# Statistical analysis of NIOZ140 september 2020.

## Herkomst data:

Excel file:

* M:\Molecular\_lab\ngs\_projects\_processed\NIOZ140\maaike\New runs dual barcode\statistics HW\ N140\_MG\_OTU\_processed\_belangrijk.xlsx

ADA:

* /export/lv4/projects/foils\_mg/CASCABEL/foils\_statia\_2019/runs/bc\_correction/otu/taxonomy\_vsearch/otuTable\_noSingletons.txt

PRIMER:

* M:\Molecular\_lab\ngs\_projects\_processed\NIOZ140\maaike\New runs dual barcode\statistics HW\ Otus and orders dual barcode sept 2020.pwk

## Werkproces:

* OTUtabel ingelezen in Excel sheet: otuTable\_noSingletons
* Gemodificeerd in sheet: otuTable modified.
  + OTUs <10 reads in totaal en OTUs aanwezig in <2 samples verwijderd
  + Taxonomy tabel gemodificeerd:
    - Tm Order niveau
    - Unknown taxons, \*’s, en lege taxons systematisch aangevuld met

taxon\_unkn class\_unkOrder bv:

* Gemodificeerde OTUtabel ingelezen in Primer:
  + Op OTUniveu
    - nMDS
    - ANOSIM
    - PERMANOVA
  + ORDER niveau
    - nMDS
    - ANOSIM
    - PERMANOVA
    - SIMPER

## Reads

Linear

Of dezelfde data maar dan logaritmisch uitgezet (focus op lage aantallen)

Bijzonderheden :

* Vrij grote verschillen in aantal reads per sample:
* NC hebben veel reads
* Drie samples met 0 read, 10 samples met weinig reads

|  |  |  |
| --- | --- | --- |
| **sample** | **treatment** | **reads** |
| NIOZ140.14 | negative control | 102310 |
| NIOZ140.62 | T1\_a\_PE\_UV | 0 |
| NIOZ140.65 | T1\_a\_PET | 4697 |
| NIOZ140.71 | negative control | 347810 |
| NIOZ140.76 | T1\_b\_PET | 4086 |
| NIOZ140.77 | T1\_b\_PET\_UV | 3502 |
| NIOZ140.82 | Negative control | 135540 |
| NIOZ140.83 | T1\_c\_PE | 6058 |
| NIOZ140.87 | T1\_c\_PET | 3386 |
| NIOZ140.89 | T1\_c\_PS | 4180 |
| NIOZ140.90 | T1\_c\_PS\_UV | 7 |
| NIOZ140.91 | T1\_c\_Nylon | 3885 |
| NIOZ140.92 | T1\_c\_Nylon\_UV | 3490 |
| NIOZ140.108 | T6\_b\_PET\_UV | 1074 |
| NIOZ140.123 | negative control | 155091 |
| NIOZ140.126 | T3\_PP | 2078 |
| NIOZ140.127 | T3\_PP\_UV | 0 |
| NIOZ140.128 | T3\_PET | 0 |
|  |  |  |
|  |  |  |

* is there a relation between nr of reads and brightness of bands on gel??
* Relation between material and nr of reads??

## Wat doen we met chloroplasts?

Making nMDS plots in general:

## Steps

* Standardize nr of reads of Otus per sample based on total nr of reads per sample (to give all samples the same weight)
* Transformation of the standardized reads.
  + I did a mild transformation (square root)
  + Alternative transformations are possible (from none to log(x+1), and possibly even present/absent. I did not do this, but I would be surpised if the conclusion will be different.
* Bray-Curtis similarity index (often used for “count” data, with a lot of zero’s)
* nMDS to visualize the Br-C matrix., to visualize the relation between samples based on distribution of the OTU’s



### Conclusie:

Chloroplasten (algen) groeien in de loop van de dagen, aantallen op T6 veel hoger dan op T1.

Dit kan interfereren met de conclusies van het microbiome 🡪 analyse uitvoeren zonder chloroplasts



### Opvallend:

* Kleine cluster met samples 10,11,13 and 14(NC)
* NC’s liggen tussen andere samples??

## Focus op T1 en T6

* N140.90 verwijderd (7 counts)



* Is sample N140.113 bijzonder?





## Statistical tests

There are basically two way to test the significance of the factors time, material and UV treatment

* ANOSIM: Analysis of similarities provides a way to test statistically whether there is a significant difference between two or more groups of sampling units
* Permanova: multivariate statistical tes comprable with Anova

# Results

## Results of multivariate analysis.

### All multivariate analysis was carried out in Primer-E.

.

#### Schedule of the Experimental set up: (2 missing experimental units) 🡪 almost fully balanced

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| day | T1 | T1 | T6 | T6 |
| UV treatment | UV | No-UV | UV | No-UV |
| material |  |  |  |  |
| PET | A B C | A B C | A B C | A B C |
| PS | A B | A B C | A B C | A B C |
| Nylon | A B C | A B C | A B C | A B C |
| PE | B C | A B C | A B C | A B C |
| PP | A B C | A B C | A B C | A B C |

## Statistical Tests

PERMANOVA

Permutational MANOVA

*Resemblance worksheet*

Name: OTUs T1 T6 N90 weg

Data type: Similarity

Selection: 7-15,17-26,28-34,36-67

Standardise Samples by Total

Transform: Square root

Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)

Fixed effects sum to zero for mixed terms

Permutation method: Permutation of residuals under a reduced model

Number of permutations: 99999

*Factors*

Name Abbrev. Type Levels

Time Ti Random 2

Plastic Pl Fixed 5

UV\_exp UV Fixed 2

*Excluded terms*

PlasticxUV\_exp 🡪 not significant

TimexPlasticxUV\_exp 🡪not significant

*PERMANOVA table of results*

Unique

Source df SS MS Pseudo-F P(perm) perms

Ti 1 40016 40016 15.291 1e-5 90063

Pl 4 14536 3634 1.1892 0.1513 65416

UV 1 2729.3 2729.3 0.59111 0.499 6

TixPl 4 12223 3055.8 1.1677 0.037 75755

TixUV 1 4617.2 4617.2 1.7644 0.0036 86204

Res 46 1.2038E+05 2616.9

Total 57 1.9451E+05

Dus bij het volledige model zijn de factor Time en de interacties Ti X Pl en Ti X UV significant , de hoofdfactoren Plastic en UV echter niet.

T1 en T6 afzonderlijk (onderzoeken aangezien er geen UV hoofdeffect is, maar wel een UV X Ti effect.

## T1



PERMANOVA

Permutational MANOVA

*Resemblance worksheet*

Name: OTUs T1

Data type: Similarity

Selection: 1-28

Standardise Samples by Total

Transform: Square root

Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)

Fixed effects sum to zero for mixed terms

Permutation method: Permutation of residuals under a reduced model

Number of permutations: 99999

*Factors*

Name Abbrev. Type Levels

Time Ti Random 1

Plastic Pl Fixed 5

UV\_exp UV Fixed 2

*Excluded terms*

Time

TimexPlastic

TimexUV\_exp

PlasticxUV\_exp 🡪 not significant

TimexPlasticxUV\_exp

*PERMANOVA table of results*

Unique

Source df SS MS Pseudo-F P(perm) perms

Pl 4 10661 2665.2 1.0889 0.1793 78043

UV 1 3690.7 3690.7 1.5079 0.0313 88240

Res 22 53846 2447.5

Total 27 68200

Dan blijkt er op tijdstip T1 wel een significant UV effect te zijn.

Effect van het type plastic op de microbiele samenstelling is hier niet significant

## T6



PERMANOVA

Permutational MANOVA

*Resemblance worksheet*

Name: OTUs T6

Data type: Similarity

Selection: 29-58

Standardise Samples by Total

Transform: Square root

Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)

Fixed effects sum to zero for mixed terms

Permutation method: Permutation of residuals under a reduced model

Number of permutations: 99999

*Factors*

Name Abbrev. Type Levels

Time Ti Random 1

Plastic Pl Fixed 5

UV\_exp UV Fixed 2

*Excluded terms*

Time

TimexPlastic

TimexUV\_exp

PlasticxUV\_exp 🡪 not significant

TimexPlasticxUV\_exp

*PERMANOVA table of results*

Unique

Source df SS MS Pseudo-F P(perm) perms

Pl 4 16418 4104.5 1.4806 0.0007 79523

UV 1 3654.3 3654.3 1.3182 0.0941 88083

Res 24 66532 2772.2

Total 29 86605

Opmerkelijk:

UV of geen UV is geen sterk significante factor op dag T6 (p>5%), Type plastic daarentegen we (P<0.1%)l!!!!

Probeer hier maar een goede biologische verklaring voor te geven.

# Order level

## Focus op T1 en T6, chloroplasten niet meegenomen.





## Permanova

PERMANOVA

Permutational MANOVA

*Resemblance worksheet*

Name: Resem1

Data type: Similarity

Selection: All

Standardise Samples by Total

Transform: Square root

Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)

Fixed effects sum to zero for mixed terms

Permutation method: Permutation of residuals under a reduced model

Number of permutations: 99999

*Factors*

Name Abbrev. Type Levels

Time Ti Random 2

Plastic Pl Fixed 5

UV\_exp UV Fixed 2

*Excluded terms*

TimexPlastic

PlasticxUV\_exp

TimexPlasticxUV\_exp

*PERMANOVA table of results*

Unique

Source df SS MS Pseudo-F P(perm) perms

Ti 1 30639 30639 38.552 1e-5 93481

Pl 4 4713 1178.2 1.4825 0.0312 87980

UV 1 619.98 619.98 0.19739 0.4985 6

TixUV 1 3140.9 3140.9 3.9521 0.0011 92703

Res 50 39738 794.75

Total 57 78292

In deze test (order level, no chloroplast, T1 en T6 alleen)

Zeer sterk Tijdseffect, klein Plastics effect, en een sterk interactie Ti x UV effect ondanks dat main factor UV niet significant is

## Alleen T1

PERMANOVA

Permutational MANOVA

*Resemblance worksheet*

Name: order T1

Data type: Similarity

Selection: 1-28

Standardise Samples by Total

Transform: Square root

Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)

Fixed effects sum to zero for mixed terms

Permutation method: Permutation of residuals under a reduced model

Number of permutations: 99999

*Factors*

Name Abbrev. Type Levels

Time Ti Random 1

Plastic Pl Fixed 5

UV\_exp UV Fixed 2

*Excluded terms*

Time

TimexPlastic

TimexUV\_exp

PlasticxUV\_exp 🡪 not significant in expanded model

TimexPlasticxUV\_exp

*PERMANOVA table of results*

Unique

Source df SS MS Pseudo-F P(perm) perms

Pl 4 3271.3 817.83 1.0335 0.4071 88916

UV 1 1822.5 1822.5 2.3031 0.0268 93339

Res 22 17409 791.34

Total 27 22478

UV is a significant factor in time T1 , Plastic not

## Alleen T6

PERMANOVA

Permutational MANOVA

*Resemblance worksheet*

Name: order T6

Data type: Similarity

Selection: 29-58

Standardise Samples by Total

Transform: Square root

Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)

Fixed effects sum to zero for mixed terms

Permutation method: Permutation of residuals under a reduced model

Number of permutations: 99999

*Factors*

Name Abbrev. Type Levels

Time Ti Random 1

Plastic Pl Fixed 5

UV\_exp UV Fixed 2

*Excluded terms*

Time

TimexPlastic

TimexUV\_exp

PlasticxUV\_exp 🡪 not significant in expanded model

TimexPlasticxUV\_exp

*PERMANOVA table of results*

Unique

Source df SS MS Pseudo-F P(perm) perms

Pl 4 4359 1089.7 1.3474 0.0794 88516

UV 1 1936 1936 2.3937 0.0263 93292

Res 24 19411 808.79

Total 29 25706

Op tijdstip T6 is UV dus een significante factor, plastic op het randje