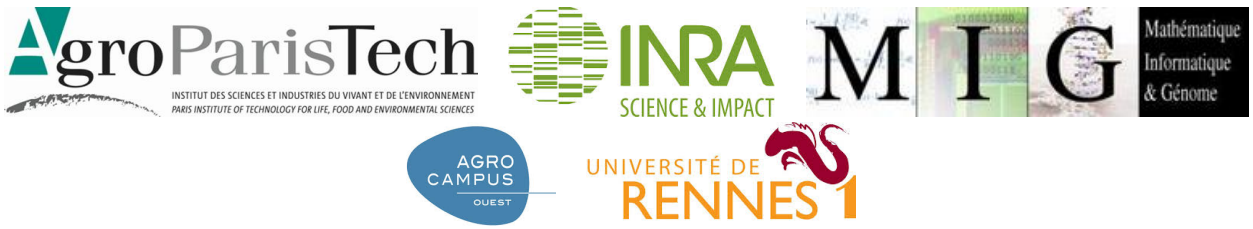
Metagenomics data analysis using a latent block model: application to plant-microbial communities interactions in the rhizosphere .

Julie Aubert, Sophie Schbath, Béatrice Laroche

Metagenomic data analysis using a LBM

Application to plant-microbial communities interactions in the rhizosphere

J. Aubert, C. Mougel, S. Schbath, S. Robin
julie.aubert@agroparistech.fr



Outline

Metagenomic data

Latent Block Model
- Model and inference
- Variational inference
- Poisson and Zero-Inflated Poisson Latent Block Model

Application to plant-microbial communities interactions in the rhizosphere

Conclusions and perspectives

Motivating example

Metagenomics : Study of genetic material recovered directly from environmental samples

Some applications

- Sequencing of the genomes of all the life on earth
- Monitoring the impact of pollutants on ecosystems
- Understanding the correlation between human health and changes in the human microbiome

One possible aim

Study the relationships between bacteria communities and environmental samples
Idea : **Simultaneous clustering** of the bacteria and samples

Metagenomic data

	S1	S2	S3	...	Sj	...	Sd
Bact. 1	0	0	0	...	y_{1j}	...	3
Bact. 2	1	15	0	...	y_{2j}	...	0
Bact. 3	59	17	43	...	y_{3j}	...	3
...	...	...	...	...	...	...	...
Bact. i	y_{i1}	y_{i2}	y_{i3}	...	y_{ij}	...	y_{id}
...	...	...	...	...	...	...	...
Bact. n	90	120	123	...	y_{nj}	...	95
Seq. depth	4738	5157	6010	...	$\sum_{i=1}^{n} y_{ij}$	...	5916

y_{ij} = number of sequences from sample j assigned to bacteria i.

Data characteristics

Count data with **excess of zeros** dependent from the sequencing effort

Latent Block Model (LBM) (Govaert et Nadif. 2003)

Assumptions

Identical distribution resp. for all rows and columns :
$(Z_i) \sim \mathcal{M}(1, \pi = (\pi_1, \ldots, \pi_g))$ $(W_j) \sim \mathcal{M}(1, \rho = (\rho_1, \ldots, \rho_m))$
Latent variables (Z_i) and (W_j) are independent
Latent class assumption : the random variables Y_{ij} are independent conditionally on labels

Model

$(Y_{ij}), i = 1, \ldots, n$ et $j = 1, \ldots, d$ matrix of observations.

$$Y_{ij}|(Z_{ik} = 1, W_{j\ell} = 1) \sim f(.; \alpha_{k\ell})$$

$\alpha = (\alpha_{k\ell})$ parameters for the distribution f.
$\theta = (\alpha, \pi, \rho)$ vector of parameters.

Inference - ML approach

Based on the observed data $Y = (Y_{ij})$, we want to infer :

- the parameters $\theta = (\alpha, \pi, \rho)$
- the hidden status $Z = (Z_i)$ and $W = (W_j)$

Direct approach

log-Likelihood : $logP(Y;\theta) = log \sum_{Z,W} P(Y, Z, W; \theta)$

Can not be computed : $g^n * m^d$ *terms*

Ex : $g = 2, m = 2, n = 100, m = 10 \rightarrow 1.29e^{33}$ *terms to calculate.*

E-M algorithm (Dempster et al. 1977)

Aims at maximizing the log-likelihood

$$logP(Y;\theta)$$

through the alternation of two steps

EM-trick

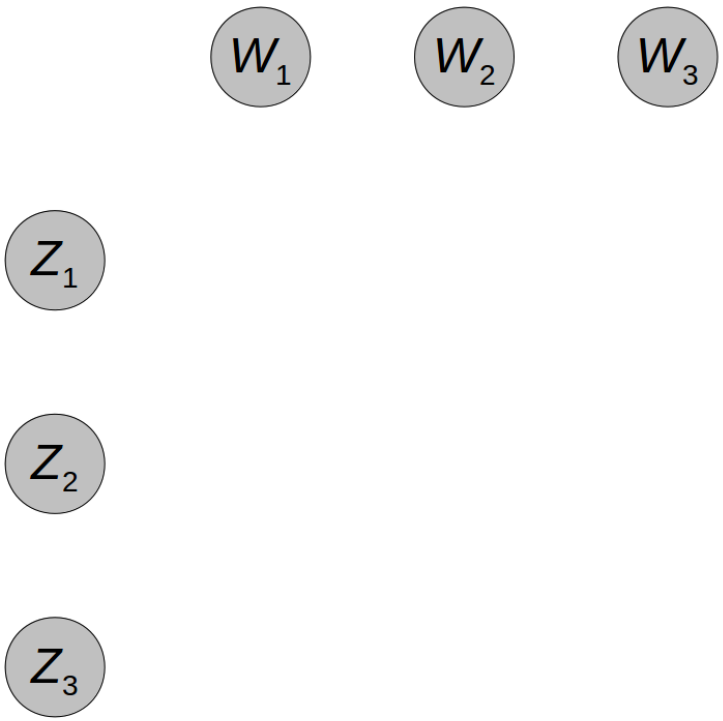
$$logP(Y;\theta) = logP(Y,H;\theta) - logP(H|Y;\theta)$$

$$logP(Y;\theta) = \mathbb{E}[logP(Y,H;\theta)|Y] + \mathcal{H}[P(H|Y;\theta)]$$

where $\mathcal{H}$ stands for the entropy and $H = (Z, W)$

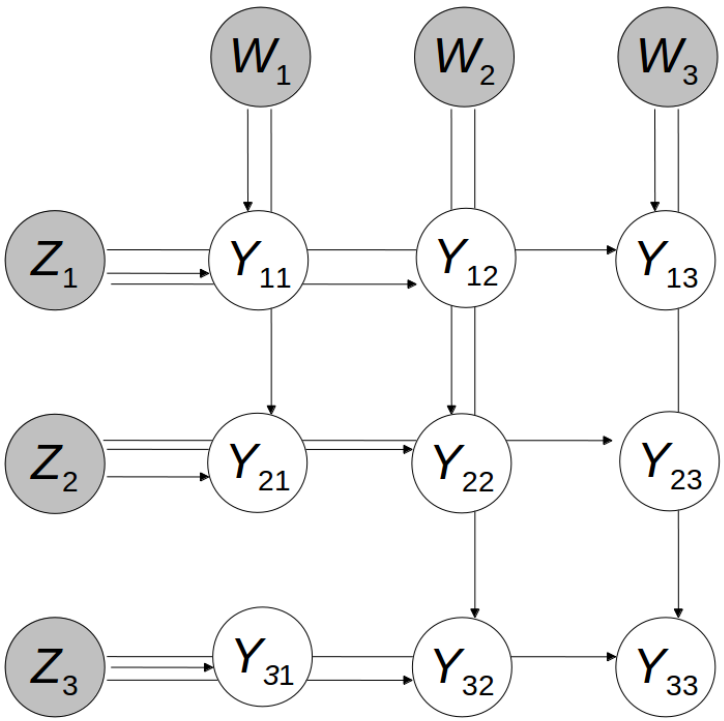
1. Expectation-step :
   calculate $P(H|Y) \rightarrow$ sometimes impossible
2. Maximization-step :
   maximize $\mathbb{E}[logP(Y,H;\theta)|Y]$ with respect to θ
   $\rightarrow$ generally similar to standard MLE.

Graphical representation



- Z_i and W_j unobserved labels of row i and column j

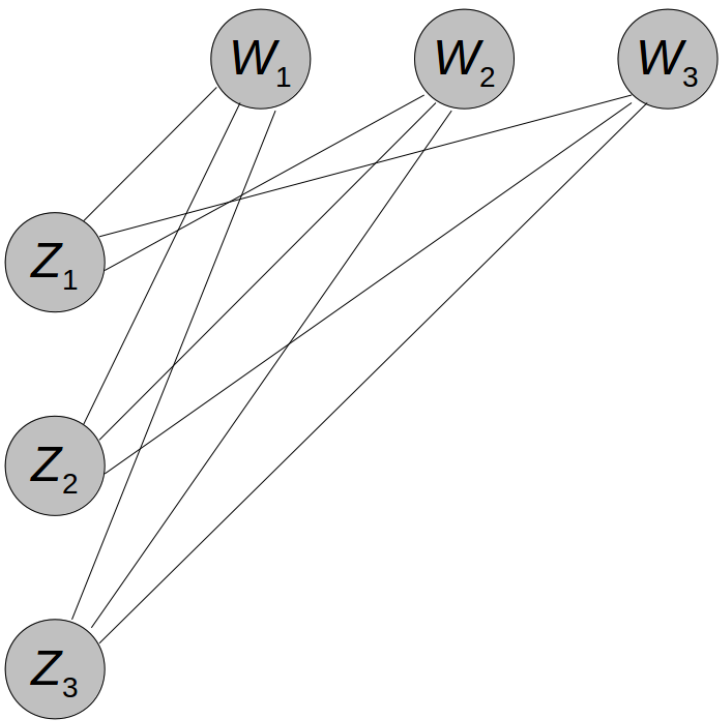
Graphical representation



- Z_i and W_j unobserved labels of row i and column j
- Y_{ij} depends on the labels.

Graphical representation

Conditional distribution of $H = (Z, W)$



- Z_i and W_j unobserved labels of row i and column j
- Y_{ij} depends on the labels.
- Z_i and W_j are not independent conditionally on Y_{ij}
  $\rightarrow P(H|Y)$ intractable
  Regular EM algorithm cannot be used

Variational approximation - VEM (Wainwright and Jordan 2008)

Notations : $H = (Z, W)$

Lower bound of the log-likelihood

For any distribution Q(H) :

$$logP(Y) \geq logP(Y) - KL[Q(H), P(H|Y)]$$

$$logP(Y) \geq \mathbb{E}_Q[logP(Y, H)] + \mathcal{H}(Q(H))$$

Link with EM :

$$logP(Y) = \mathbb{E}[logP(Y, H)] + \mathcal{H}(P(H|Y))$$

replacing $P(H|Y)$ with $Q(H)$

Variational EM algorithm

Variational E-step : replace the calculation of $P(H|Y)$ with the search of

$$Q^*(H) = \underset{Q \in \mathcal{Q}}{argmin} KL[Q(H), P(H|Y)]$$

M-step : compute

$$\hat{\theta} = \underset{\theta}{argmax} \mathbb{E}_{Q^*}[logP(Y, H; \theta)]$$

Mean-field approximation

$\mathcal{Q}$ = set of factorisable distr. with independence of the labels :

$$\mathcal{Q} = Q : Q(H) = \prod_{i=1}^{n} Q_i(Z_i) \prod_{j=1}^{d} Q_j(W_j),$$

with $\prod_i Q_i(Z_i) = \prod_i \prod_k s_{ik}^{Z_{ik}}, \prod_j Q_j(W_j) = \prod_j \prod_l t_{j\ell}^{W_{j\ell}}$,
where $s_{ik} = \mathbb{E}_Q(Z_{ik})$, $t_{j\ell} = \mathbb{E}_Q(W_{j\ell})$, $s_{ik}\, t_{j\ell} = \mathbb{E}_Q(Z_{ik} W_{j\ell})$

Generalized Variational EM (Govaert et Nadif 2010)

Plug-in of s_{ik}, $t_{j\ell}$ in place of Z_{ik}, $W_{j\ell}$, $s_{ik}t_{j\ell}$ in place of $Z_{ik}W_{j\ell}$.

$$F(s,t,\theta) = \mathbb{E}_Q log P(Y,Z,W;\theta) + \mathcal{H}(s) + \mathcal{H}(t)$$

$$(*) = \sum_{i,k} s_{ik} log \pi_k + \sum_{j,\ell} t_{j\ell} log \rho_\ell + \sum_{i,j,k,\ell} s_{ik}t_{j\ell} log(f(Y_{ij};\alpha_{k\ell})$$

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4. Iterate steps 2 and 3 until convergence.

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Prop : the lower bound increases after each iteration.

Poisson and Zero-Inflated Poisson Latent Block Model

Poisson LBM $Y_{ij} \in \mathbb{N},\ Y_{ij}|k,\ell \sim \mathcal{P}(\mu_i \nu_j \alpha_{k\ell})$

- μ_i : mean level of presence of one particular bacteria
- ν_j : scale factor correcting for sequencing depth (around 1)
- $\alpha_{k\ell}$: interaction level between bacteria and environmental samples within the (k, ℓ) group

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Zero-inflated Poisson LBM $Y_{ij} \in \mathbb{N},\ Y_{ij}|k,\ell \sim ZIP(.; \alpha_{k\ell})$

$$Y_{ij}|(Z_{ik} = 1, W_{j\ell} = 1) \sim ZIP(.; \alpha_{k\ell})$$

$$\begin{cases} P(Y_{ij}|(Z_{ik} = 1, W_{j\ell} = 1) = 0) = \delta_{k\ell} + (1 - \delta_{k\ell}) e^{-\alpha_{k\ell}} \\ P(Y_{ij}|(Z_{ik} = 1, W_{j\ell} = 1) = y_{ij}) = (1 - \delta_{k\ell}) \frac{\alpha_{k\ell}^{y_{ij}} e^{-\alpha_{k\ell}}}{y_{ij}!} \text{ for } y_{ij} \geq 1 \end{cases}$$

Zero-Inflated Latent Block Model - Inference

Similar to Poisson Latent Block Model.
Part of the M-step :

$$\widehat{\delta_{k\ell}} = \frac{\bar{y_{k\ell}}}{\sum_{i,j} s_{ik} t_{j\ell} \widehat{\alpha_{k\ell}}}$$

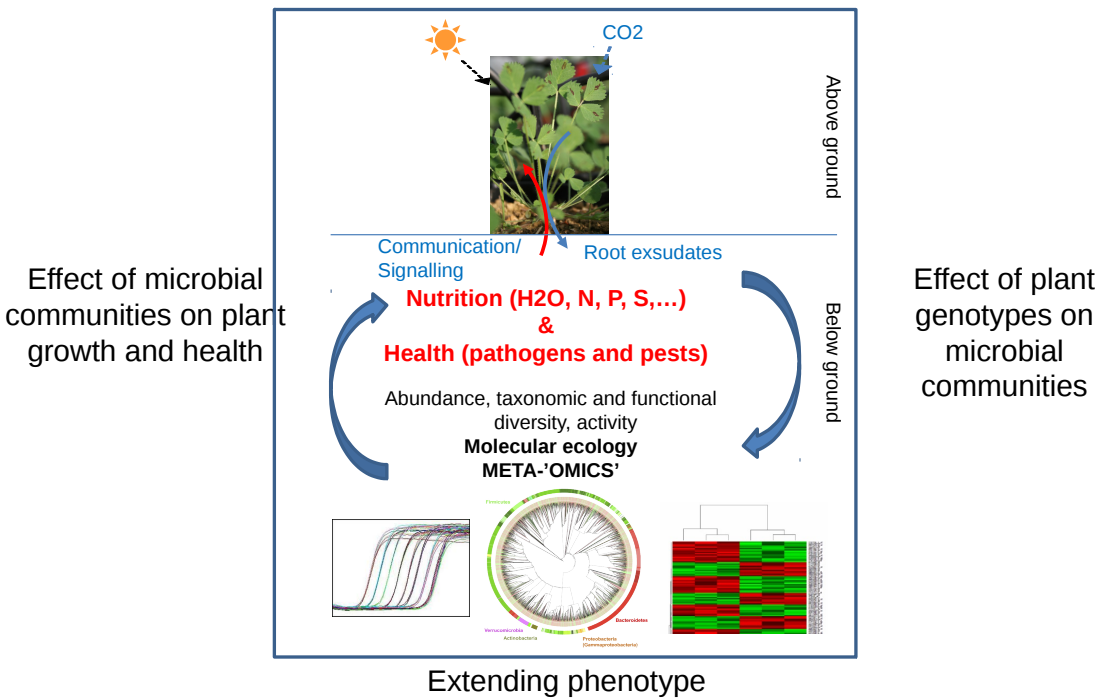
where $\bar{y_{k\ell}} = \frac{\sum_{i,j} s_{ik} t_{j\ell} y_{i,j}}{\sum_{i,j} s_{ik} t_{j\ell}}$

A fixed-point equation to solve :

$$\widehat{\alpha_{k\ell}} = \frac{\bar{y_{k\ell}}(1 - e^{1-\widehat{\alpha_{k\ell}}})}{1 - n_0^{k\ell}/n^{k\ell}} = G(\widehat{\alpha_{k\ell}})$$

Application - MétaRhizo project (C. Mougel)

Hyp : Plant genotype modifies the structure of the bacteria community in the rhizosphere (the region of soil directly influenced by root secretions and associated soil microorganisms).

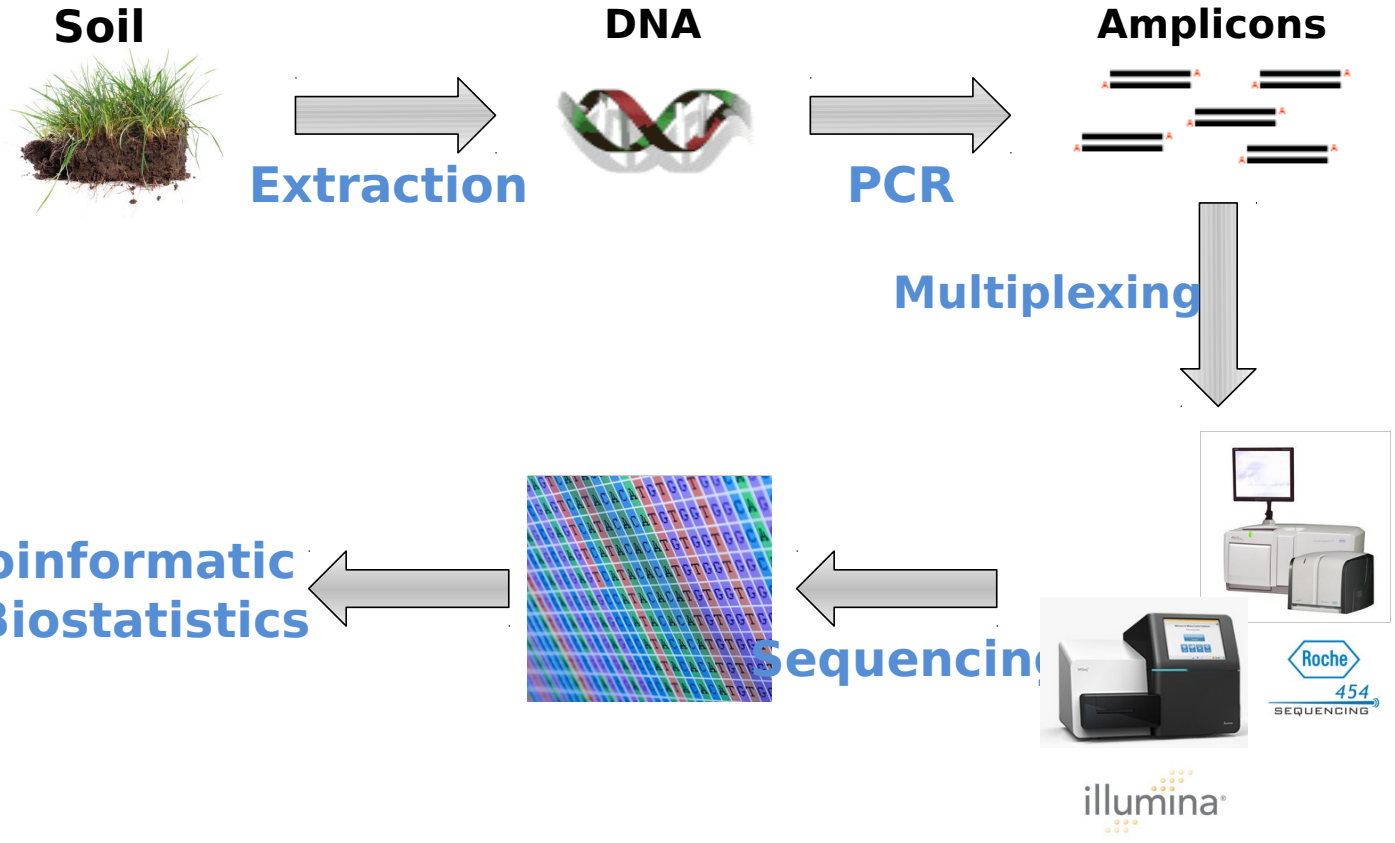


Medicago truncatula

Small annual legume : model organism in genomic research.
Forms symbioses with nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi.
$\rightarrow$ **Reduction of nitrogen inputs** responsible for various pollution (substainable agriculture)

Sampling strategy of the M. truncatula core collection, Ronfort et al. (2006)

Standard operating procedures from soil collection to mathematical analysis



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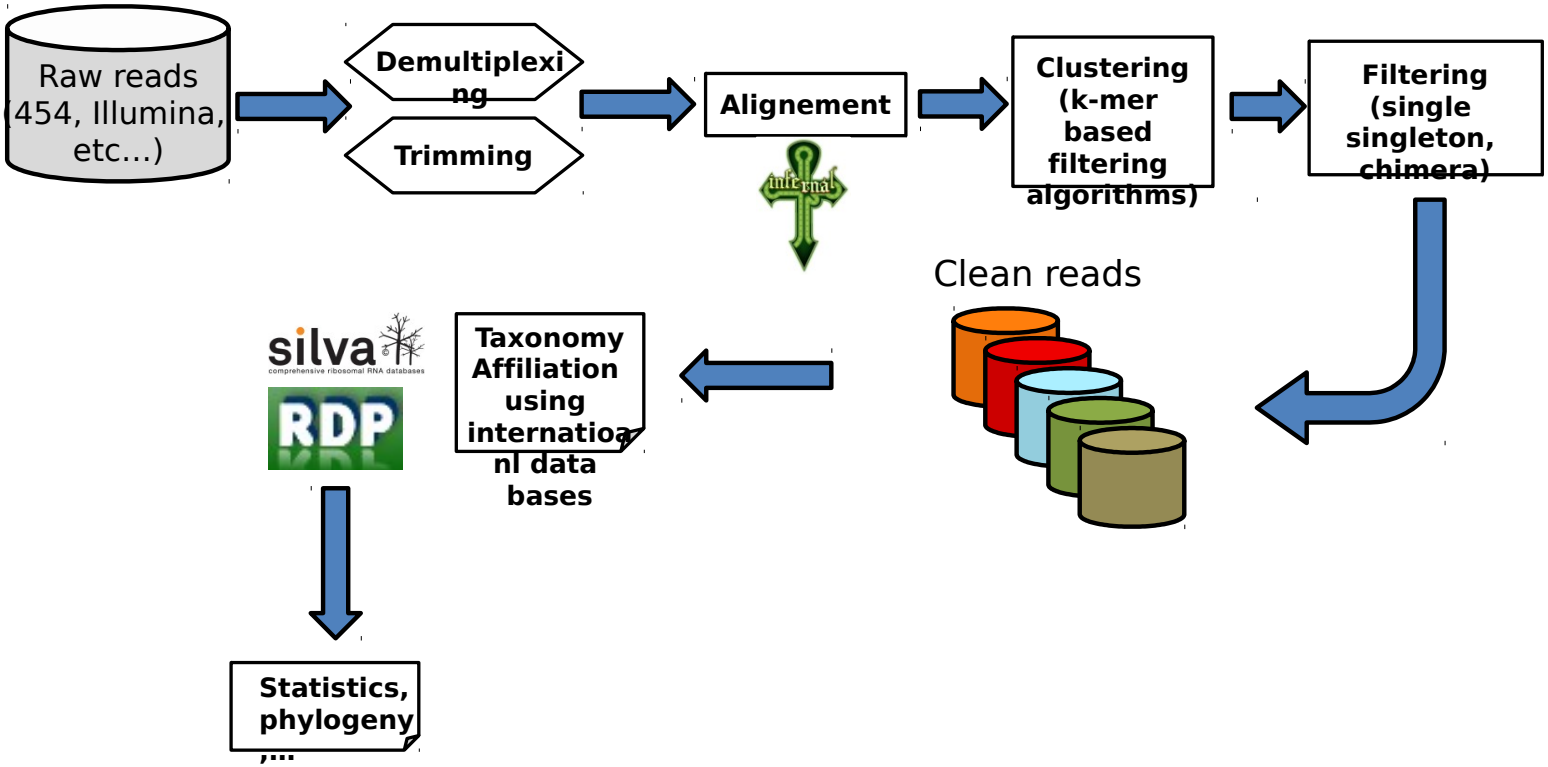
A dedicated bioinformatic workflow for microbial communities diversity analysis: the GnS-PIPE



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First results

After filtering : 442 bacteria * 508 samples

Poisson LBM with g=5 and m=6

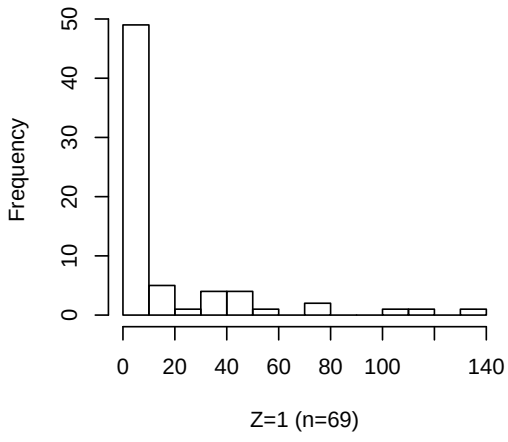
	1	2	3	4	5	6
1	-0.01	-0.00	-0.09	0.01	0.14	0.01
2	-0.06	-0.04	0.10	-0.14	1.67	-0.17
3	0.41	-0.13	0.16	-0.07	-0.38	0.06
4	-0.49	0.02	-0.54	0.19	-0.62	0.02
5	-0.25	0.15	0.26	-0.13	-0.51	-0.08

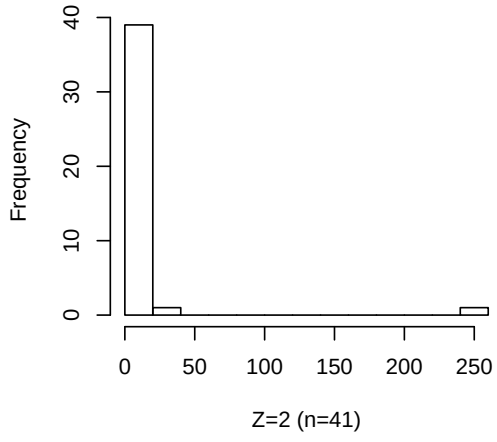
TABLE: $log(\widehat{\alpha_{k\ell}})$

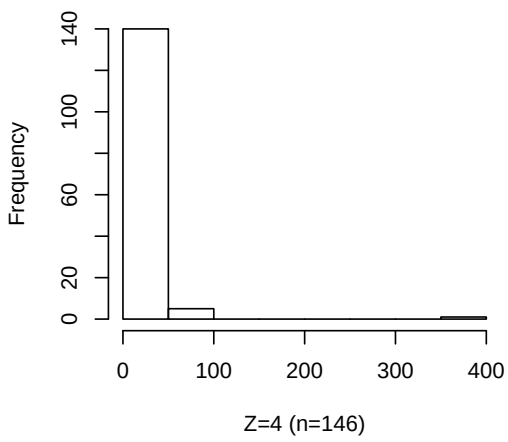
First results

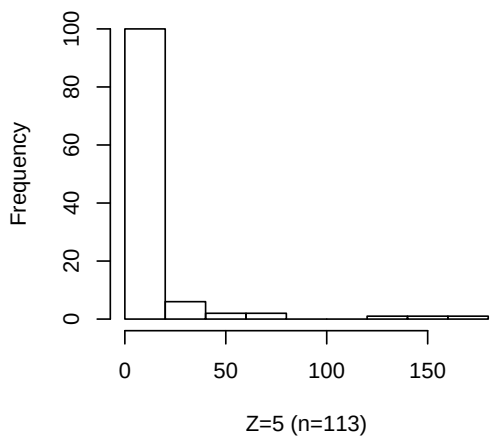
Group $(2, 5)$: 8 plants et 41 bacteria.

Groups are not correlated with the abundance level of bacteria.









First results

Plants

originate from mediterranean Middle-East + Tunisia and Libya,
belongs to 2 genomics group among 4,
but nothing obvious on the ecophysiology of plants.

Bacteria

With one of these following ecophysiological traits :

- acidophilus
- anaerobic
- metabolism of single carbon
- nitrogen metabolism

Conclusions and perspectives

- Parameters biologically interpretable
- First encouraging results
- LBM : parcimonious and complex model able to reduce data dimension

Short-term perspectives

- Application of $ZIP(\mu_i \nu_j \alpha_{k\ell})$ LBM on the data : what advantage ?
- Discussion with my favorite biologist in order to understand the group deciphered by LBM

Perspectives

- Choice of the number of groups
- Variance for $\hat{\alpha_{k\ell}}$

Model with covariates

$$Y_{ij}|(Z_{ik} = 1, W_{j\ell} = 1) \sim \mathcal{P}(\lambda_{k\ell} e^{\beta^\intercal x_{ij}})$$

with x_{ij} vector of covariates.

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S. Robin S. Schbath

References

[1] Govaert, G. et Nadif, M. (2010), **Latent Block Model for contingency table**, *Communications in Statistics - Theory and Methods*, 39(3), 416–425.

[2] Dempster, A. P., Laird, N. M. and Rubin, D. B. (1977), **Maximum likelihood from incomplete data via the EM algorithm**, *Journal of the Royal Statistical Society, B* 39(1), 1-38.

[3] Ronfort, J. et al. (2006), **Microsatellite diversity and broad scale geographic structure in a model legume : building a set of nested core collection for studying naturally occurring variation in Medicago truncatula**, BMC Plant Biology, 6(1), 28.

[4] Wainwright, M. J. and Jordan, M. I. (2008), **Graphical models, exponential families, and variational inference. Found**, *Trends Mach. Learn.*,1, 1–305.