

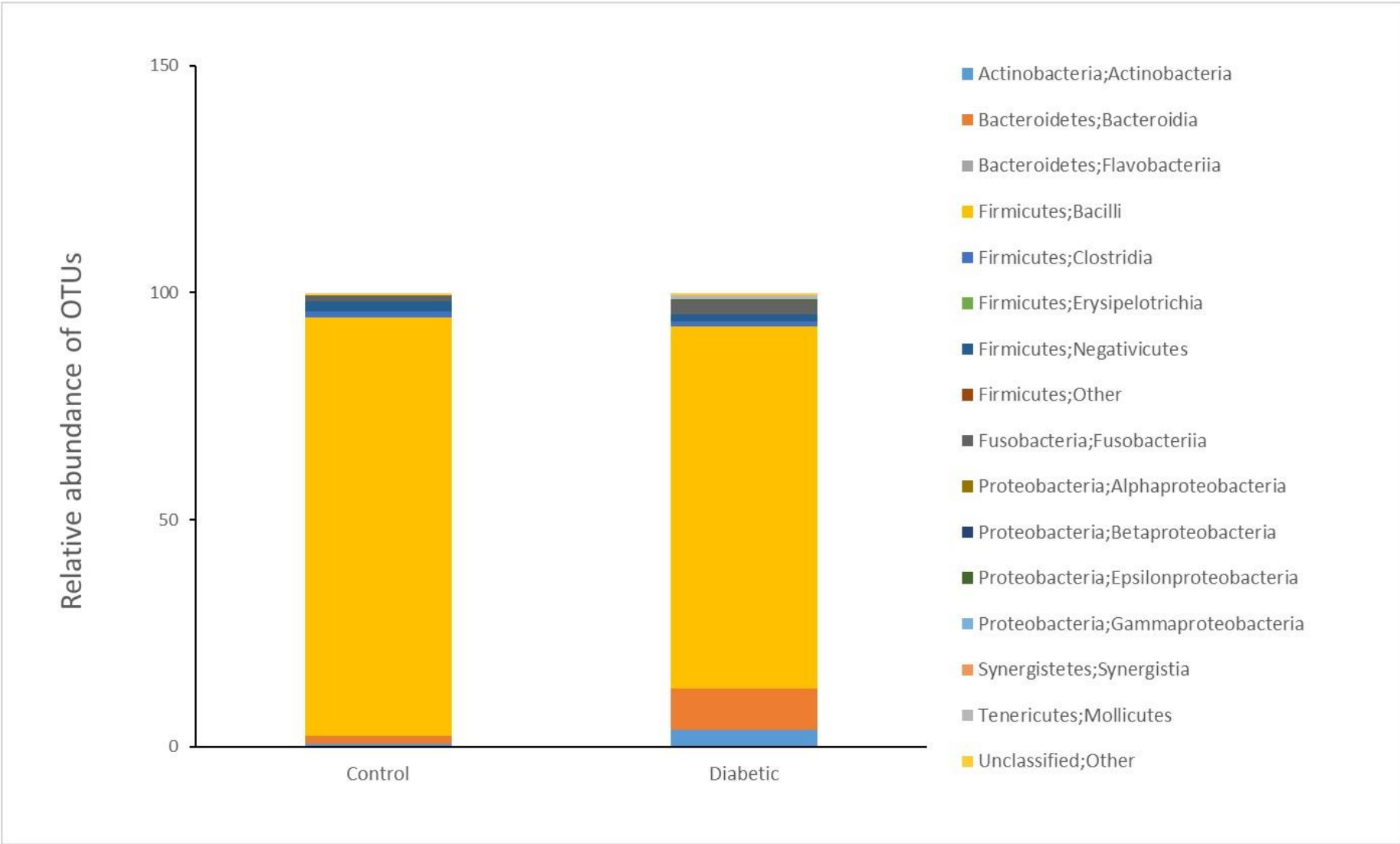
Supplementary information

Association of prevalent vaginal microbiome of mother with occurrence of type I diabetes in child

Mysore V Tejesvi, Ritva Nissi, Karita Saravesi, Anna Maria Pirttilä, Annamari Markkola, Anne Talvensaari-Mattila, Anna Liisa

Ruotsalainen

Supplementary information 1.



Supplementary information 2.

R scripts, outputs and diagnostic plots of analysis of variance (ANOVA) and linear mixed effects (LME) models.

1. Analysis of the impact of swab type on Chao1, Shannon and Simpson index and OTU relative abundance by using 1-way analysis of variance (ANOVA). Explanatory factor: swab type (levels: cotton and Dacron). Testing carried out for control and diabetic groups separately.
2. Testing the impact of mother group (= diab, levels: control = no diabetic child/children, diabetic = 1 or more T1D child/children).

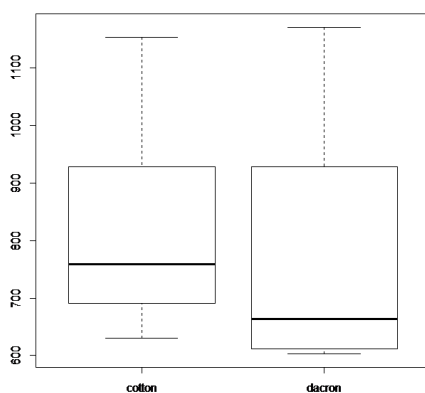
1. Impact of swab:

Impact of swab type within the control group:

```
> model<-aov(chao~swab)
```

```
> summary(model)
```

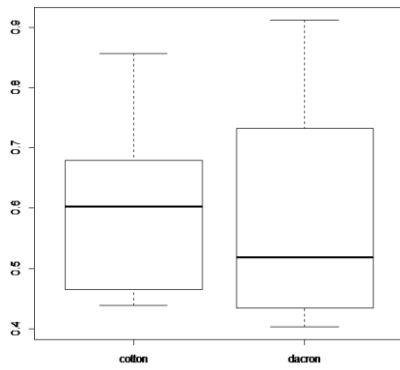
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	8066	8066	0.181	0.677
Residuals	14	622179	44441		



```
> model<-aov(simpson~swab)
```

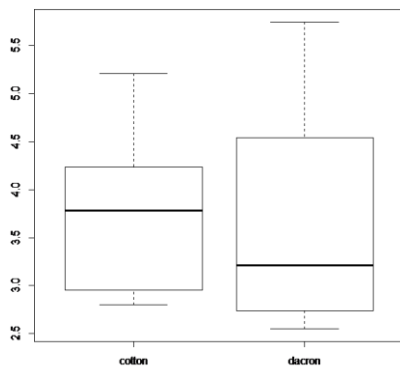
```
> summary(model)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	0.0007	0.000711	0.026	0.875
Residuals	14	0.3901	0.027863		



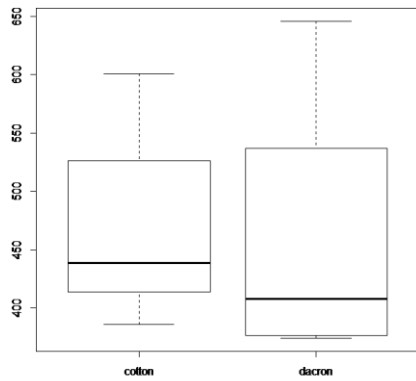
```
> model<-aov(shannon~swab)
> summary(model)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	0.027	0.0275	0.026	0.873
Residuals	14	14.522	1.0373		



```
> model<-aov(otus~swab)
> summary(model)
```

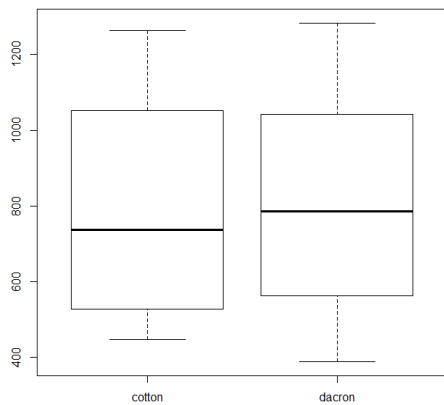
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	420	420	0.045	0.835
Residuals	14	130976	9355		



Impact of swab type within the diabetic -group:

Chao mary(model)

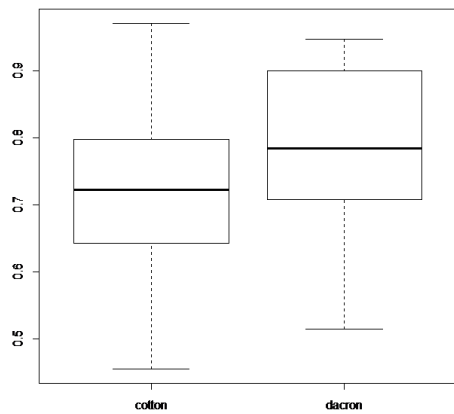
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	773	773	0.008	0.93
Residuals	14	1370532	97895		



```
> model<-aov(simpson~swab)
```

```
> summary(model)
```

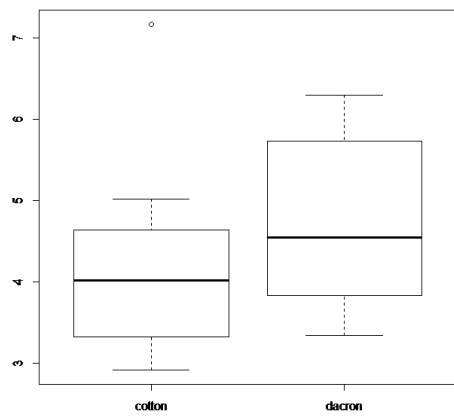
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	0.01512	0.01512	0.708	0.414
Residuals	14	0.29889	0.02135		



```
> model<-aov(shannon~swab)
```

```
> summary(model)
```

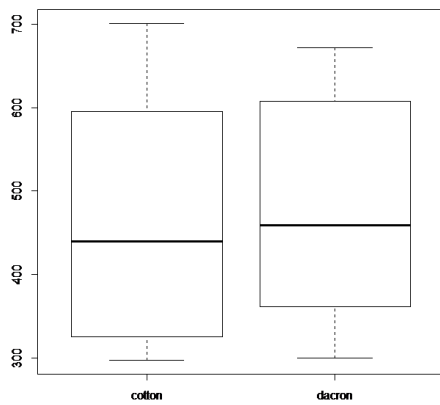
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	0.903	0.9026	0.579	0.459
Residuals	14	21.806	1.5576		



```
> model<-aov(otus~swab)
```

```
> summary(model)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
swab	1	743	743	0.035	0.855
Residuals	14	298727	21338		



2. LME analysis with swab type nested within mother as random factors:

chao1:

```
> model<-lme(chao1~diab, random=~1|mother/swab)
```

```
> summary(model)
```

Linear mixed-effects model fit by REML

Data: NULL

AIC	BIC	logLik
402.0949	409.1009	-196.0475

Random effects:

Formula: ~1 | mother

(Intercept)

StdDev: 257.4674

Formula: ~1 | swab %in% mother

(Intercept) Residual

StdDev: 56.79128 40.285

Fixed effects: chao1 ~ diab

Value Std.Error DF t-value p-value

(Intercept) 795.0355 92.67789 16 8.578480 0.0000

diaby 5.2664 131.06633 14 0.040181 0.9685

Correlation:

(Intr)

diaby -0.707

Standardized Within-Group Residuals:

Min Q1 Med Q3 Max

-0.86810772 -0.29023812 -0.04126476 0.28423593 0.82173538

Number of Observations: 32

Number of Groups:

mother swab %in% mother

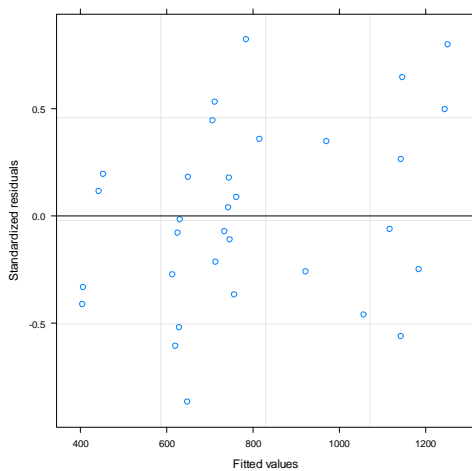
16 32

> anova(model, test="F")

numDF denDF F-value p-value

(Intercept) 1 16 148.15720 <.0001

diab 1 14 0.00161 0.9685



Shannon:

```
> model<-lme(log(shannon)~diab, random=~1 | mother/swab)
```

```
> summary(model)
```

Linear mixed-effects model fit by REML

Data: NULL

AIC BIC logLik

4.983506 11.98949 2.508247

Random effects:

Formula: ~1 | mother

(Intercept)

StdDev: 0.2358537

Formula: ~1 | swab %in% mother

(Intercept) Residual

StdDev: 0.1091228 0.05912714

Fixed effects: log(shannon) ~ diab

	Value	Std.Error	DF	t-value	p-value
--	-------	-----------	----	---------	---------

(Intercept)	1.2770332	0.08897251	16	14.353122	0.0000
-------------	-----------	------------	----	-----------	--------

diaby	0.1928538	0.12582613	14	1.532701	0.1476
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Correlation:

(Intr)

diaby -0.707

Standardized Within-Group Residuals:

Min	Q1	Med	Q3	Max
-----	----	-----	----	-----

-0.840098596	-0.177552409	-0.004972501	0.142179216	0.822314774
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Number of Observations: 32

Number of Groups:

mother swab %in% mother

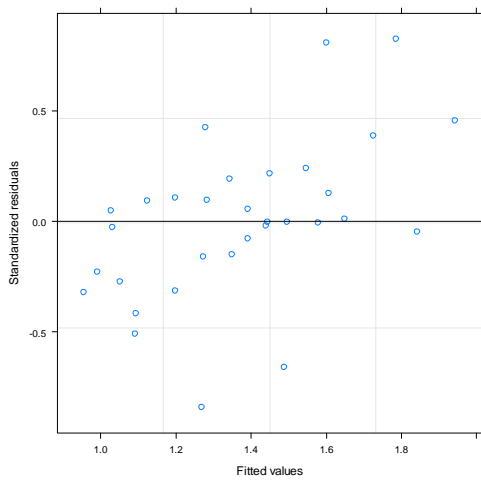
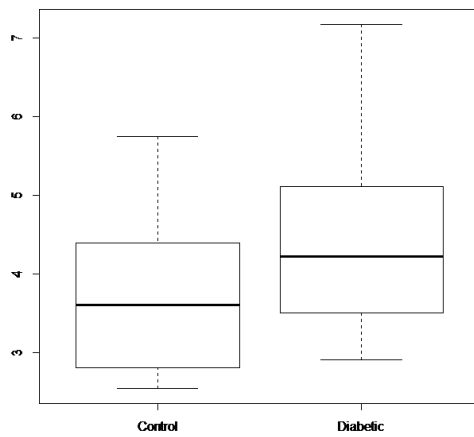
16 32

```
> anova(model, test="F")
```

	numDF	denDF	F-value	p-value
--	-------	-------	---------	---------

(Intercept)	1	16	476.5961	<.0001
-------------	---	----	----------	--------

diab	1	14	2.3492	0.1476
------	---	----	--------	--------



Simpson:

```
> model<-lme(simpson~diab, random=~1 | mother/swab)
```

```
> summary(model)
```

```
Linear mixed-effects model fit by REML
```

```
Data: NULL
```

```
    AIC    BIC logLik
```

```
-30.84982 -23.84383 20.42491
```

```
Random effects:
```

```
Formula: ~1 | mother
```

```
(Intercept)
```

```
StdDev: 0.1445313
```

```
Formula: ~1 | swab %in% mother
```

```
(Intercept) Residual
```

```
StdDev: 0.05461761 0.03184253
```

```
Fixed effects: simpson ~ diab
```

```
    Value Std.Error DF  t-value p-value
```

```
(Intercept) 0.5921033 0.0534881 16 11.069813 0.0000
```

```
diaby      0.1580268 0.0756436 14  2.089096 0.0554
```

```
Correlation:
```

```
(Intr)
```

```
diaby -0.707
```

```
Standardized Within-Group Residuals:
```

```
    Min    Q1    Med    Q3    Max
```

```
-0.878460163 -0.205815669 0.006344226 0.164438159 0.898140447
```

```
Number of Observations: 32
```

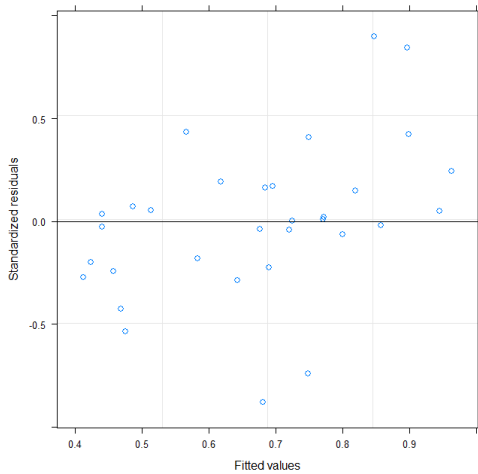
```
Number of Groups:
```

```
    mother swab %in% mother
```

```
    16     32
```

```
> anova(model, test="F")
```

	numDF	denDF	F-value	p-value
(Intercept)	1	16	314.85578	<.0001
diab	1	14	4.36432	0.0554



OTUs:

```
> model<-lme(observed_otus~diab, random=~1|mother/swab)
```

```
> summary(model)
```

Linear mixed-effects model fit by REML

Data: NULL

AIC	BIC	logLik
-----	-----	--------

351.3529	358.3589	-170.6765
----------	----------	-----------

Random effects:

Formula: ~1 | mother

(Intercept)

StdDev: 120.6659

Formula: ~1 | swab %in% mother

(Intercept) Residual

StdDev: 22.15855 16.78072

Fixed effects: observed_otus ~ diab

	Value	Std.Error	DF	t-value	p-value
(Intercept)	463.0000	43.22408	16	10.71162	0.000
diaby	8.6875	61.12808	14	0.14212	0.889

Correlation:

(Intr)

diaby -0.707

Standardized Within-Group Residuals:

Min	Q1	Med	Q3	Max
-0.85536882	-0.34326499	-0.06487676	0.31265093	1.01254874

Number of Observations: 32

Number of Groups:

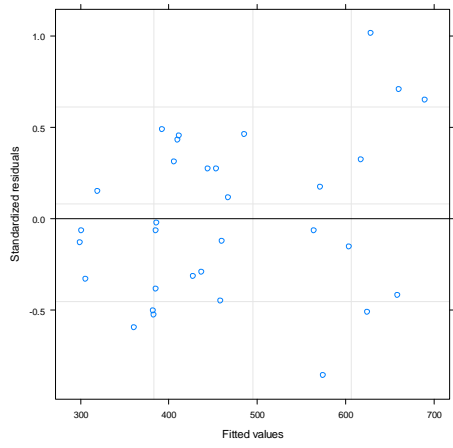
mother swab %in% mother

16 32

> anova(model, test="F")

	numDF	denDF	F-value	p-value
(Intercept)	1	16	233.8037	<.0001
diab	1	14	0.0202	0.889

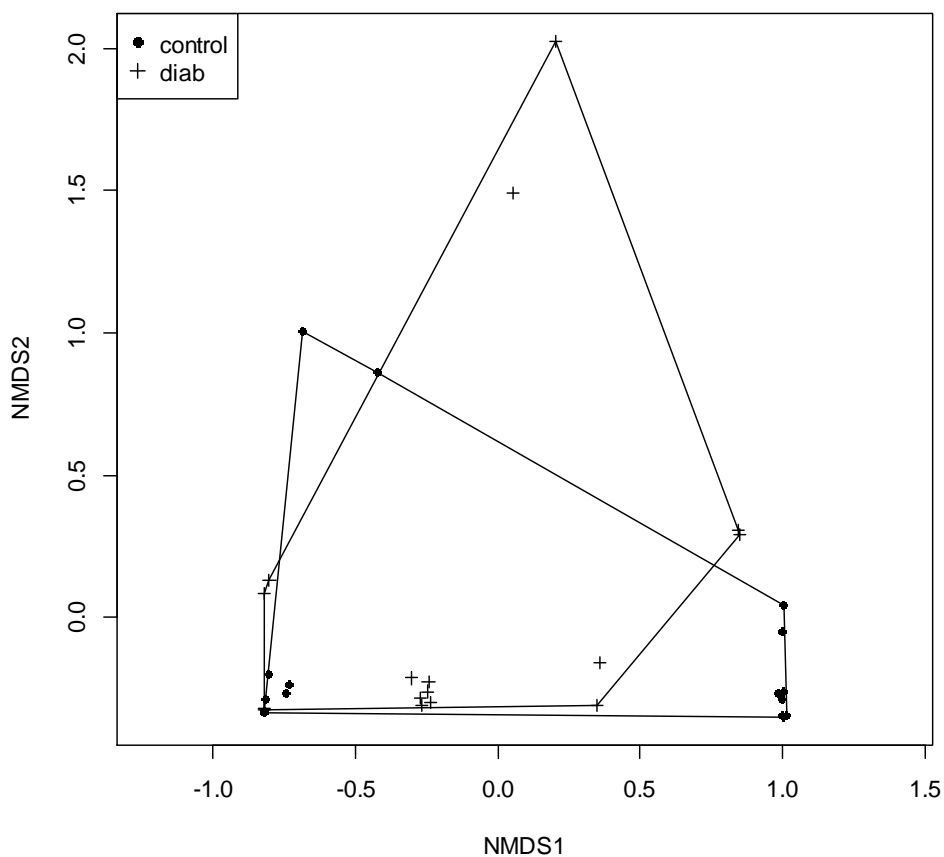
> plot(model)



Supplementary information 3.

R scripts and outputs of NMDS ordination and PERMANOVA (function *adonis* in R-program) analyses based on the relative abundance of OTUs. A: All OTUs. B: Lactobacillus OTUs. Degrees of freedom, F and p values are in bold.

- A. NMDS ordination of the microbiomes of control and diabetic samples. N = 8 (16 microbiomes based on swab type duplicates).



```
> attach(aineisto)
```

```
> diss<-vegdist(aineisto[,4:187])
```

```
> model<-adonis(diss~Treatment, strata=Swab, data=aineisto, permutations=9999)
```

```
> model
```

Call:

```
adonis(formula = diss ~ Treatment, data = aineisto, permutations = 9999, strata = Swab)
```

Blocks: strata

Permutation: free

Number of permutations: 9999

Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Treatment	1	0.2739	0.27386	1.2735	0.04072	0.2615
Residuals	30	6.4513	0.21504		0.95928	
Total	31	6.7251		1.00000		

B. PERMANOVA and NMDS ordination of *Lactobacillus* OTUs based on their relative abundances

```
> diss <- vegdist(aineisto[,4:17])
```

```
> mod <- adonis(diss ~ Treatment, strata=Swab, data=aineisto, permutations=9999)
```

```
> mod
```

Call:

```
adonis(formula = diss ~ Treatment, data = aineisto, permutations = 9999, strata = Swab)
```

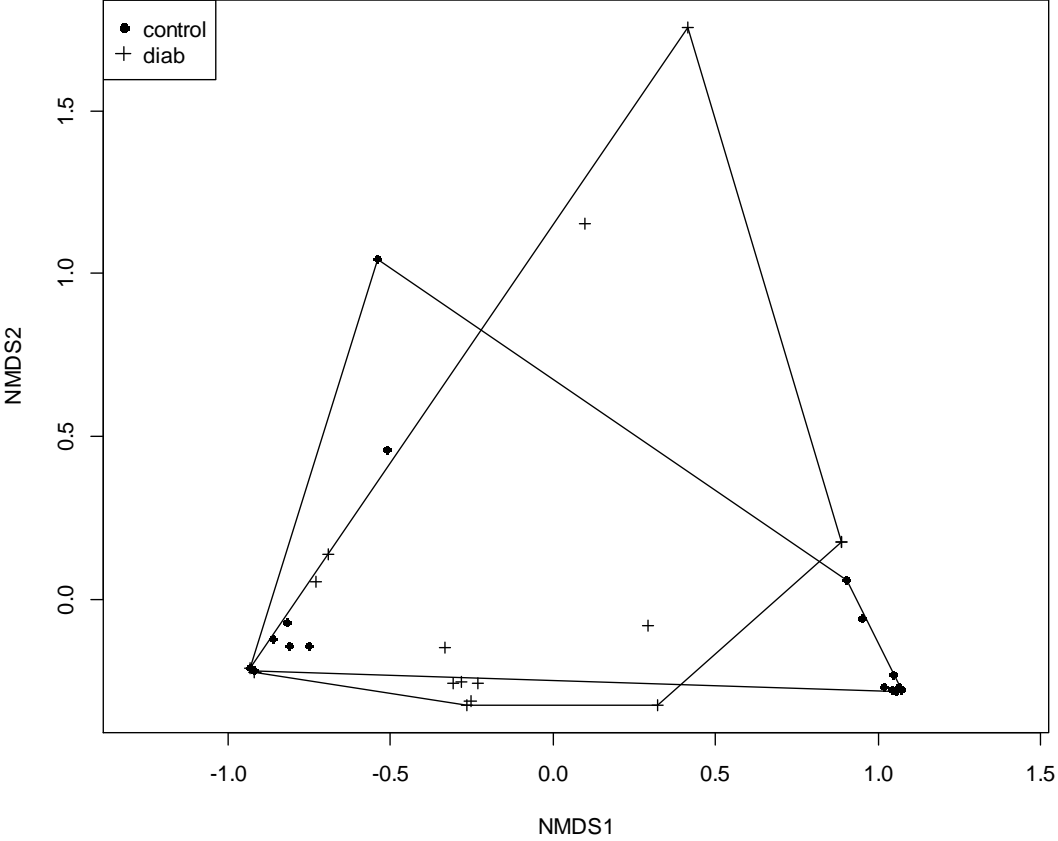
Blocks: strata

Permutation: free

Number of permutations: 9999

Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Treatment	1	0.2489	0.24894	1.3046	0.04167	0.2592
Residuals	30	5.7247	0.19082		0.95833	



Supplementary information 4.

Twenty most abundant OTUs in the vaginal microbiomes of mothers who have not delivered a child/children with type 1 diabetes (Control) and mothers who have at least one child with type 1 diabetes (Diabetic).

Control	%	SD	Diabetic	%	SD
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Other	0.468	0.379	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;OTU199 NN=Lactobacillus_johnsonii_HE573915 D=94.9	0.416	0.288
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;OTU199 NN=Lactobacillus_johnsonii_HE573915 D=94.9	0.322	0.373	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Other	0.281	0.185
Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus;Streptococcus_agalactiae	0.064	0.147	Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus;Streptococcus_agalactiae	0.048	0.128
Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Olsenella;Other	0.022	0.022	Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Olsenella;Other	0.030	0.080
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;OTU1294 NN=Lactobacillus_gasseri_JQ805634 D=96.7	0.017	0.045	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Lactobacillus_gasseri	0.024	0.037
Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;Prevotella_bivia	0.016	0.018	Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;Prevotella_timonensis	0.023	0.061
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;OTU1032 NN=Lactobacillus_johnsonii_HE573915 D=95.8	0.013	0.034	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Lactobacillus_johnsonii	0.020	0.026
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Lactobacillus_gasseri	0.012	0.033	Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;Prevotella_bivia	0.017	0.049
Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;Prevotella_timonensis	0.010	0.023	Fusobacteria;Fusobacteriia;Fusobacteriales;Fusobacteriaceae;Fusobacterium;OTU23 NN=Fusobacterium_periodonticum_AB910749 D=96.5	0.014	0.042
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Lactobacillus_johnsonii	0.005	0.005	Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Other;Other	0.012	0.040
Firmicutes;Bacilli;Lactobacillales;Aerococcaceae;Aerococcus;Aerococcus_viridans	0.005	0.014	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;OTU1294 NN=Lactobacillus_gasseri_JQ805634 D=96.7	0.011	0.024
Fusobacteria;Fusobacteriia;Fusobacteriales;Fusobacteriaceae;Fusobacterium;OTU23 NN=Fusobacterium_periodonticum_AB910749 D=96.5	0.005	0.006	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;OTU1032 NN=Lactobacillus_johnsonii_HE573915 D=95.8	0.009	0.011
Fusobacteria;Fusobacteriia;Fusobacteriales;Leptotrichiaceae;Sneathia;Sneathia_amnionii	0.005	0.005	Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Lactobacillus_oris	0.008	0.020

Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Other;Other	0.004	0.009	Fusobacteria;Fusobacteriia;Fusobacteriales;Fusobacteriaceae;Fusobacterium;Other	0.007	0.023
Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Megasphaera;OTU301 NN=Megasphaera_elsdenii_DQ146765 D=93.8	0.004	0.004	Tenericutes;Mollicutes;Mycoplasmatales;Mycoplasmataceae;Mycoplasma;Mycoplasma_hominis	0.006	0.018
Unclassified;Other;Other;Other;Other;Other	0.003	0.009	Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Dialister;OTU1084 NN=Dialister_succinatiphilus_AB370249 D=93.2	0.006	0.019
Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus;Lactobacillus_oris	0.003	0.008	Unclassified;Other;Other;Other;Other;Other	0.005	0.004
Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Dialister;OTU1084 NN=Dialister_succinatiphilus_AB370249 D=93.2	0.003	0.005	Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus;Streptococcus_anginosus	0.004	0.010
Fusobacteria;Fusobacteriia;Fusobacteriales;Fusobacteriaceae;Fusobacterium;Other	0.002	0.006	Bacteroidetes;Bacteroidia;Bacteroidales;Other;Other;Other	0.003	0.009
Sum	0.982	1.143	Sum	0.944	1.073