#### Pit latrine fill; key lessons learnt

JeroenEnsink

- To analyse pit latrine contents for the identification of major targets for accelerated decomposition
- to identify the influence of pit design, pit usage, environmental conditions and location on decomposition rates and pit lifetime

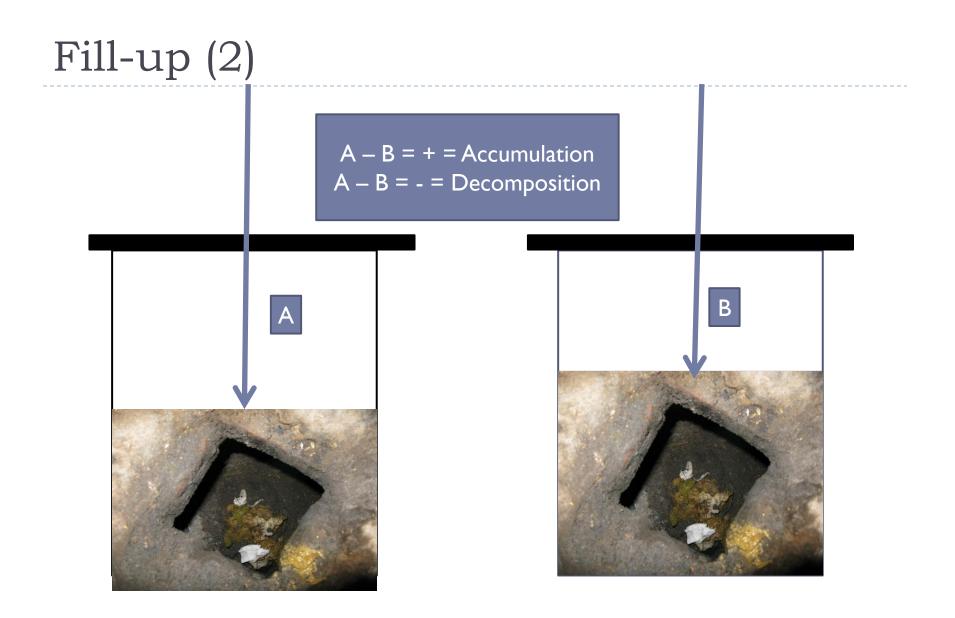
# Summary

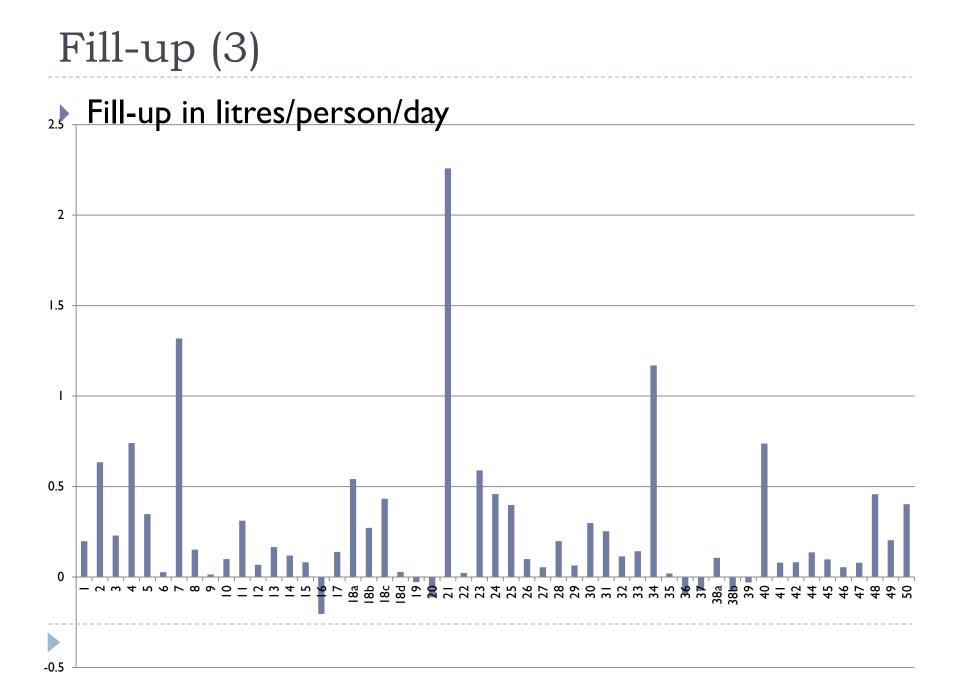
#### Cross-sectional survey (35 latrines)

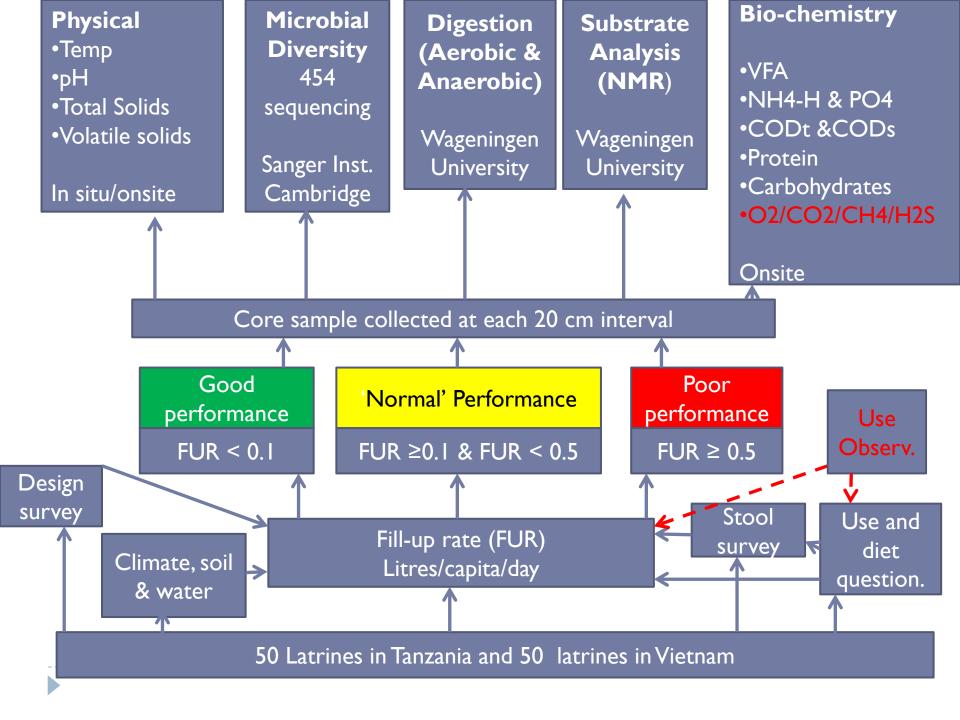
- Design+Management
- Chemical
- Physical
- Biodegradation
- Microbiology
- Longitudinal survey (80 latrines)
  - Design+Management
  - Use/fill-up -> Performance
  - Chemical
  - Physical
  - Biodegradation
  - Microbiology
  - Source material information + Diet
  - Climate
  - Drainage

## Latrine fill-up (1)







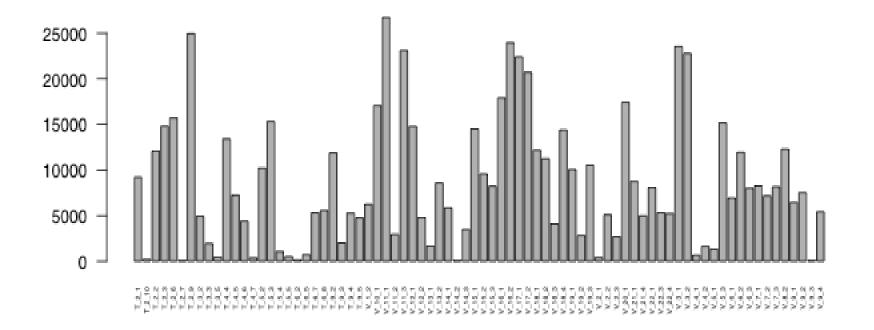


## Microbial analysis (1)

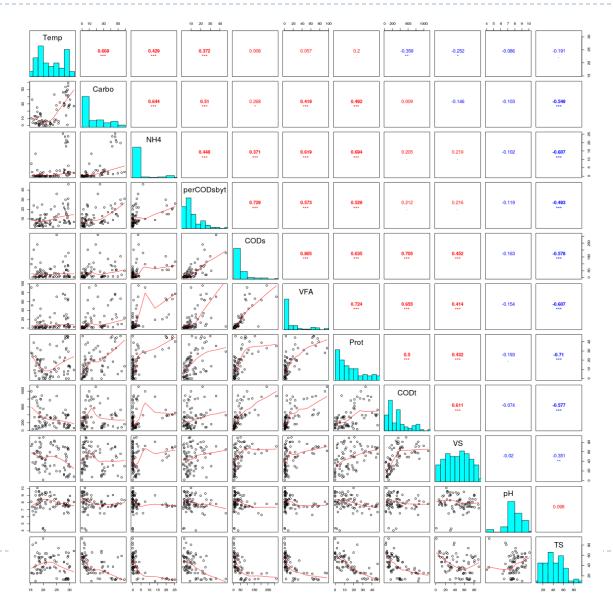
- ▶ 112 samples were sequenced
- Flowgrams were extracted insisting on exact barcode and primer matches
- Run through AmpliconNoise-Perseus pipeline
- Only 88 remained other 24 had less than 50 reads
- Assigned reads using RDP stand alone classifier
- Constructed OTUs using exact pairwise comparisons

## Microbial analysis (2)

 Substantial variation in read number (min = 53, lq = 2680, median = 6818, upper = 11980, max = 26730)

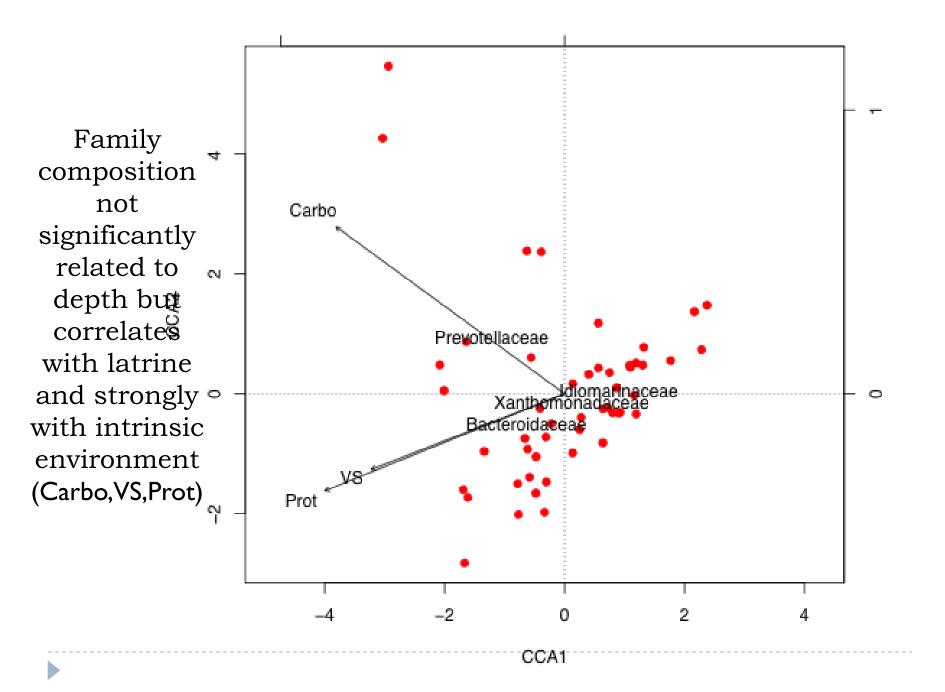


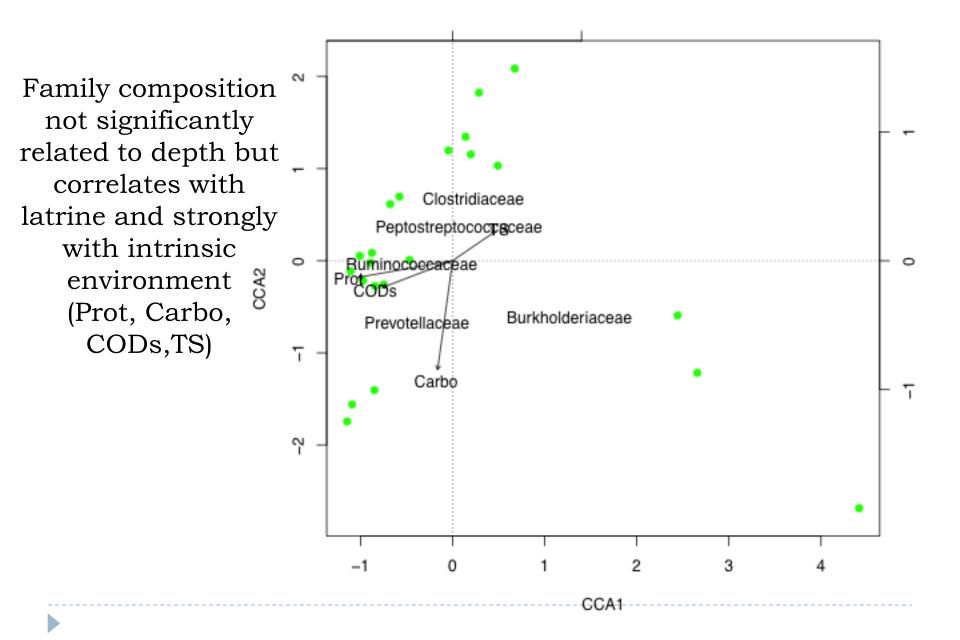
## Microbial analysis (3)



## Microbial analysis (4)

	Phylum	Both	Tanzania	Vietnam	Diff	C. Diff
1	Firmicutes	39.63	37.3-50.09-67.2	29.3-35.30-42.6	14.8	28.3
2	Proteobacteria	13.1	4.3-6.36-9.3	15.1-18.56-22.8	12.2	51.7
3	Bacteroidetes	22.17	12.8-17.88-24.9	18.8-22.96-28.0	5.1	61.4
4	Synergistetes	1.02	2.9-4.27-6.4	0.2-0.37-0.6	3.9	68.9
5	Unknown	4.52	4.5-6.58-9.6	2.5-3.28-4.3	3.3	75.2
6	Actinobacteria	6.41	2.8-4.18-6.2	5.7-7.17-9.1	3	80.9
7	Deinococcus-Thermus	2.36	0.5-0.93-1.7	2.3-3.01-3.9	2.1	84.9
8	Spirochaetes	1.26	1.8-2.71-4.1	0.5-0.69-1.0	2	88.8
9	Verrucomicrobia	1.08	0.0-0.14-0.5	0.9-1.27-1.7	1.1	90.9
10	Tenericutes	2.12	0.9-1.43-2.3	1.5-2.03-2.7	0.8	92.4
11	Fibrobacteres	0.31	0.5-0.82-1.5	0.0-0.09-0.2	0.7	93.8
12	Chloroflexi	1.96	1.2-1.87-3.0	1.2-1.59-2.1	0.5	94.7
13	TM7	0.43	0.0-0.07-0.5	0.3-0.45-0.7	0.4	95.4
14	Planctomycetes	0.71	0.1-0.34-0.8	0.5-0.72-1.1	0.4	96.2
15	Fusobacteria	0.68	0.1-0.34-0.8	0.4-0.65-1.0	0.4	96.9
16	Acidobacteria	0.72	0.5-0.89-1.6	0.3-0.52-0.8	0.4	97.6
17	Lentisphaerae	0.41	0.0-0.14-0.5	0.2-0.40-0.6	0.3	98.1
18	Euryarchaeota	0.06	0.1-0.20-0.6	0.0-0.00-0.0	0.2	98.5
19	Nitrospira	0.16	0.0-0.00-0.0	0.1-0.17-0.3	0.2	98.8
20	Thermotogae	0.04	0.0-0.14-0.5	0.0-0.00-0.0	0.1	99.1
21	OD1	0.1	0.0-0.00-0.0	0.0-0.11-0.3	0.1	99.3
22	Cyanobacteria	0.24	0.0-0.14-0.6	0.1-0.24-0.4	0.1	99.5
23	Gemmatimonadetes	0.24	0.1-0.20-0.6	0.1-0.22-0.4	0.1	99.6
24	BRC1	0.12	0.0-0.14-0.6	0.0-0.09-0.2	0.1	99.7
25	OP10	0.04	0.0-0.07-0.5	0.0-0.02-0.2	0	99.8
26	WS3	0.04	0.0-0.00-0.0	0.0-0.04-0.2	0	99.9
27	Chrysiogenetes	0.06	0.0-0.07-0.5	0.0-0.04-0.2	0	100
28	Chlamydiae	0.02	0.0-0.00-0.0	0.0-0.02-0.2	0	100
29	Acidobacteria	0.72	0.5-0.89-1.6	0.3-0.52-0.8	0	100

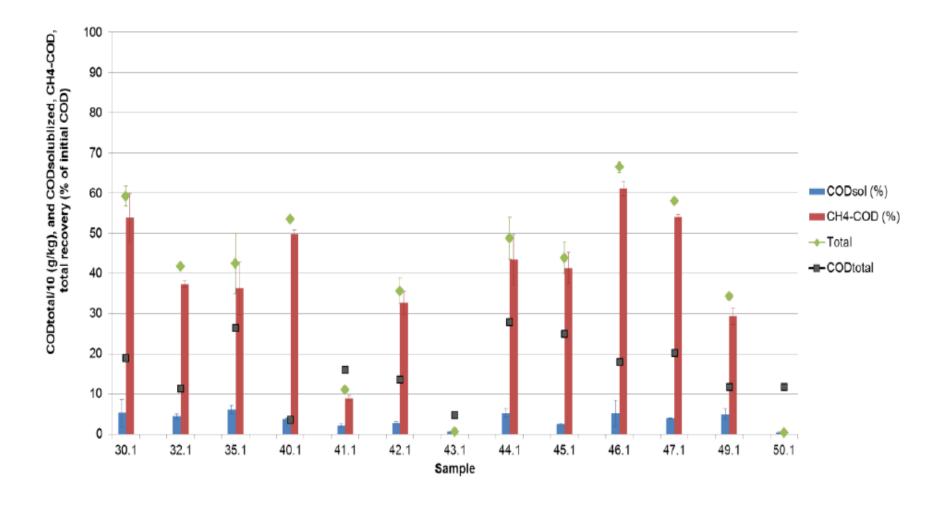




## Discussion

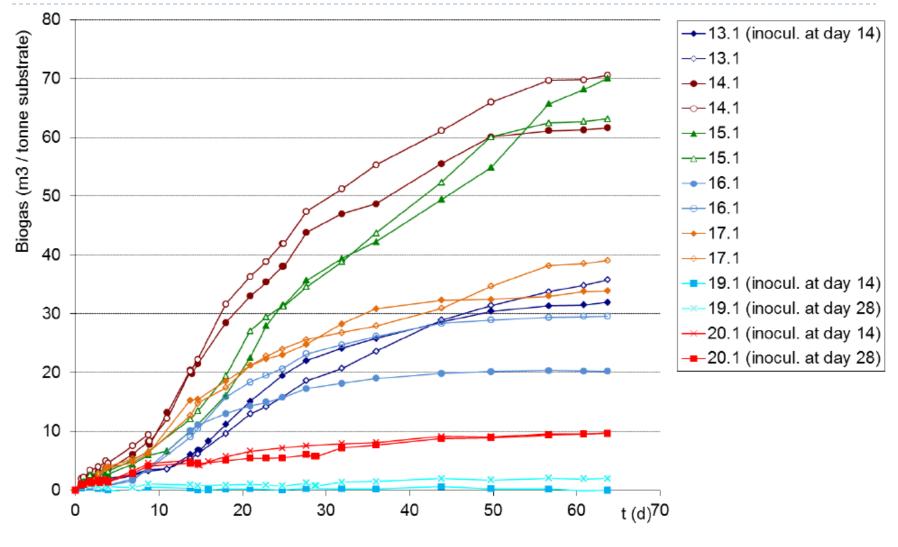
- Environment is strongly determining microbiota
- Distinct microbiota Vietnam vs Tanzania
  - human associated enriched Tanzania vs more marine halophiles Vietnam
- Family level richness positively correlated with TS in Vietnam
- Tanzania and Vietnam
  - particular groups (possibly enriched for human associated) correlated with decomposition substrates (Carbo/Prot) and environmental organisms associated with TS
- To do:
  - Add new cross-sectional sequences
  - Correlate biodegradation results with composition
  - Longitudinal study fill rate with composition

### Biodegradation (1) remaining fraction



#### Biodegradation (2) – Latrine types

D



## Fill-up modelling

#### Milestone 19 31/8/2012

- Field hydrology and column tests in Ifakara
- Using degradation and fill-up data

#### Key findings

- Pit dimensions and number of users are key determinants of fill-up
- Pit drainage main limiting factor
  - Pore clogging?
- pit fill rate is not sensitive to the breakdown rate but to the products into which it breaks down

## Assumptions of rate limiting steps

- There is a fraction of faecal matter which is difficult to degrade
  - Through characterising this material we will be able to identify whether specific organisms, or enzymes exist which could break it down and be added to the latrine
- Environmental conditions are unsuitable to support the microbial communities needed for breakdown
  - to alter them either through physical action (eg aeration, mixing, or adding water), or design (adding a roof, lining, improving drainage) or chemical addition (eg for pH control) or additives which provide a suitable "niche" for the right bacteria
- There is a lack of a key microbial family needed for biodegradation
  - /See first) + seeding of latrines with feacal material of successful pits?

## Outputs (1)

	Title	Type of data	Lead author	Proposed journal						
High	High Priority papers									
1	Pit latrine decomposition characteristics	Literature review study	Belen Torondel	Crit Rev Env Scie Techn						
2	Microbial biodiversity and environment of pit latrines	Microbial, biochemistry and physical data from cross-sectional study	Belen Torondel	ISME						
3	Latrine characteristics	Biochemistry, physical and use data (Tanzania and Vietnam)	Jeroen Ensink	Env Sci Tech						
4	Assotiation of microbial diversity and latrines filling rate	Microbial, biochemistry, physical and fill-up data from longitudinal study	Belen Torondel	ISME						
5	Meausring performance of pit latrines	Fill-up data of pit latrines, presenting data on good and poor performance +fresh stool data	Jeroen Ensink	Env SciTech						
6	Modeling pit latrines	Results of hydrological testing and modelling	Lindsay Todman	Water Research						
7	Biodegradation of pit latrines	Results of tests conducted by LeAF and Wageningen University	Miriam van Eekert	Water Research						
8	Assotiation of methanogens diversity and latrines filling rate	Methanogens data combined with all other data from longitudinal study	Ozan Gundogdo							
9	Extending the life of the pitlatrine	Summary paper, bringing all results together	Walter Gibson	Nature/Science						
	Lower Priority Papers									
10	Bacteria and methanogens present in latrines and their association with diet	Cross-sectional and longitudinal data	Ozan Gundogdu							
11	Importance of pit latrines as source of fly breeding	student project	Kristen Knudson	Vect & Para						
12	Is simple enough? Evaluating pit latrines	student project	Sarah Baker	Trans Royal Soc Trop Med						
13	Sampling pit latrines	Practitions paper highlighting sampling and findings	Jeroen Ensink	Waterlines						

## Outputs (2)

G Model TRSTMH-1665; No. of Pages 2

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Short Communication

#### Helminth transmission in simple pit latrines

Sarah M. Baker, Jeroen H.J. Ensink\*