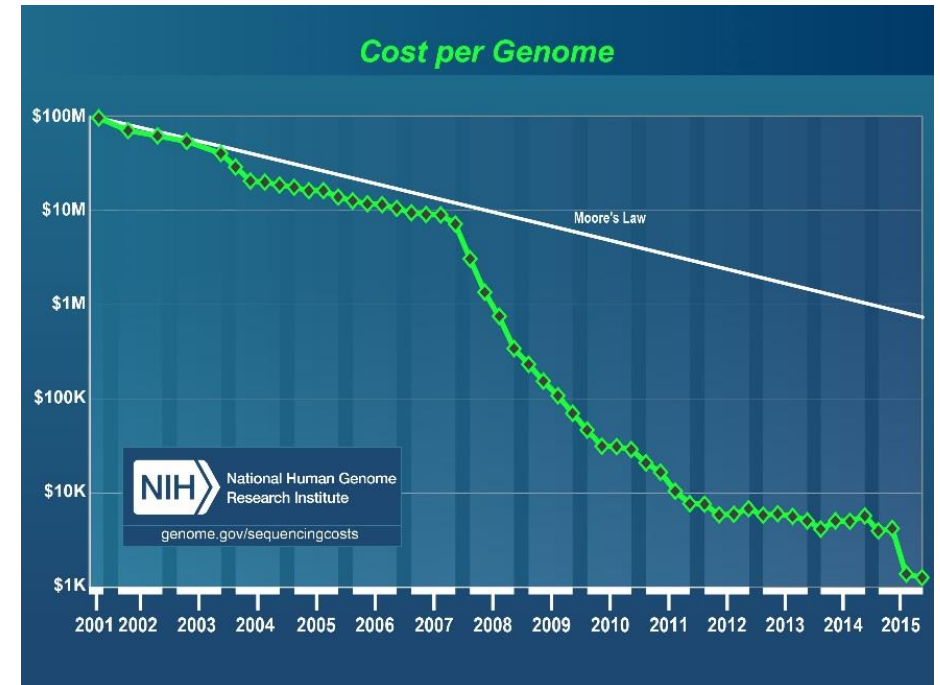
# "The great plate count anomaly"

- Omtrent 1% av alle mikroorganismer i miljøet er kultiverbare -

# DNA sekvensering og bioinformatikk

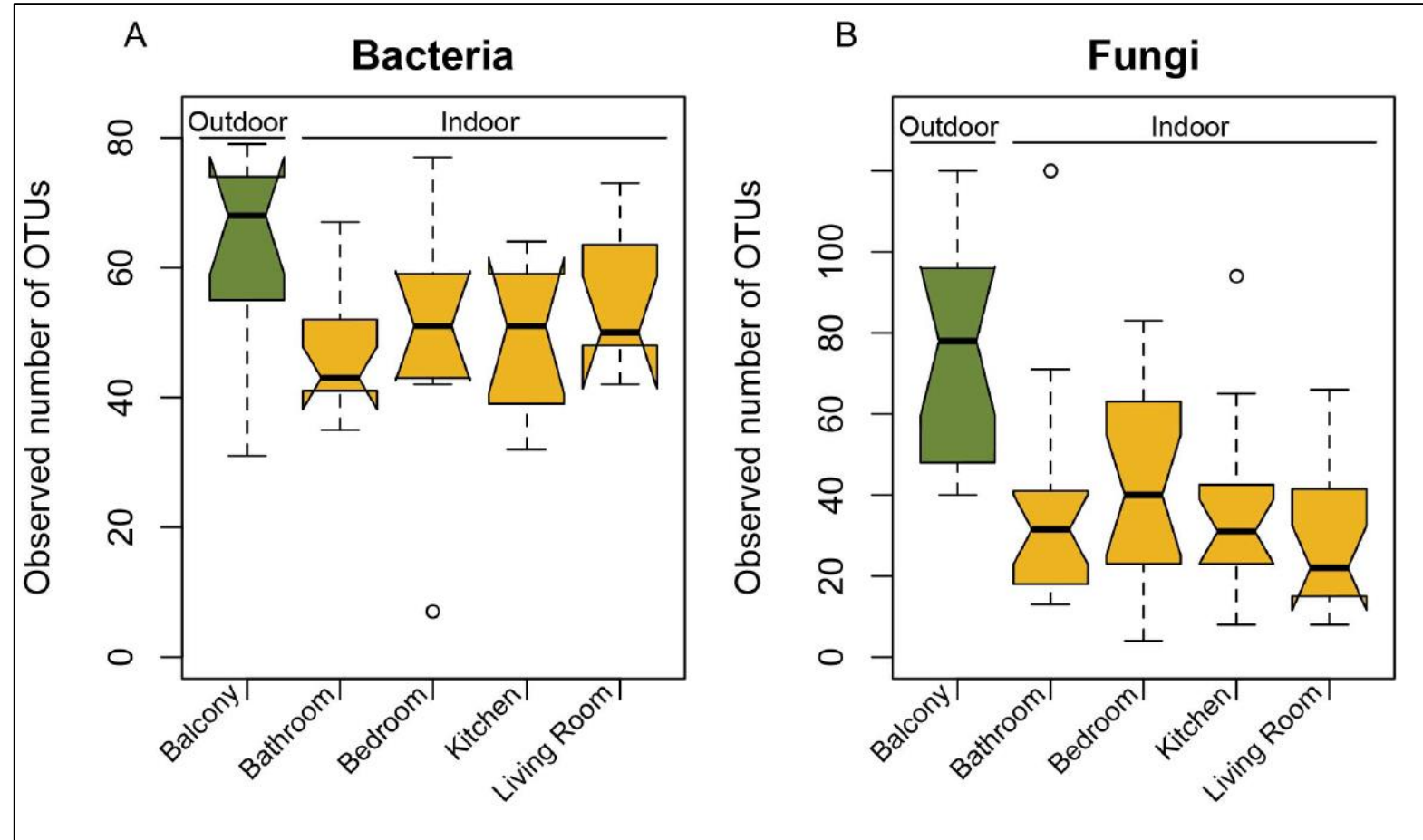
- Arvemateriale i celler
- Fremskritt i sekvenseringsteknologi muliggjør ny forskning
  - Mikrobiomer
  - Miljømikrobiologi
- Big Data

## Bygningsmikrobiomer



# Hva kan vi måle

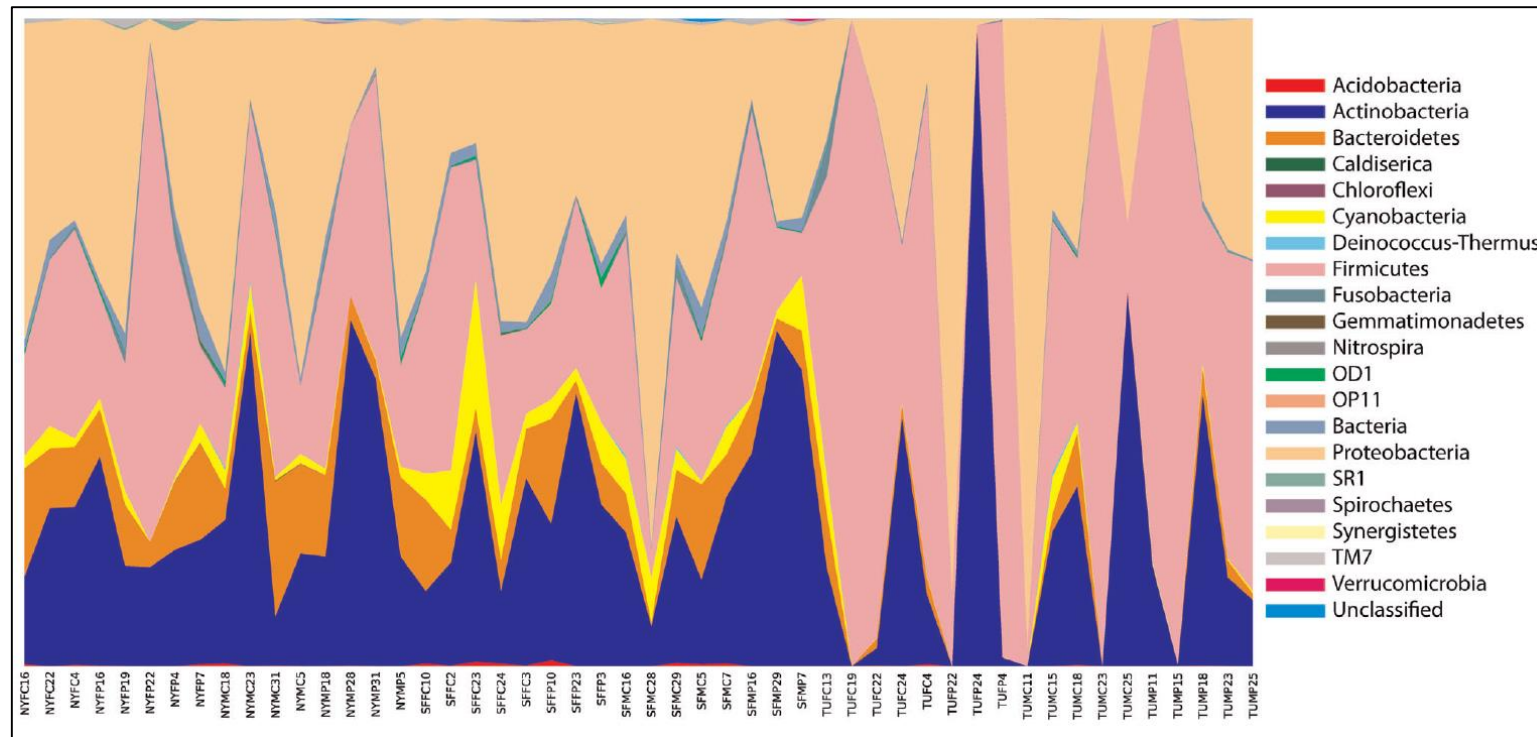
- Artsrikhet





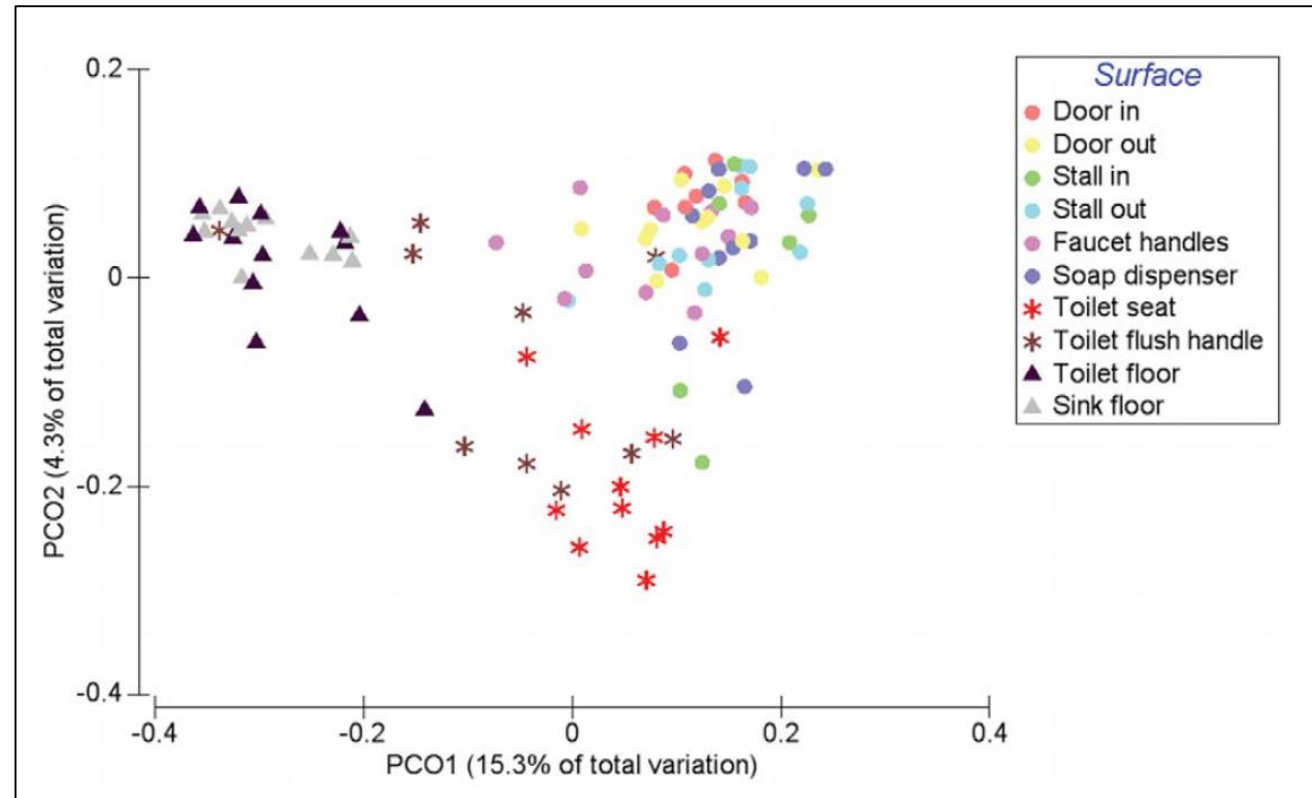
# Hva kan vi måle

- Artsrikhet
- Sammensetning



# Hva kan vi måle

- Artsrikhet
- Sammensetning

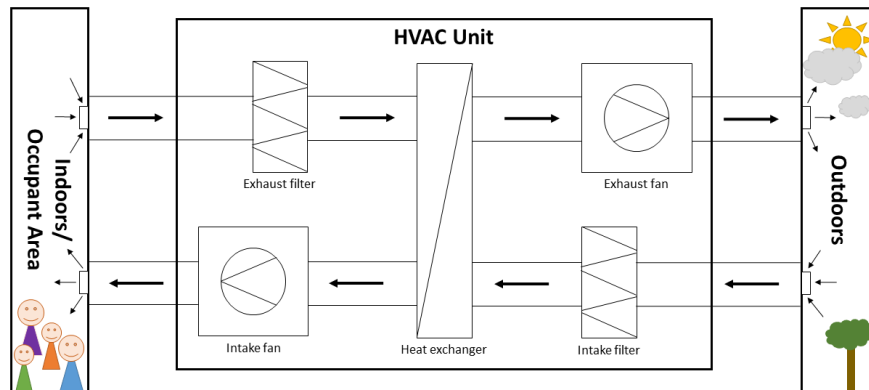


- "Community" sammenligning

# Studier

## Ventilasjon i barnehager

- Ventilasjonstøv
  - Avtrekk og tilluft,
  - Ni ventilasjonsanlegg
- Andre analyser
  - Endotoksiner
  - CFU-målinger



## Longitudinell kartlegging av inneklima

- Gulvstøv
  - Nybygd barnehage
  - Tre rom
  - Fem ganger, et år
- Andre analyser
  - Antibiotika-resistens
  - Petrifilm
  - Overflate-RLU

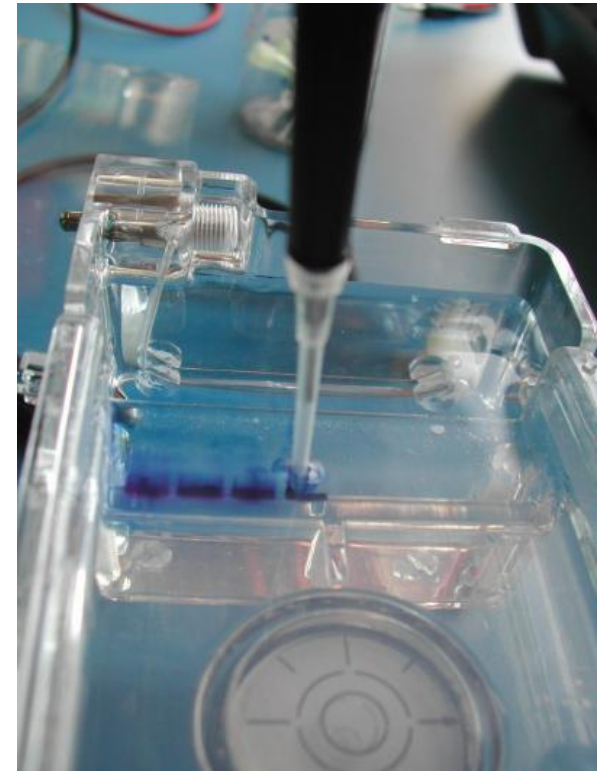
# Prøveinnsamling

- Støvprøver samlet i sterile filterkassetter
  - Ventilasjonsfilter
  - Gulv



# DNA prosessering og sekvensering

- DNA ekstraksjon
  - Powerwater DNA isolation kit (MoBio)
- Amplicon library prep
  - 16S rRNA primers 27F - 338R
  - V<sub>1</sub>-V<sub>2</sub> region
- Sekvensering
  - Roche 454 GS junior



# Data behandling

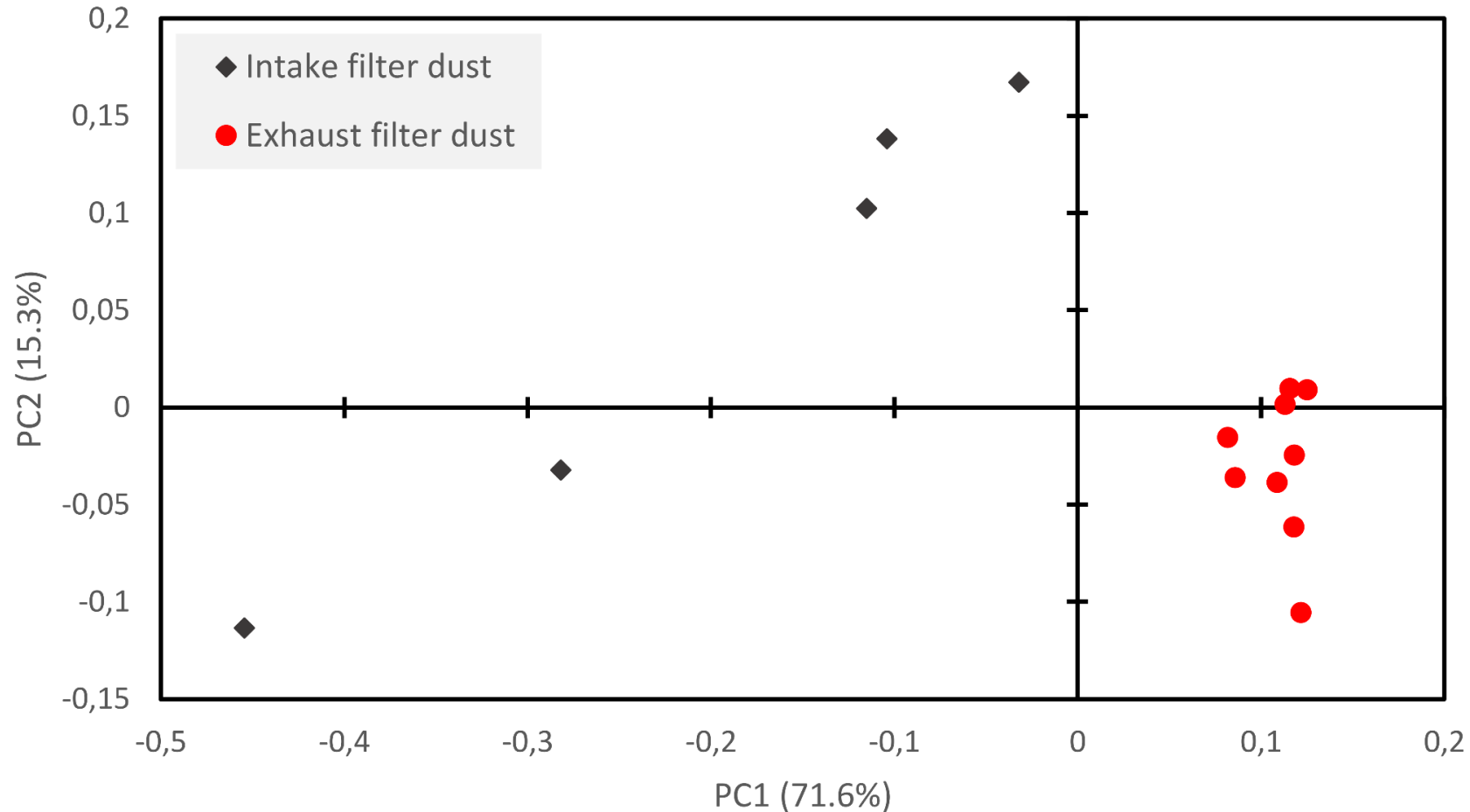
- 1,2 million gensekvenser
- Sekvensbehandling i Qiime
  - Computing server at HiOA
    - Dell R815
    - 64 cores
    - 254 GB ram
    - Ubuntu 14.04.3 Desktop
    - AMD 64 Bit
- Statistikk i R
  - Phyloseq
  - Vegan
- Ca. 15 000 Operational Taxonomic Units (OTUs)  
("Bakteriearter")



# Diversitet og rikhet

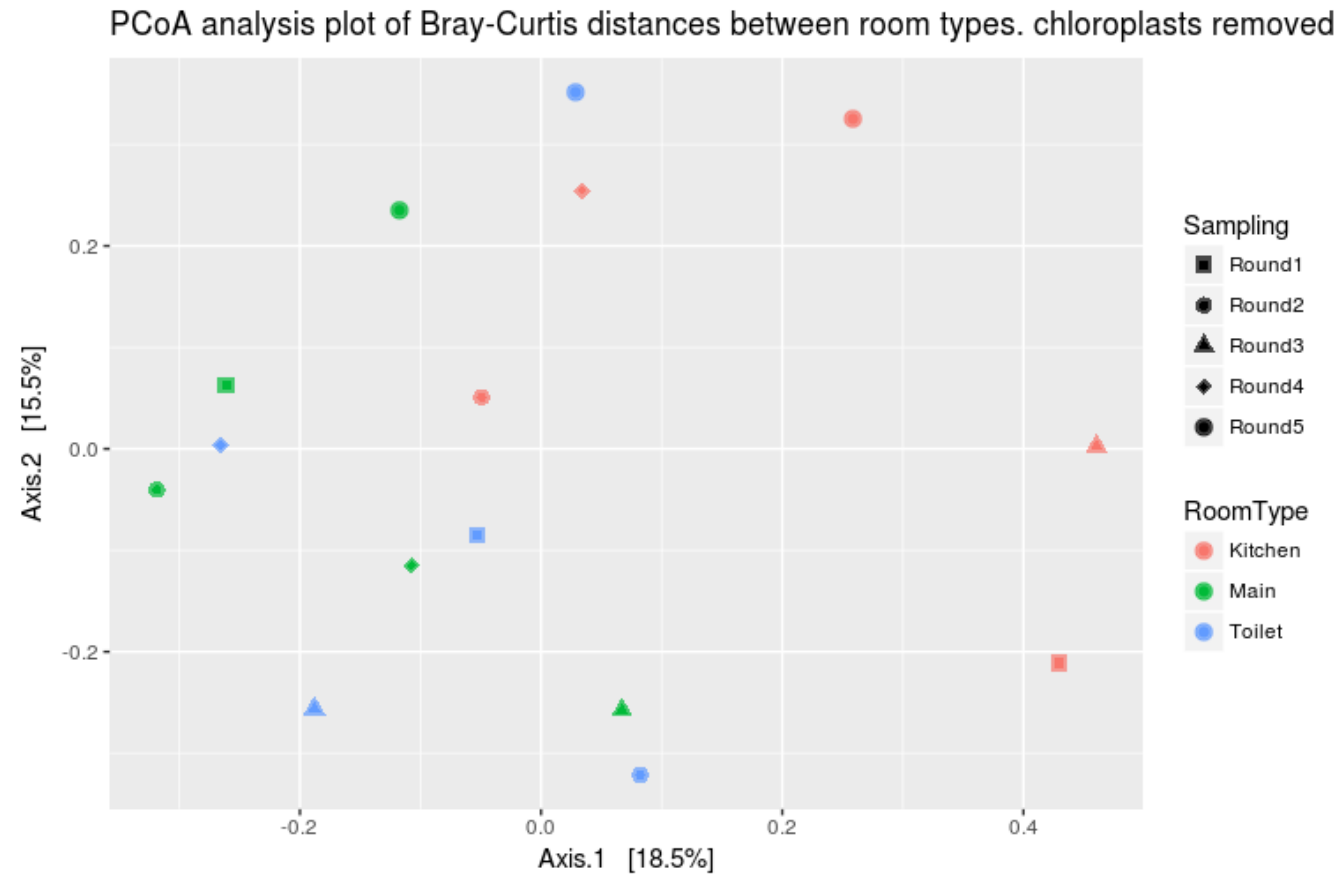
Nursery (N)		Alpha diversity metrics at 19 000 sequences		
Sample	Site	Number of OTUs	CHAO1	Faith's Phylogenetic Diversity
<b>Exhaust</b>	<b>N1</b>	2703	3697	110
		2787	3685	115
		2824	3737	114
	<b>N2</b>	2231	3162	96
		2577	3552	104
	<b>N3</b>	2244	3183	106
<b>N4</b>	2346	3111	111	
<b>N5</b>	2273	3194	110	
<b>N6</b>	1753	2352	88	
<b>Exhaust average</b>		<b>2415</b>	<b>3297</b>	<b>106</b>
<b>Intake</b>	<b>N1</b>	2748	3872	109
	<b>N2</b>	2382	3403	96
	<b>N3</b>	1523	2305	81
	<b>N5</b>	2281	3129	91
	<b>N6</b>	240	456	21
	<b>Intake average</b>		<b>1835</b>	<b>2633</b>

# Bakterielle "Communities" i ventilasjonsfilter



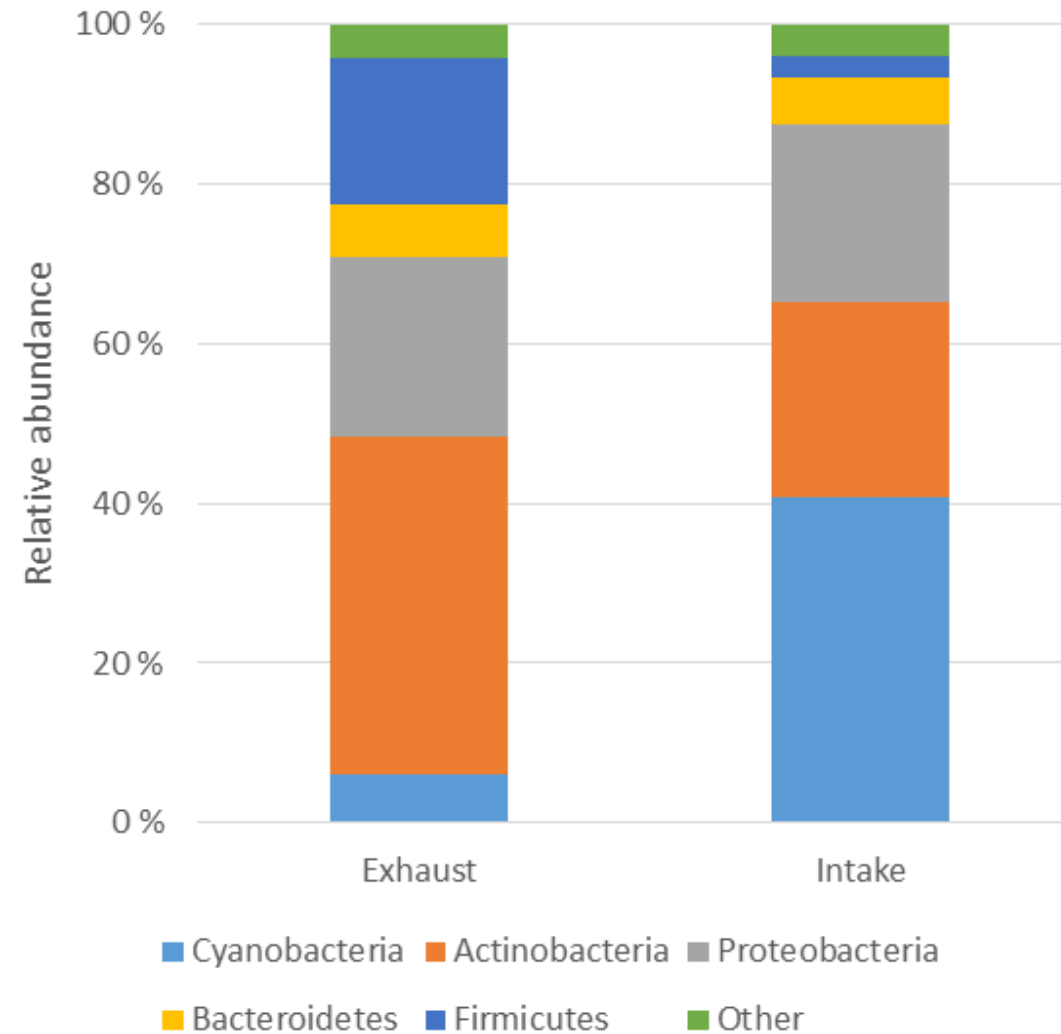


# Bakterielle "Communities" i gulvstøv



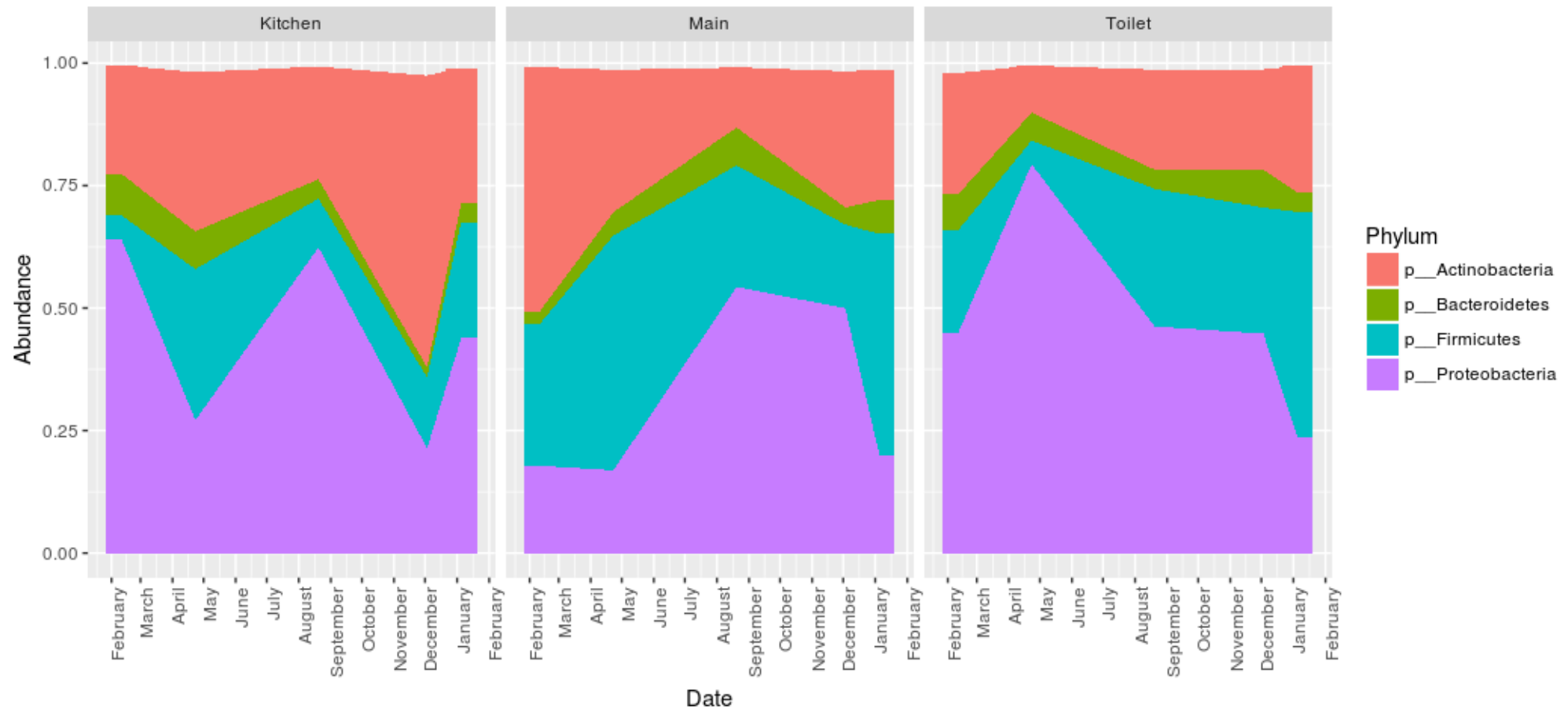
- PERMANOVA: sampling round ( $R^2_{\text{Adonis}} = 0.326$ ,  $P_{\text{Adonis}} = 0.018$ ), room type ( $R^2_{\text{Adonis}} = 0.191$ ,  $P_{\text{Adonis}} = 0.008$ )

# Bakteriesammensetning: Ventilasjonsfilter



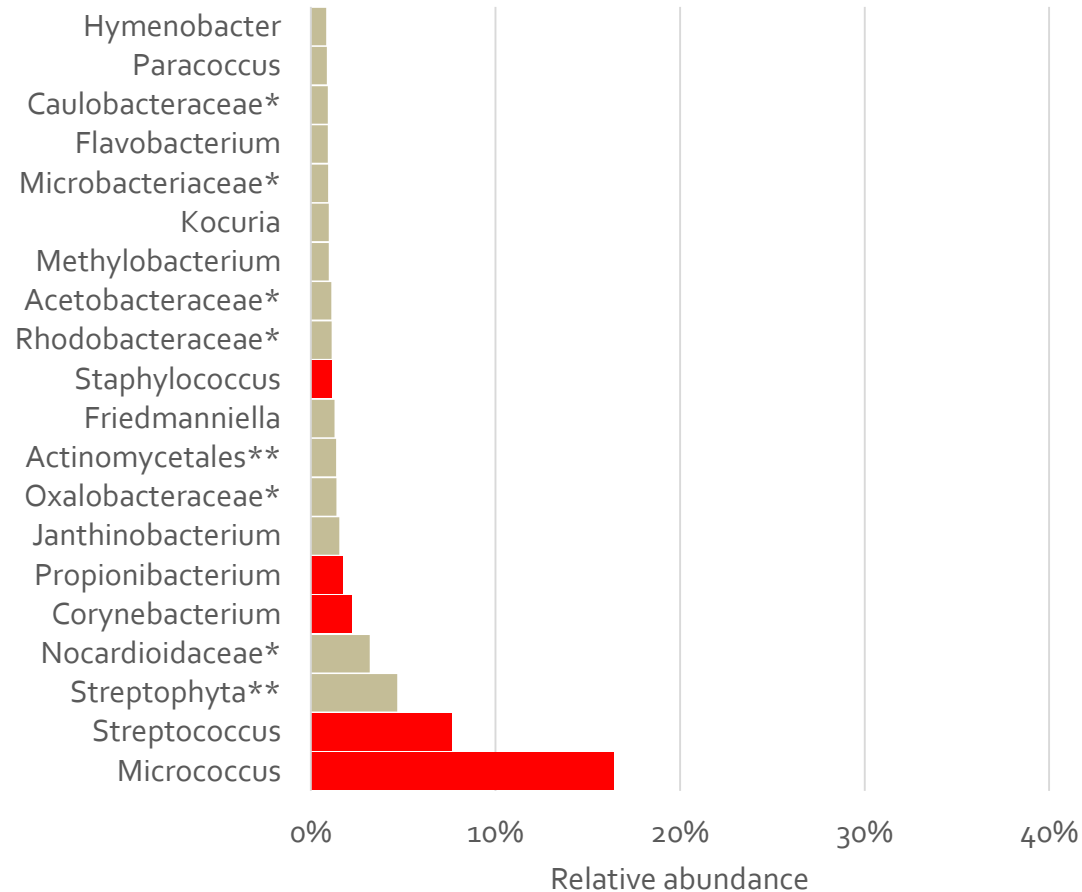
# Bakteriesammensetning: Gulvstøv

Proportion of top 4 phyla without chloroplasts

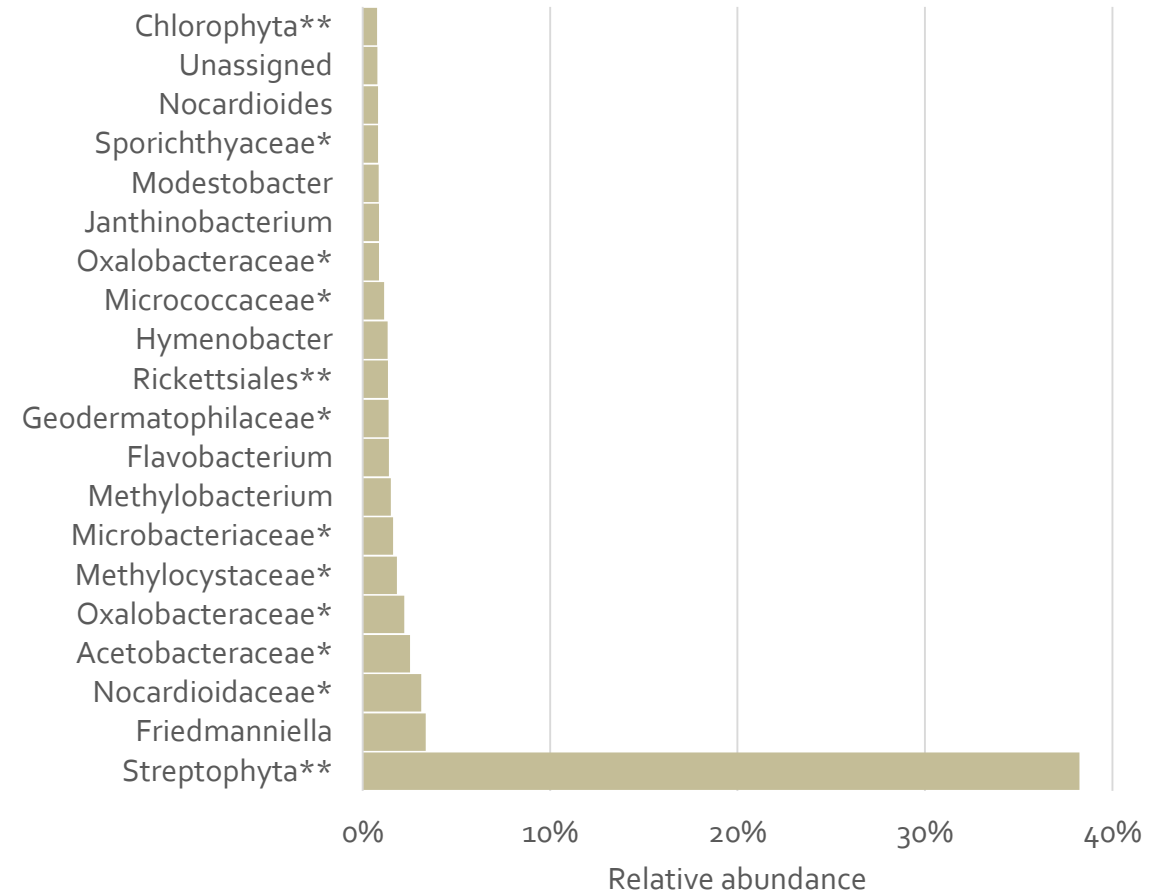


# Topp 20 bakterier i ventilasjonsfilter

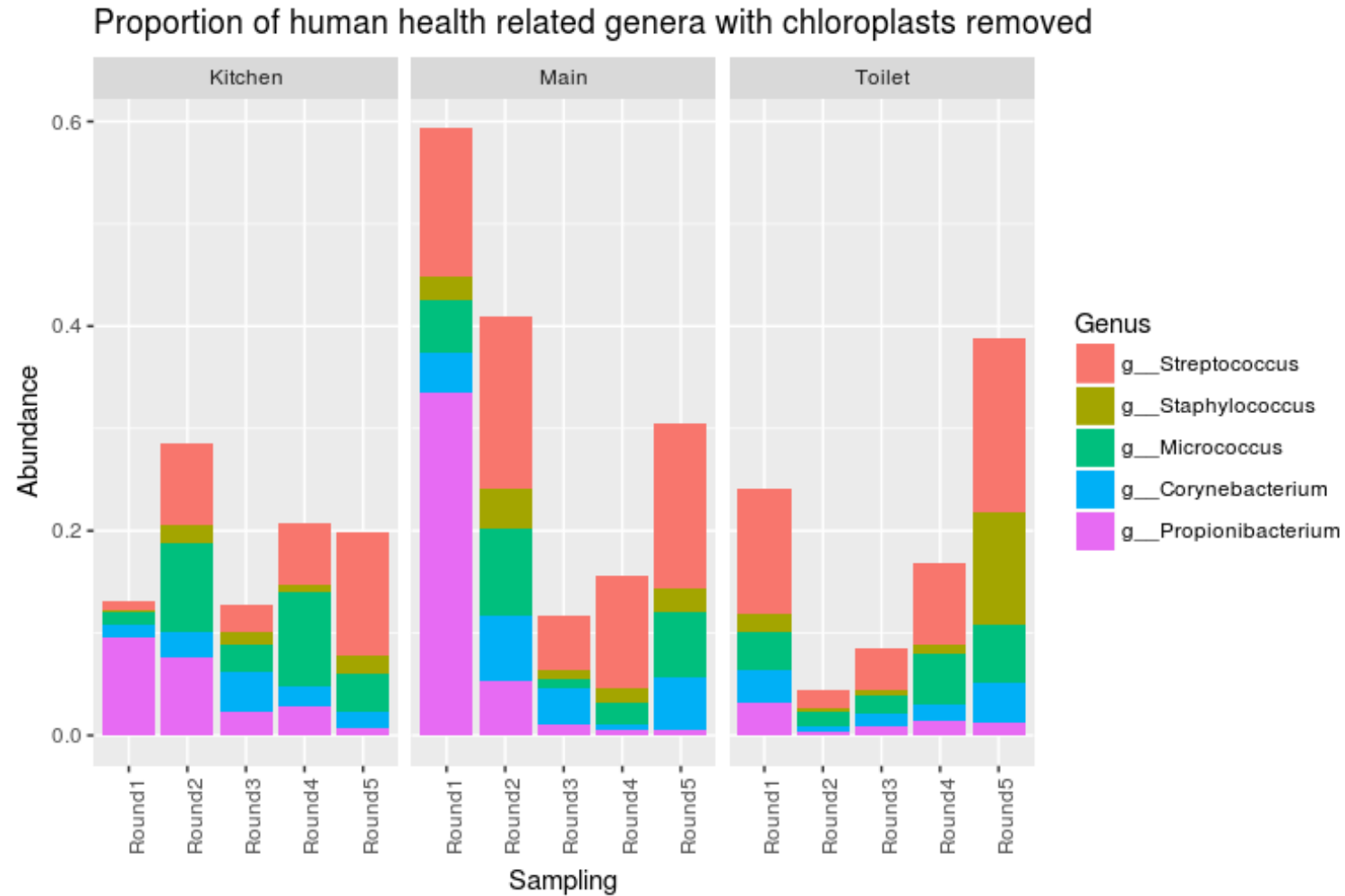
## Exhaust



## Intake



# Menneskeassosierte bakterier i gulvstøv

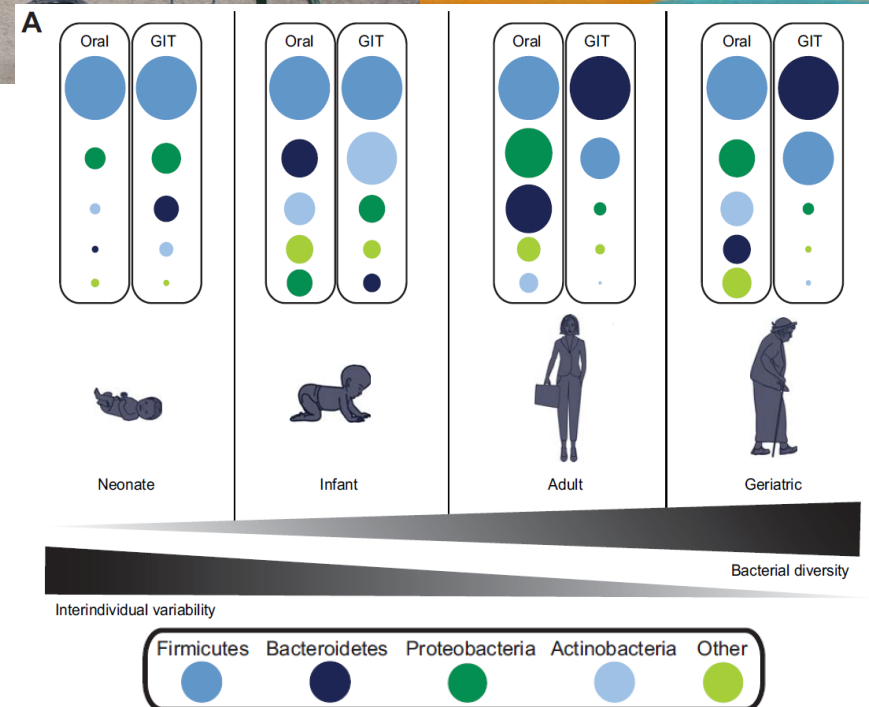
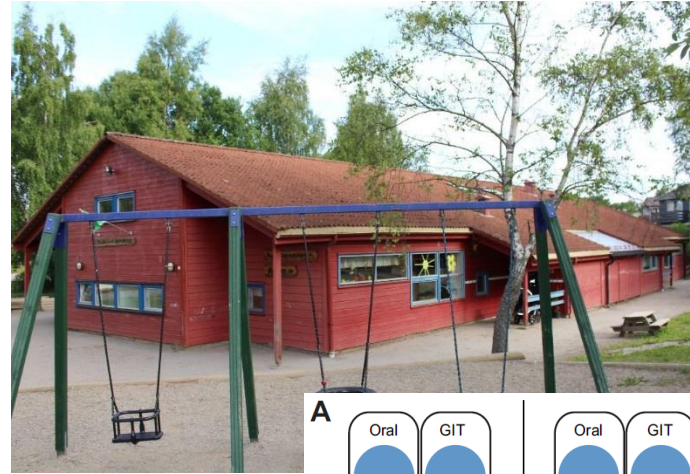


# Antibiotikaresistens

Resistance gene	Antibiotic resistance	Species	Room type (presence or absence of gene)			Detection limit range (pg DNA)
			Main	Toilet	Kitchen	
mecA	Methicillin resistance	<i>Staphylococcus</i>	+	+	+	2181.7 - 218.87 pg
ermA	Macrolide resistance	<i>Staphylococcus</i>	+	+	+	1.206 - 0.121 pg
aac(6')-aph(2'')	Aminoglycoside resistance	<i>Enterococcus</i>	+	+	+	9.35 - 0.935 pg
vanA	Vancomycin resistance	<i>Staphylococcus</i> <i>/Enterococcus</i>	-	-	-	261.1- 26.11 pg

# Hvorfor barnehager?

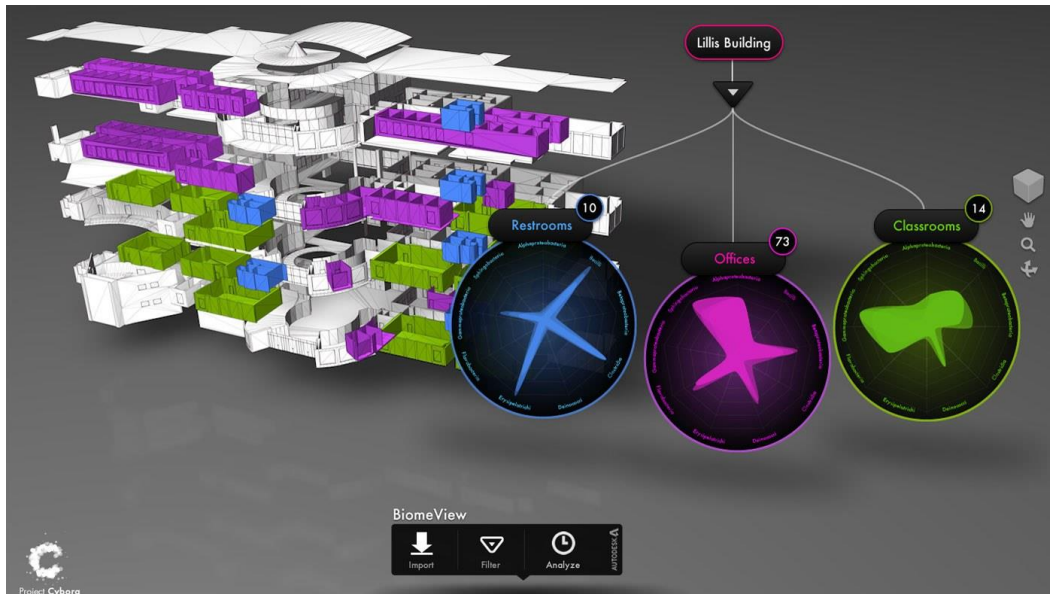
- Barnehager som bygg
- Støv
- Barns helse
  - Bakteriernes rolle i kroppen
  - Miljøets innvirkning på helse



# Muligheter fremover



- Bioteknologi
  - Metagenomikk
  - Metatranskriptomikk
- Minlon
- BIM
  - Mekanistisk tilnærming
  - Simulering av biomer





# Andre muligheter

- Tracking sources

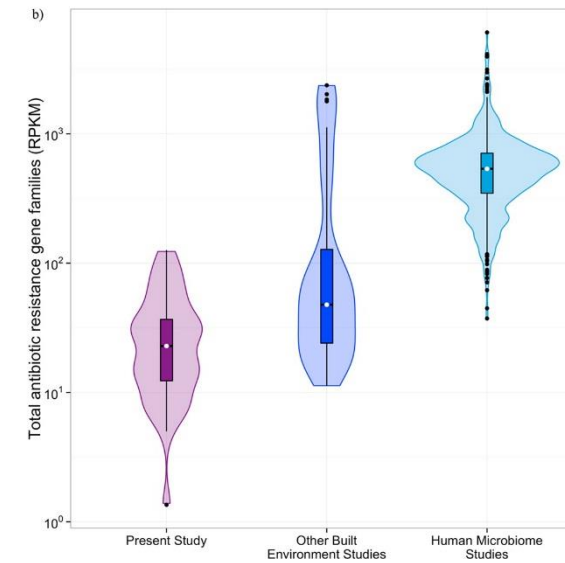
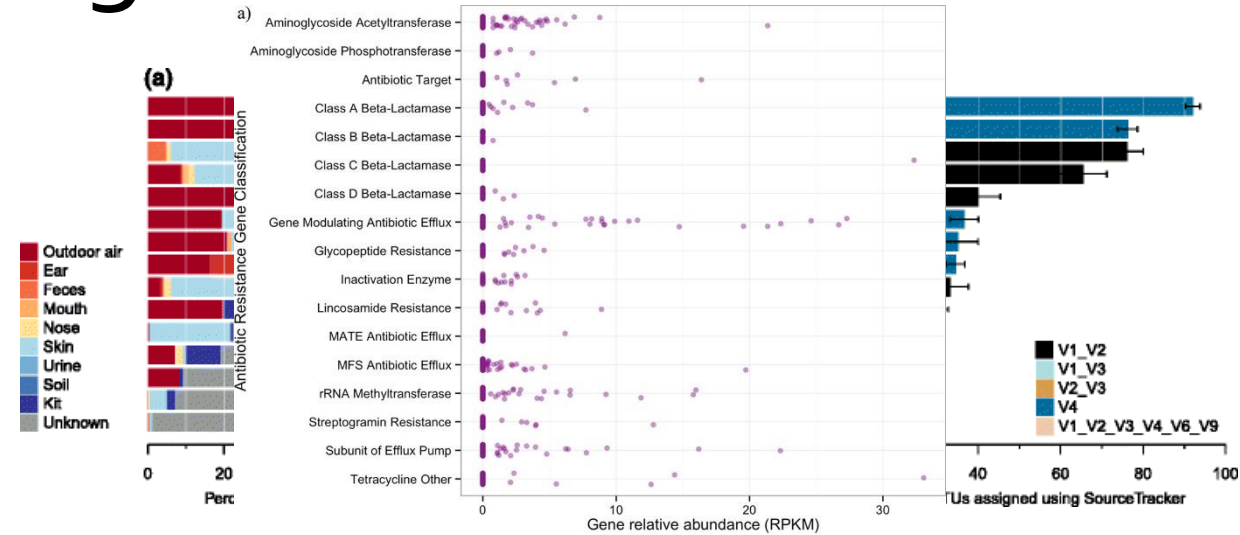
  - Sourcetracker, Knights Lab

    - Adams, R. I., et. al. (2015). "Microbiota of the indoor environment: a meta-analysis." Microbiome 3.

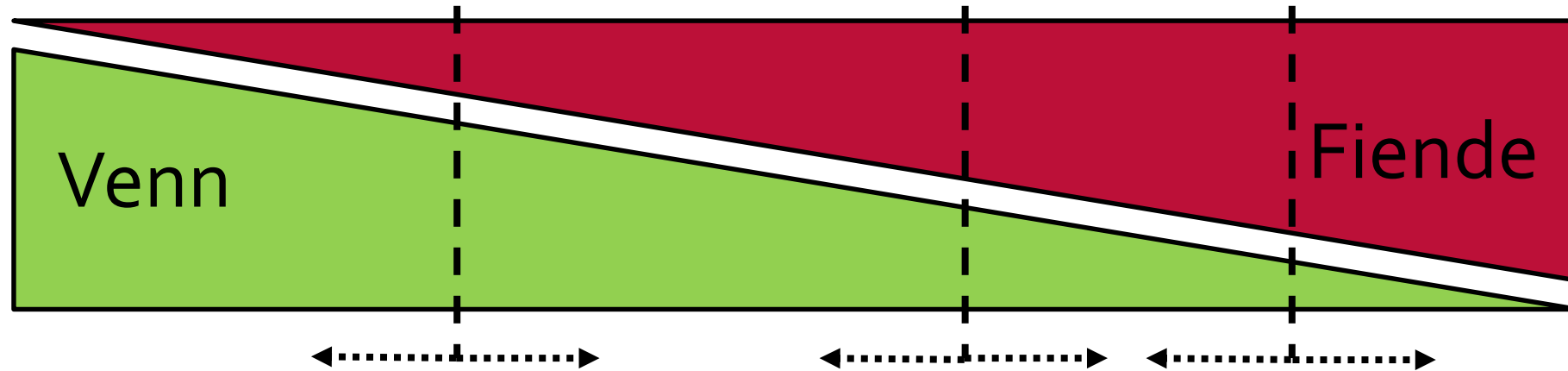
- Antibiotic resistance screening

  - ShortBRED, Huttenhower Lab

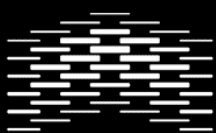
    - Hartmann, E. M., et. al. (2016). "Antimicrobial Chemicals Are Associated with Elevated Antibiotic Resistance Genes in the Indoor Dust Microbiome." Environmental Science & Technology 50(18): 9807-9815.



# Veien fremover



- Paradigmeskifte i mikrobiologi
- Bygg = økosystem
  - (U)naturlig seleksjon
- Energi vs. Helse
- Retningslinjer
- "Helsefremmende" bygg

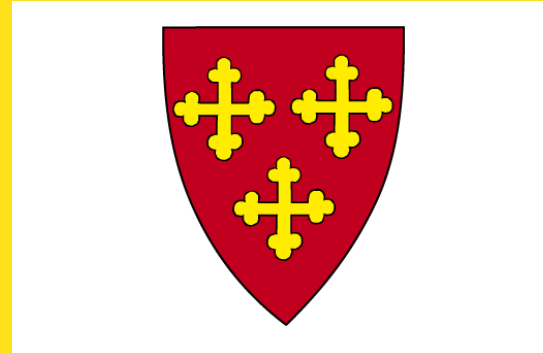


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Takk til:



Takk for oppmerksomheten



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