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Metagenomic data analysis using a LBM

Application to plant-microbial communities interactions in the
rhizosphere

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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	...	S _j	...	S _d
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 :

$$(Z_i) \sim \mathcal{M}(1, \pi = (\pi_1, \dots, \pi_g)) \quad (W_j) \sim \mathcal{M}(1, \rho = (\rho_1, \dots, \rho_m))$$

Latent variables (Z_i) and (W_j) are independent

Latent class assumption : the random variables Y_{ij} are independent conditionally on labels

Model

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

$$Y_{ij} | (Z_{ik} = 1, W_{jl} = 1) \sim f(., \alpha_{kl})$$

$\alpha = (\alpha_{kl})$ 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 : $\log P(Y; \theta) = \log \sum_{Z, W} P(Y, Z, W; \theta)$

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

$$\log P(Y; \theta)$$

through the alternation of two steps

EM-trick

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

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

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 $\mathbb{E}[\log P(Y, H; \theta) | Y]$ with respect to θ

\rightarrow generally similar to standard MLE.

Graphical representation



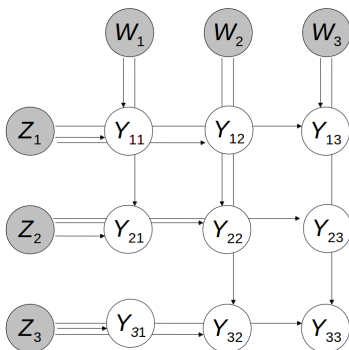
- Z_i and W_j unobserved labels of row i and column j

Z_1

Z_2

Z_3

Graphical representation

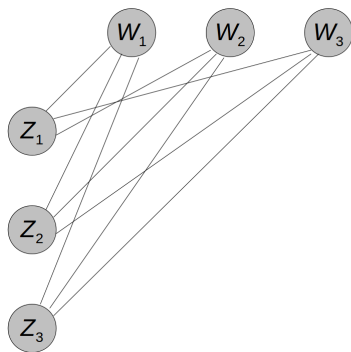


- Z_i and W_j unobserved labels of row i and column j
- Y_{ij} depends on the labels.

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}
→ $P(H|Y)$ 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 $Q(H)$:

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

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

Link with EM :

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

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

Variational EM algorithm

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

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

M-step : compute

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

Mean-field approximation

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

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

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

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 $Z_{ik}W_{j\ell}$.

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

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

1. Initialization of the unknown s, t, π, ρ, α

Generalized Variational EM (Govaert et Nadif 2010)

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1. Initialization of the unknown s, t, π, ρ, α
2. VEM on rows : Max $F(s, t, \theta)$ with respect to s, π and α with fixed ρ and t .

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4. Iterate steps 2 and 3 until convergence.

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4. Iterate steps 2 and 3 until convergence.

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

Poisson and Zero-Inflated Poisson Latent Block Model

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- 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

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 :

$$\widehat{\delta}_{kl} = \frac{\bar{y}_{kl}}{\sum_{i,j} s_{ik} t_{jl} \widehat{\alpha}_{kl}}$$

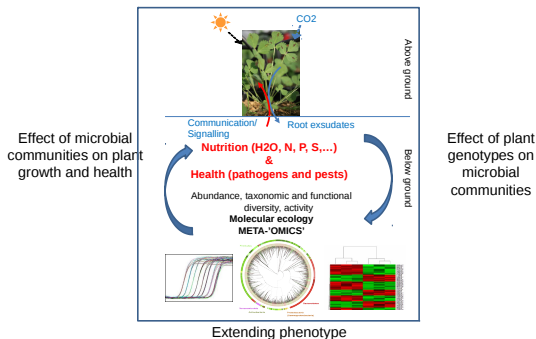
where $\bar{y}_{kl} = \frac{\sum_{i,j} s_{ik} t_{jl} y_{i,j}}{\sum_{i,j} s_{ik} t_{jl}}$

A fixed-point equation to solve :

$$\widehat{\alpha}_{kl} = \frac{\bar{y}_{kl}(1 - e^{1 - \widehat{\alpha}_{kl}})}{1 - n_0^{kl}/n^{kl}} = G(\widehat{\alpha}_{kl})$$

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

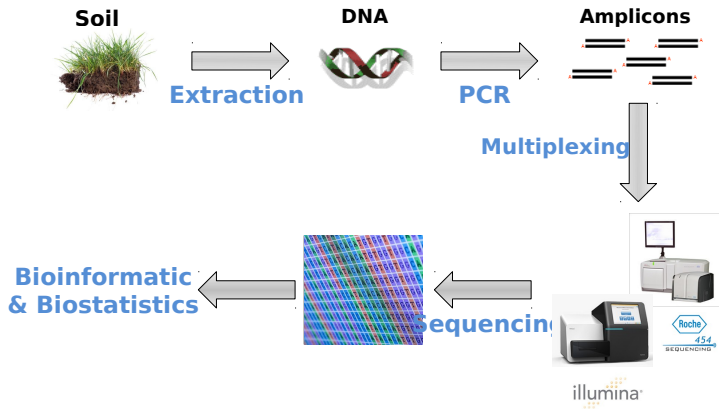
Small annual legume : model organism in genomic research.
Forms symbioses with nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi.

→ **Reduction of nitrogen inputs** responsible for various pollution
(sustainable agriculture)

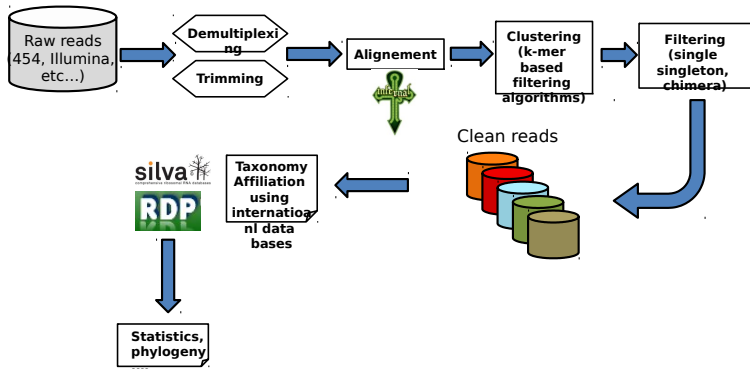


Sampling strategy of the *M. truncatula* core collection, Ronfort et al. (2006)

Standard operating procedures from soil collection to mathematical analysis



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



First results

After filtering : 442 bacteria * 508 samples

Poisson LBM with $g=5$ and $m=6$

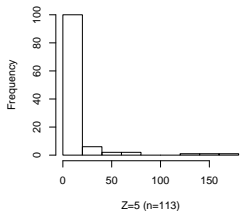
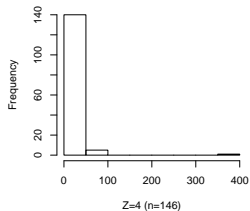
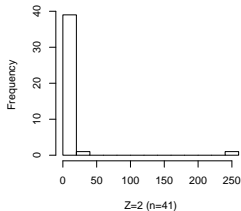
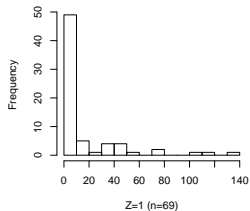
	1	2	3	4	5	6
1	-0.01	-0.00	-0.09	0.01	0.14	0.01
2	-0.06	-0.04	0.10	-0.14	1.67	-0.17
3	0.41	-0.13	0.16	-0.07	-0.38	0.06
4	-0.49	0.02	-0.54	0.19	-0.62	0.02
5	-0.25	0.15	0.26	-0.13	-0.51	-0.08

TABLE: $\log(\widehat{\alpha}_{kl})$

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 $ZIP(\mu_i \nu_j \alpha_{kl})$ 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 $\alpha_{k\ell}$

Model with covariates

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

with x_{ij} vector of covariates.

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For experiments and biological expertise

C. Mougel (INRA, IGEPP)



M2E
Meta-omics
for Microbial Ecosystems



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S. Robin



S. Schbath



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