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SUPPLEMENTARY INFORMATION

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Influence of early life exposure, host genetics and diet on the mouse gut microbiome and metabolome

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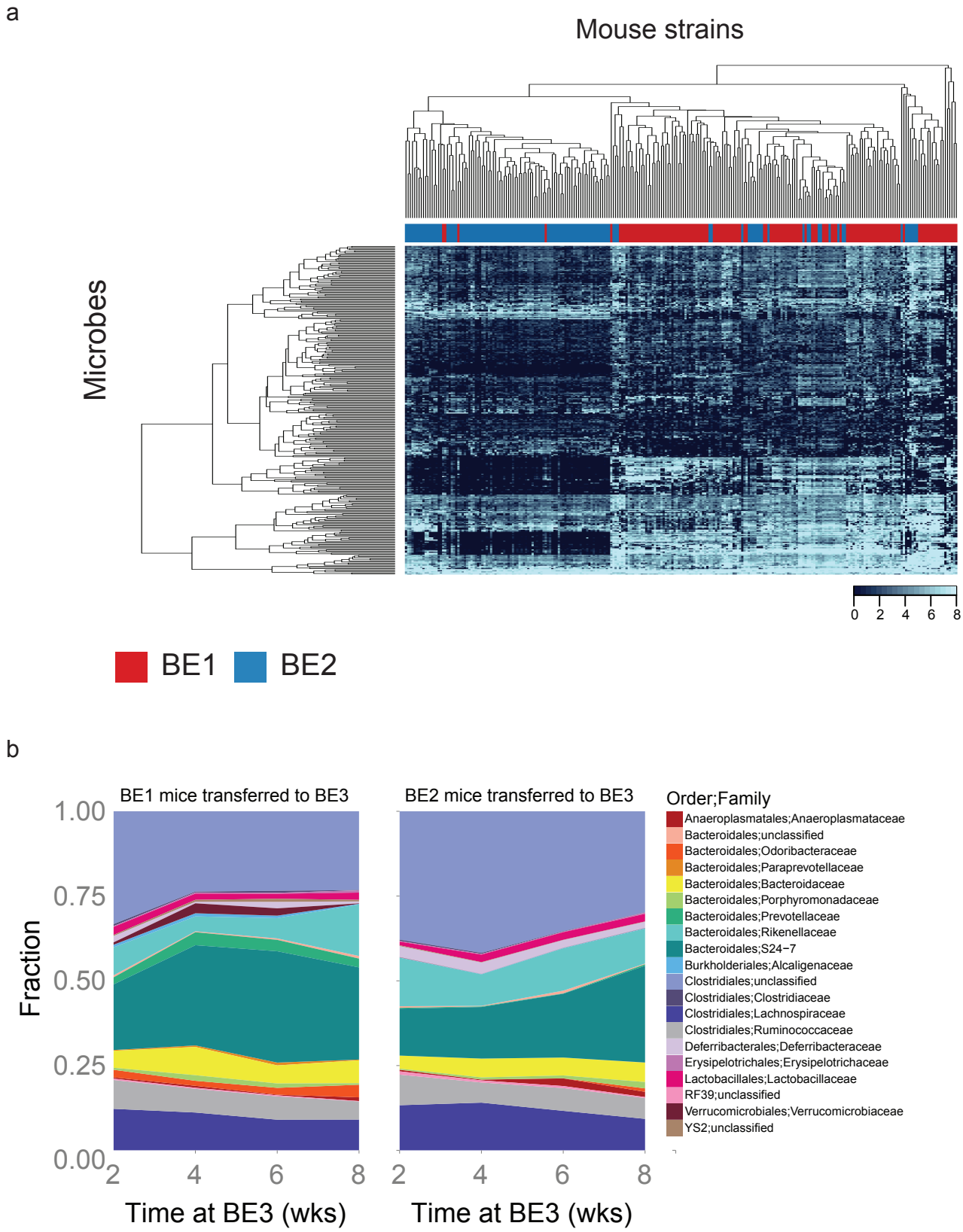
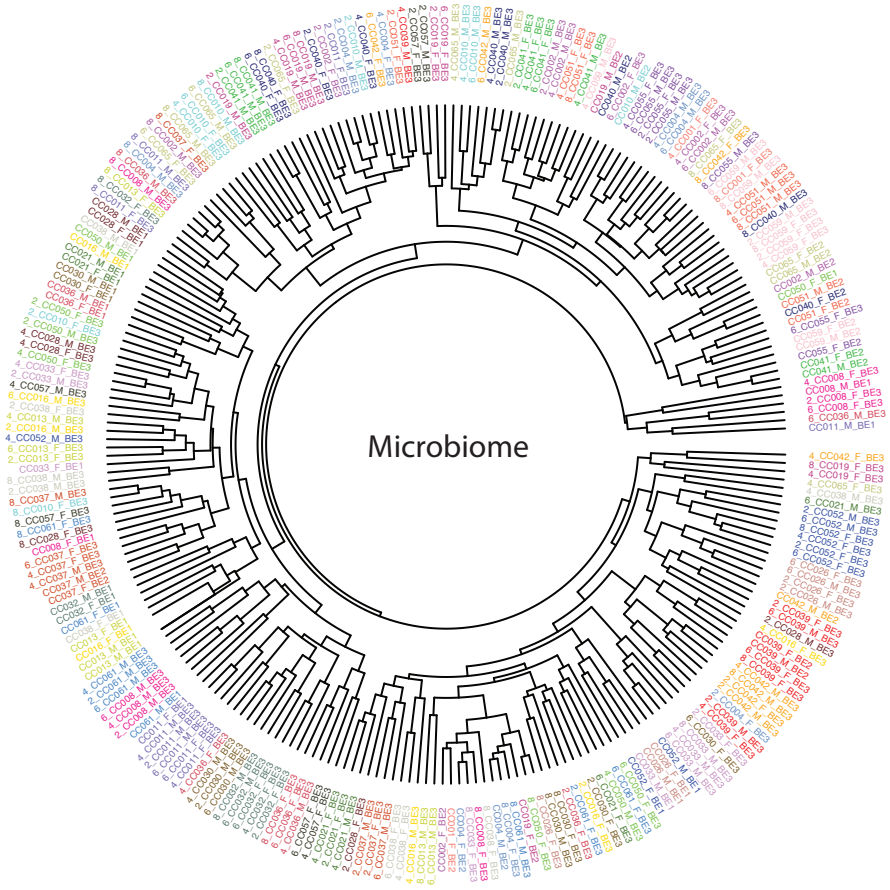
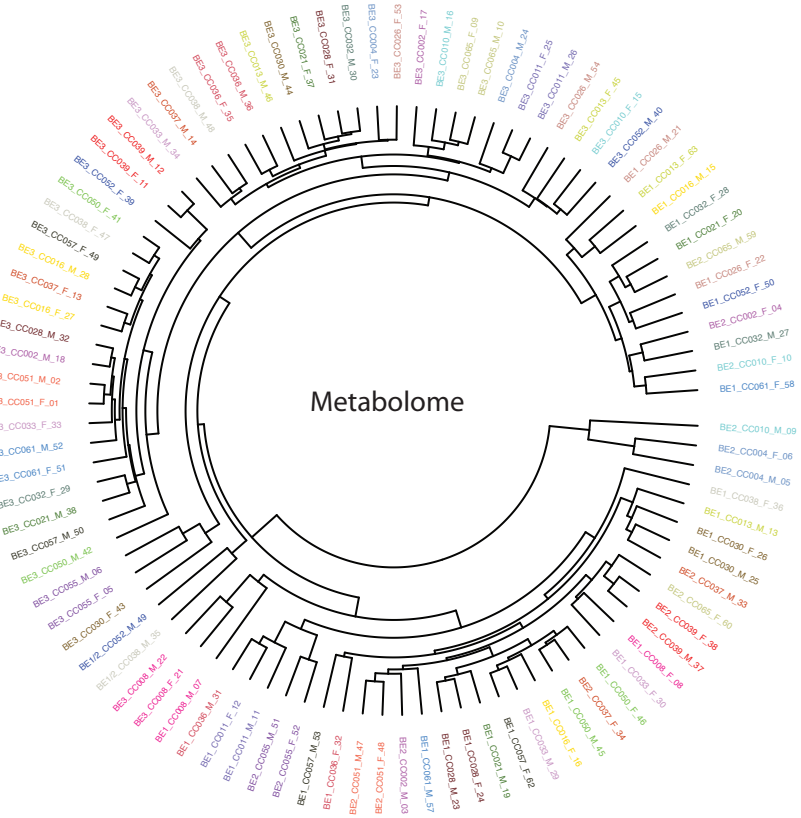
1. **Supplementary Figures 1-10**
2. **Supplementary Tables 1-12**

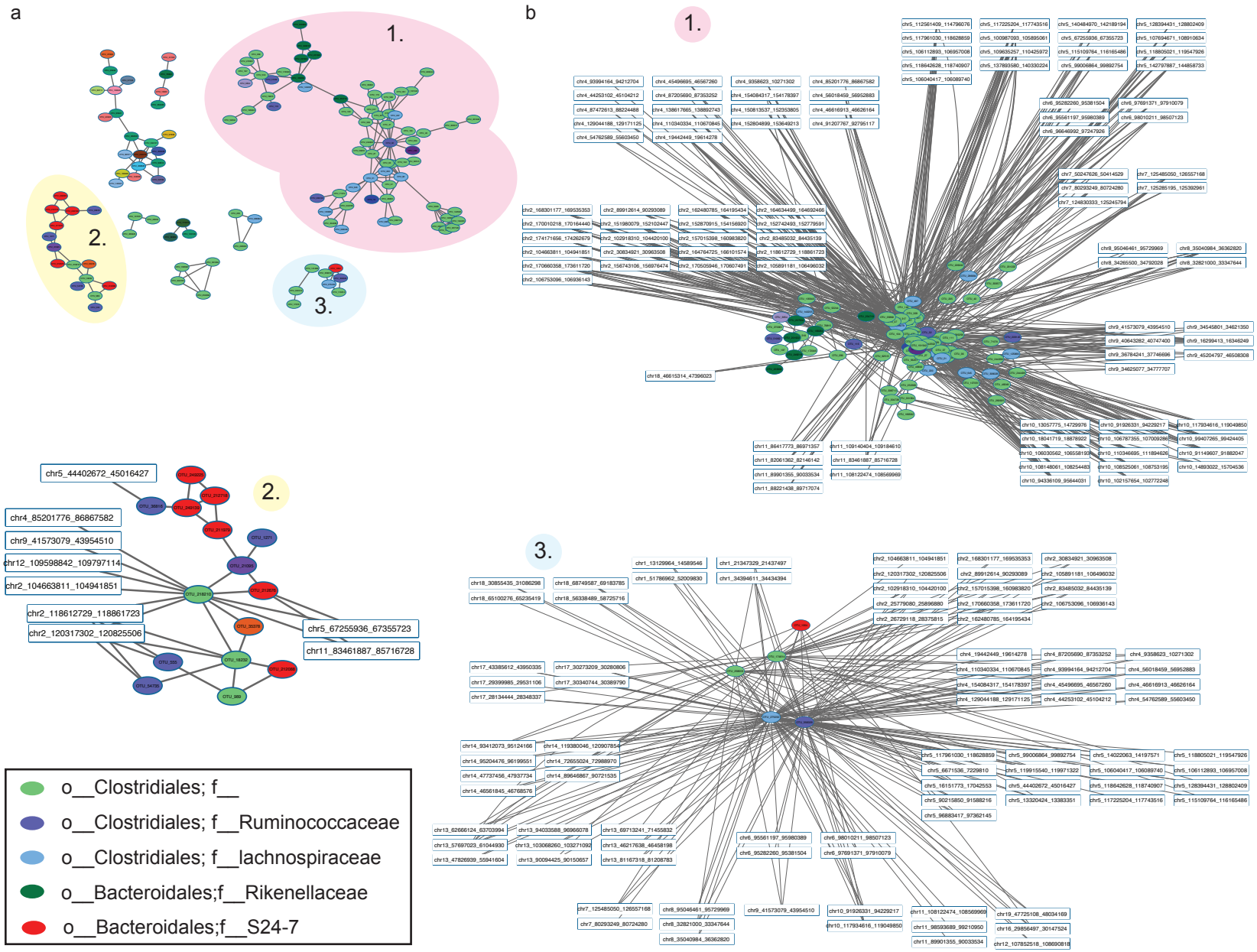


Supplementary Fig. 1. Effect of the built environment on the structure of the gut microbiome. a, Hierarchical clustering of mouse fecal microbial composition of the top 300 most abundant OTUs. Built environments (BE) are indicated in blue (BE2; barrier facility) and red (BE1; SPF facility). **b**, Stability of the gut microbial structure 2, 4, 6 and 8 weeks after transfer from BE1 and BE2 to BE3. Samples were separated by BE at birth (BE1: left; BE2: right). Relative abundances of the 20 most common families (representing >95% of the total data) over time are shown.

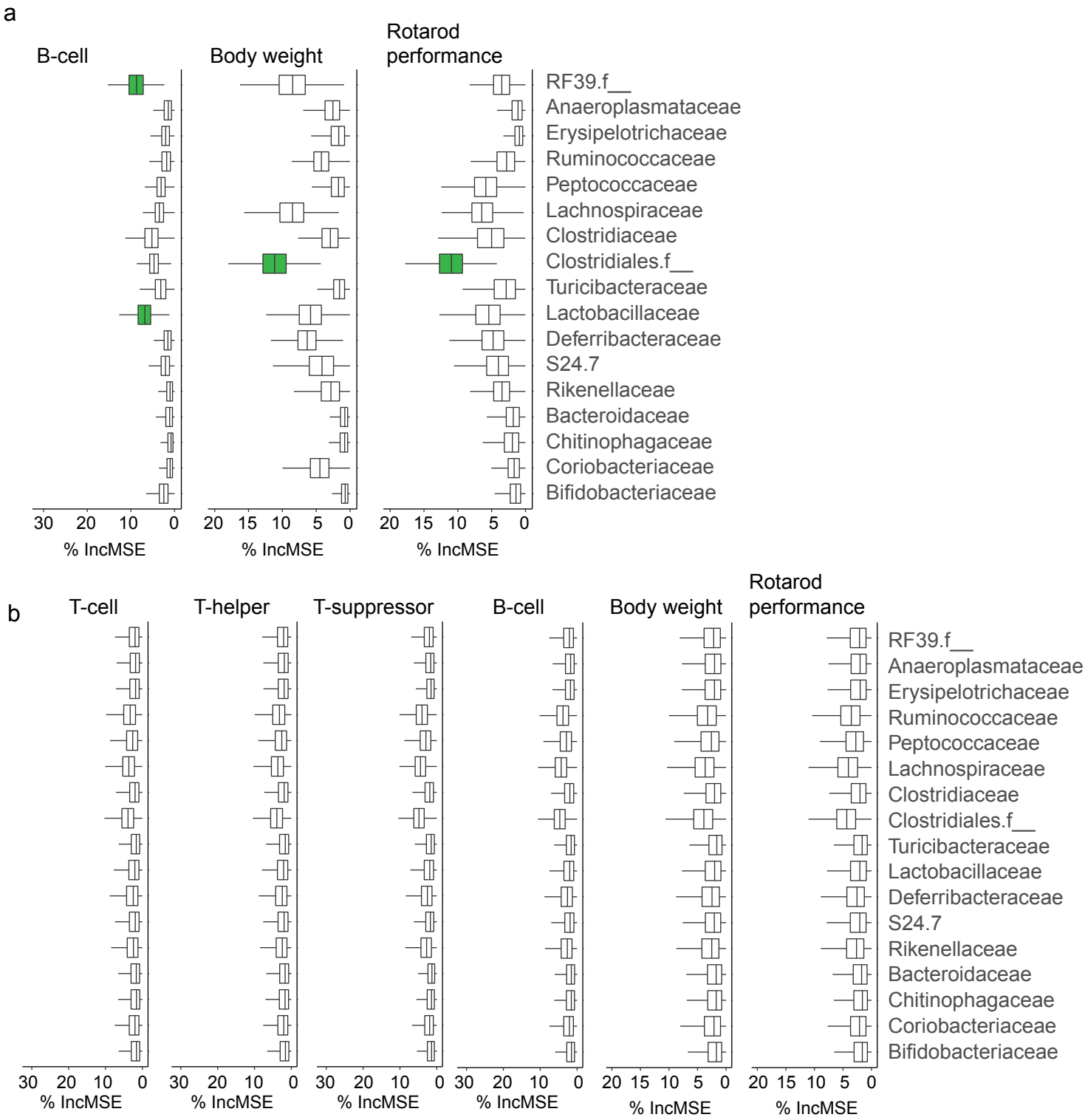




Supplementary Fig. 2. Polar dendrogram of OTU counts and metabolome profiles. Polar dendrograms using Bray-Curtis distance metric were generated for all mice based on normalized OTU counts (top) and metabolite abundance levels (bottom). Labels are colored by strain and include built environment at time of collection, the collection time-point at BE3 (2, 4, 6 or 8 weeks after arrival; microbiome only), the strain name (not including the original derivation location (Tau, Geni), but all from Unc) and sex.

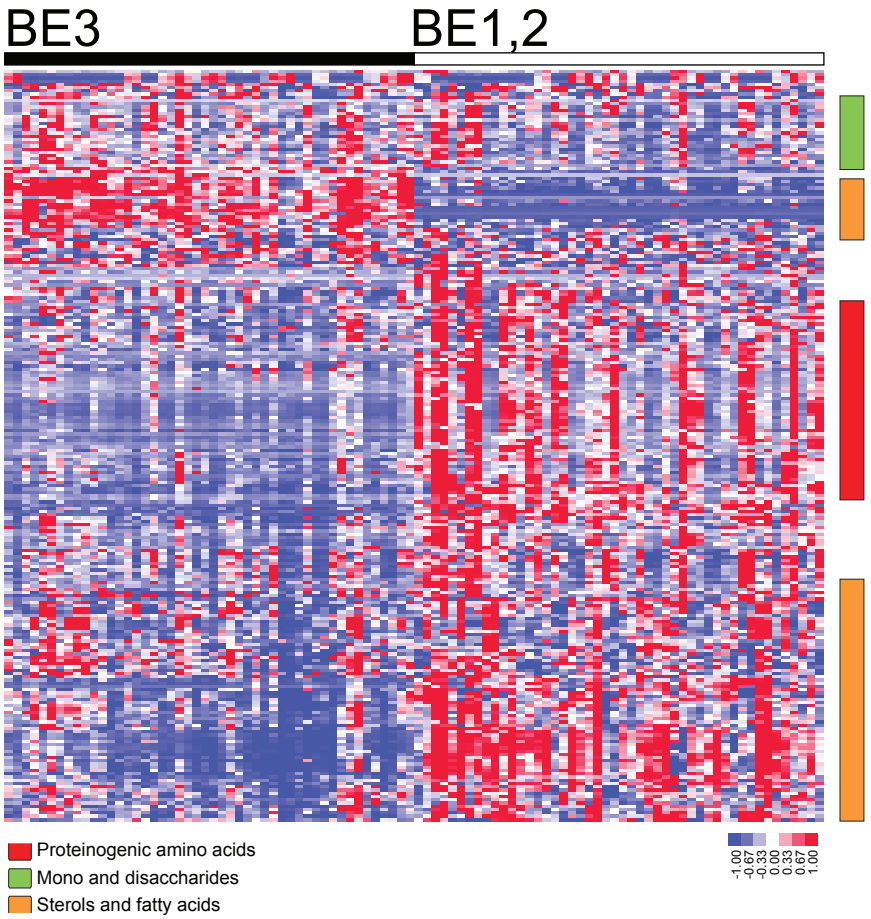


Supplementary Fig. 3. **Visual representation of OTU correlation network.** **a**, Network edges (gray lines) connect significantly correlated OTUs (ovals; colored at the family level). Correlation (>0.5) based on Spearman rank (p<1E-10). **b**, The genomic intervals with significant linkage ($-\log_{10}$ (p-value)>6) to individual OTUs are shown within three individual correlation sub-networks.

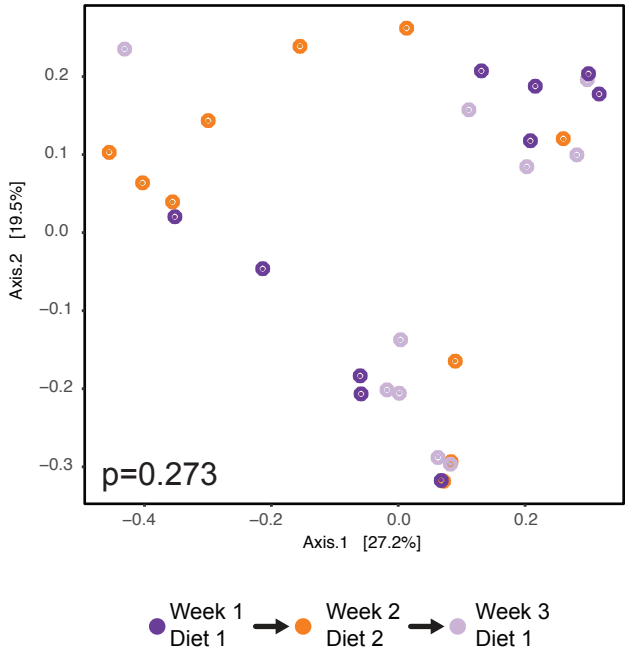


Supplementary Fig. 4. Association of microbial abundance with host phenotypes. a, Random forest analysis to assess the association between microbial abundance at the family level and mouse peripheral blood B cell counts, body weight and rotarod performance. Significant associations are indicated in green ($p<0.05$). However, these associations were not significant after adjusting for multiple comparisons using Benjamini-Hochberg **b,** Random permutation analysis to assess significance of association between microbial abundance and host phenotypes. Upper whiskers extend to the highest value that is within 1.5 * inter-quartile range (IQR). The lower whiskers represent the lowest value within 1.5 * IQR.

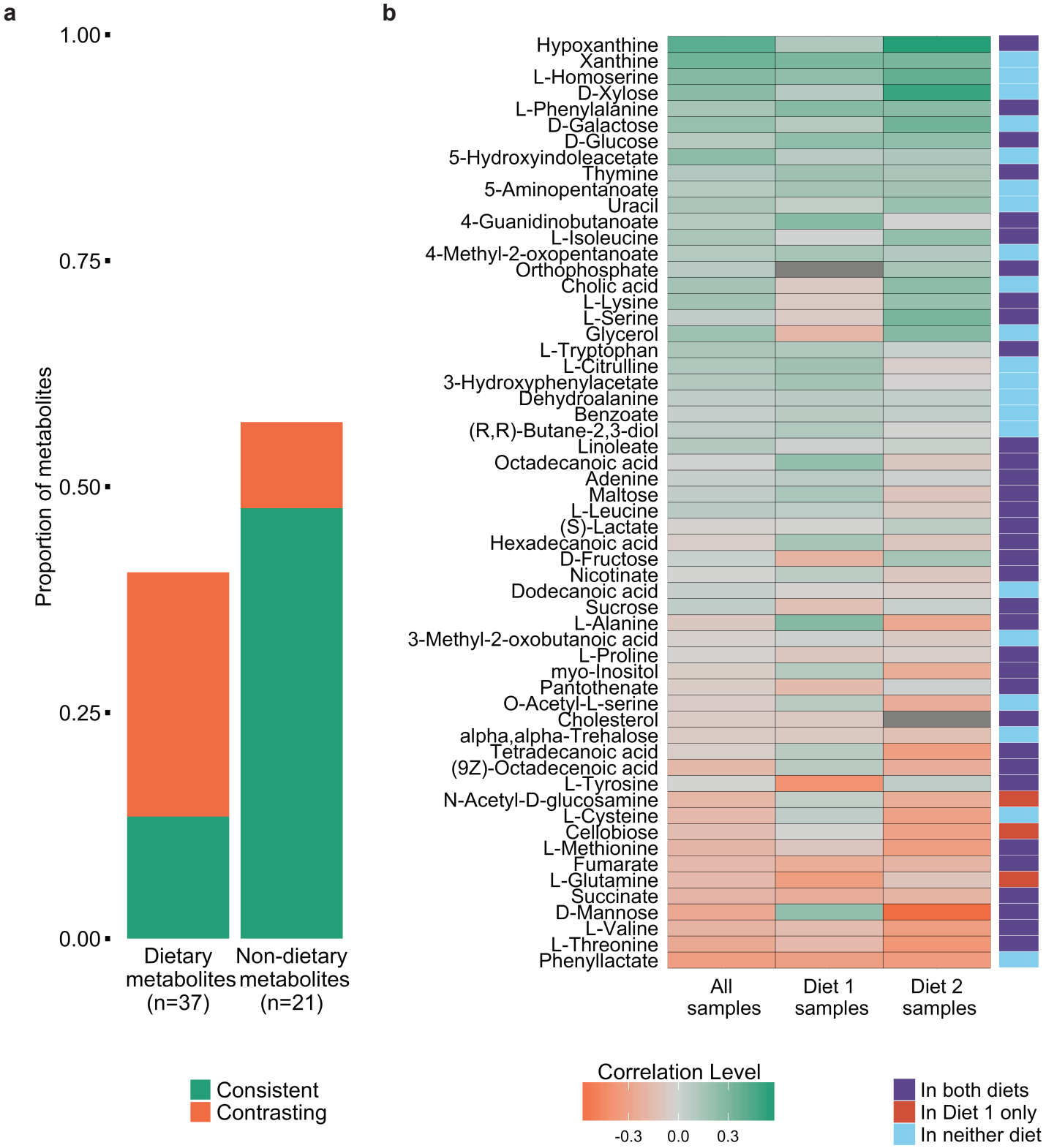
a



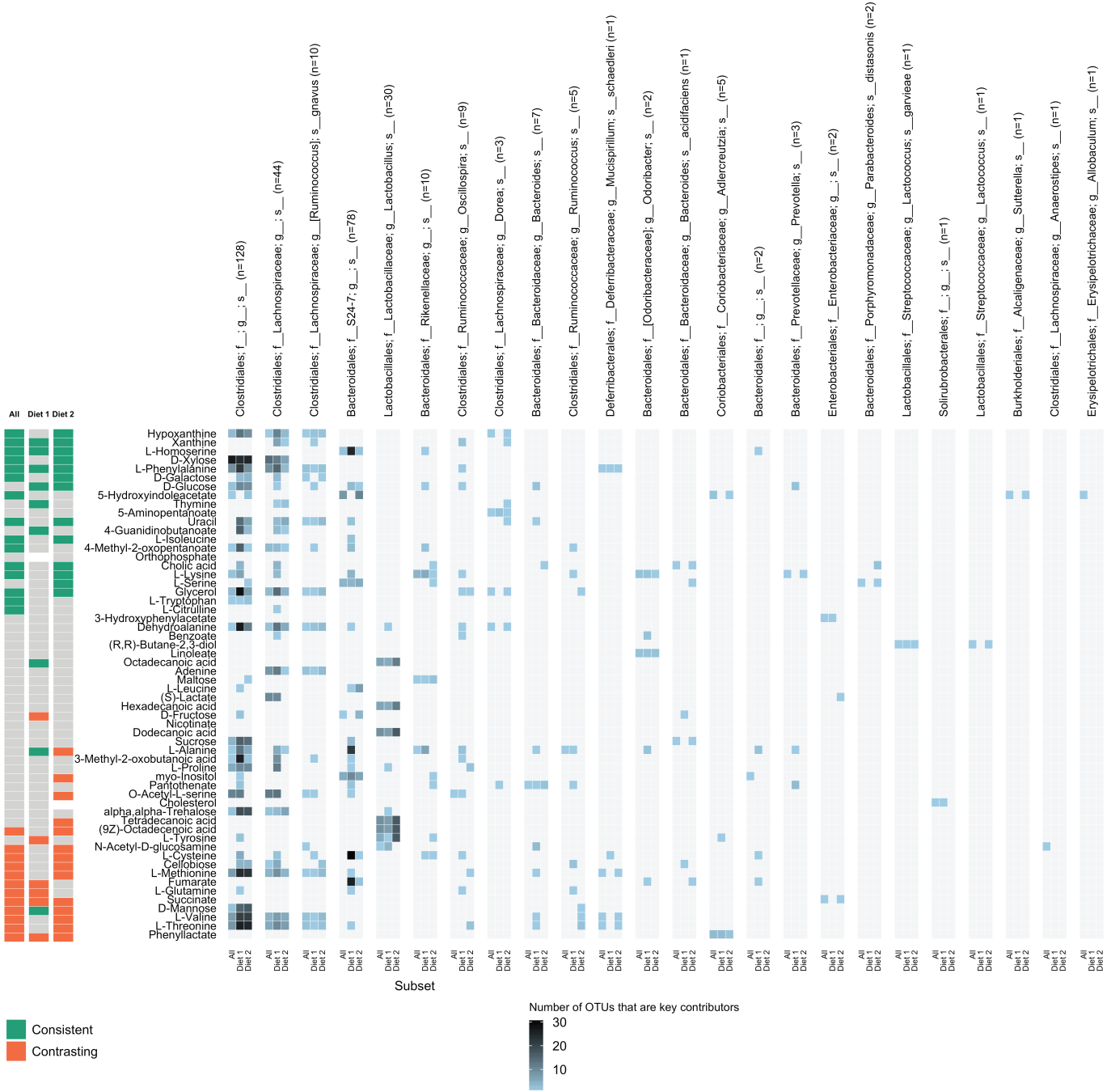
b



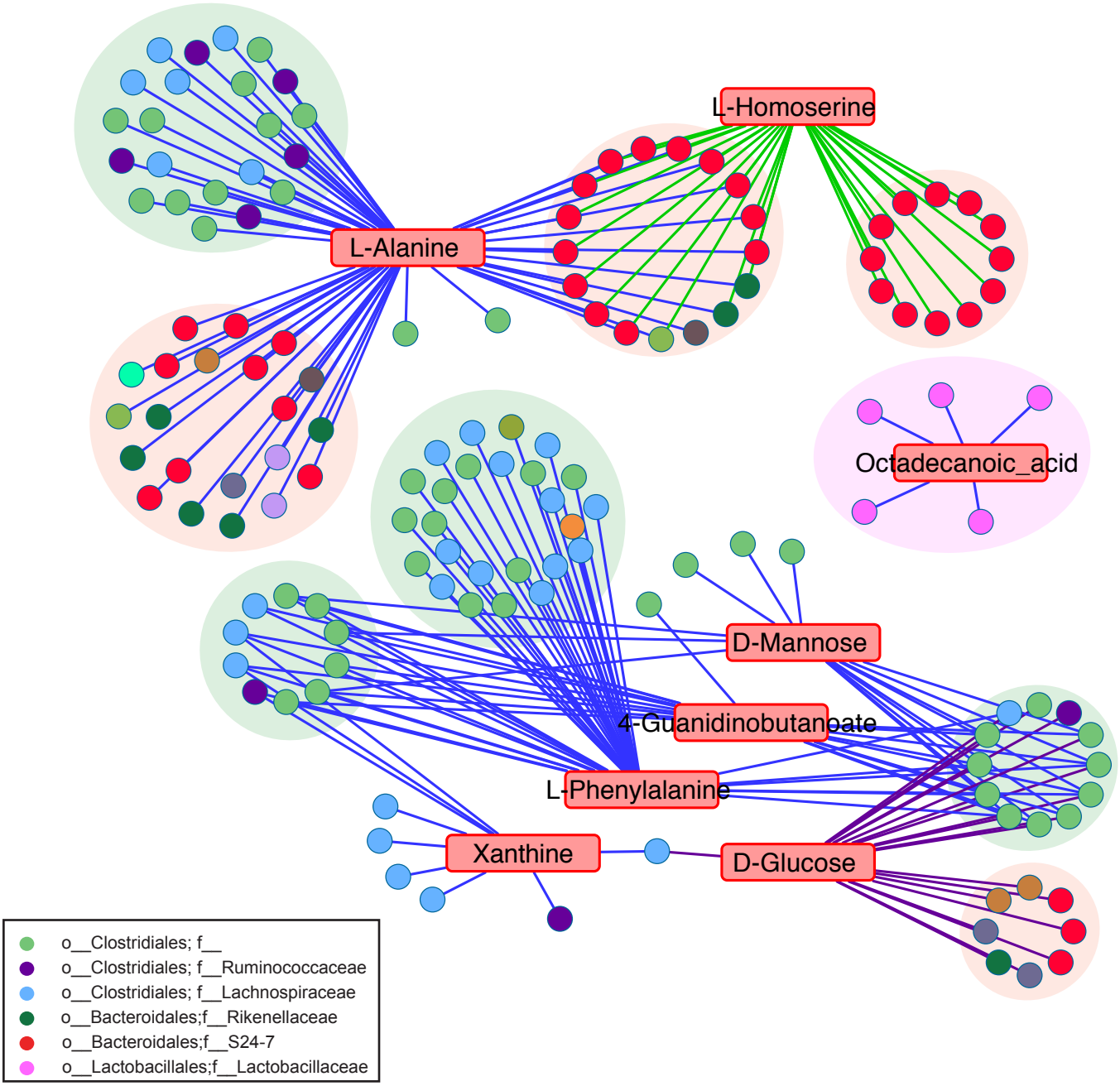
Supplementary Fig. 5. Metabolite profiles are controlled by the local diet.
a, Hierarchical clustering of fecal metabolite levels in 24 CC strains maintained on two distinct diets (BE1 and 2; Labdiet Picolab 3500 and BE3; Labdiet Prolab 5053). **b,** Microbial abundance was measured in fecal samples of five CC strains (males and females were analyzed separately for each strain) maintained for one week on standard chow (Labdiet Picolab 5053; Diet 1), one week on autoclaved chow (Labdiet Prolab 3500; Diet 2) followed by one week on standard chow (p=0.273, R2 = 0.0079 between two diets, ADONIS).



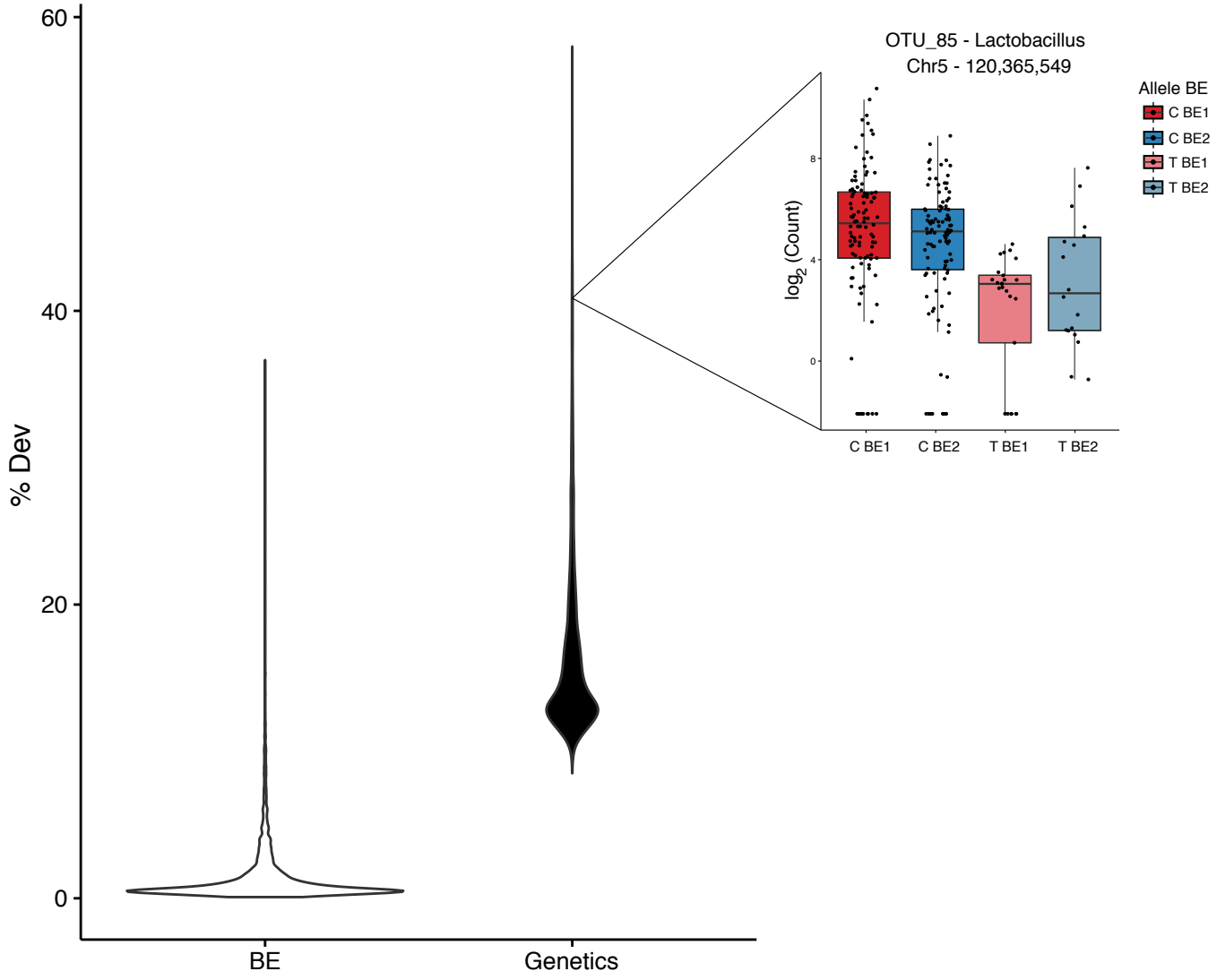
Supplementary Fig. 6. Shifts in community metabolic capacity explain observed variation in non-dietary metabolites. a, Proportions of dietary and non-dietary metabolites whose measured variation is consistent (positively correlated) or contrasting (negatively correlated) with community metabolic potential (as predicted by metabolic modeling). **b**, Correspondence between variation in community metabolic potential and variation in measured metabolite concentration across all samples and across sample subsets from each diet and facility. The far right bar indicates whether each metabolite was detected in the chow from either facility.



Supplementary Fig. 7. Potential taxonomic contributors to metabolic modeling-based metabolite predictions. The three bars on the left indicate whether each metabolite's variation was consistent or contrasting with the predicted community metabolic potential across all samples and across sample subsets from each facility (BE1 and BE2: Diet 2; BE3: Diet 1) and diet. The right grid shows the number of OTUs in each taxonomic category identified as potential contributors to community metabolism, based on consistent variation patterns and metabolic capacity.



Supplementary Fig. 8. Potential taxonomic contributors to metabolite abundance for mice housed at BE3. Potential taxonomic contributors to metabolite variation for mice maintained on Labdiet Prolab 5053 (BE3). Individual OTUs shown (circles; colored at the family level) are those whose metabolic capacity and variation across samples are consistent with the entire community metabolic potential and with measured variation in the linked metabolites (squares). Green, orange and pink clouds behind OTU sub-networks indicate Clostridiales, Bacteroidales or Lactobacillales enrichment. Edge color indicates whether a given OTU potentially impacts a certain metabolite variation via synthesis (blue edges), degradation (green edges), or both (purple edges).

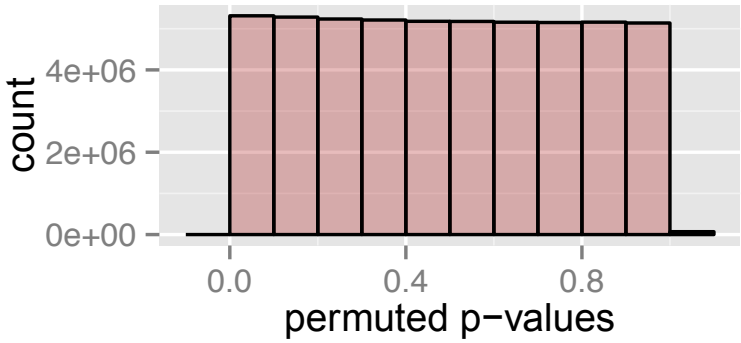


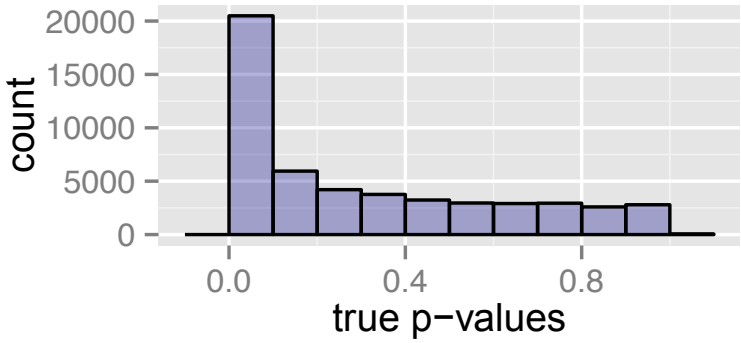
Supplementary Fig. 9. Relative contribution of built environment (BE) and genetics to microbial abundance. The percent deviance (% Dev) of microbial abundance across CC strains explained by BE and genetic factors are shown. Genetic factors contribute more than built environment (BE) to the total microbiome variation. Inset shows an example of the effect of a SNP on chromosome 5 on Lactobacillus abundance, where BE has no effect. Upper whiskers extend to the highest value that is within 1.5 * inter-quartile range (IQR). The lower whiskers represent the lowest value within 1.5 * IQR.

Supplementary Fig. 10. Summary plots from permutation of 16S data to obtain FDR estimates for QTL analyses. We performed 1,000 permutations of strain identifiers and then computed the Mann-Whitney U at each SNP for (A) data combined at the family level, (B) 15 OTUs in the lower and (C) upper quintiles of sum of p-values across all SNPs and (D) the 15 OTUs with the lowest overall sum of p-values.

A

f__Bifidobacteriaceae





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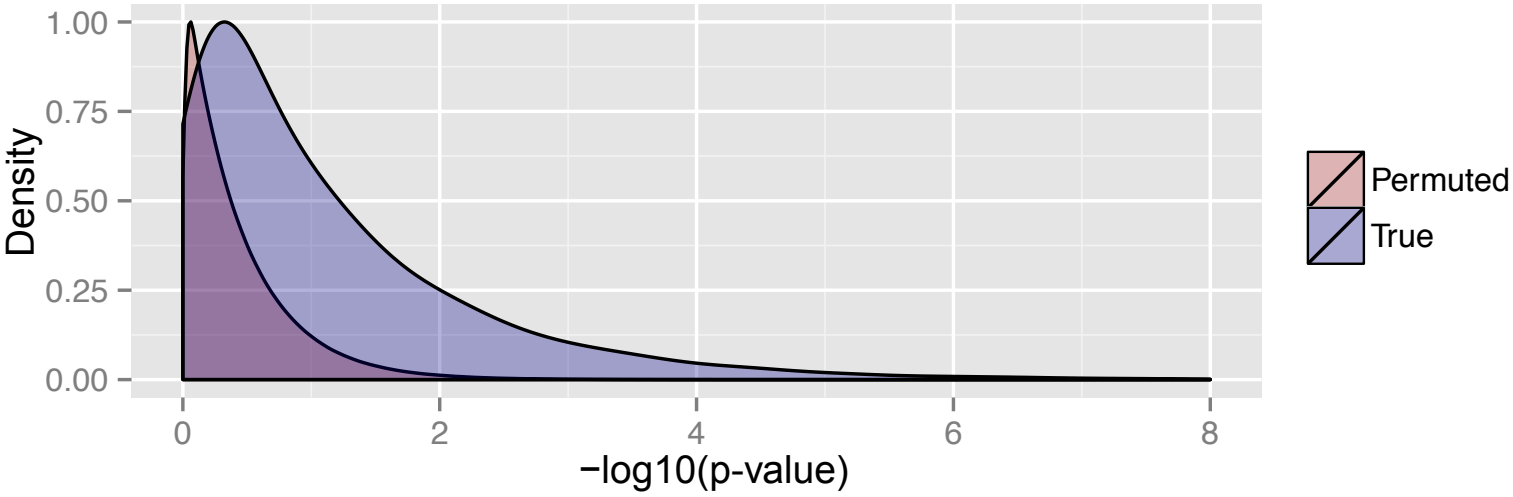

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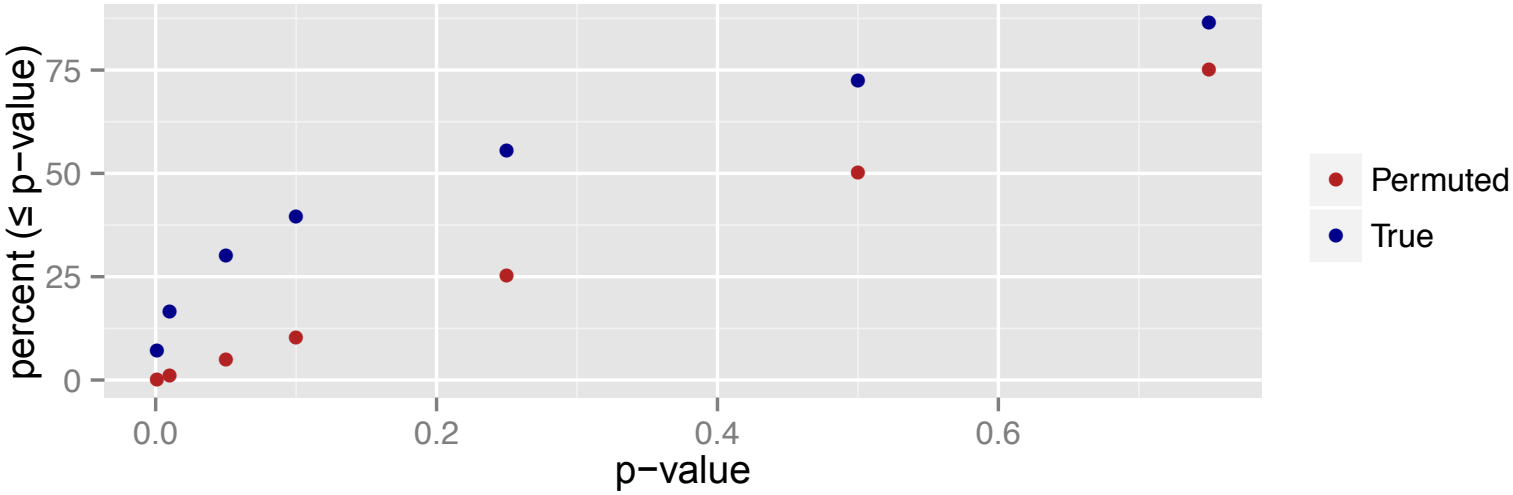
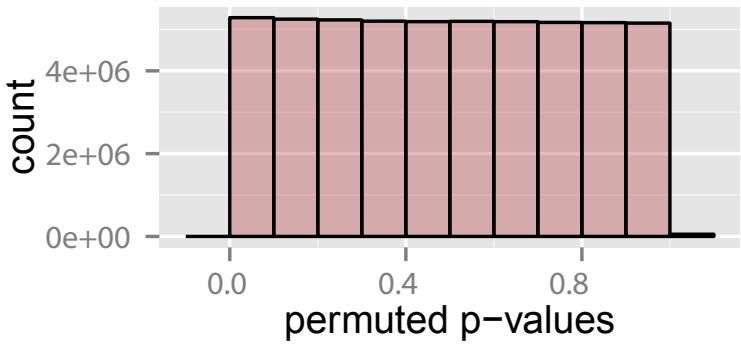
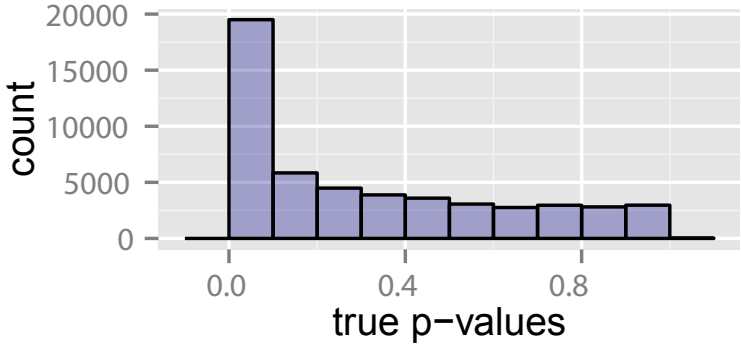
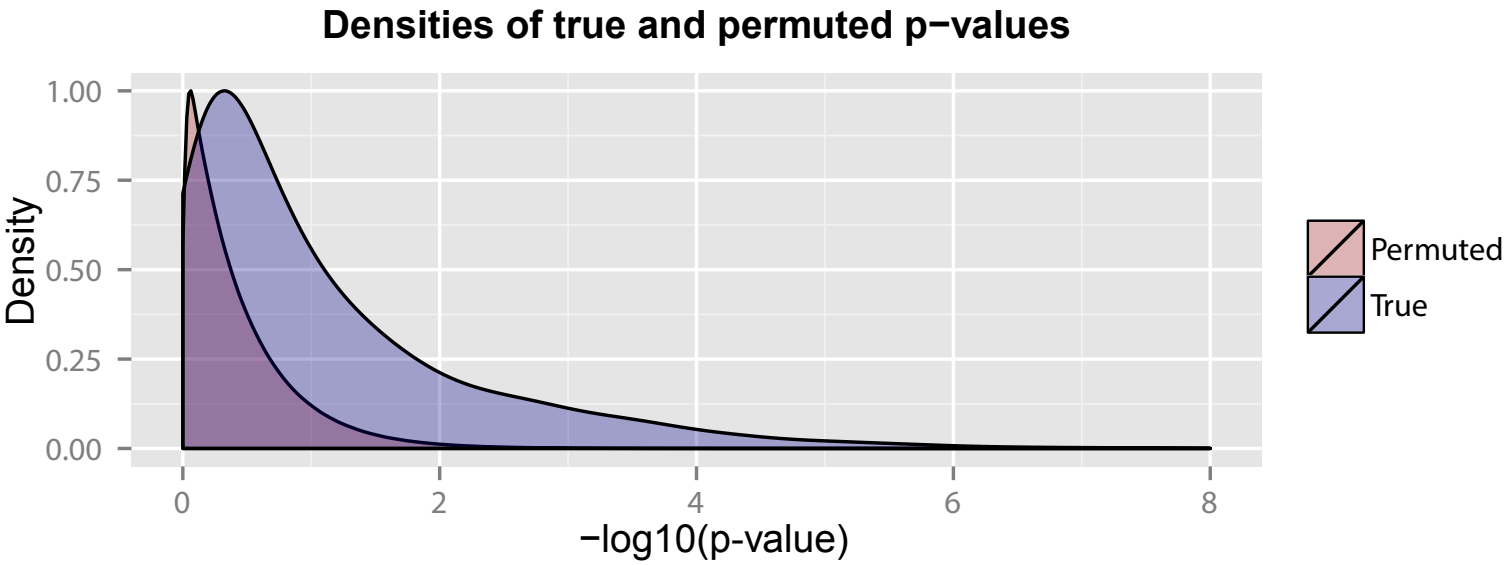


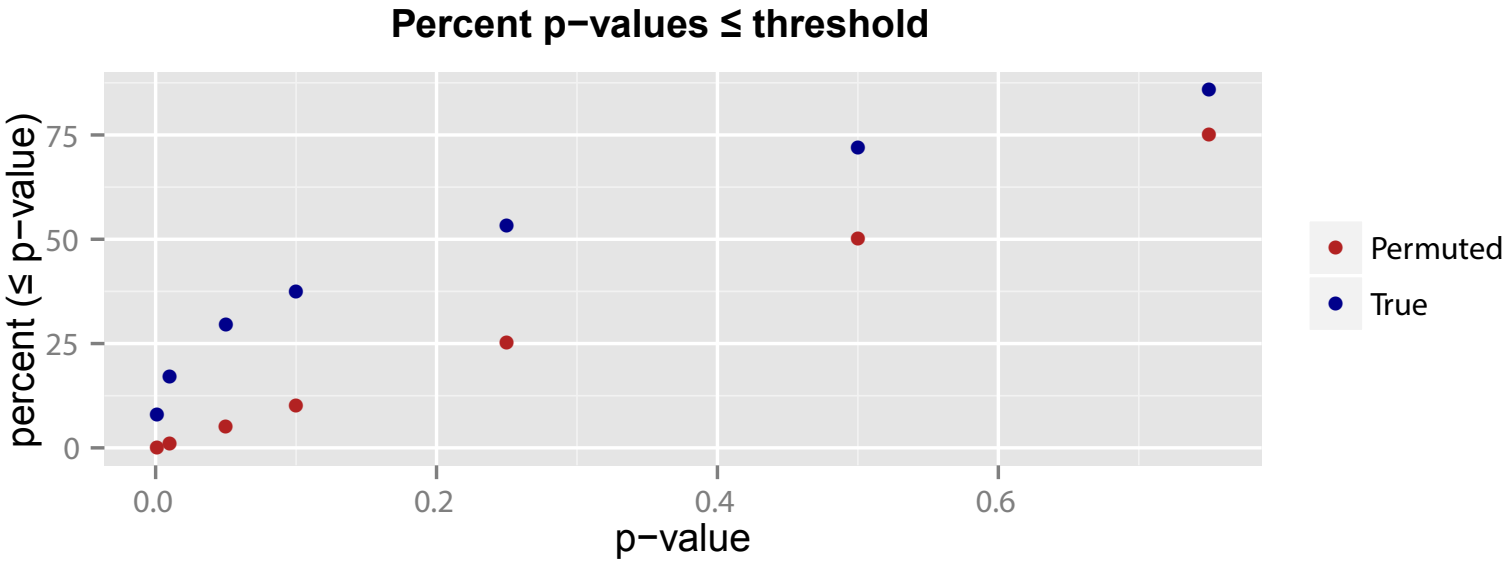
A

f__Rikenellaceae









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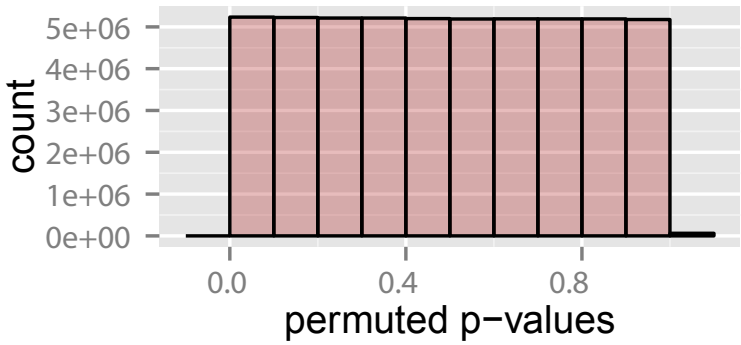

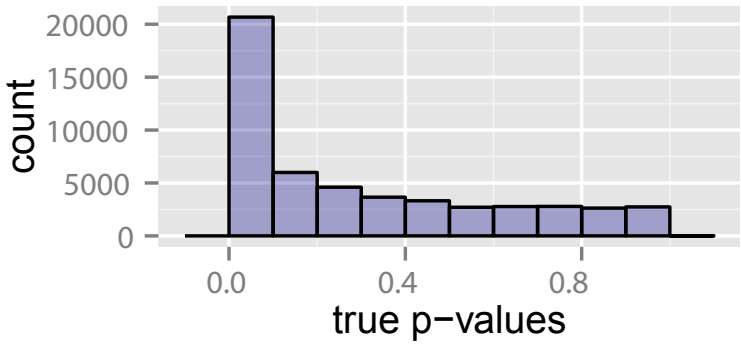

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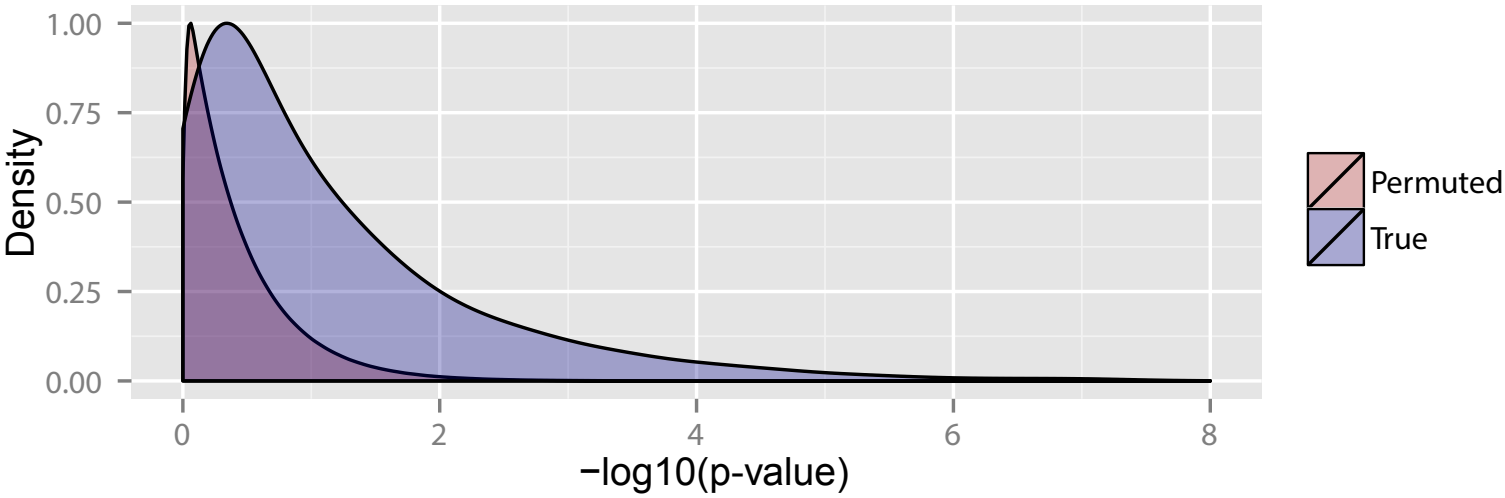

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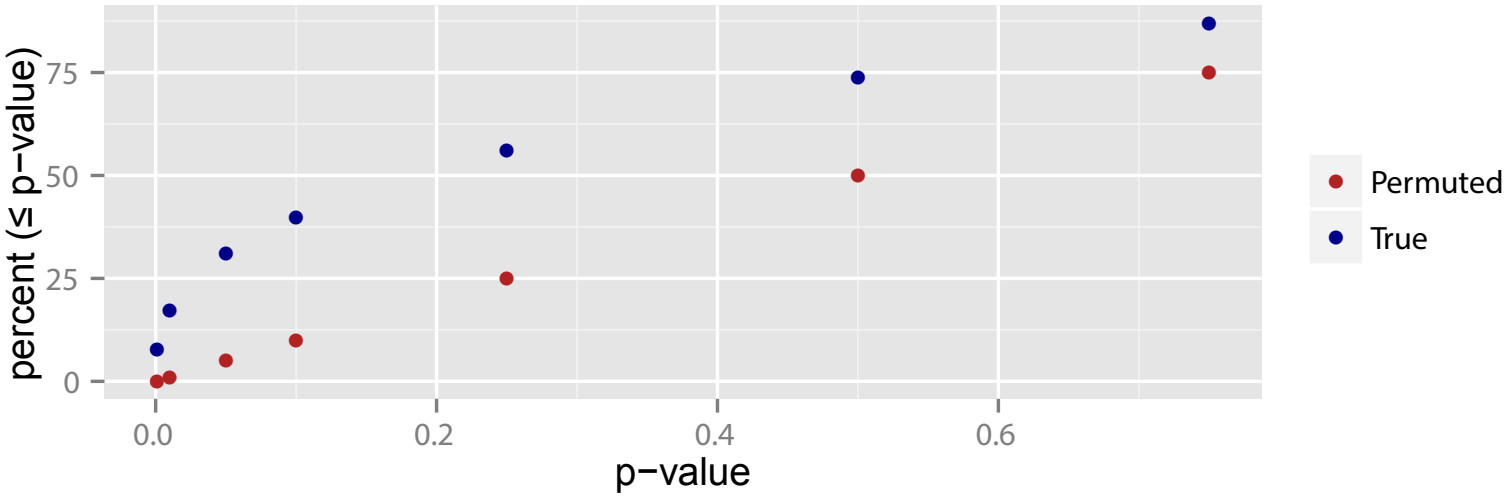

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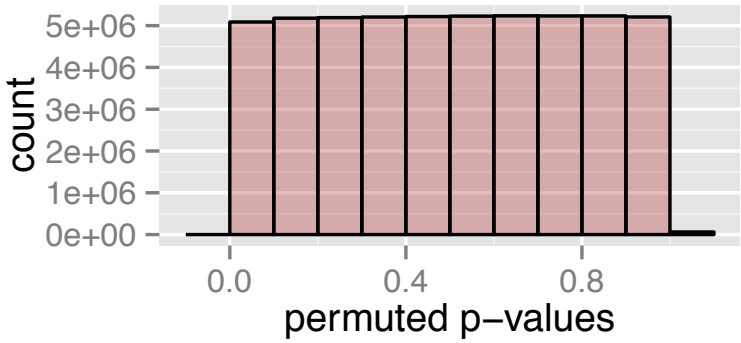

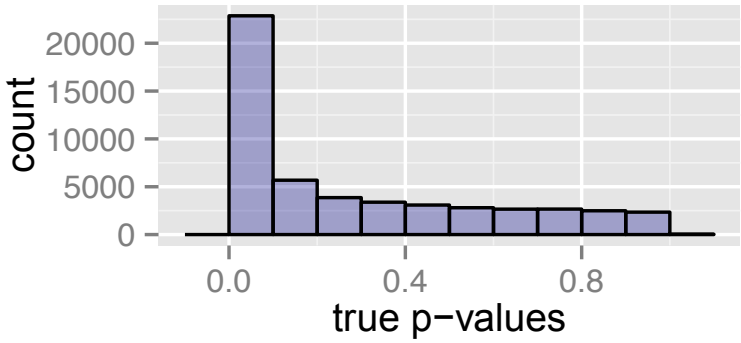

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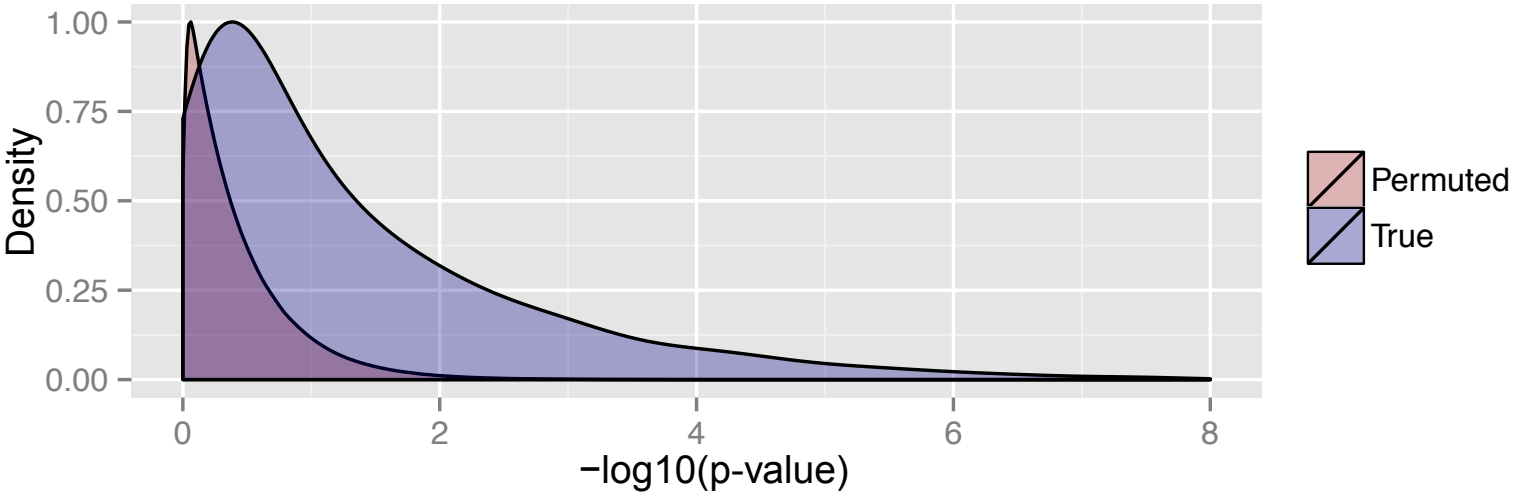

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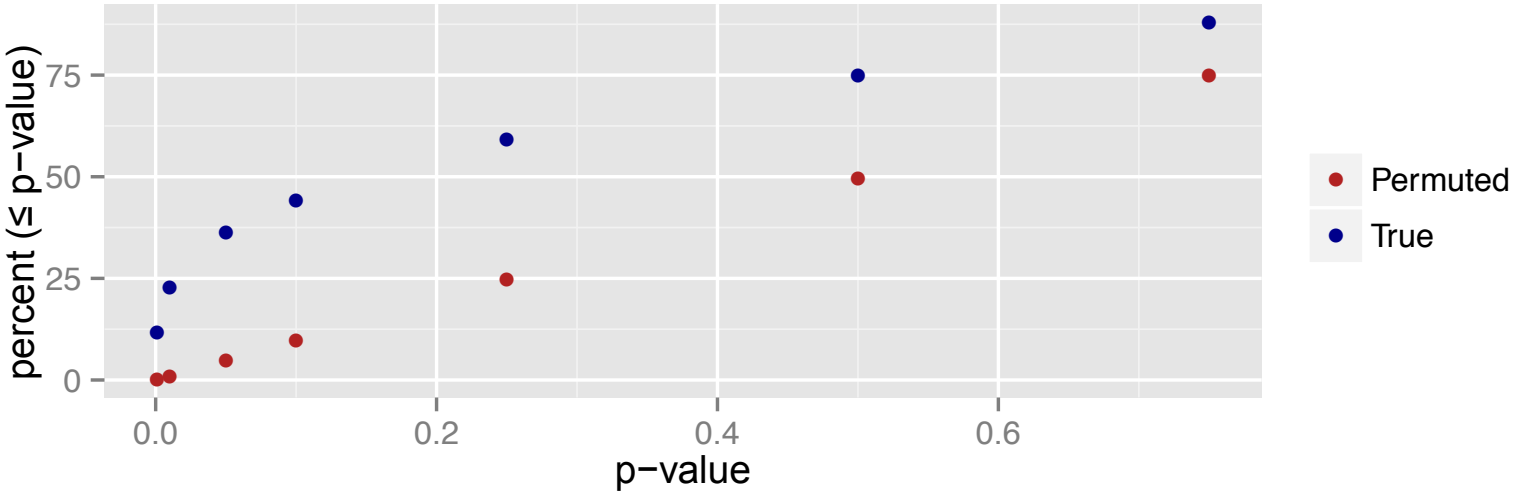

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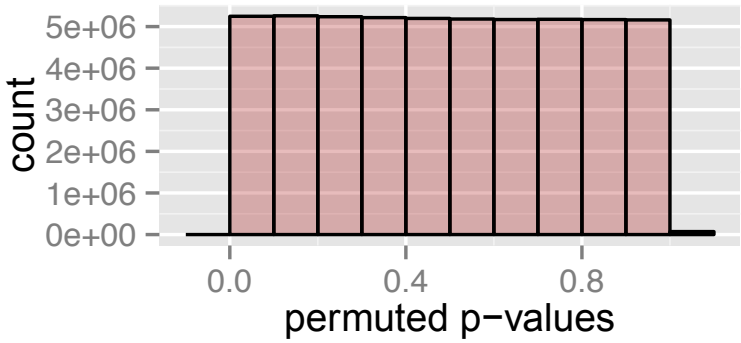

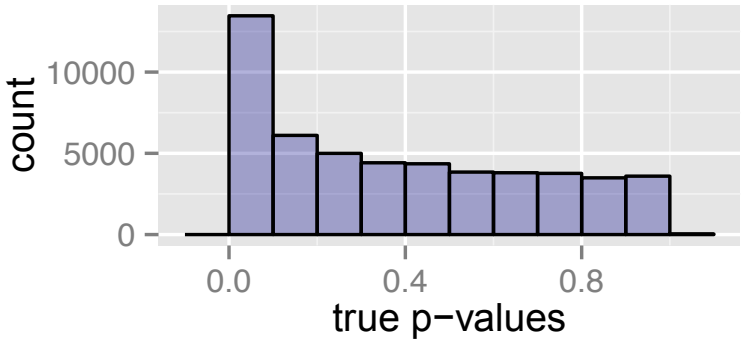

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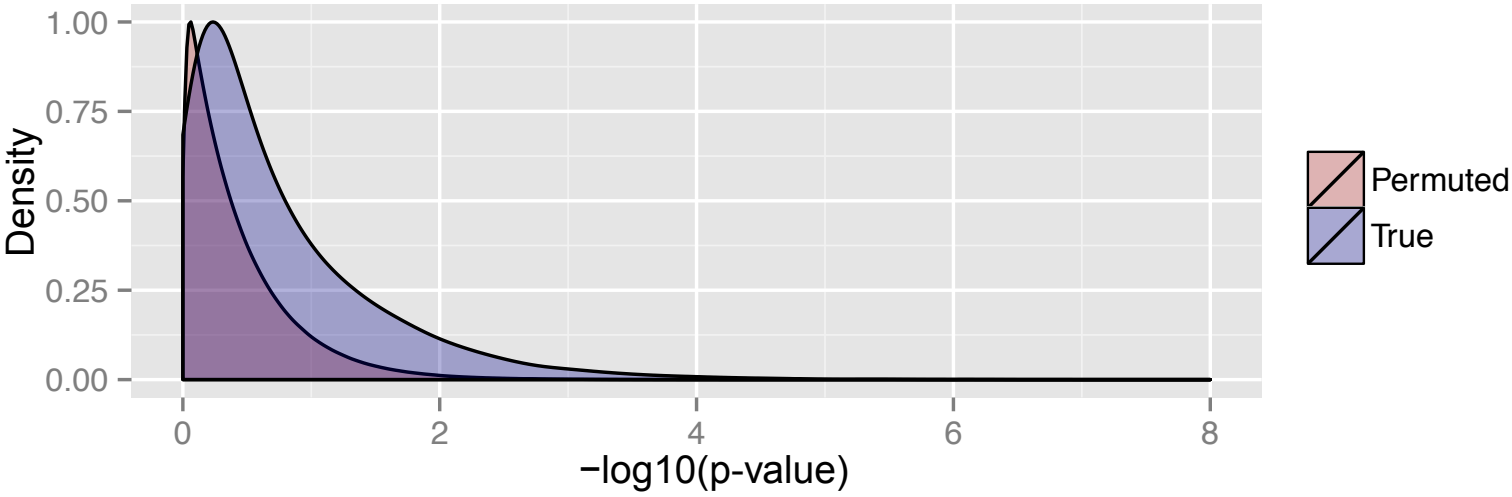

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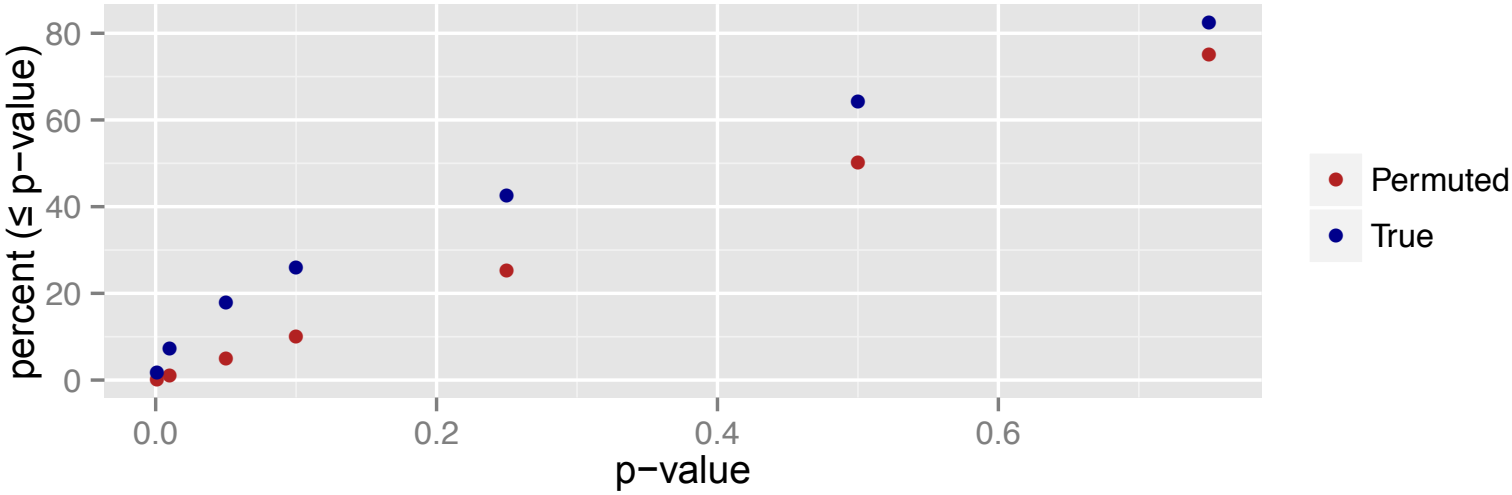

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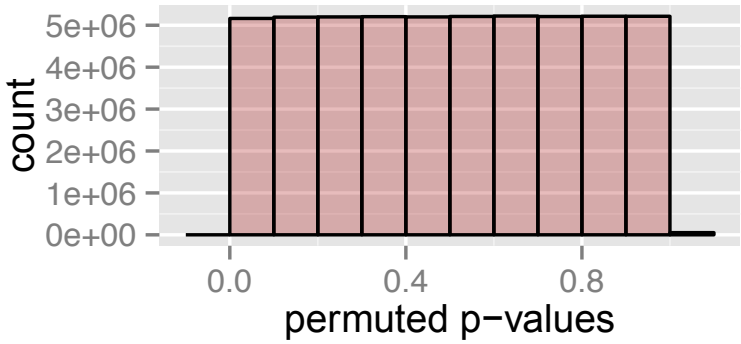

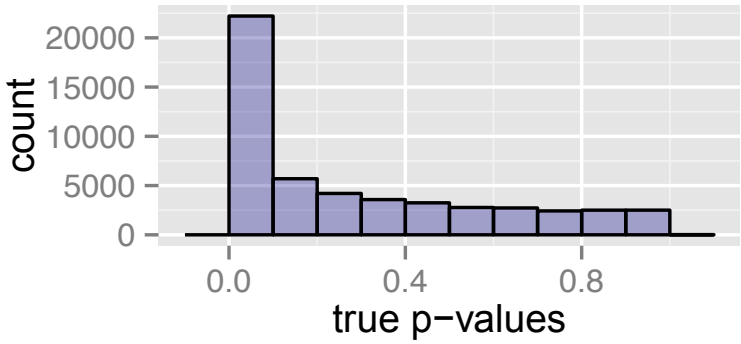

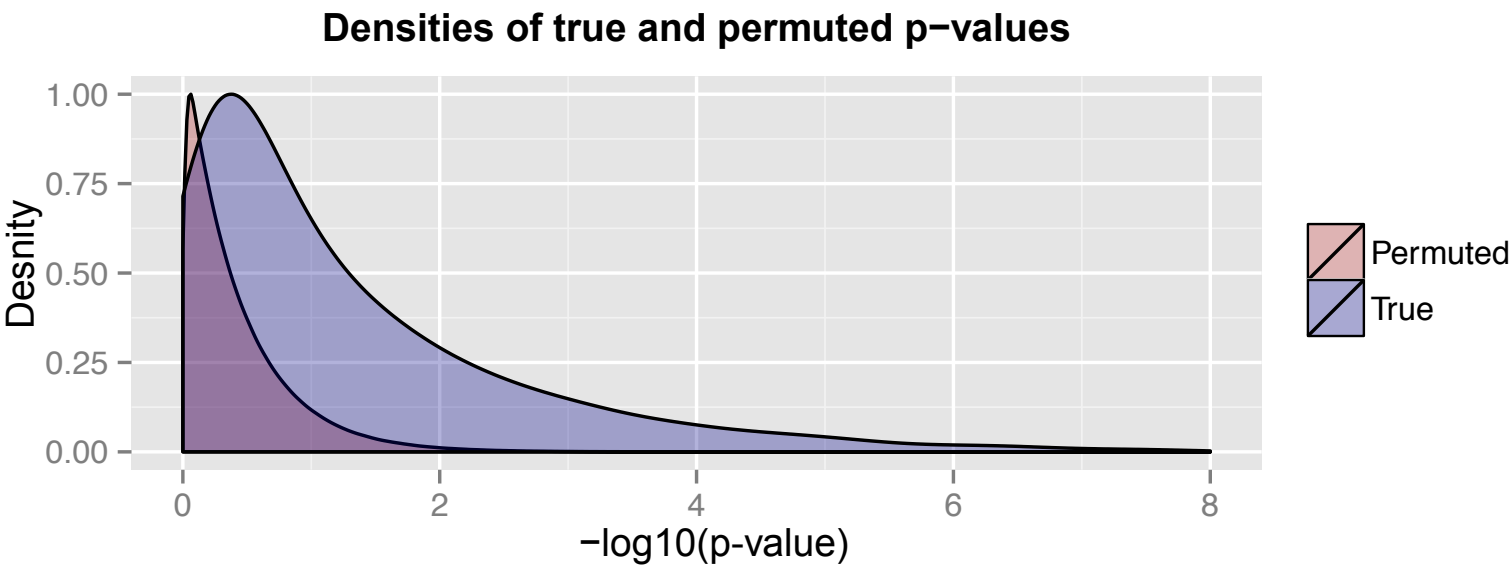

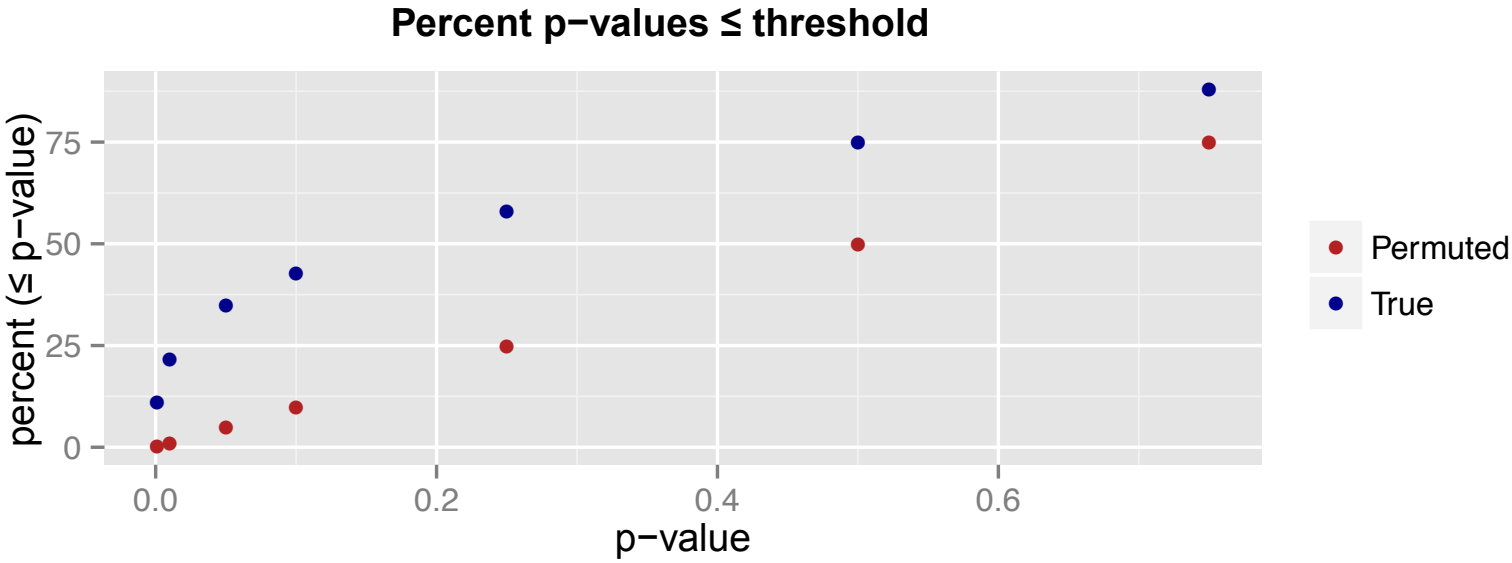

A

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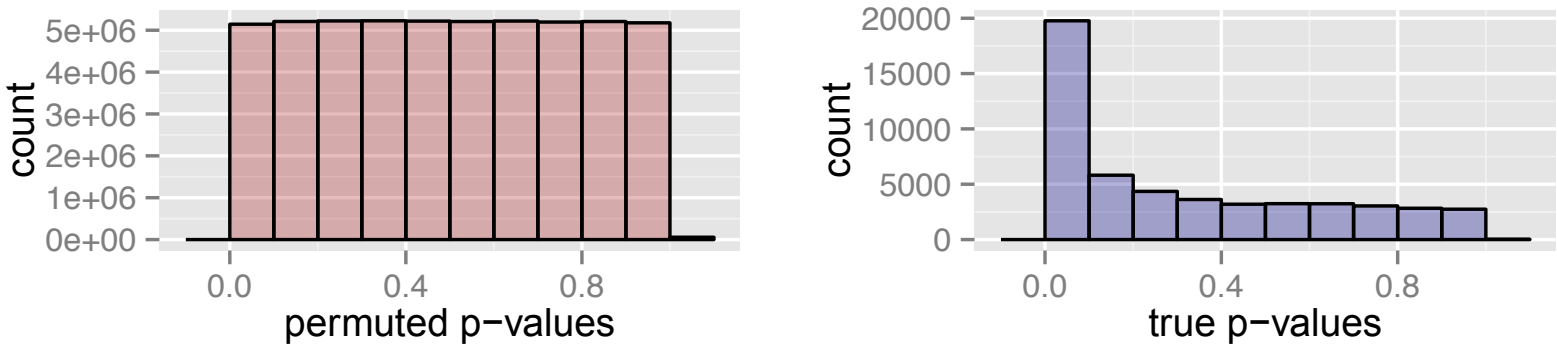

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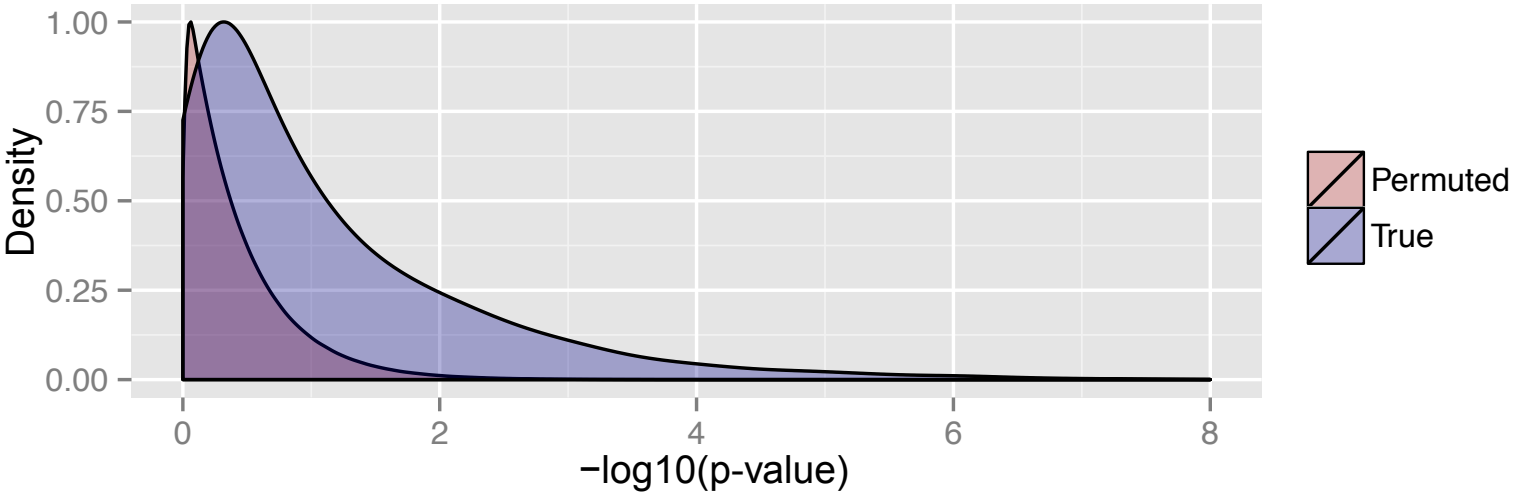

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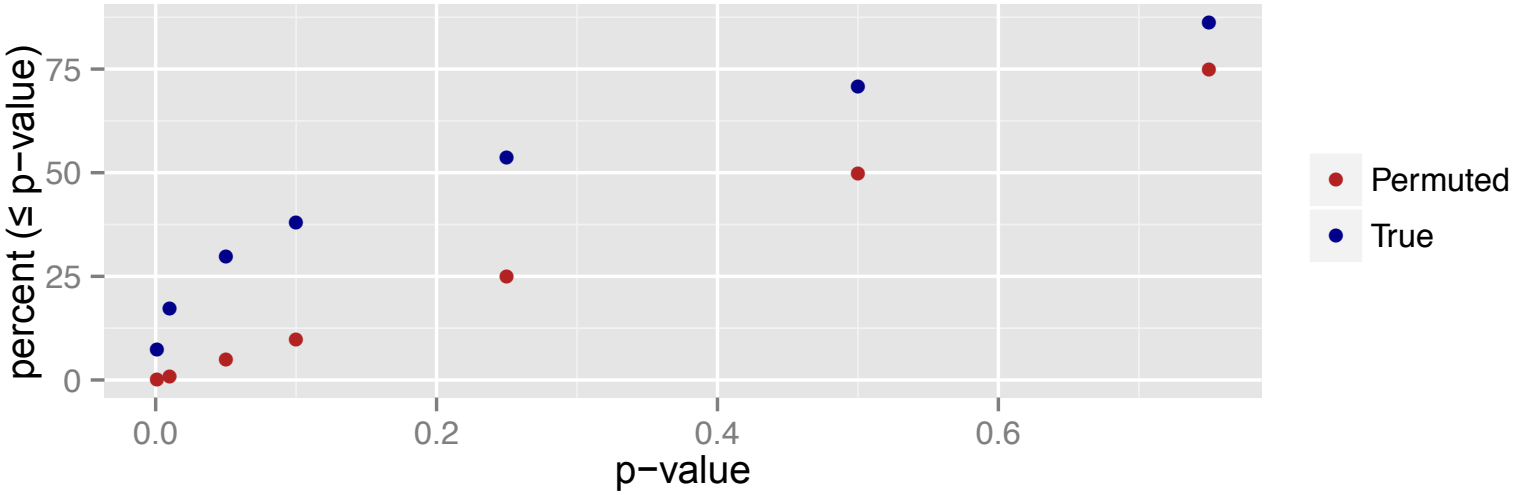
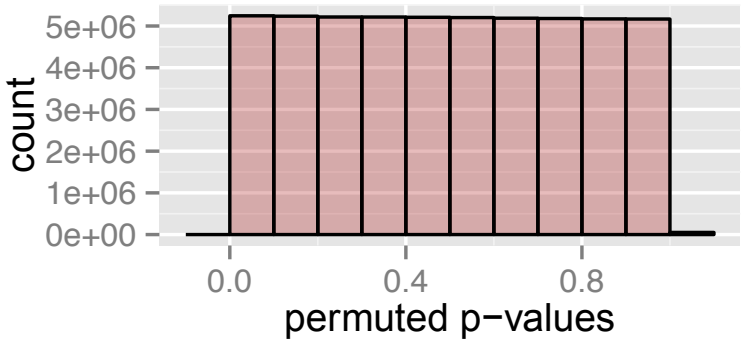
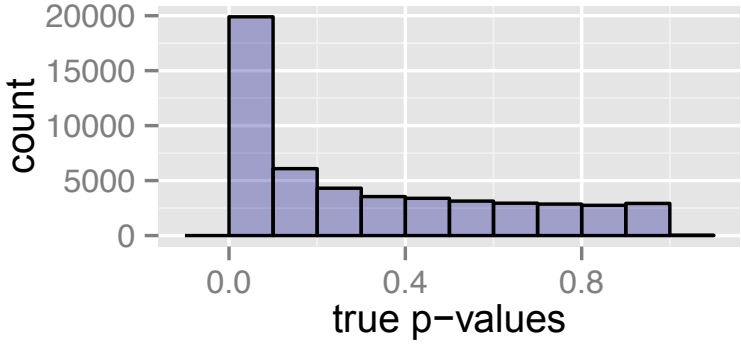


A

f__Lachnospiraceae





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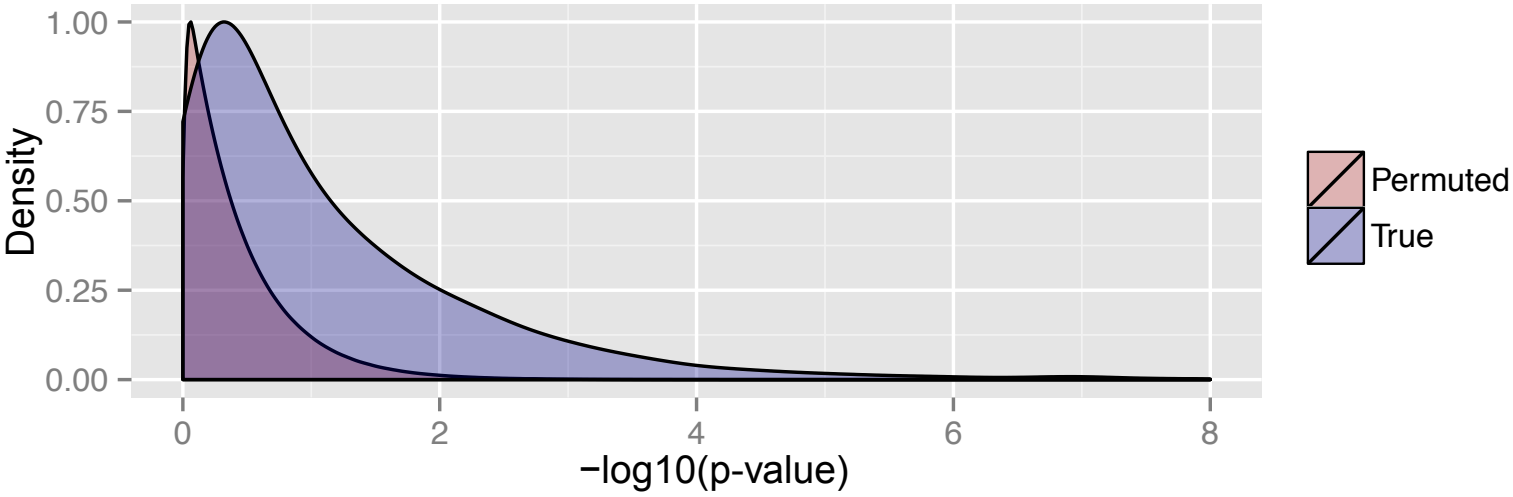

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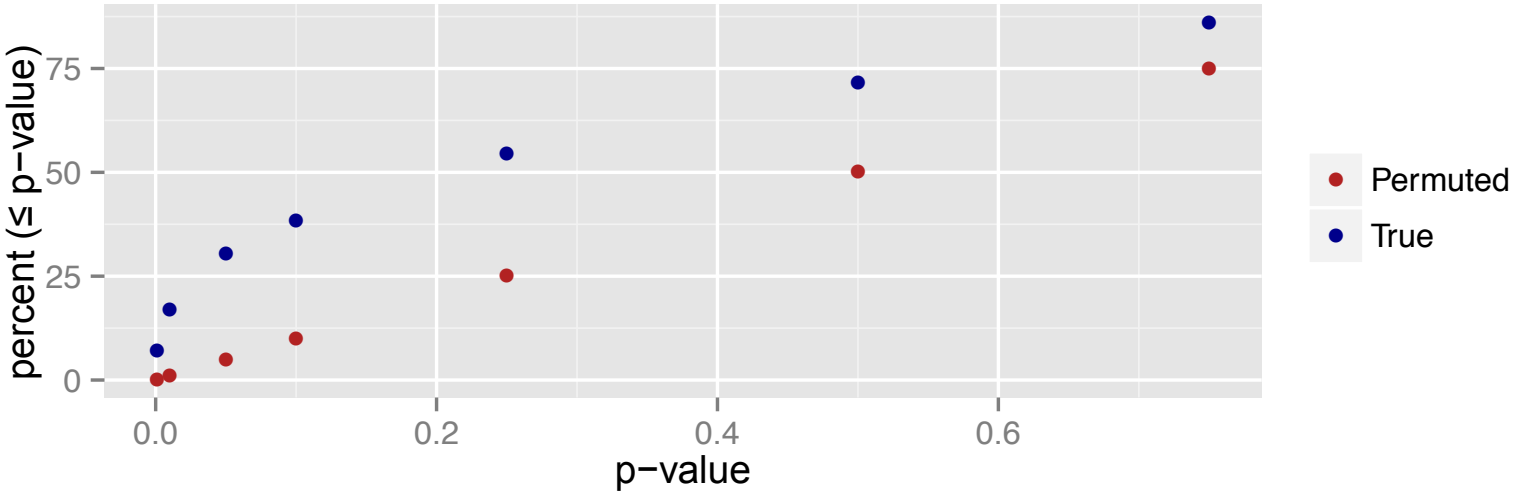
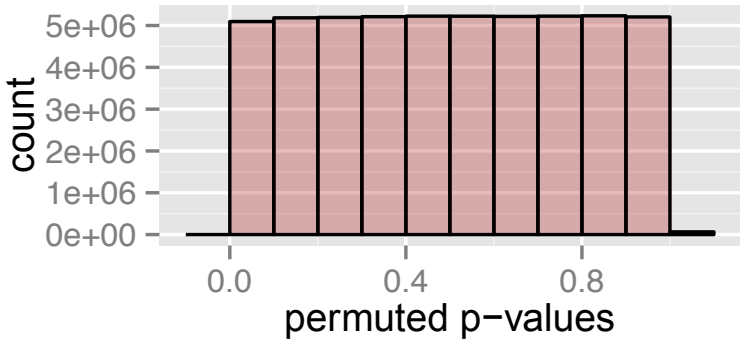
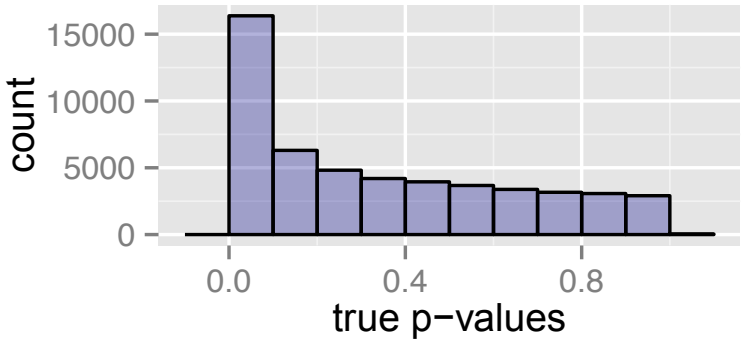


A

f__Peptococcaceae





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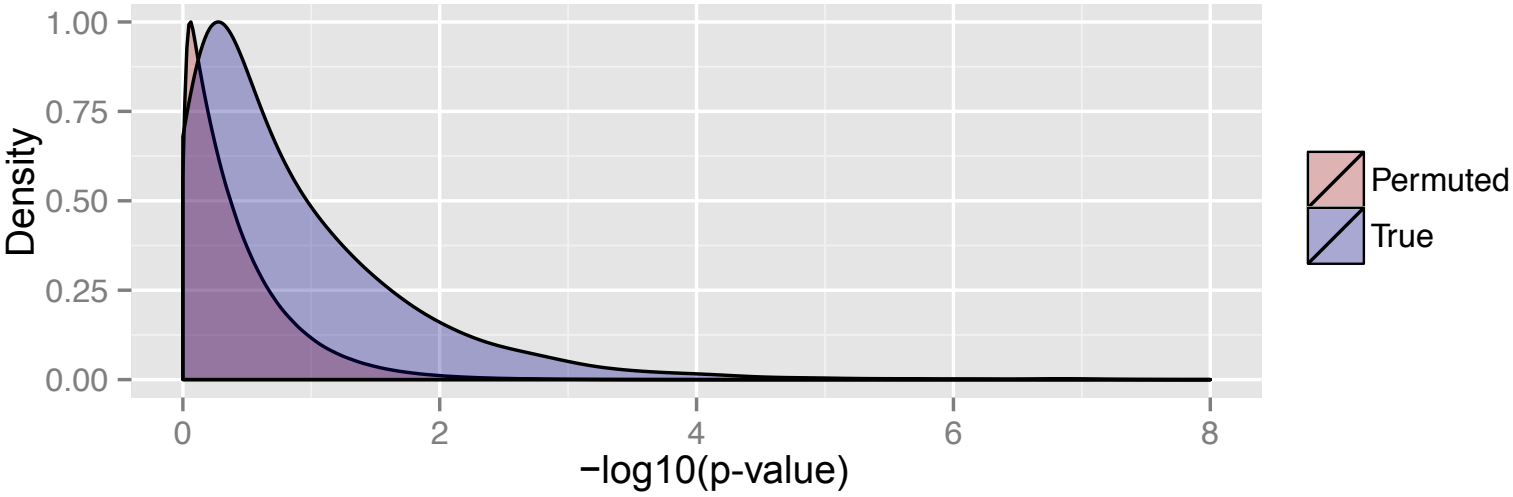

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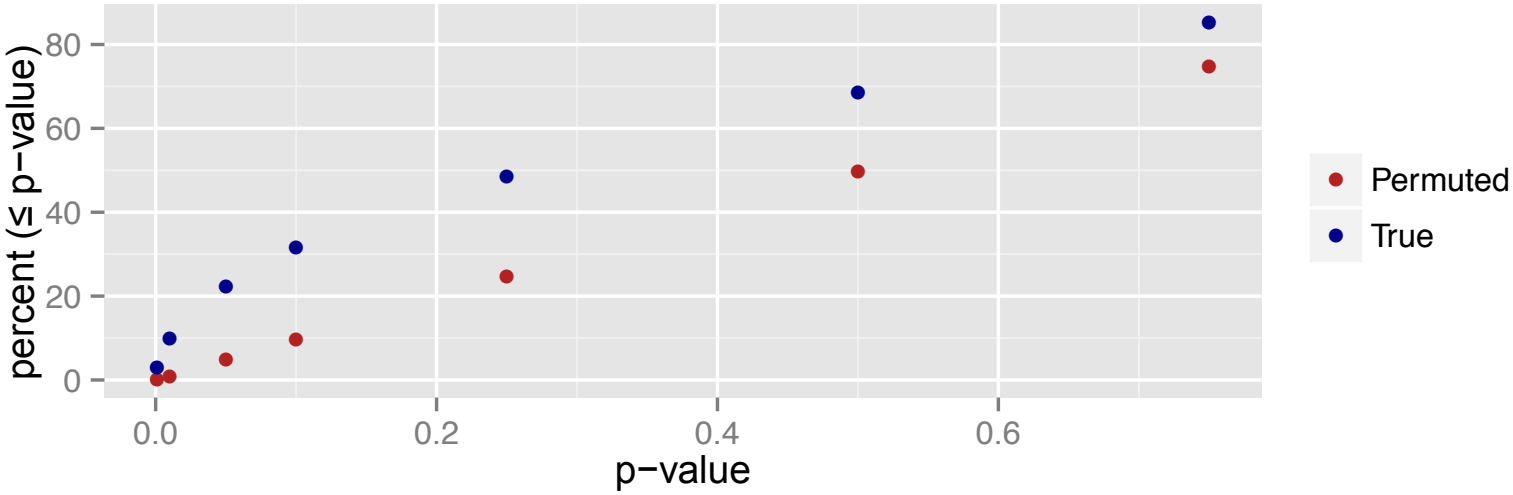
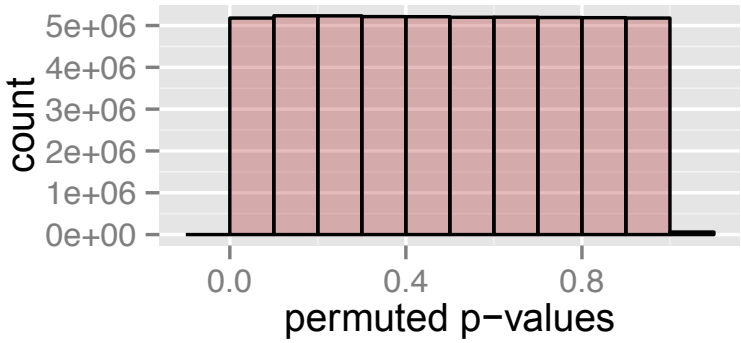
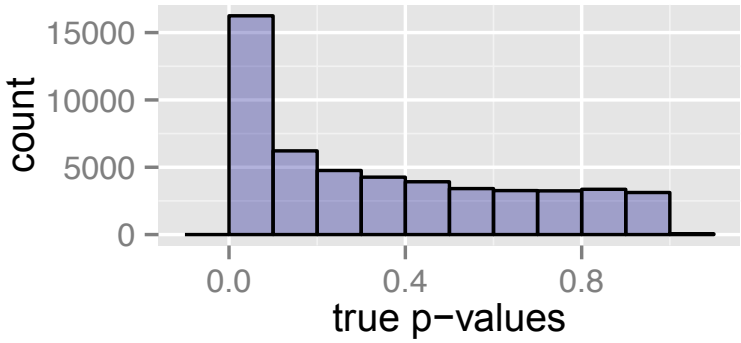


A

f__Ruminococcaceae





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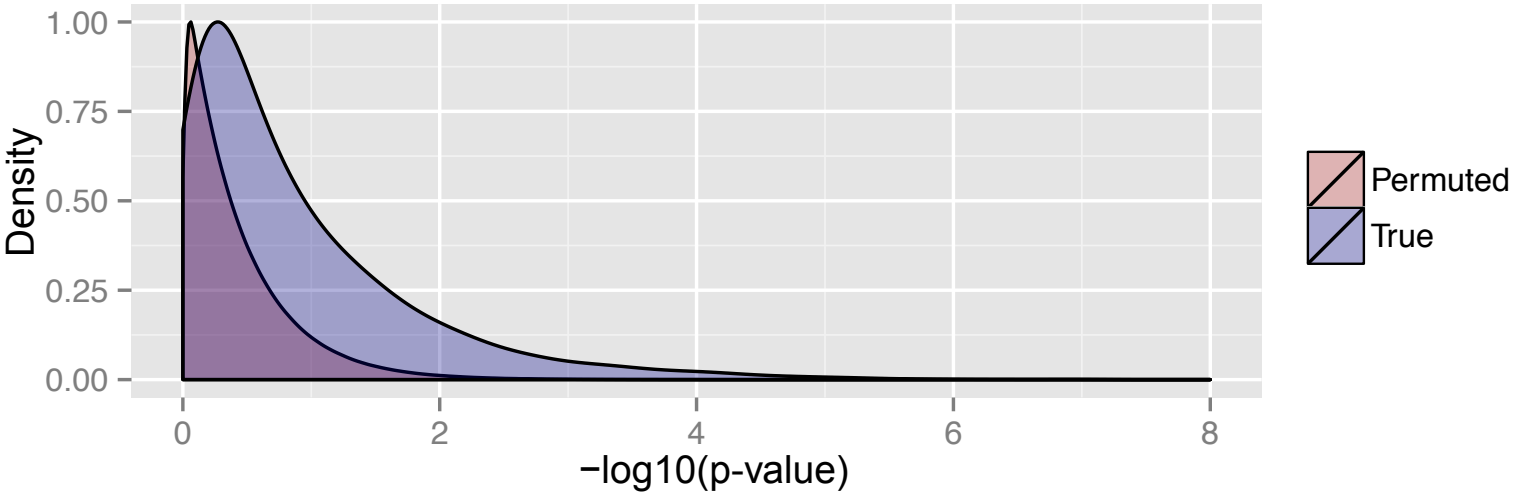

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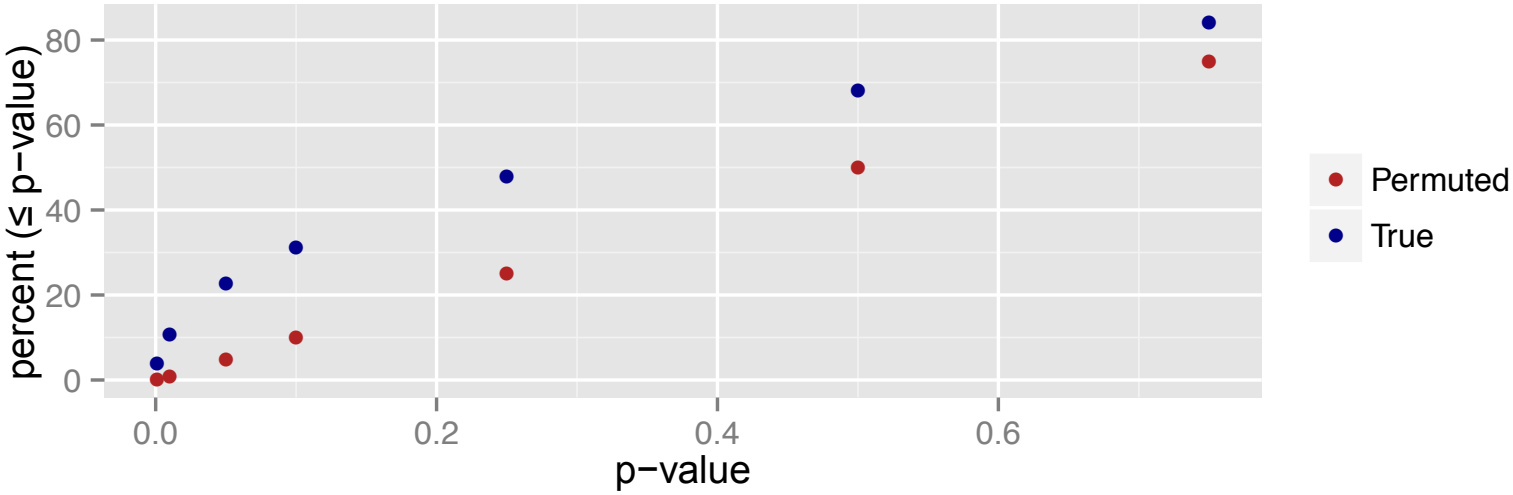
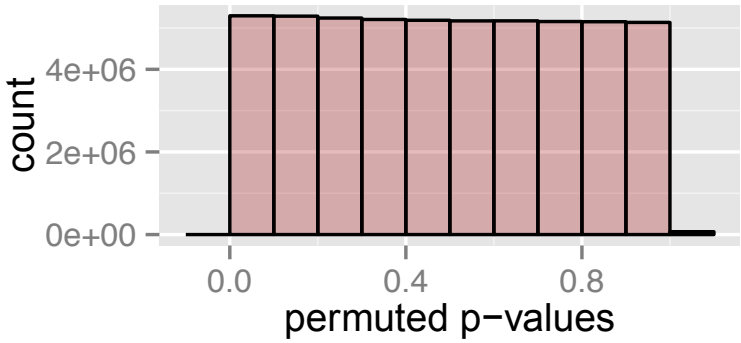
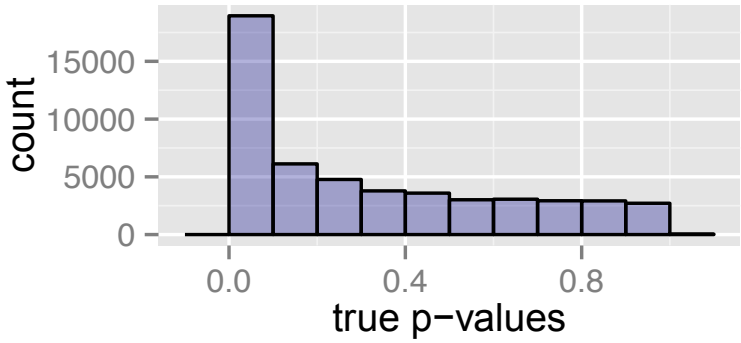
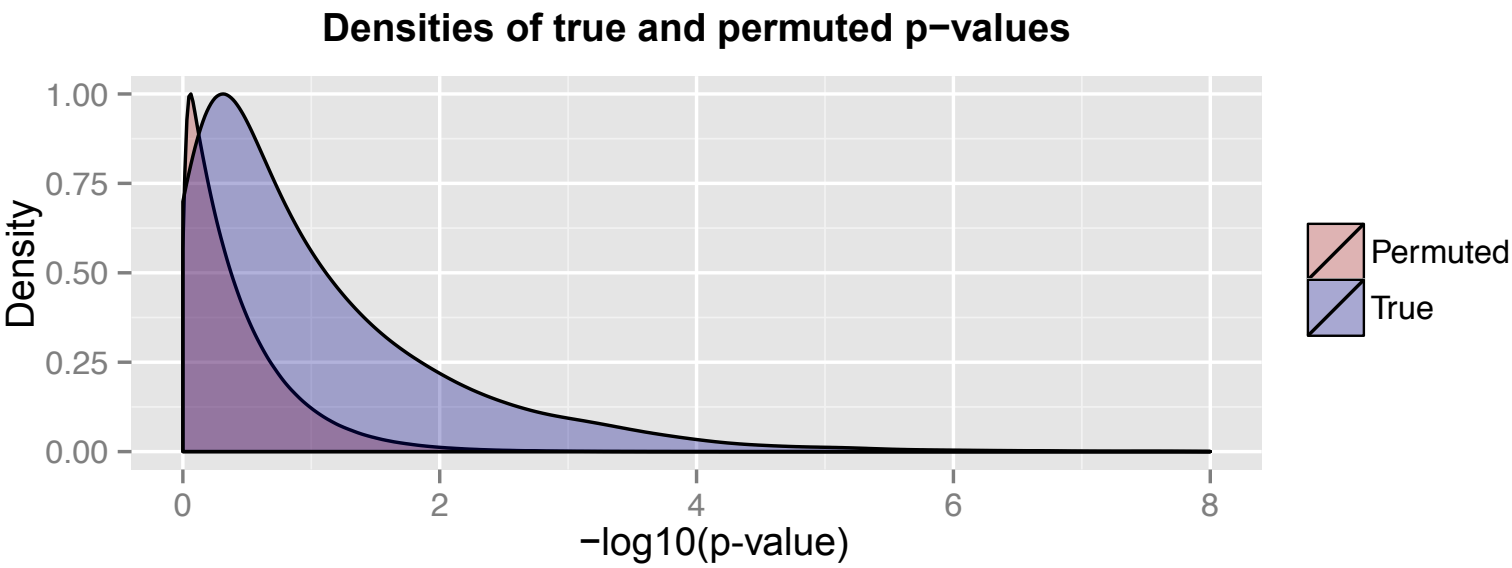


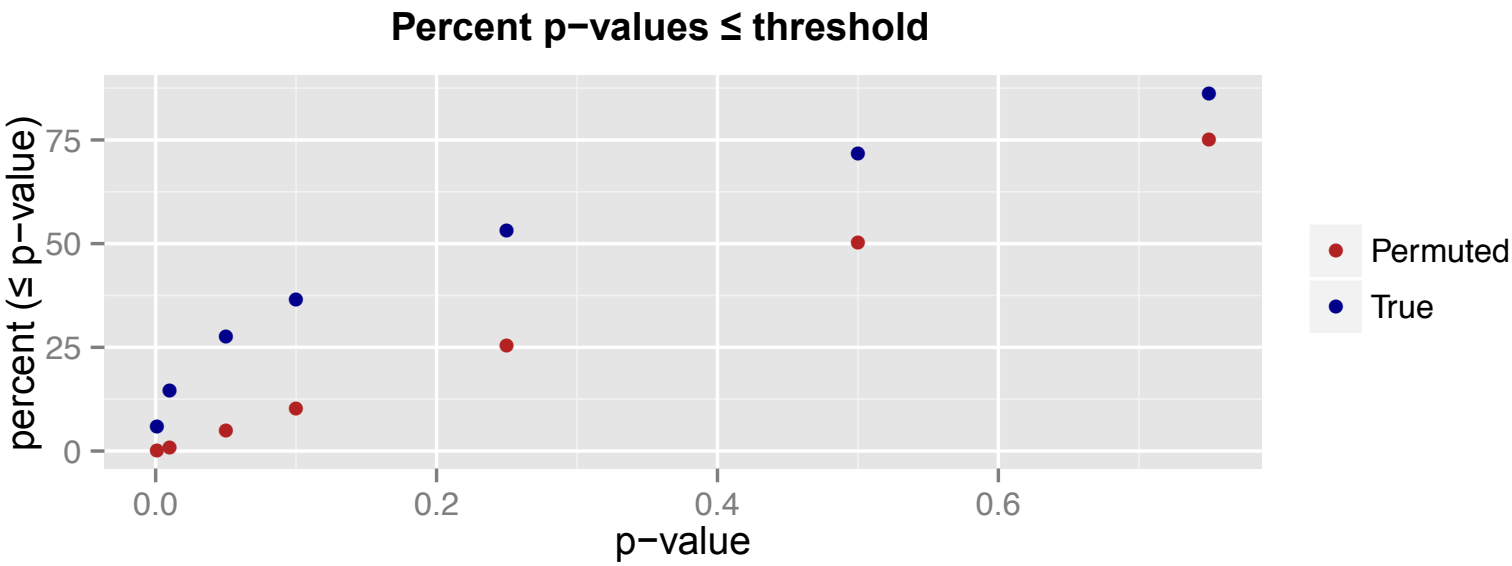
A

f__Enterobacteriaceae



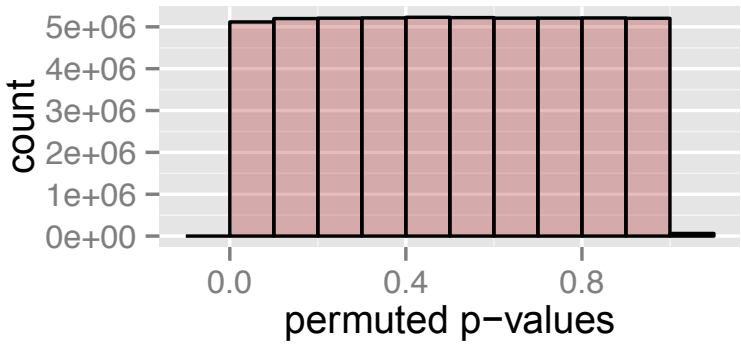


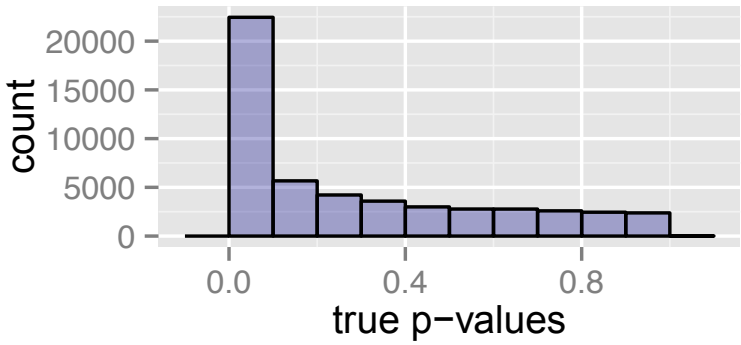


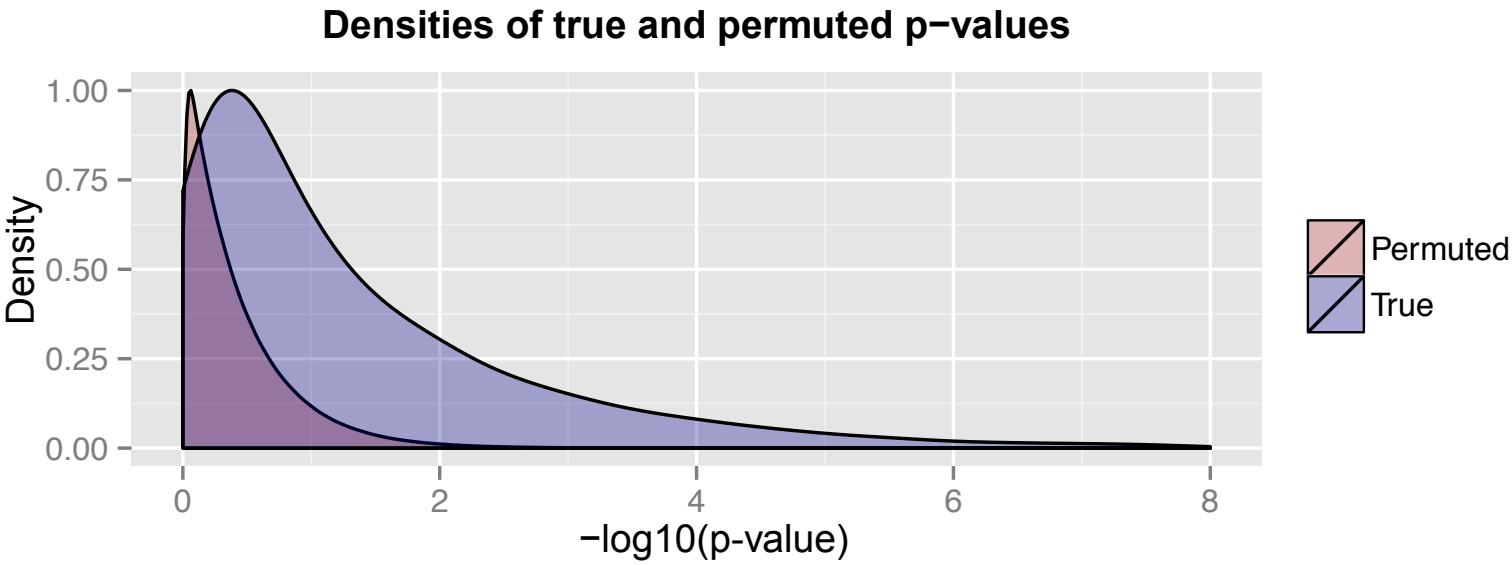


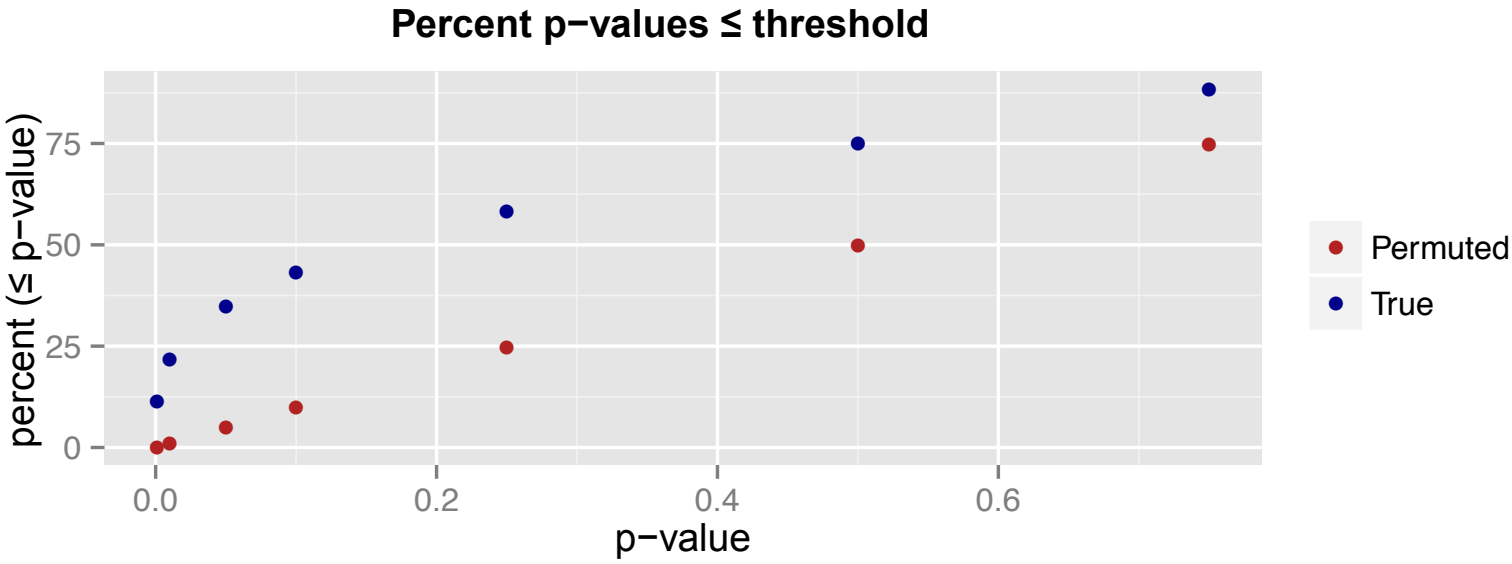
A

f__Anaeroplasmataceae



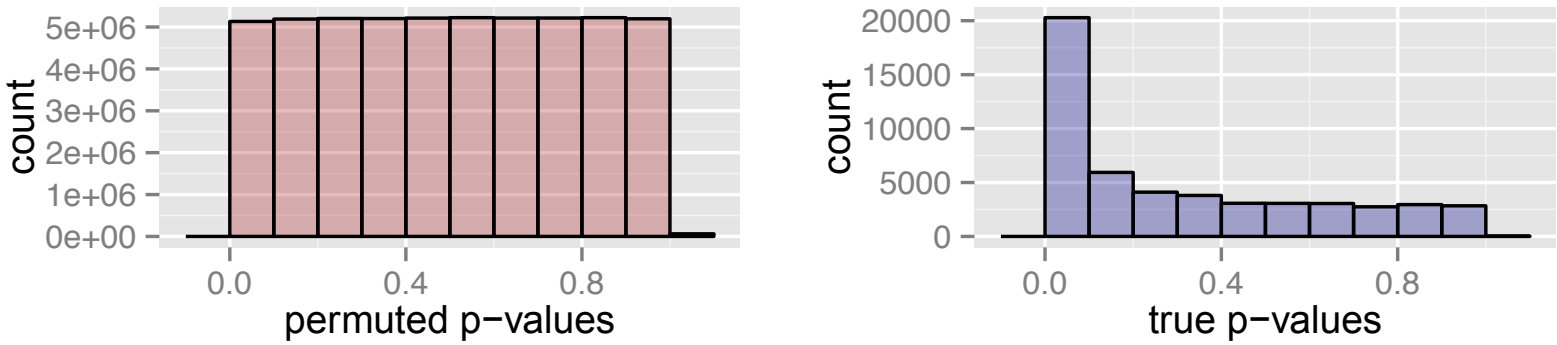


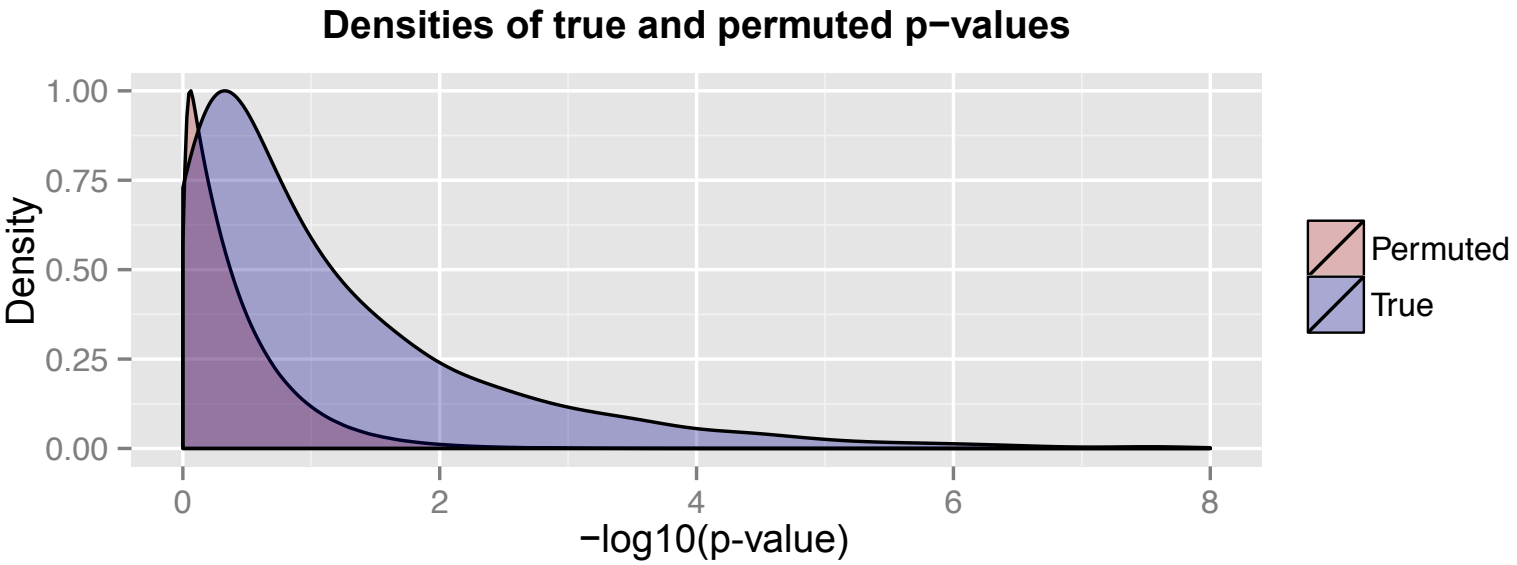


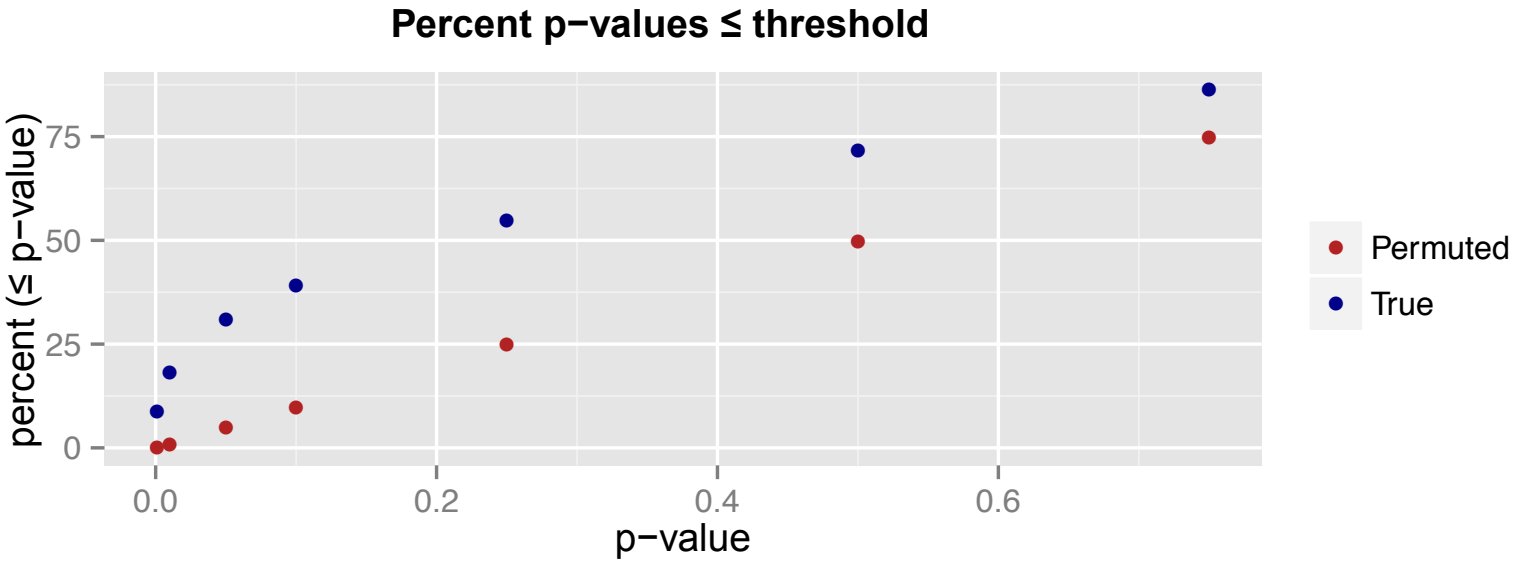


A

o__RF39, f__unknown

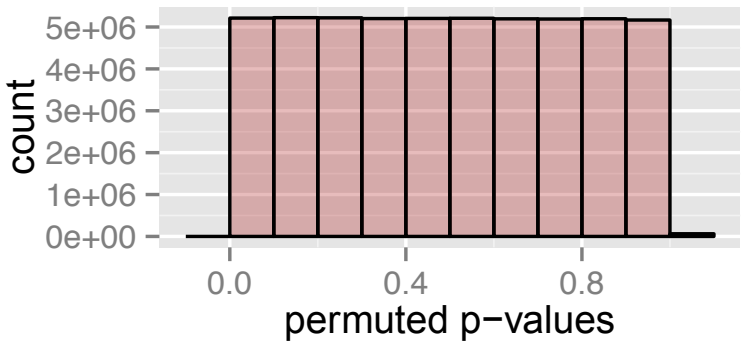


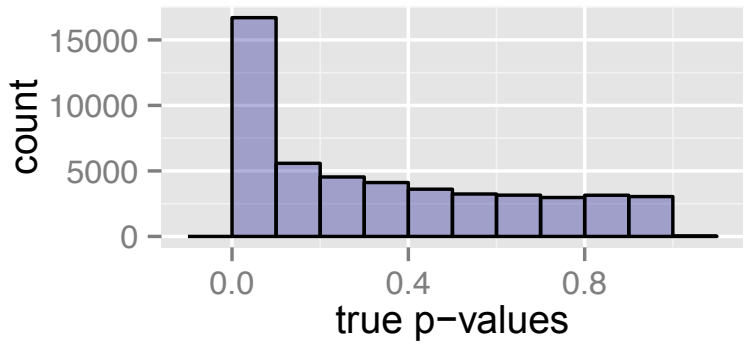




B

OTU 162


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values


count
15000
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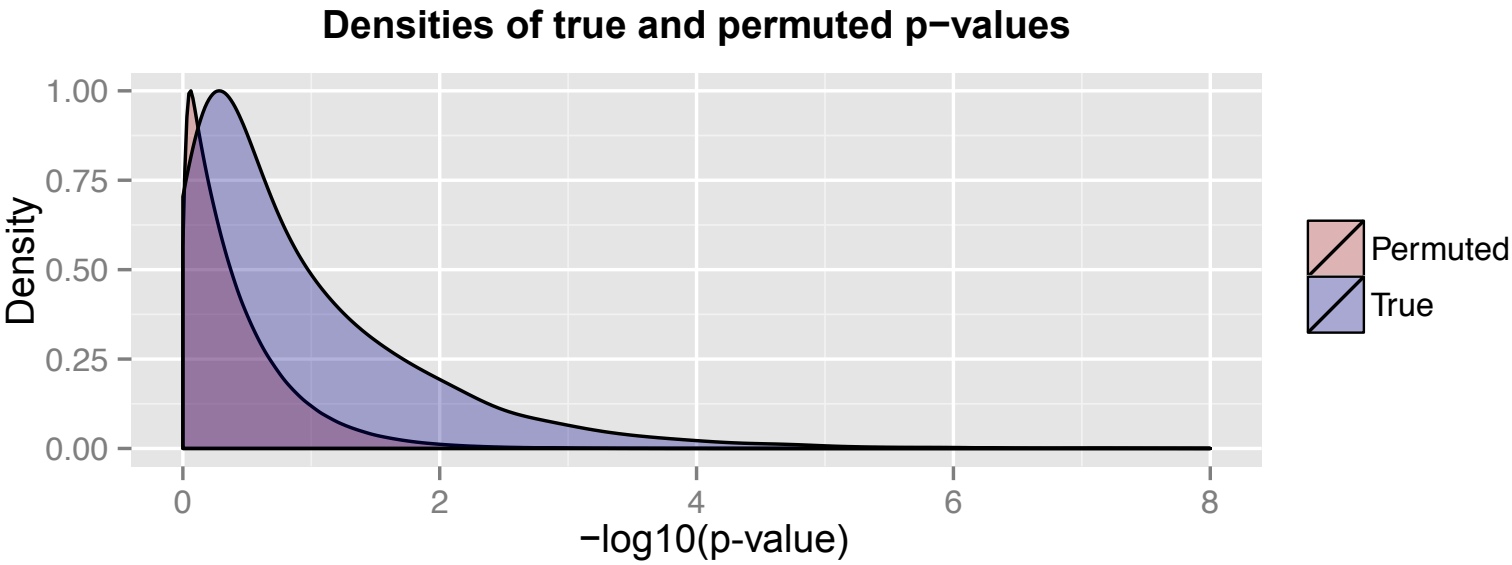
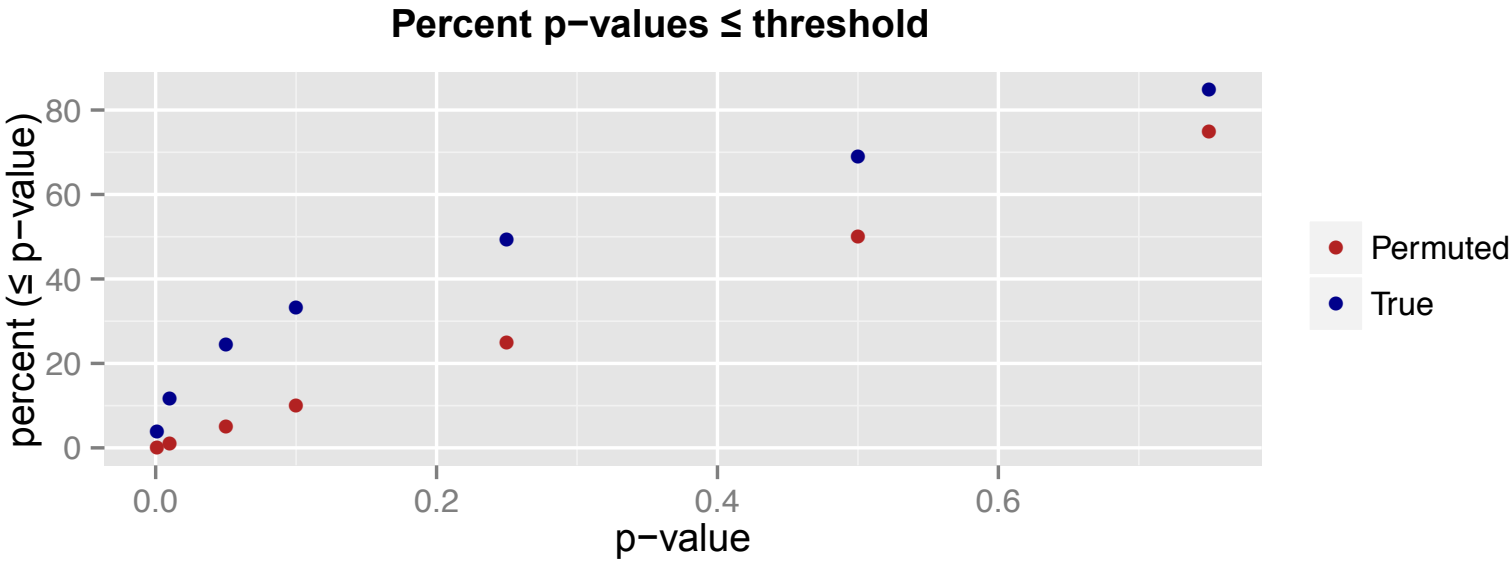
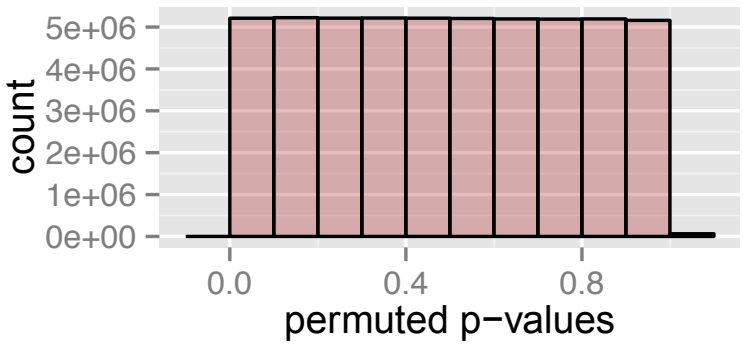
0
0.0
0.4
0.8
true p−values

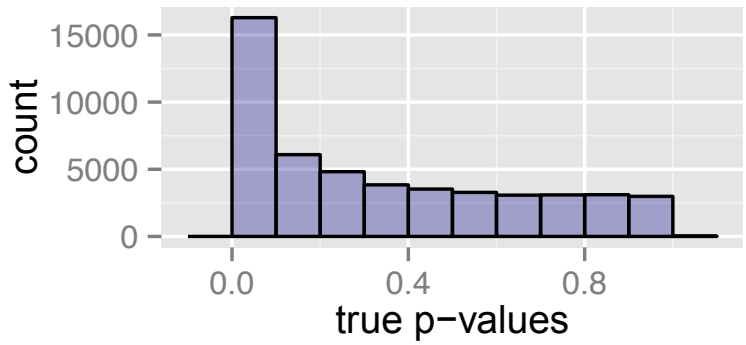

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

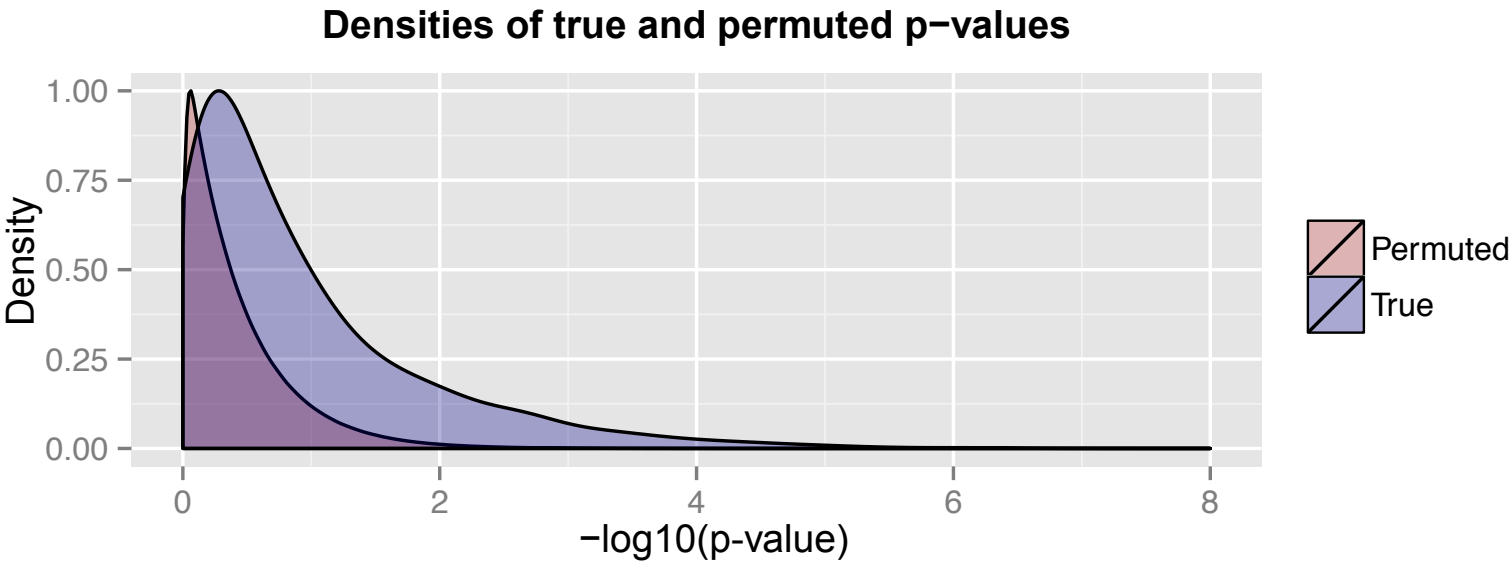

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

B

OTU 258


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values


count
15000
10000
5000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
1.00
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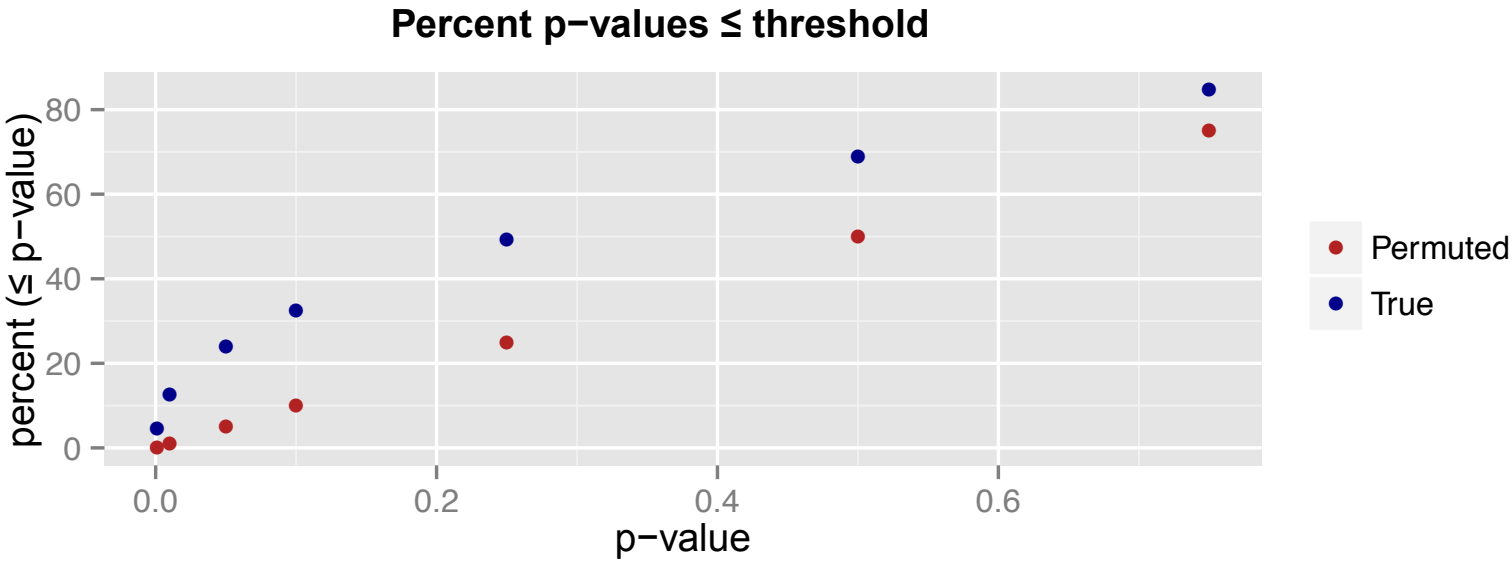
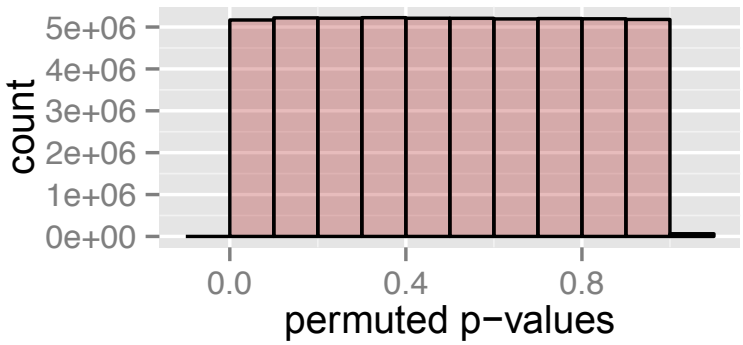
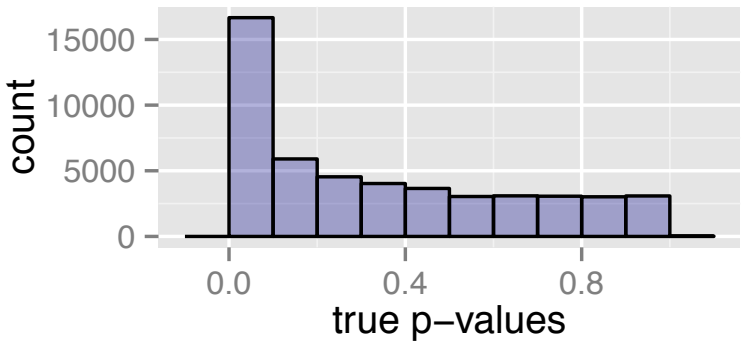
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

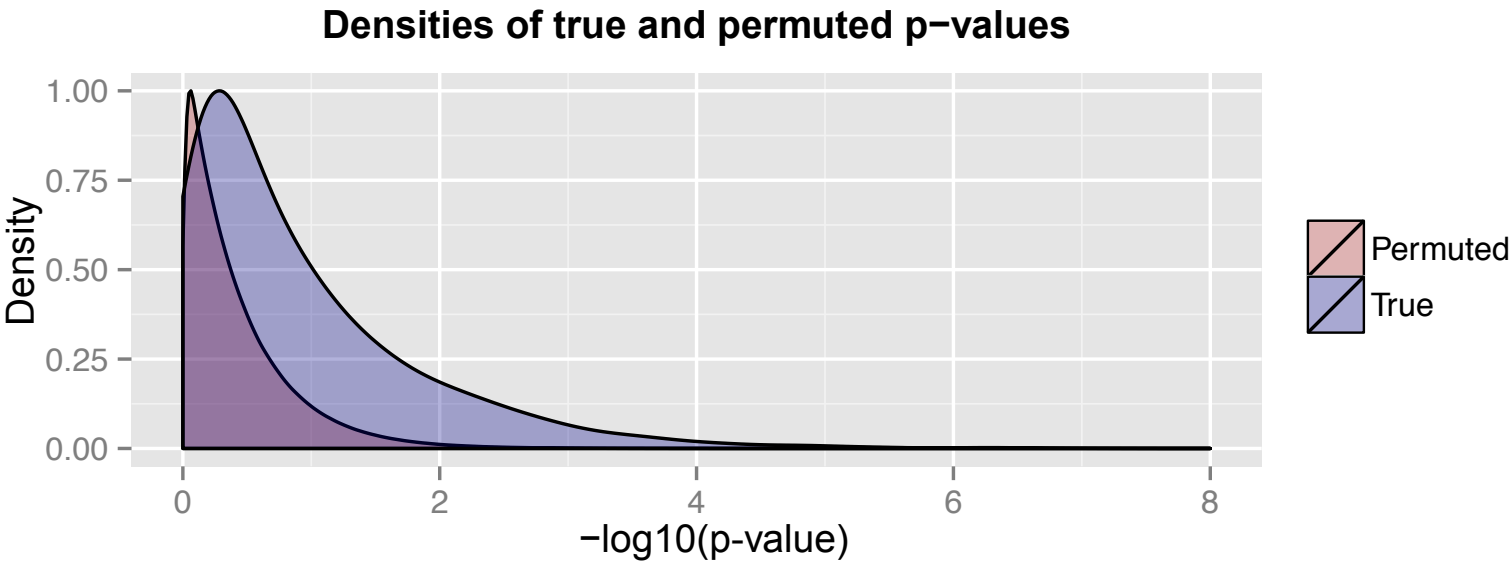

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

B

OTU 299


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values


count
15000
10000
5000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
1.00
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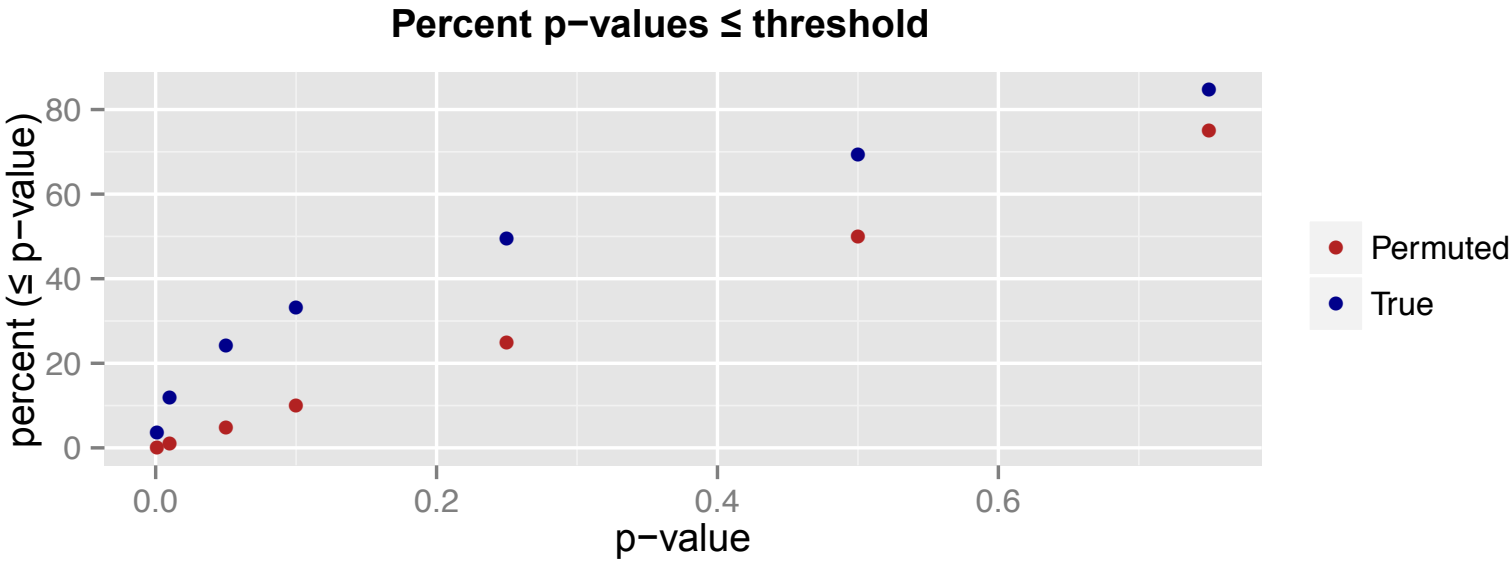
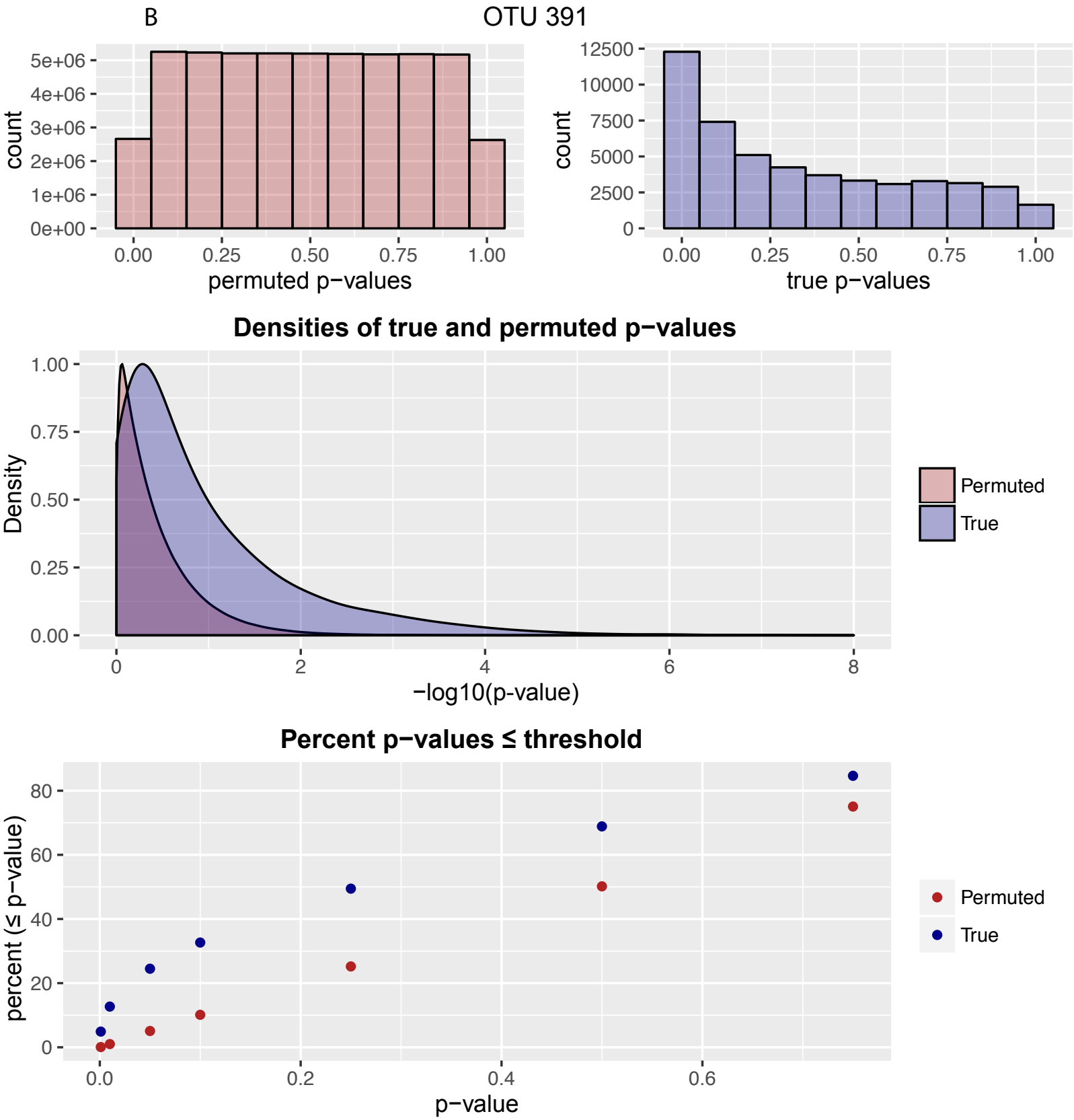
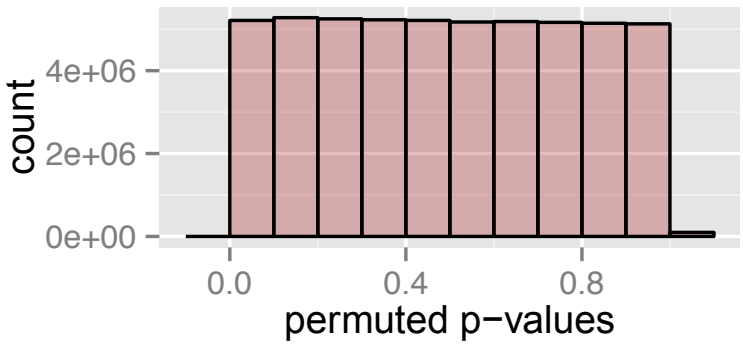
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True


B
OTU 391
count
permuted p−values
true p−values
Densities of true and permuted p−values
Density
−log10(p-value)
Permuted
True
Percent p−values ≤ threshold
percent (≤ p−value)
p−value
Permuted
True

B

OTU 438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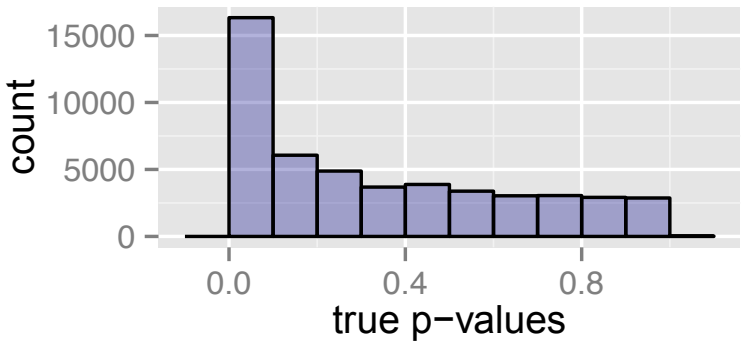
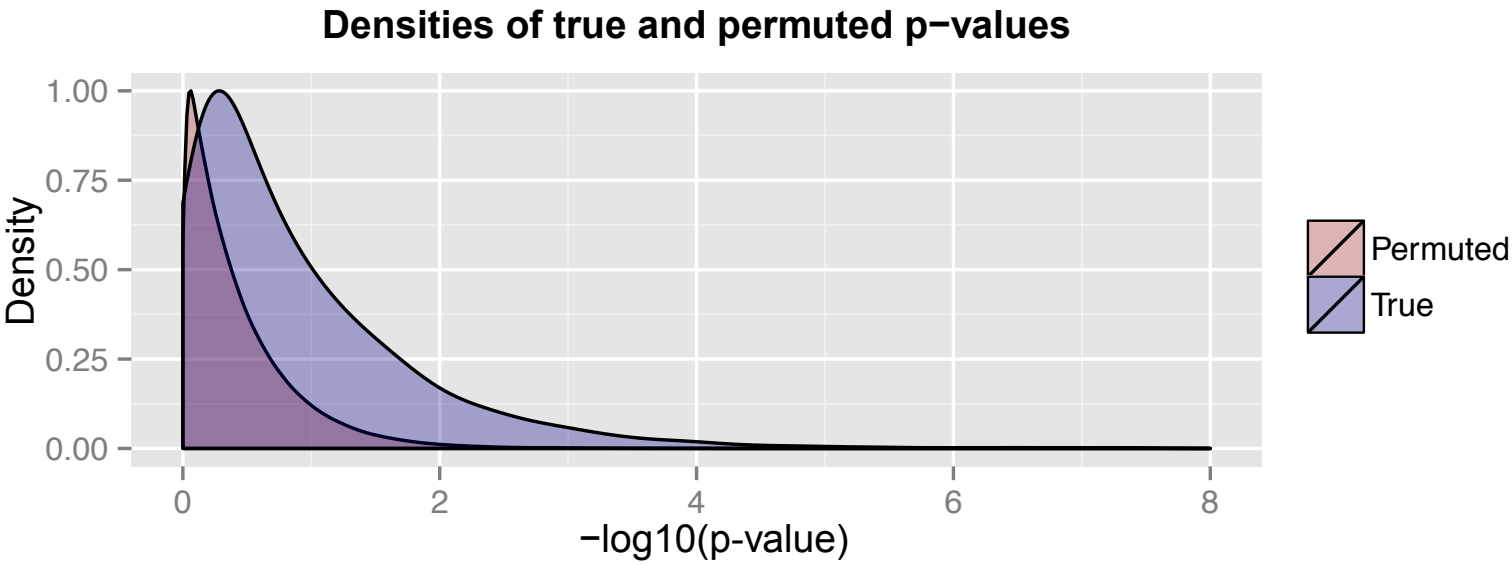
2e+06
0e+00
0.0
0.4
0.8
permuted p−values


count
15000
10000
5000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
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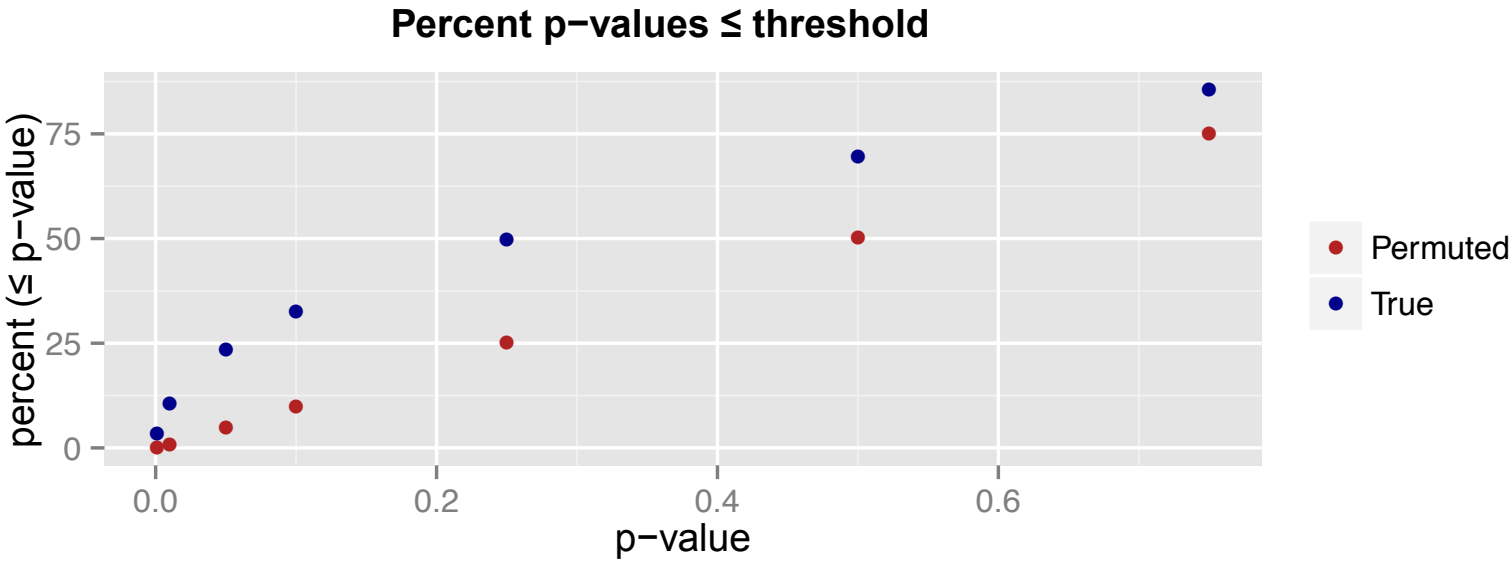
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
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50
25
0
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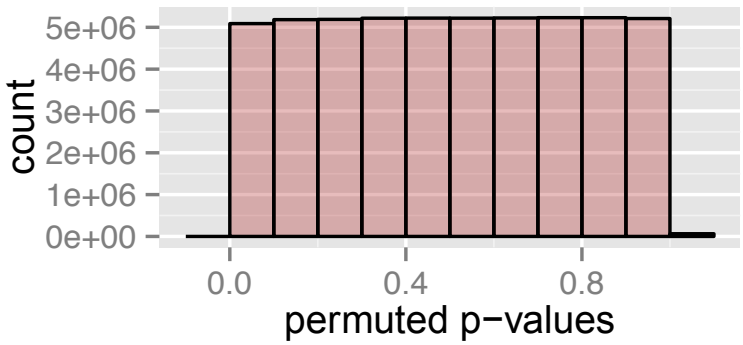
0.2
0.4
0.6
p−value
Permuted
True

B

OTU 844


count
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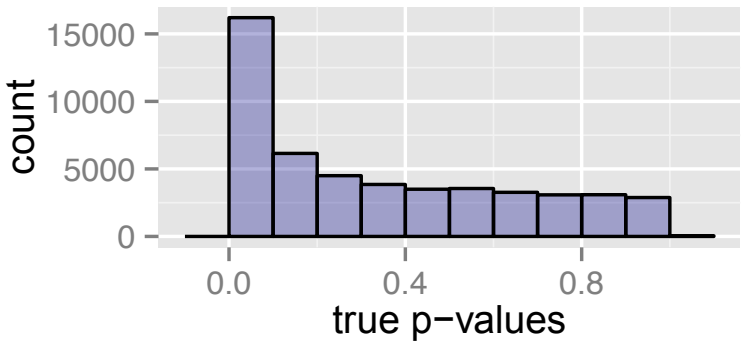
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values


count
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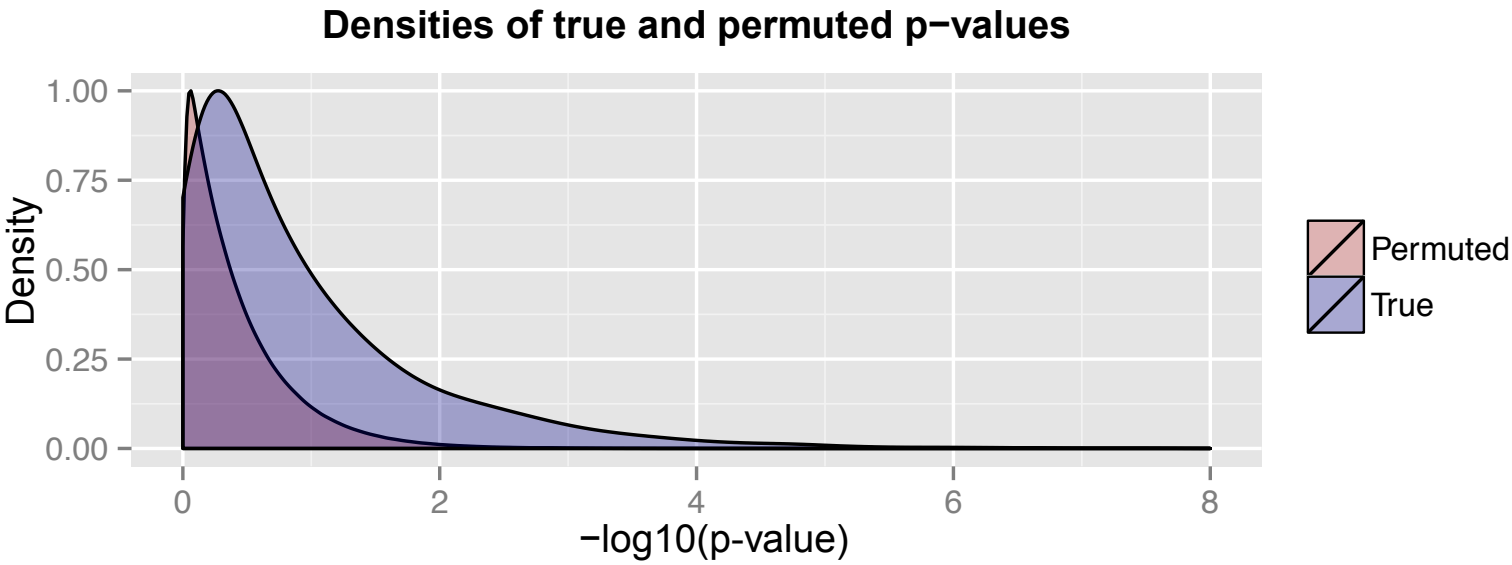
10000
5000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
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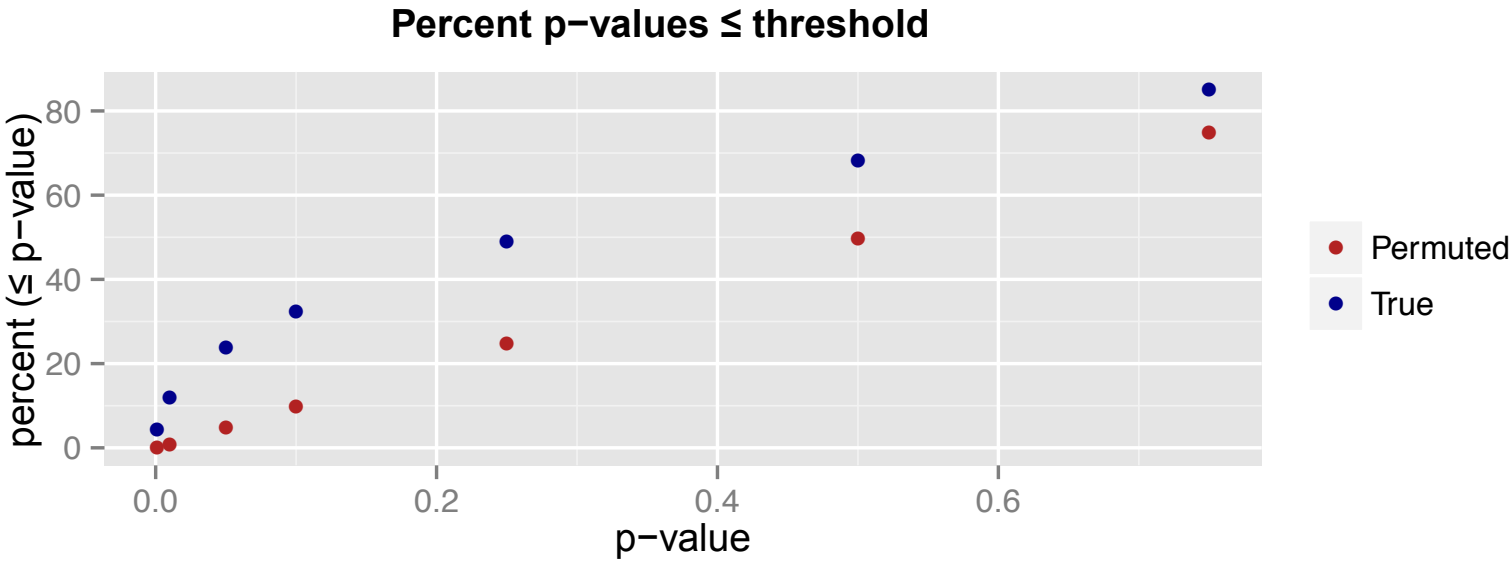
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
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20
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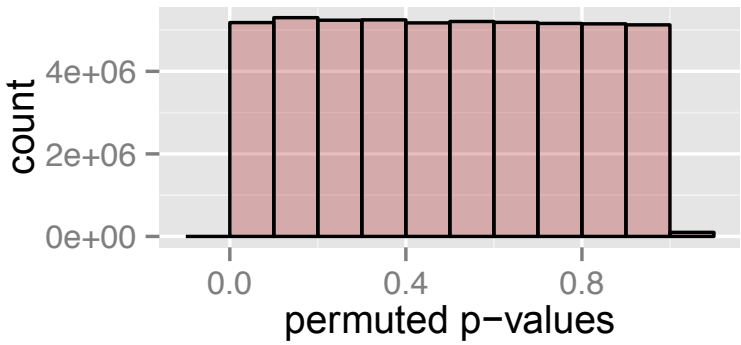
0.0
0.2
0.4
0.6
p−value
Permuted
True

B

OTU 1039


count
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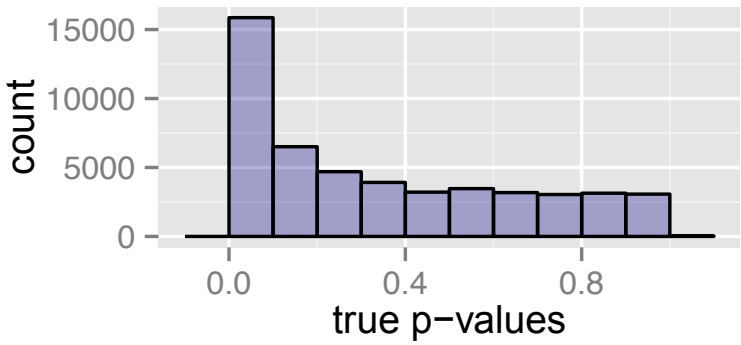
2e+06
0e+00
0.0
0.4
0.8
permuted p−values


count
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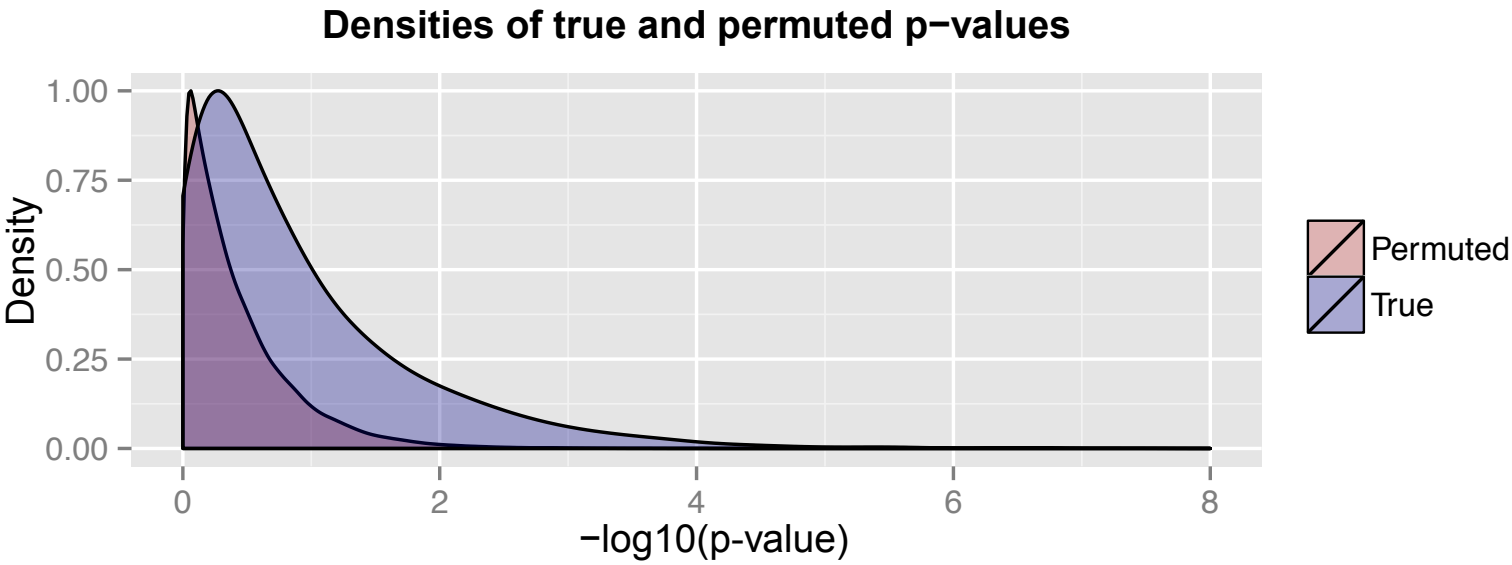
5000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
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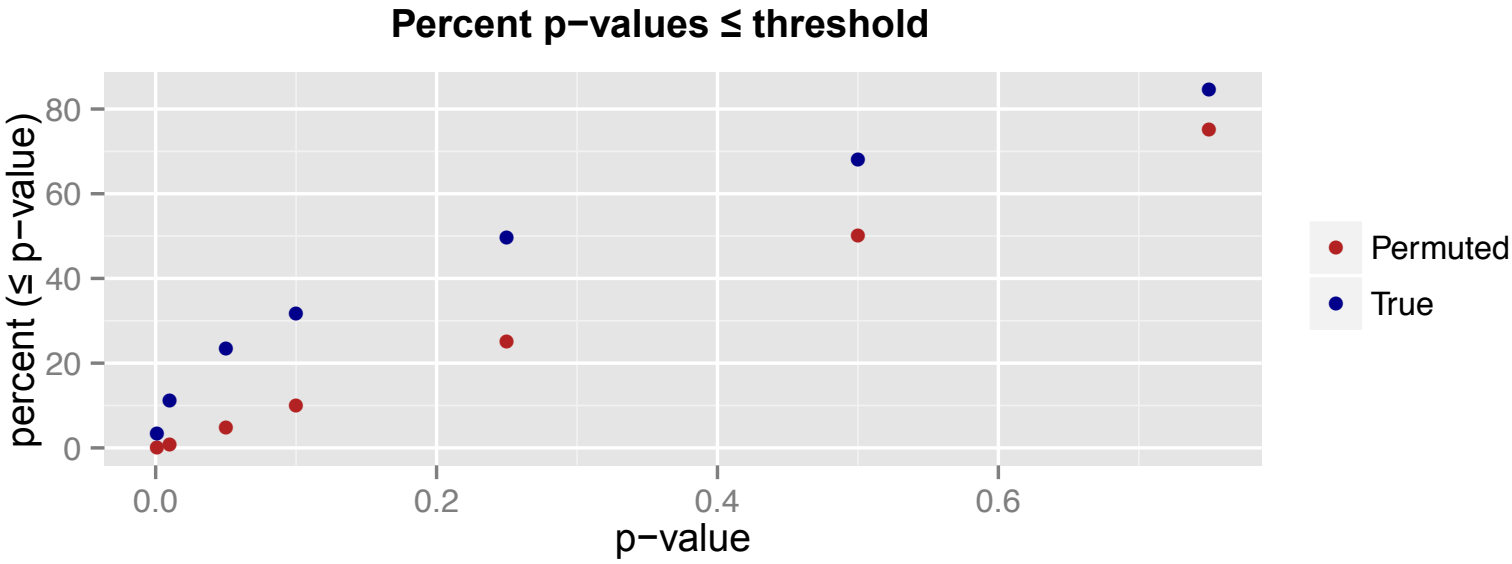
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
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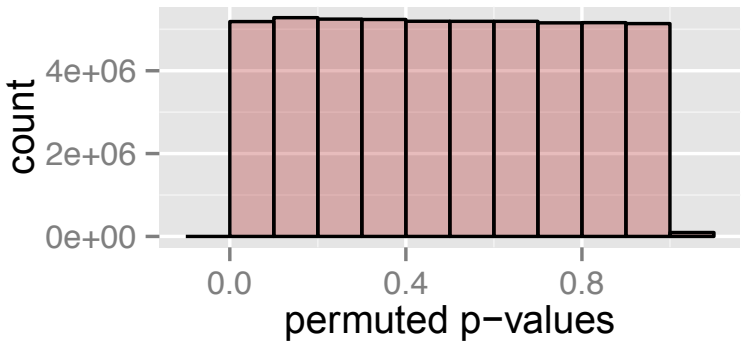
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

B

OTU 1541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2e+06
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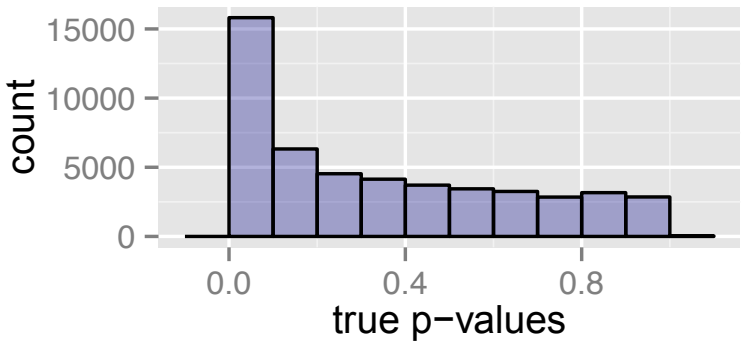
0.0
0.4
0.8
permuted p−values


count
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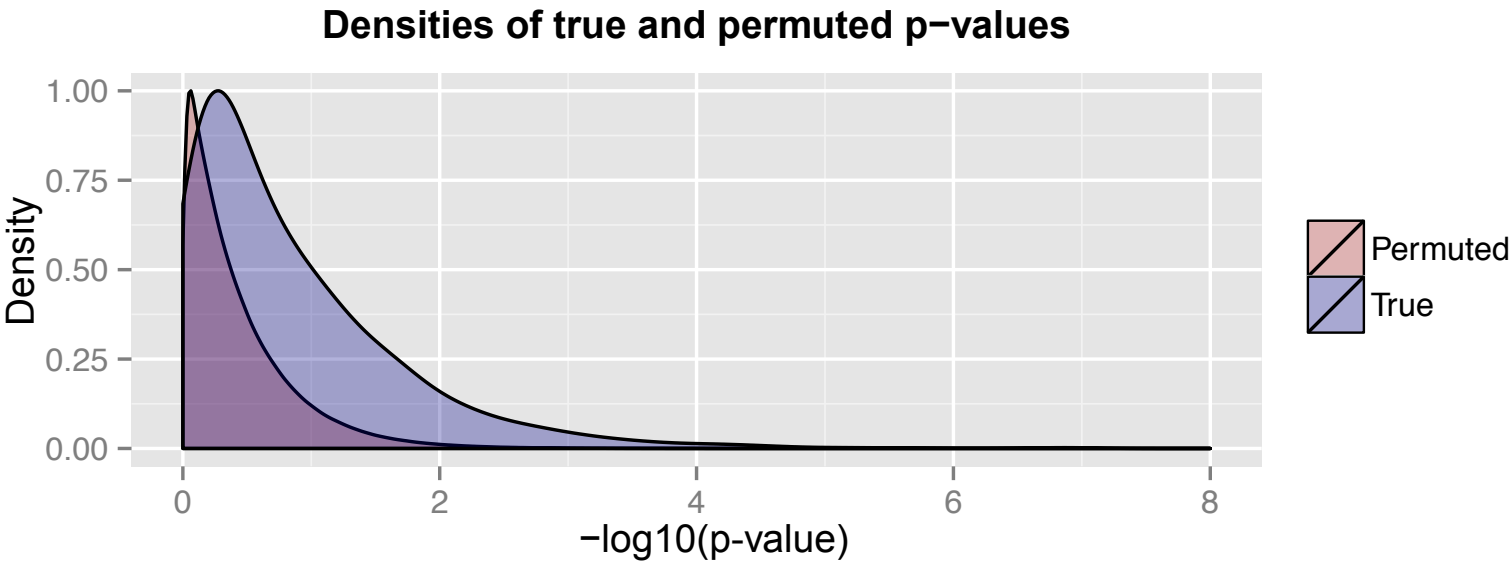
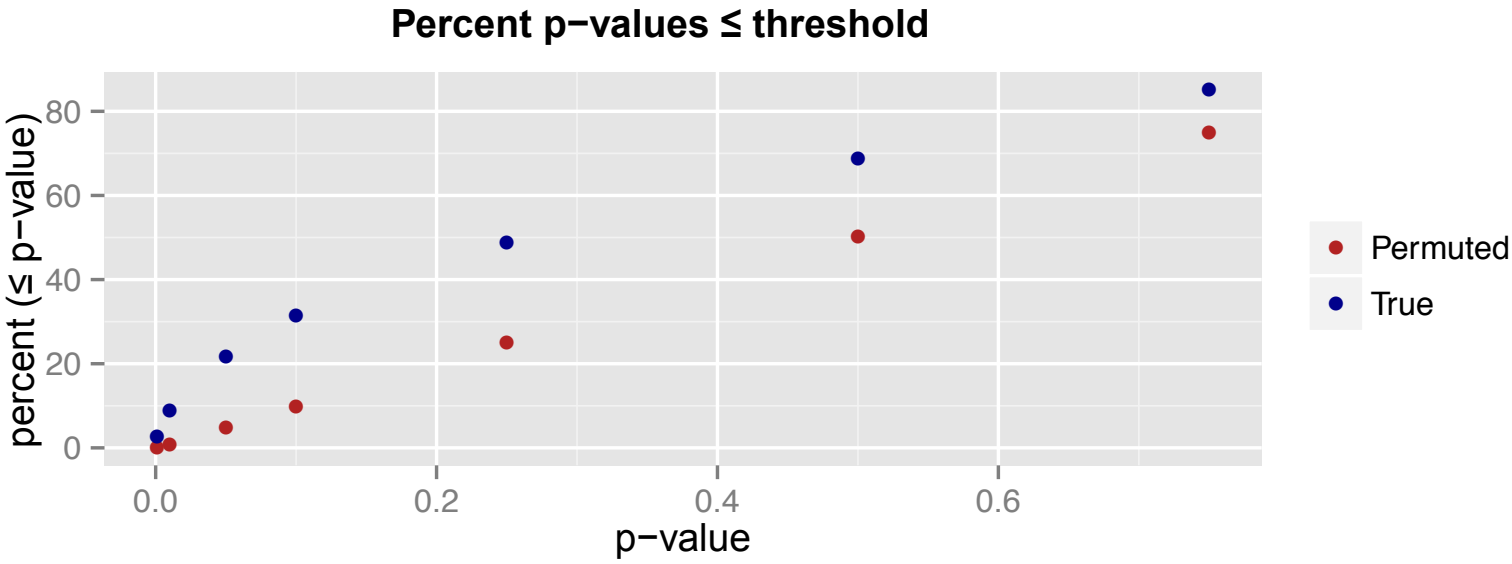
5000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
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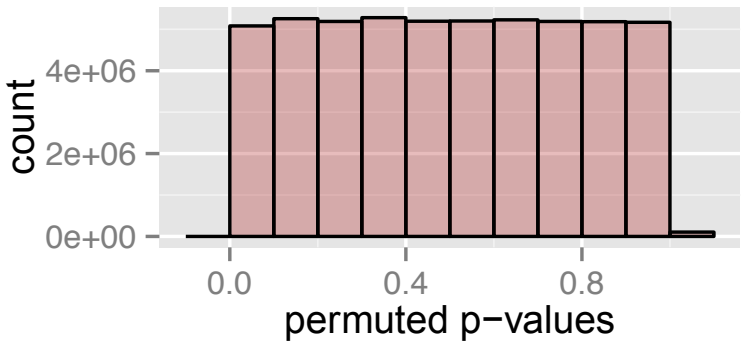
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

B

OTU 2228


count
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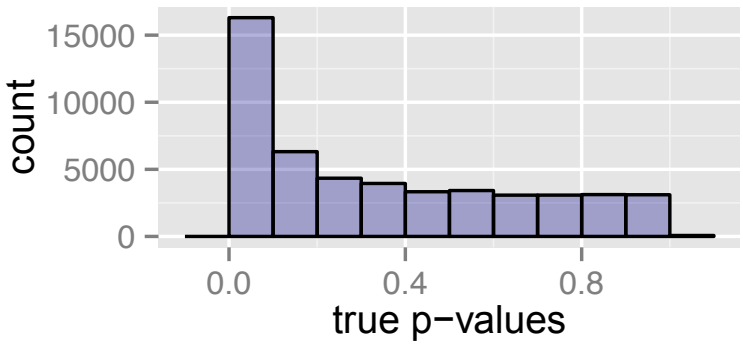
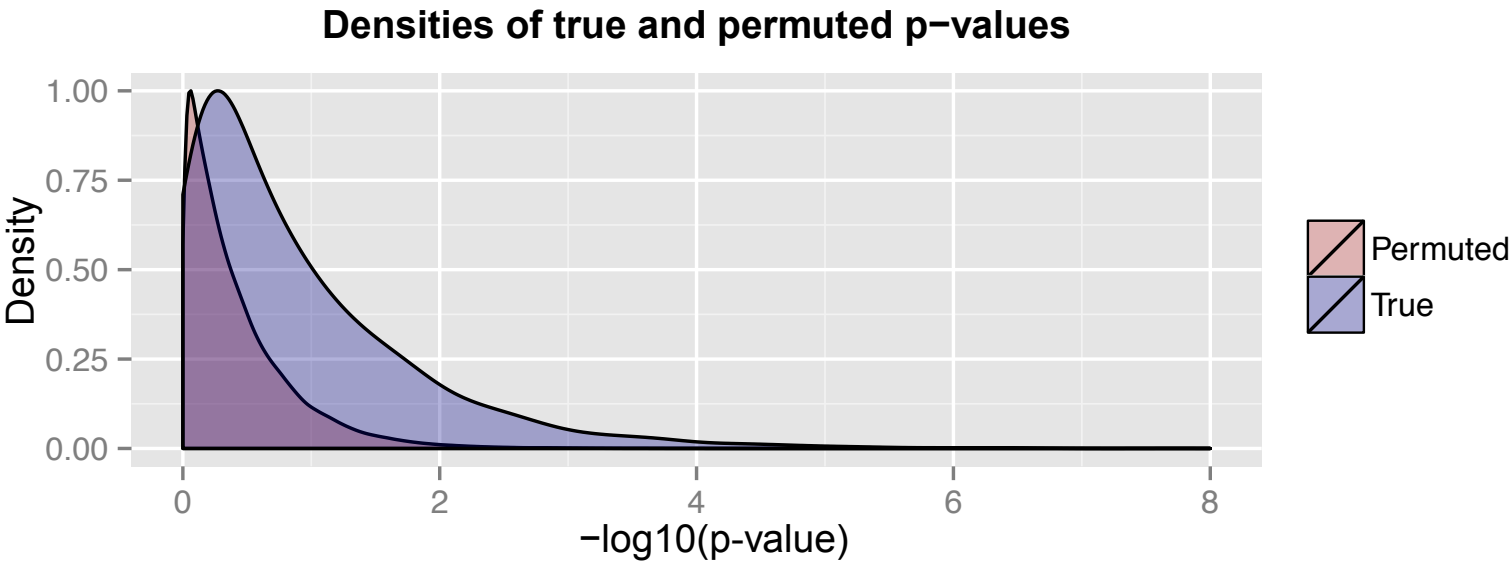
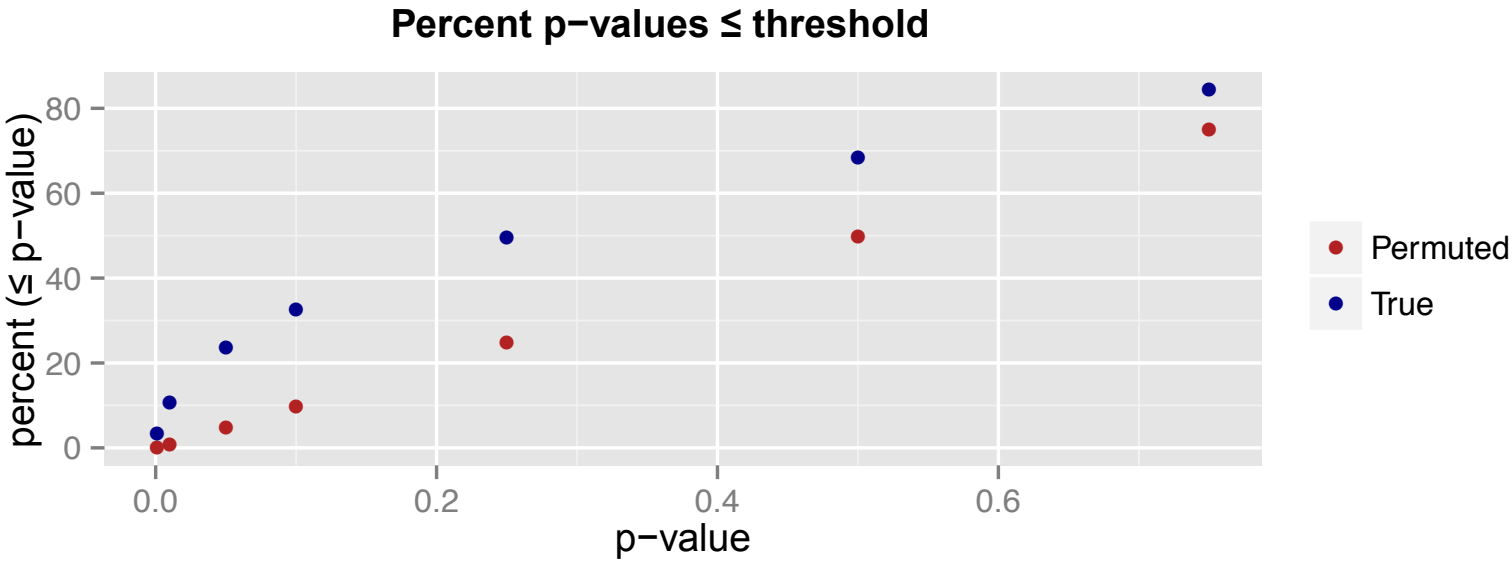
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

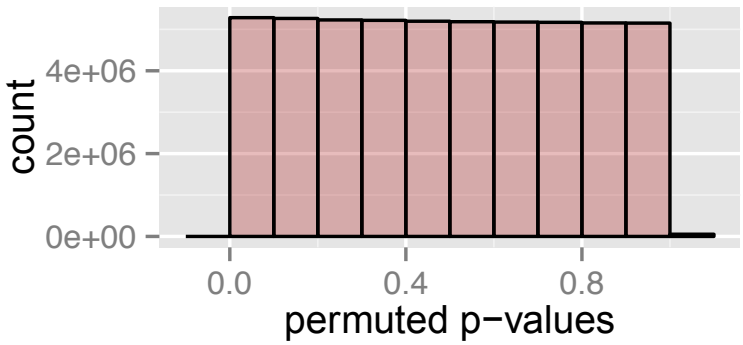

count
15000
10000
5000
0
0.0
0.4
0.8
true p−values

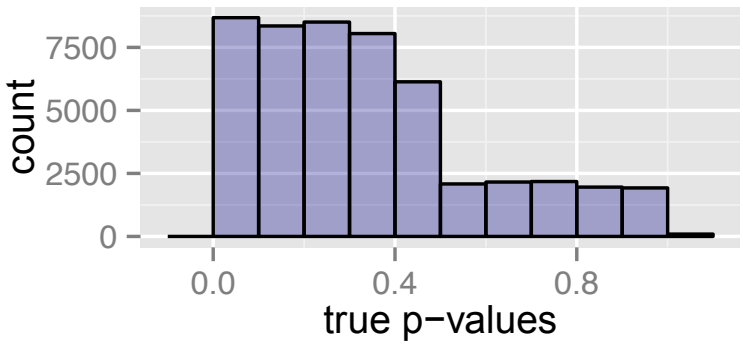

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

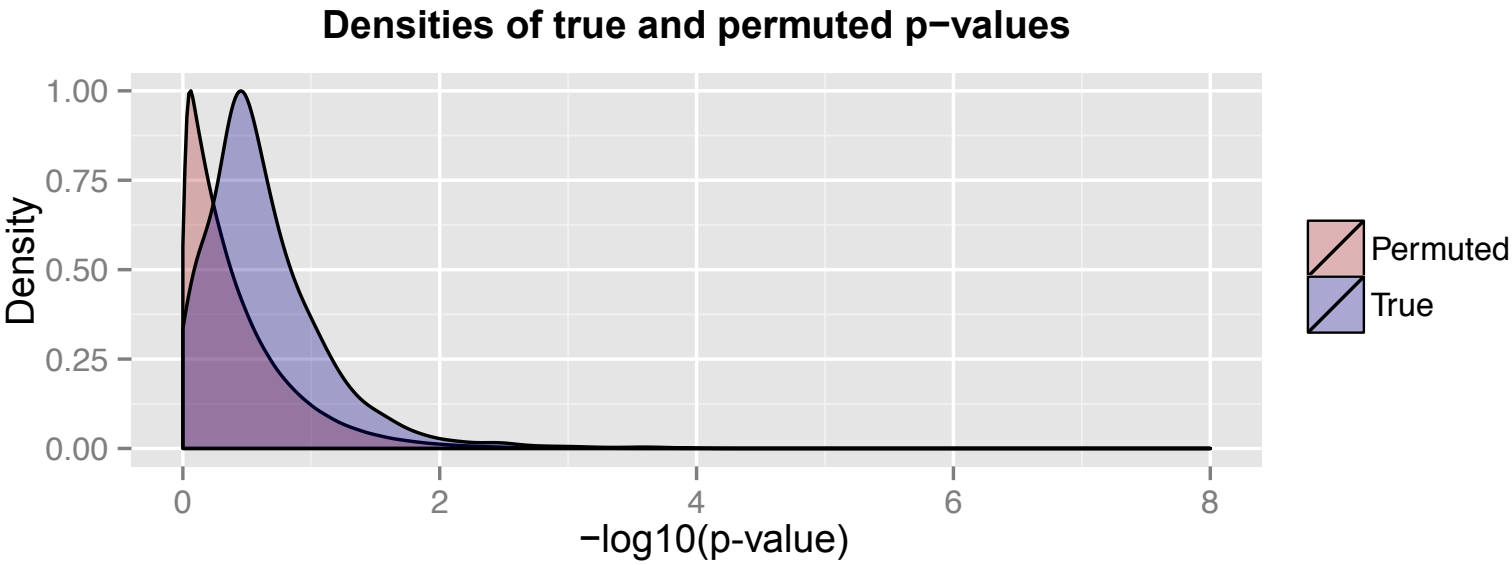

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

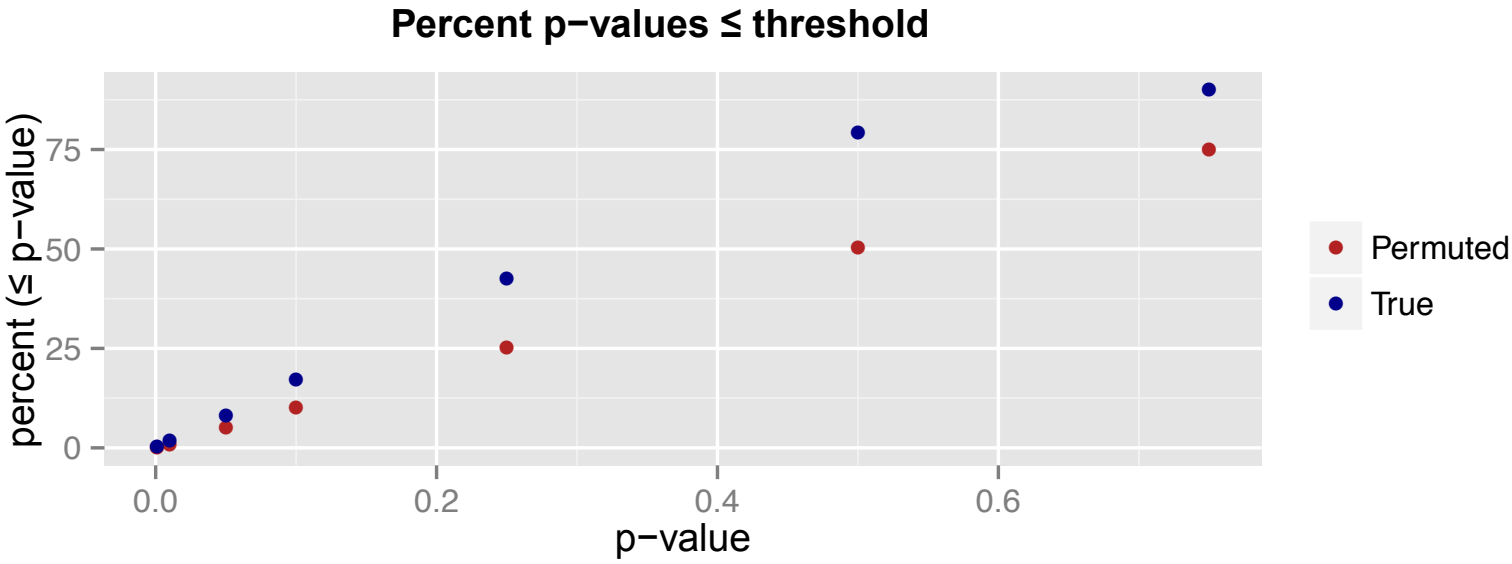
B

OTU 21095


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

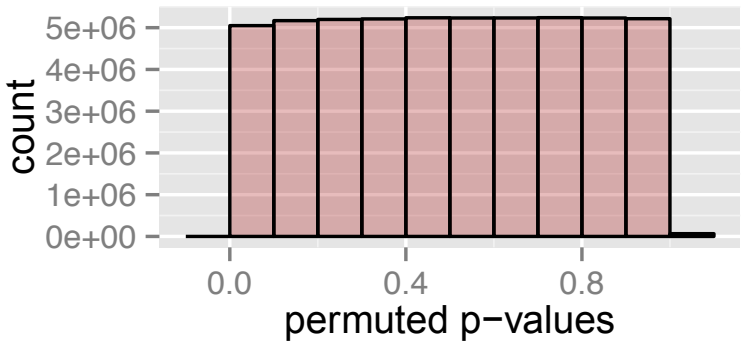

count
7500
5000
2500
0
0.0
0.4
0.8
true p−values

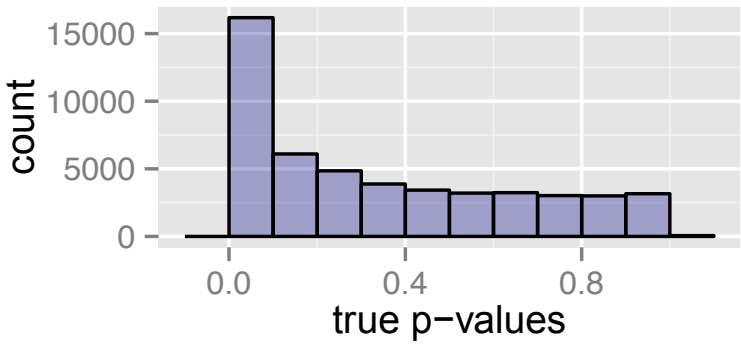

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

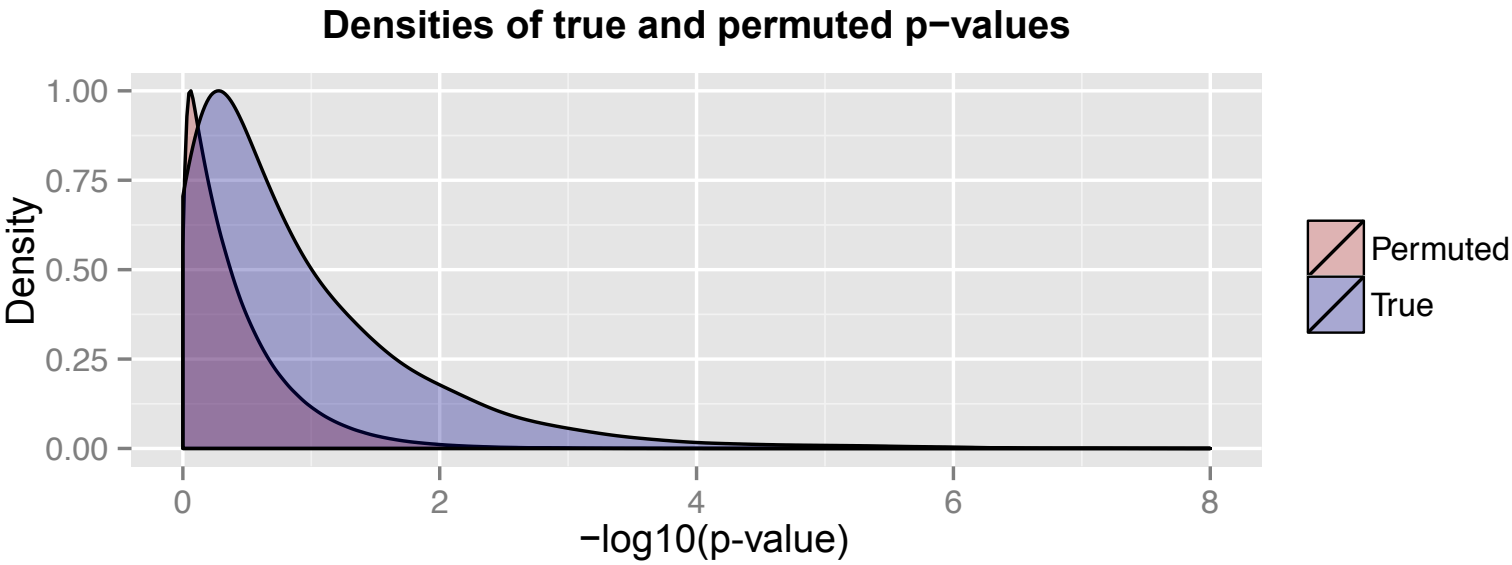

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

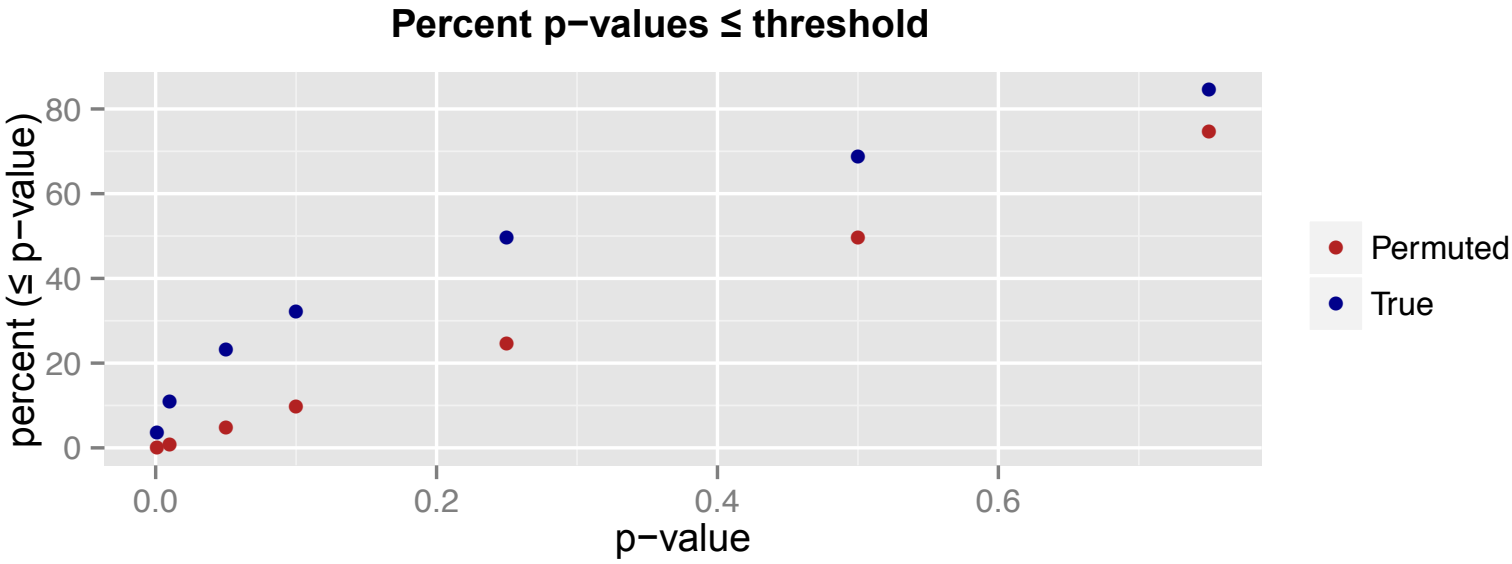
B

OTU 79815


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values

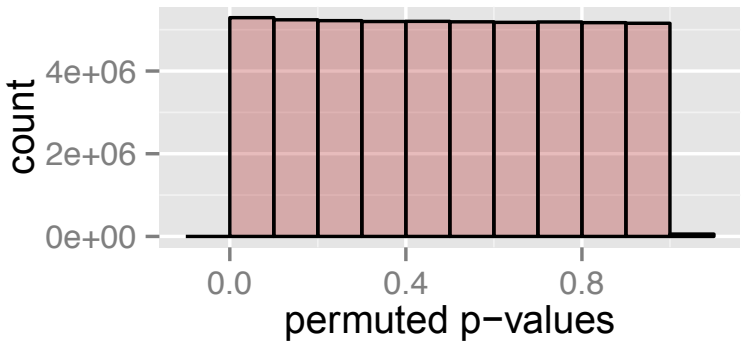

count
15000
10000
5000
0
0.0
0.4
0.8
true p−values

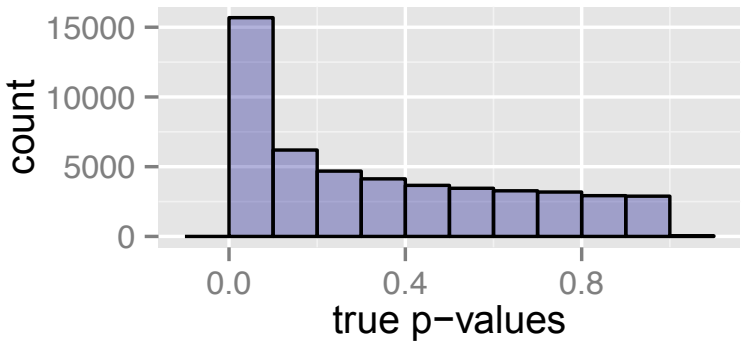

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

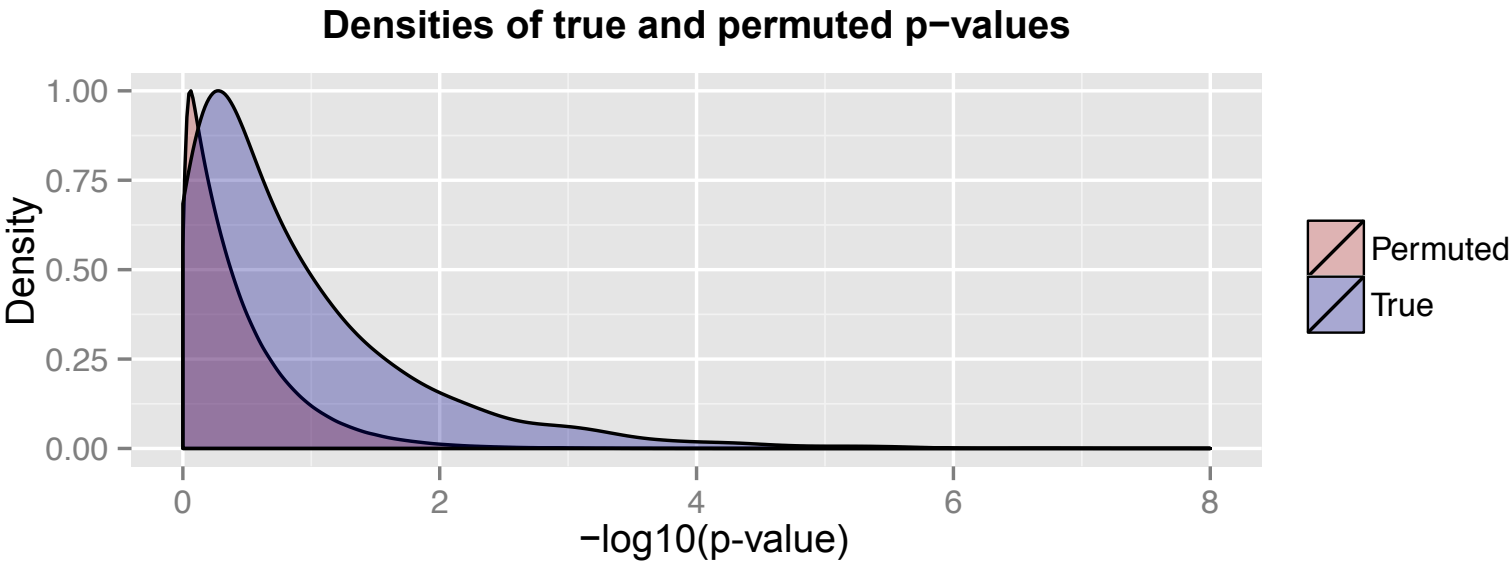

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

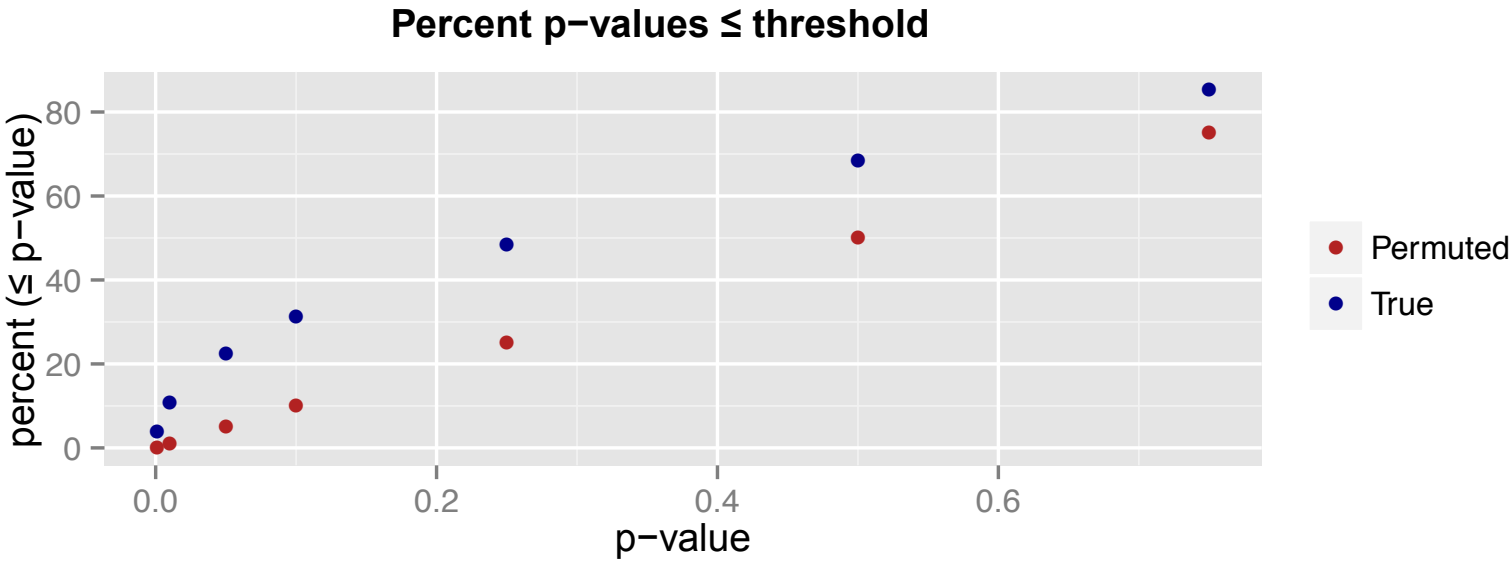
B

OTU 136366


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

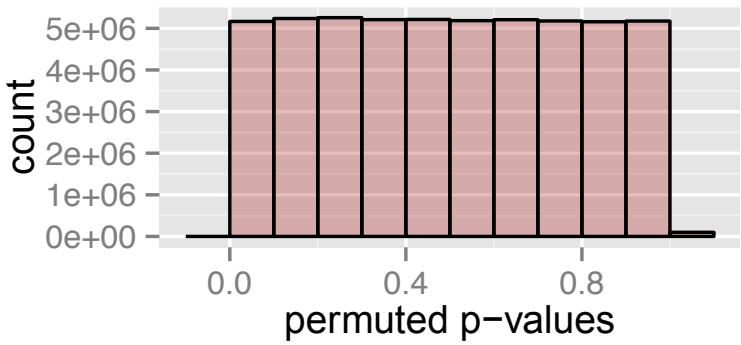

count
15000
10000
5000
0
0.0
0.4
0.8
true p−values

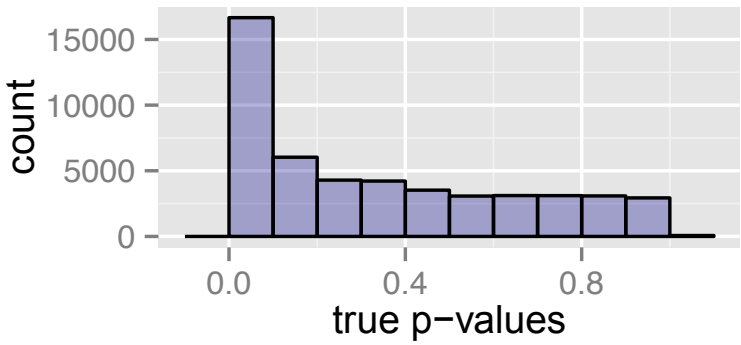

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

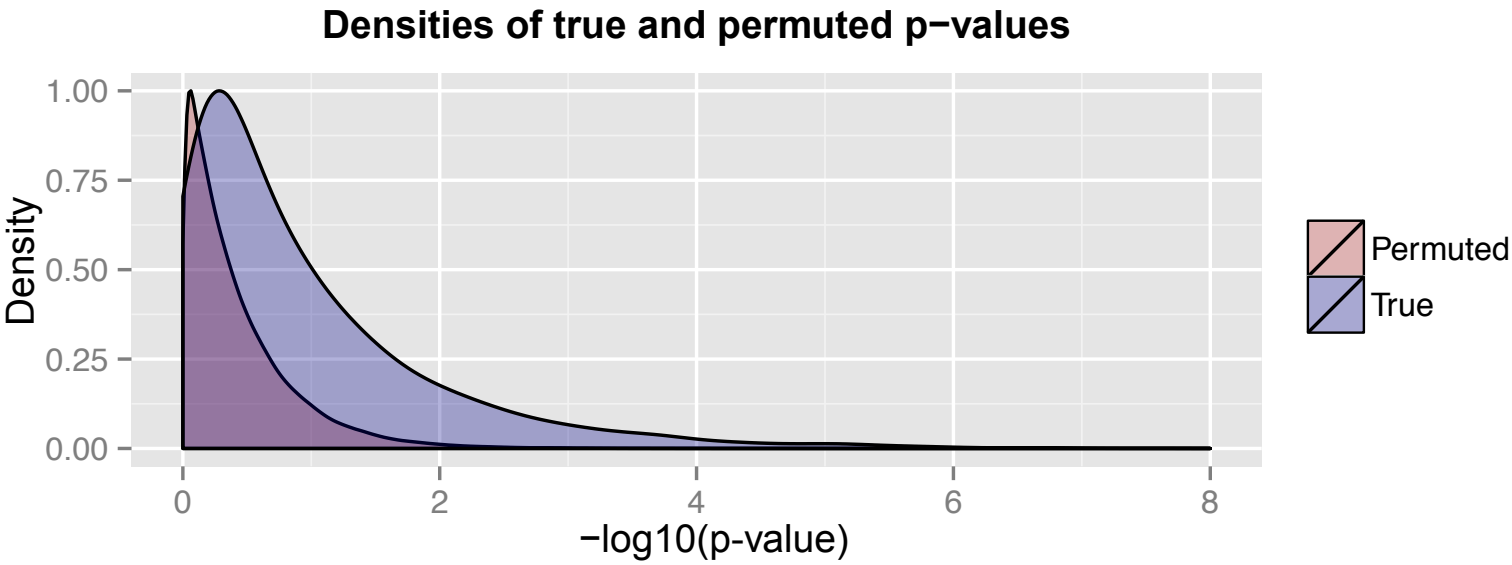

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

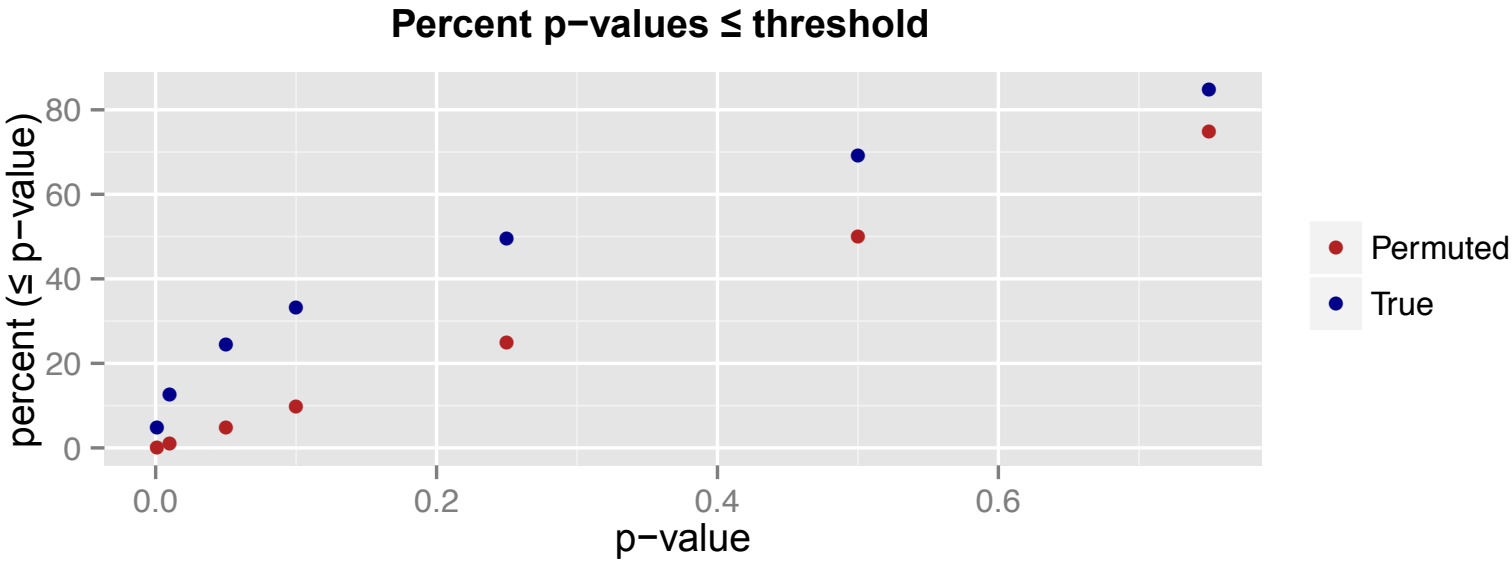
B

OTU 244358


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values

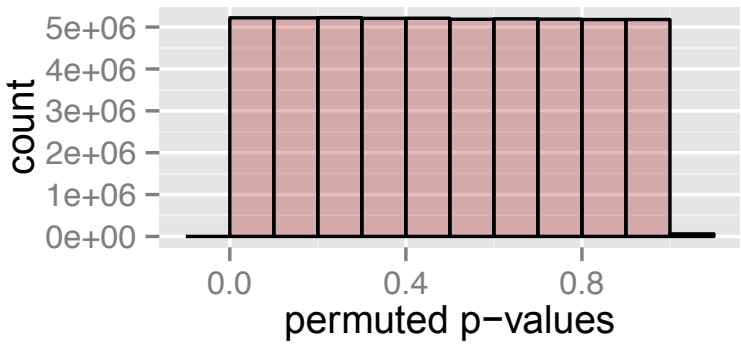

count
15000
10000
5000
0
0.0
0.4
0.8
true p−values

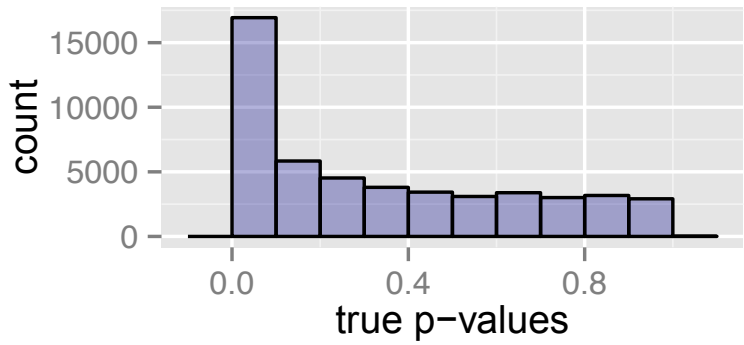

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

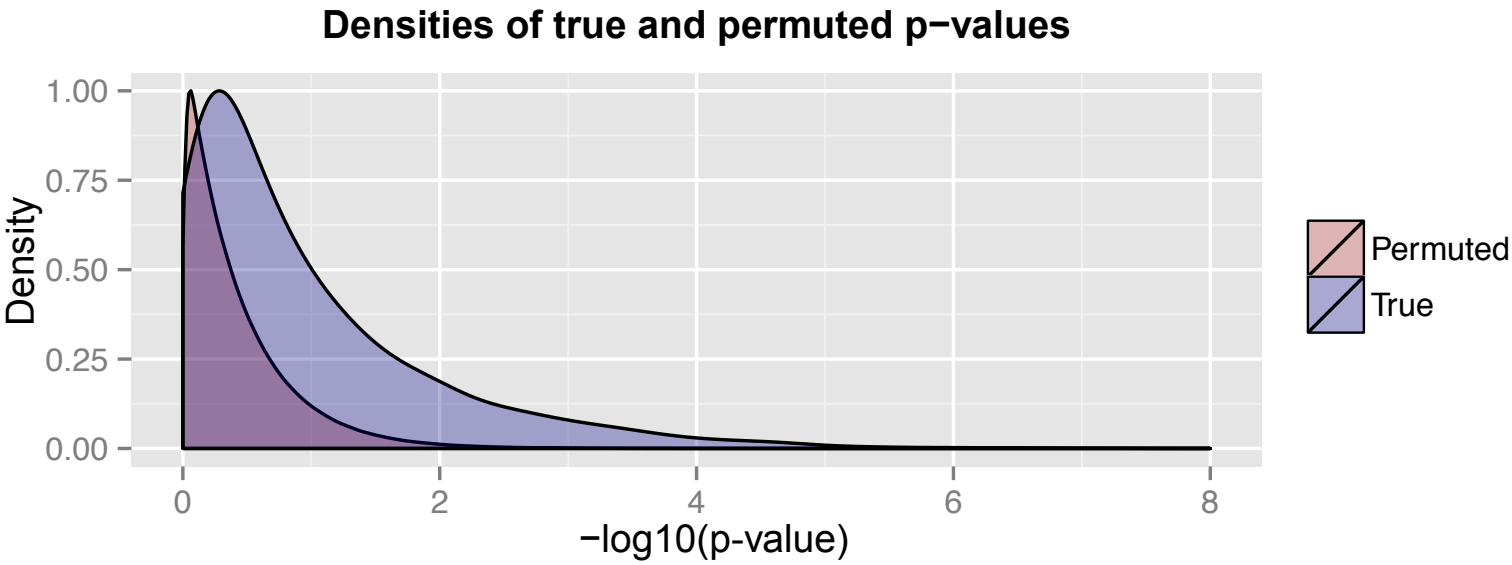

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

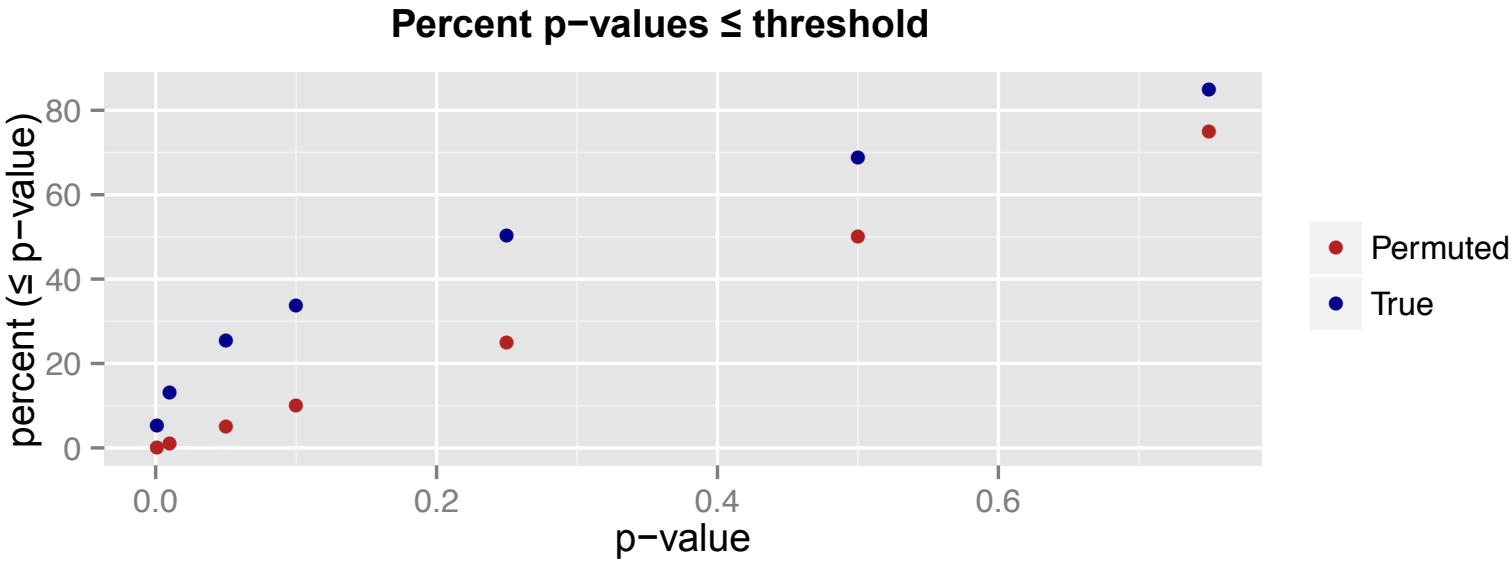
B

OTU 254719


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values

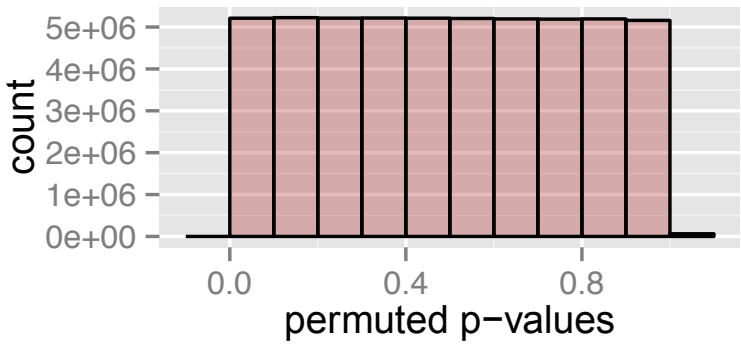

count
15000
10000
5000
0
0.0
0.4
0.8
true p−values

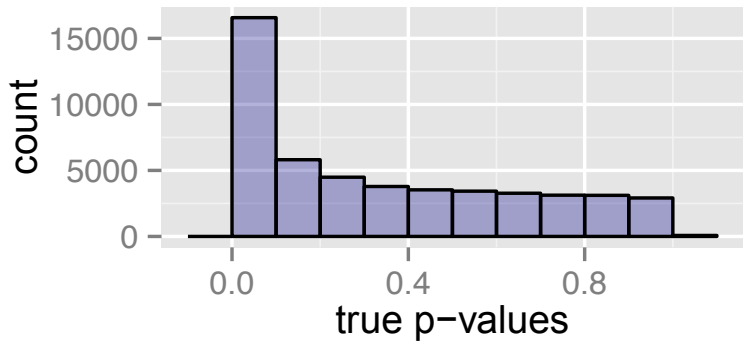

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

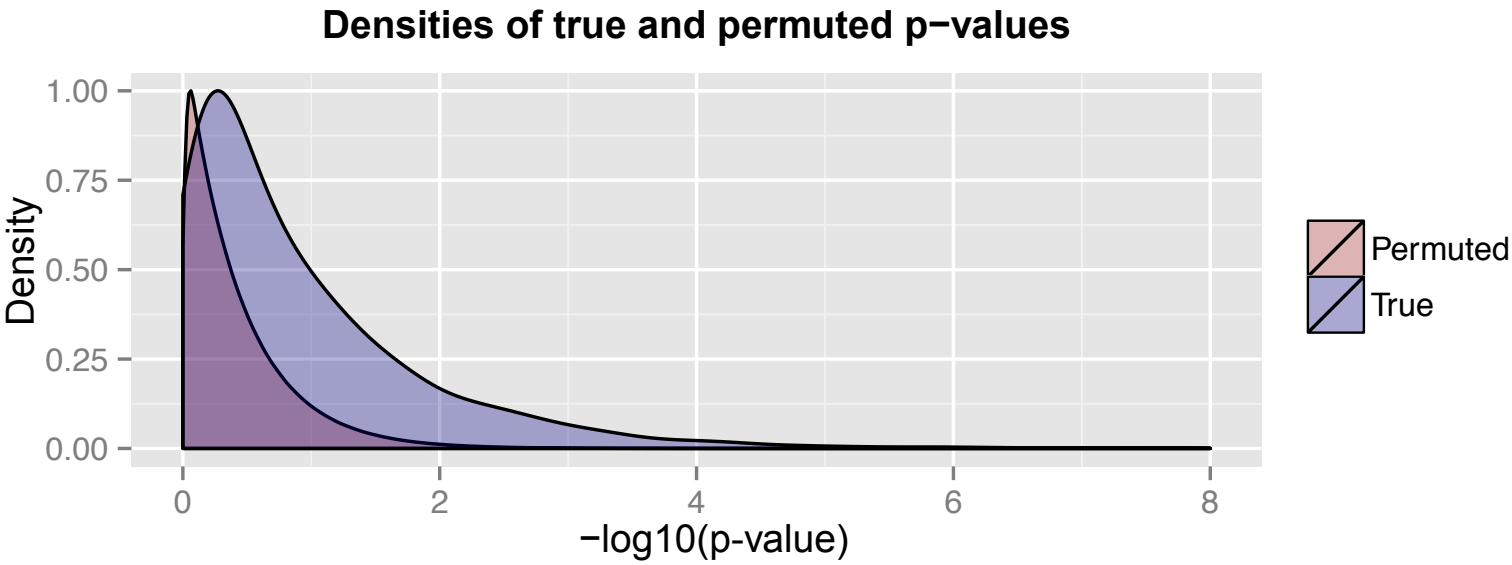

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

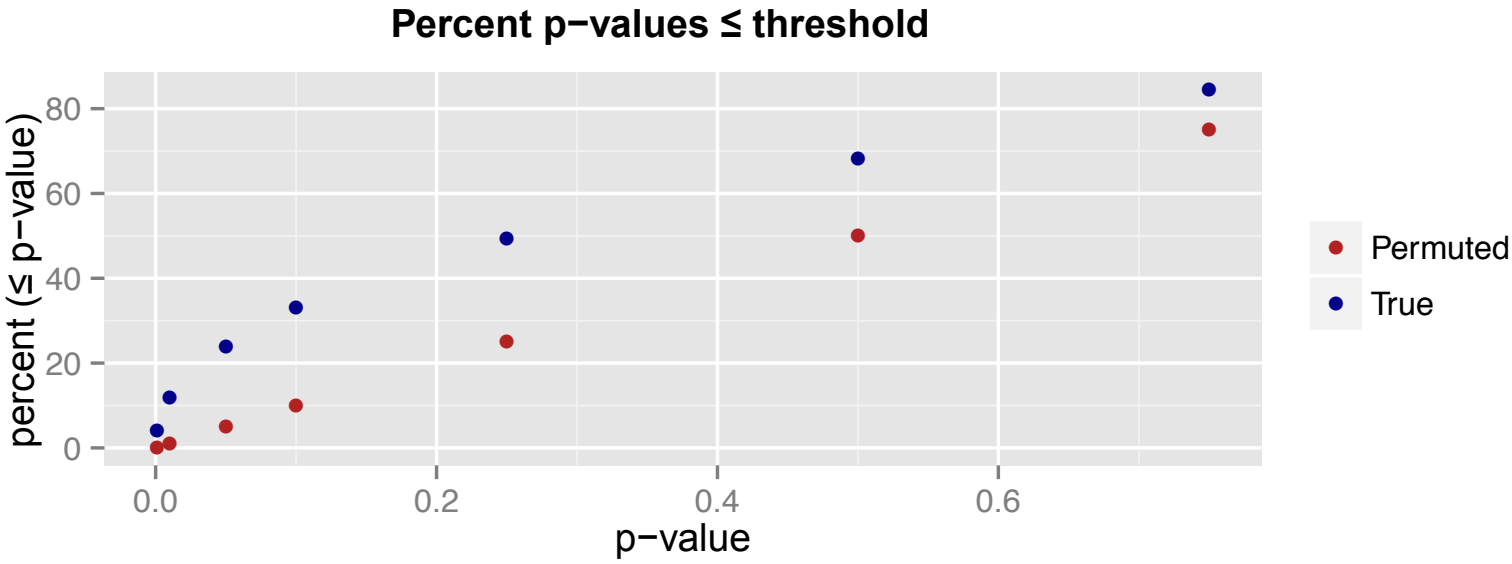
B

OTU 259097


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values

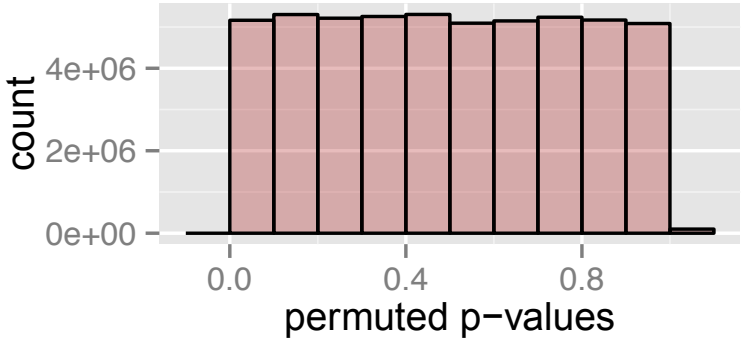

count
15000
10000
5000
0
0.0
0.4
0.8
true p−values

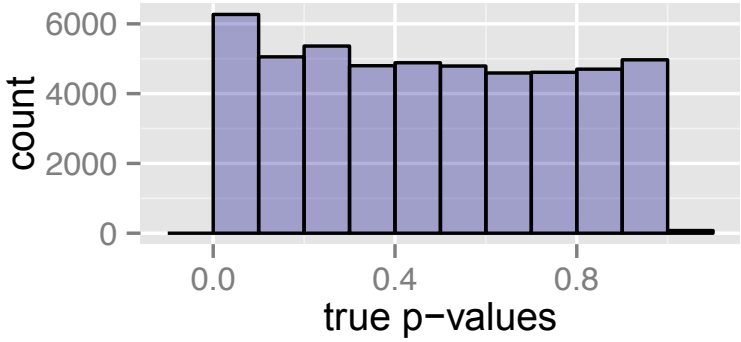

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

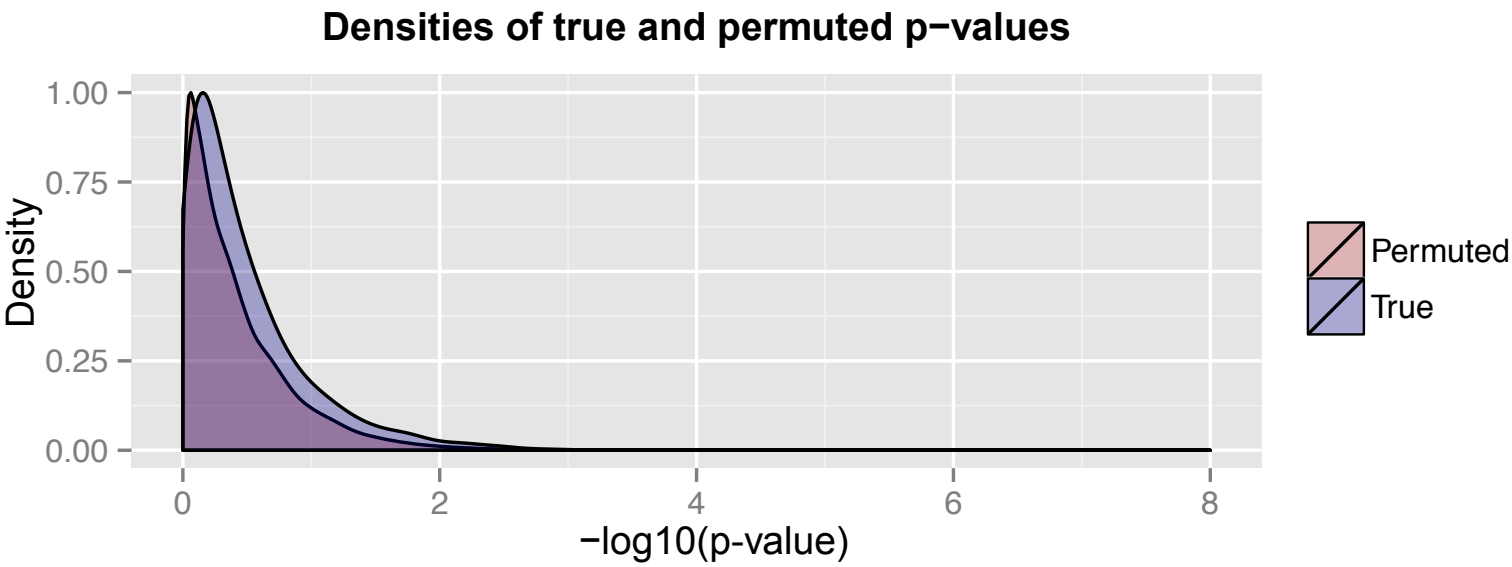

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

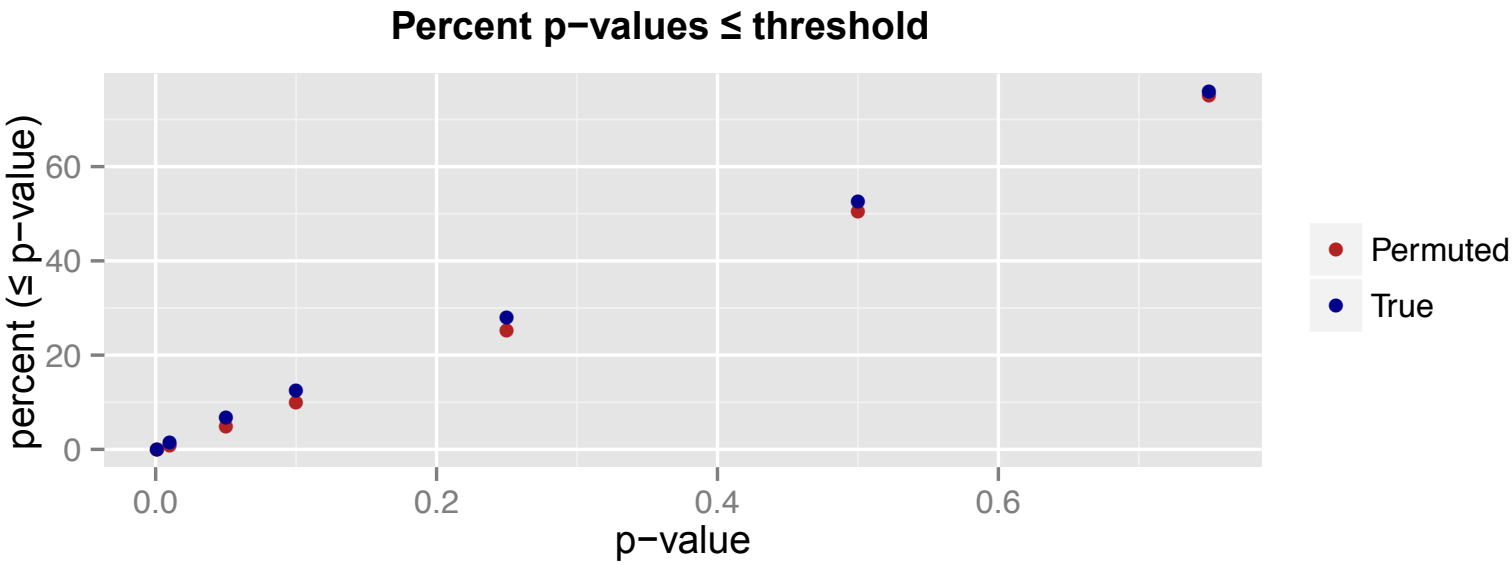
C

OTU 307


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

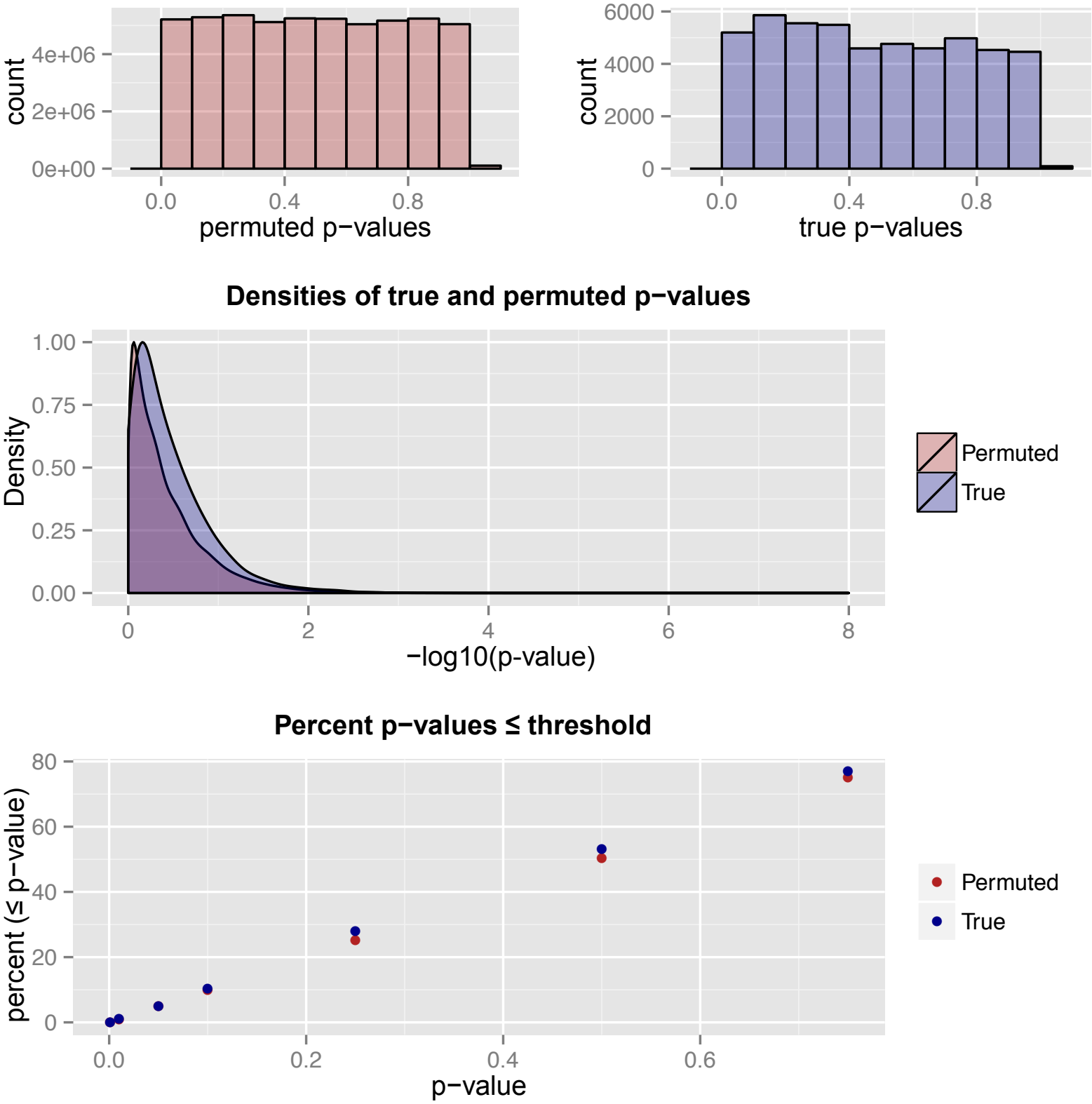

count
6000
4000
2000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

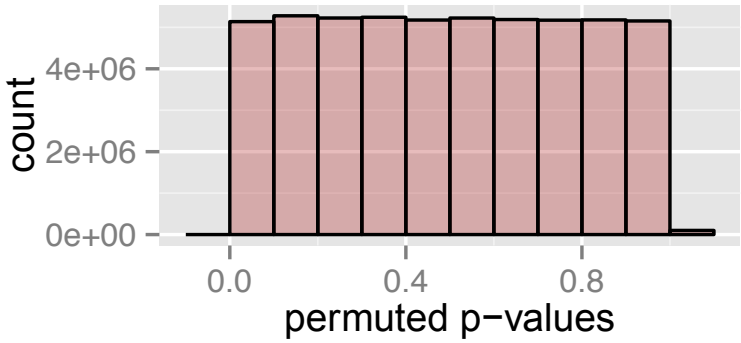
C

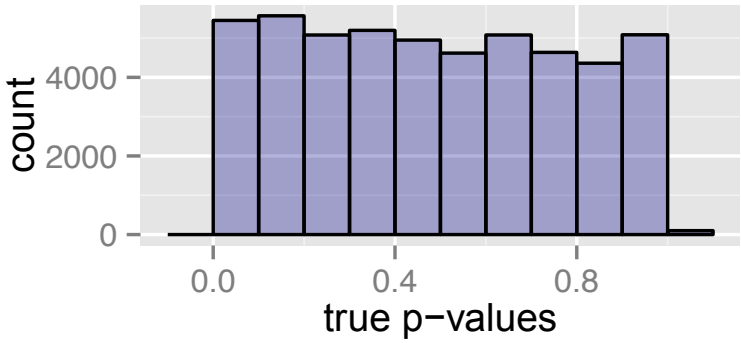
OTU 42432

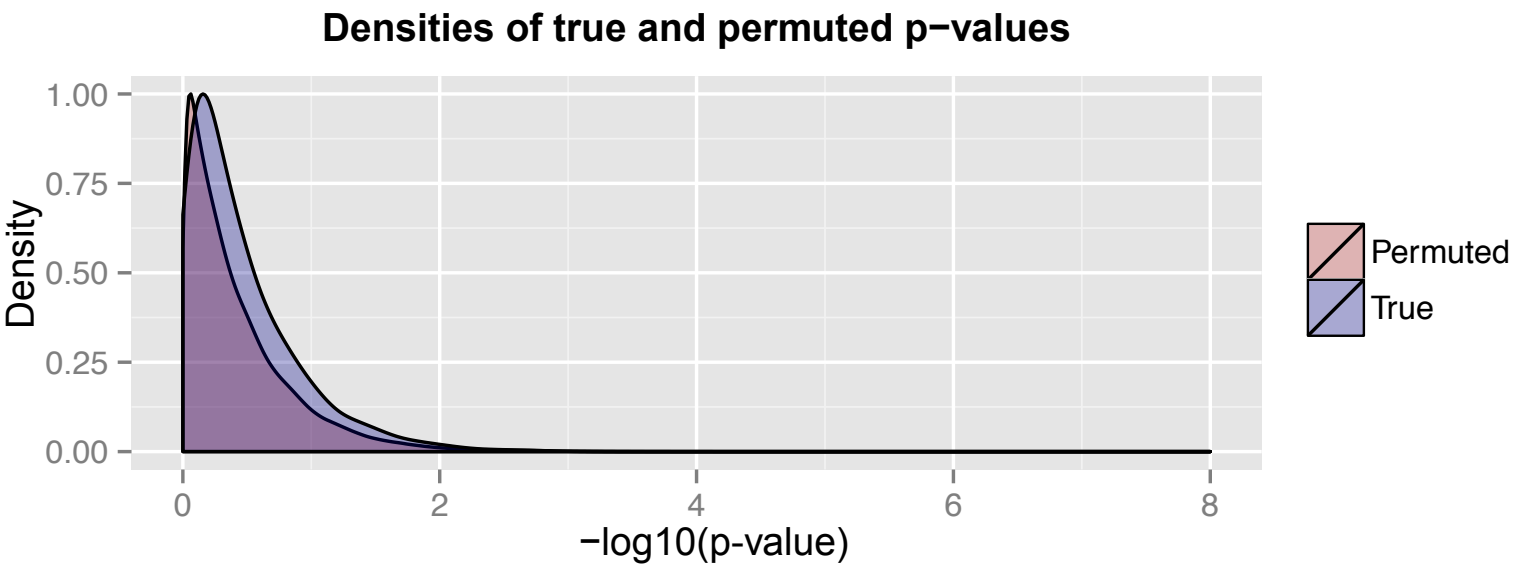

count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values
count
6000
4000
2000
0
0.0
0.4
0.8
true p−values
Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True
Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

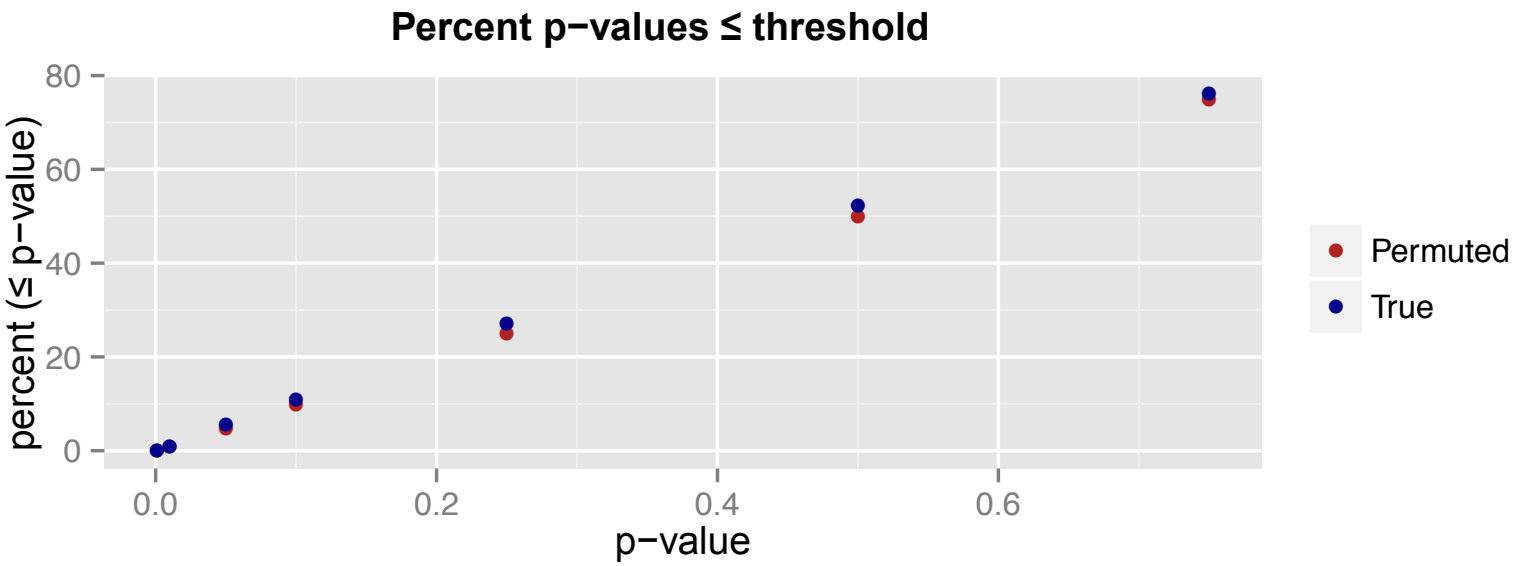
C

OTU 76682


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

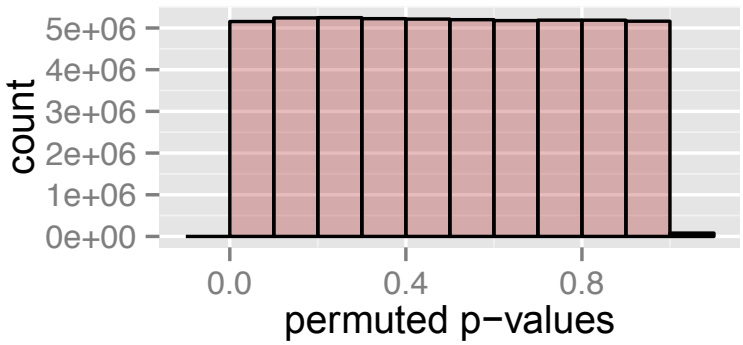

count
4000
2000
0
0.0
0.4
0.8
true p−values

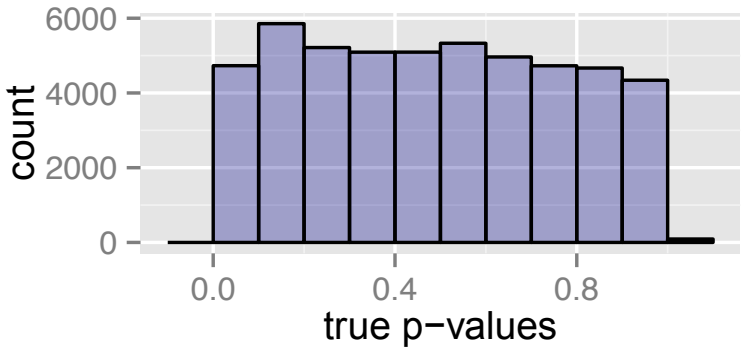

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

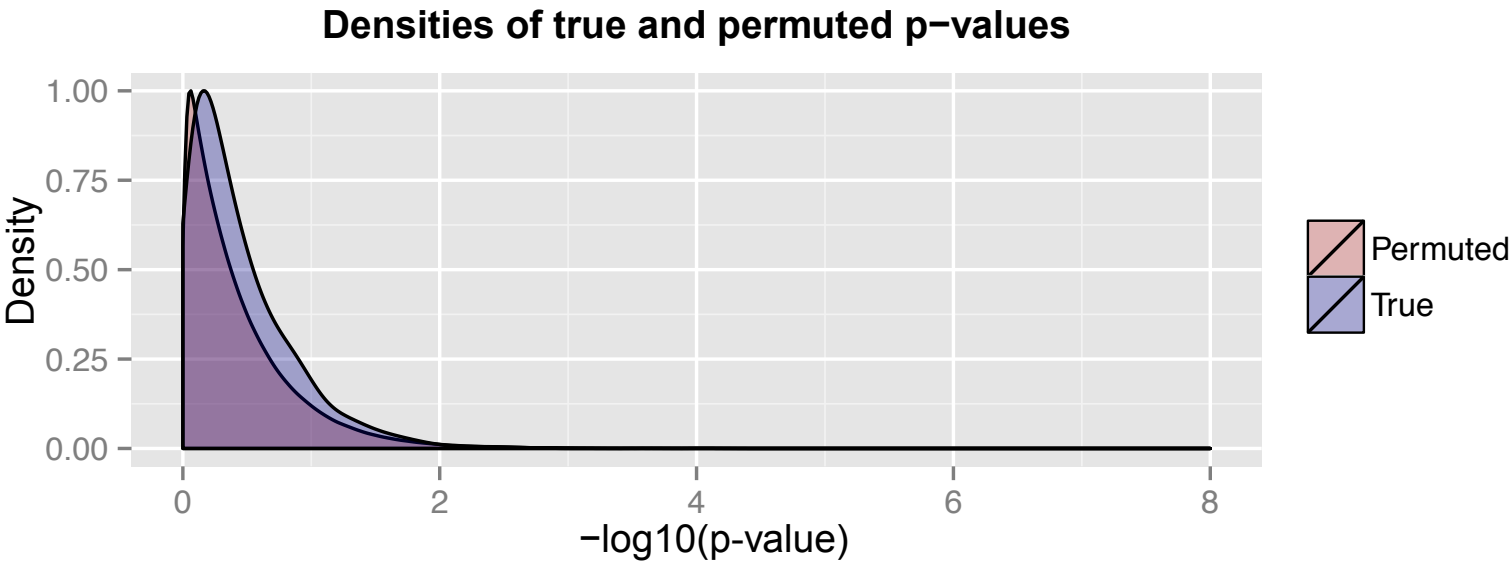

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

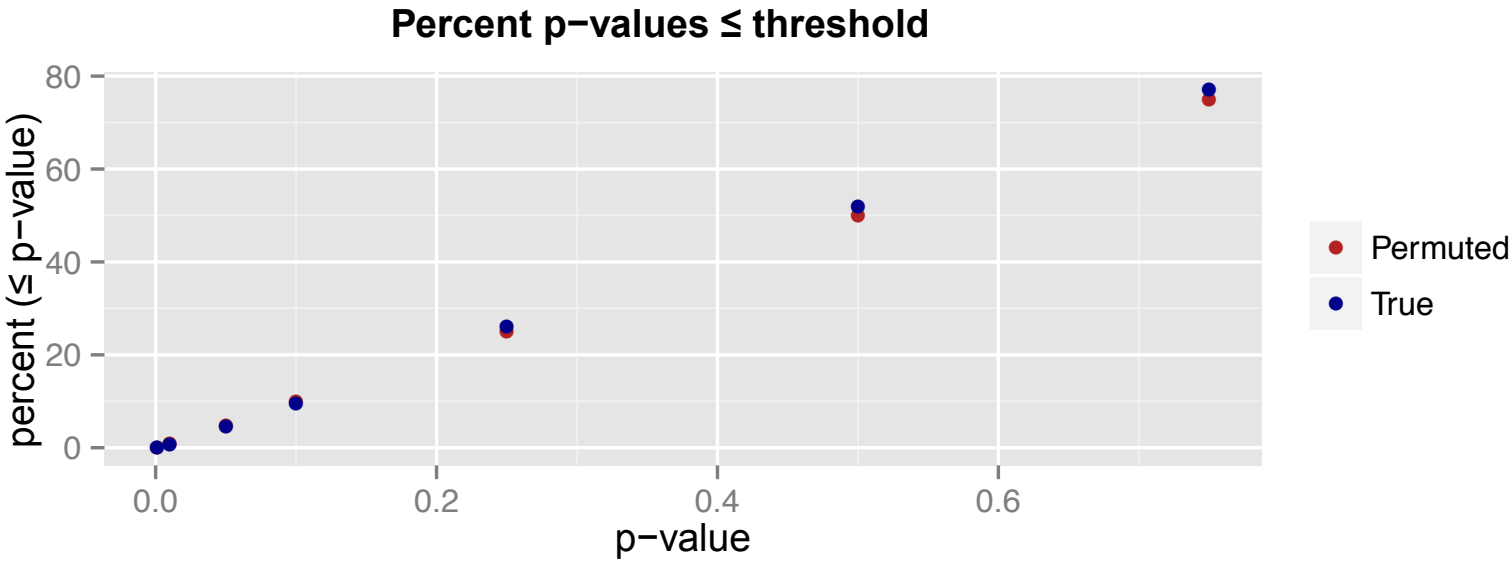
C

OTU 94966


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values

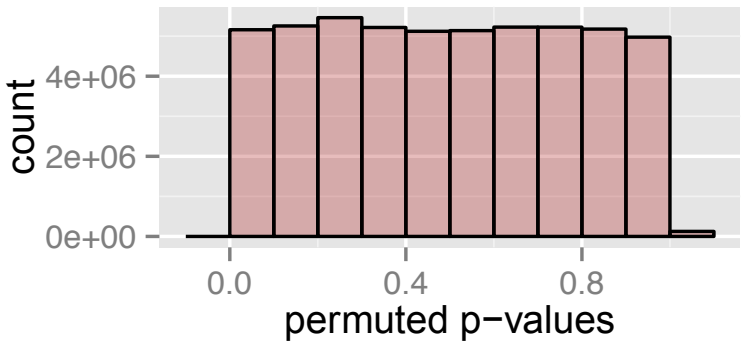

count
6000
4000
2000
0
0.0
0.4
0.8
true p−values

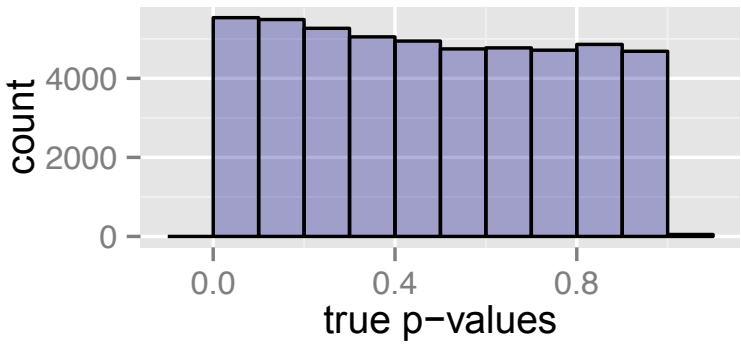

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

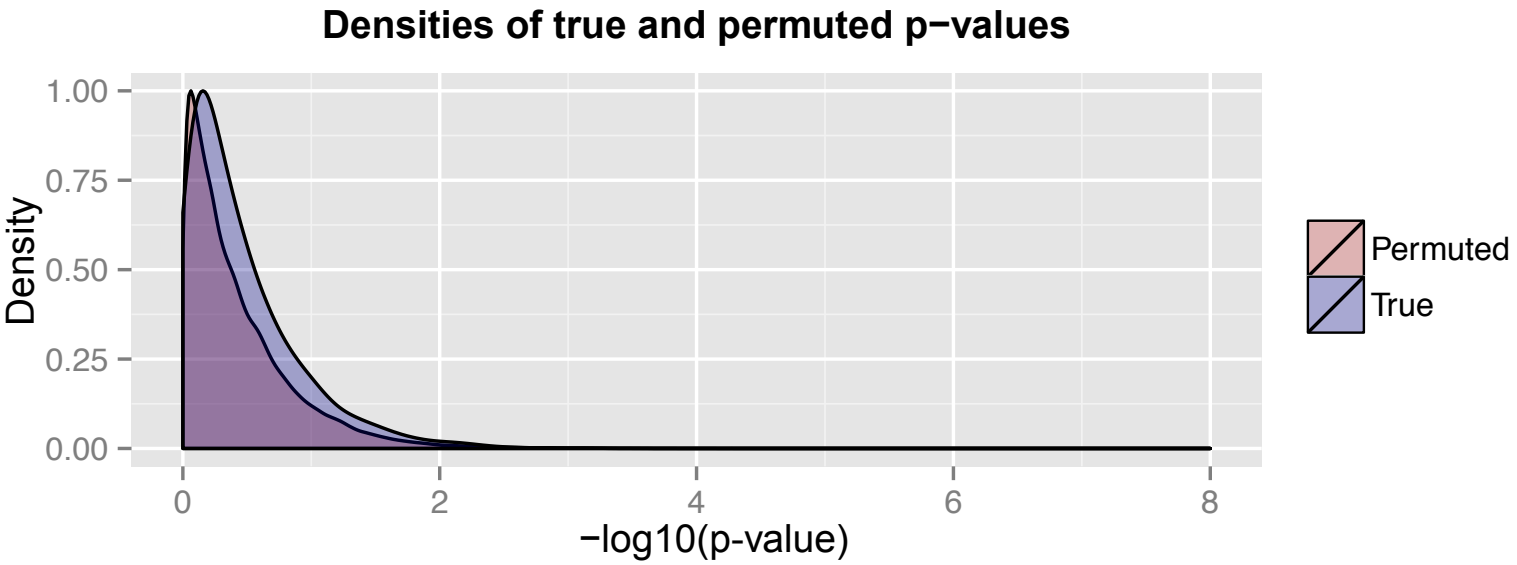

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

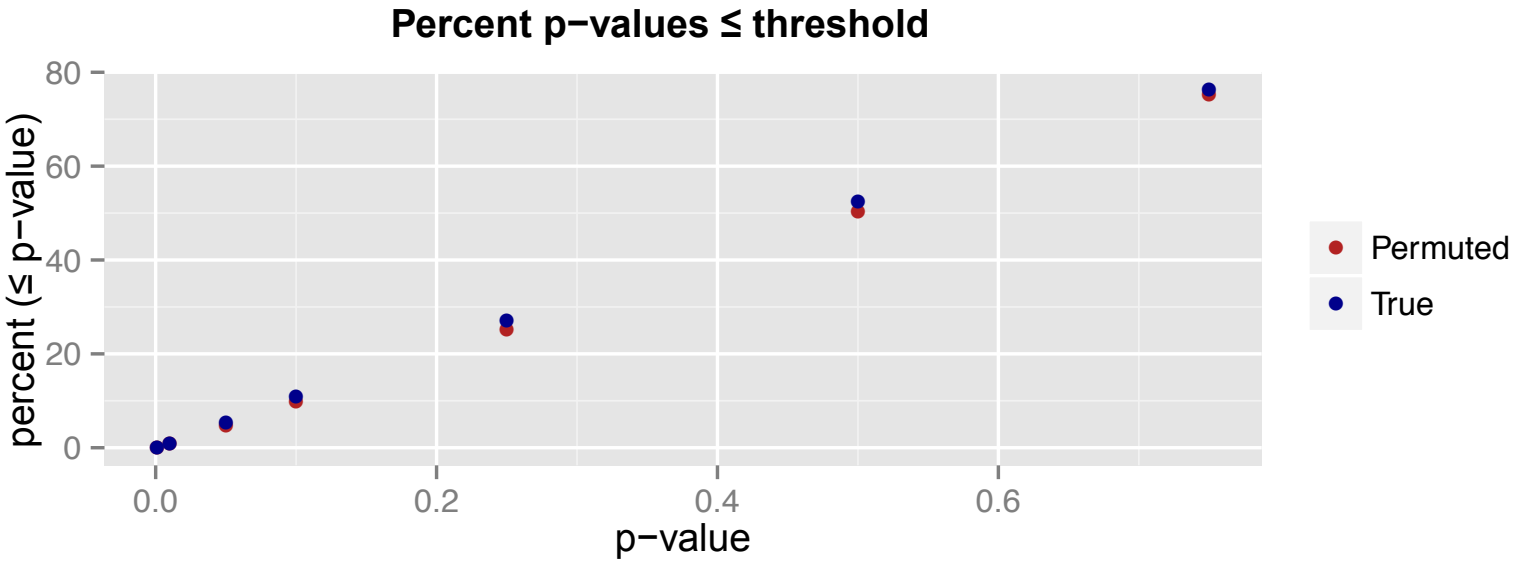
C

OTU 139345


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

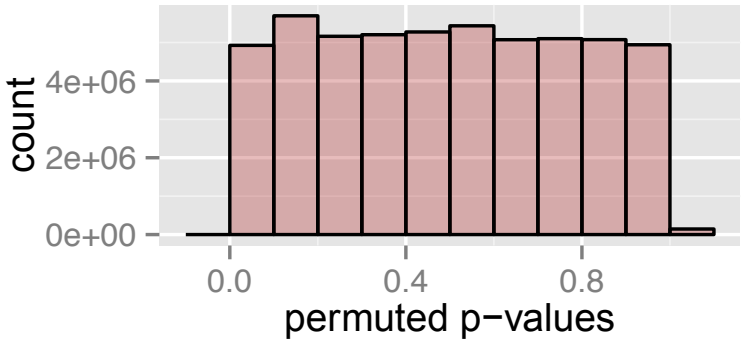

count
4000
2000
0
0.0
0.4
0.8
true p−values

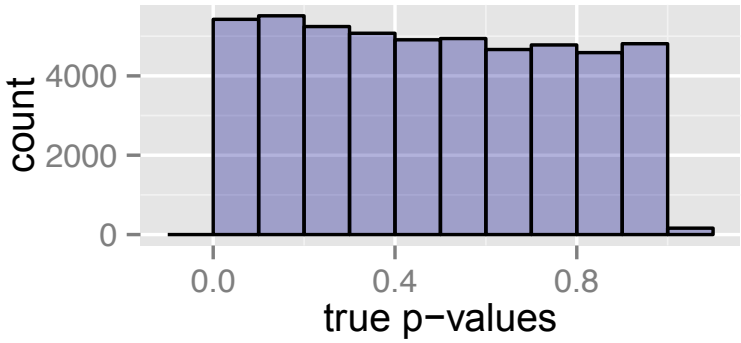

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

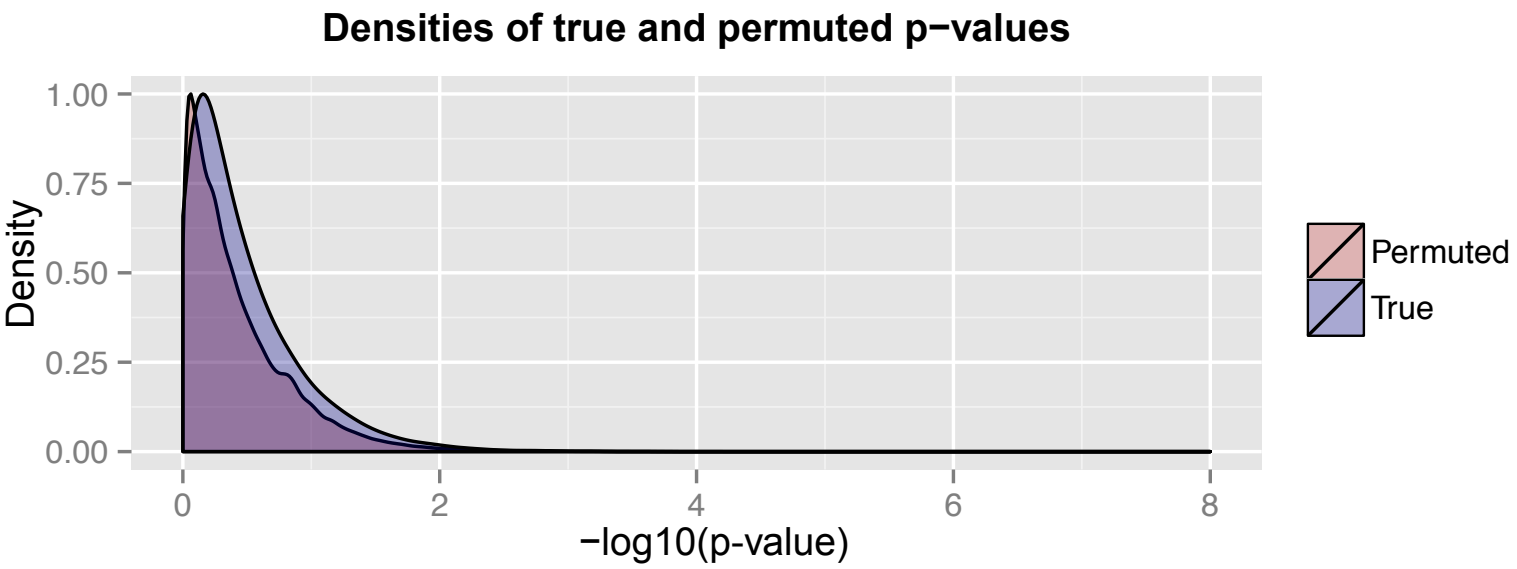

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

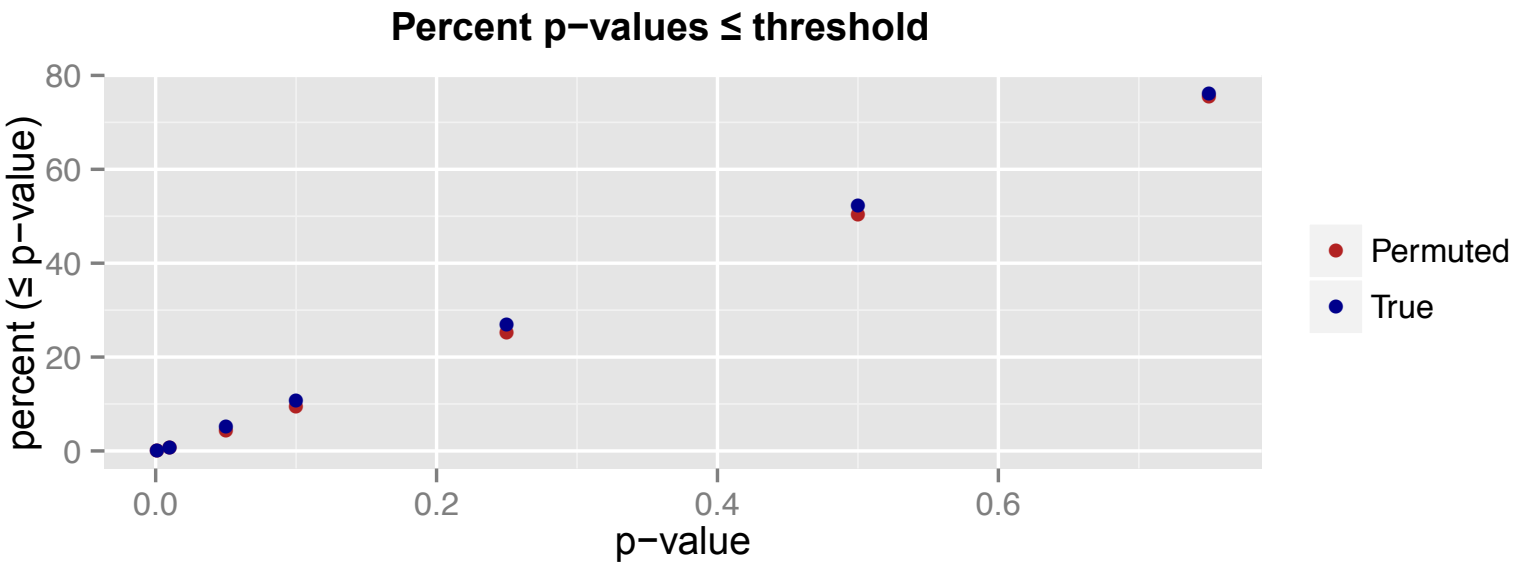
C

OTU 144586


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

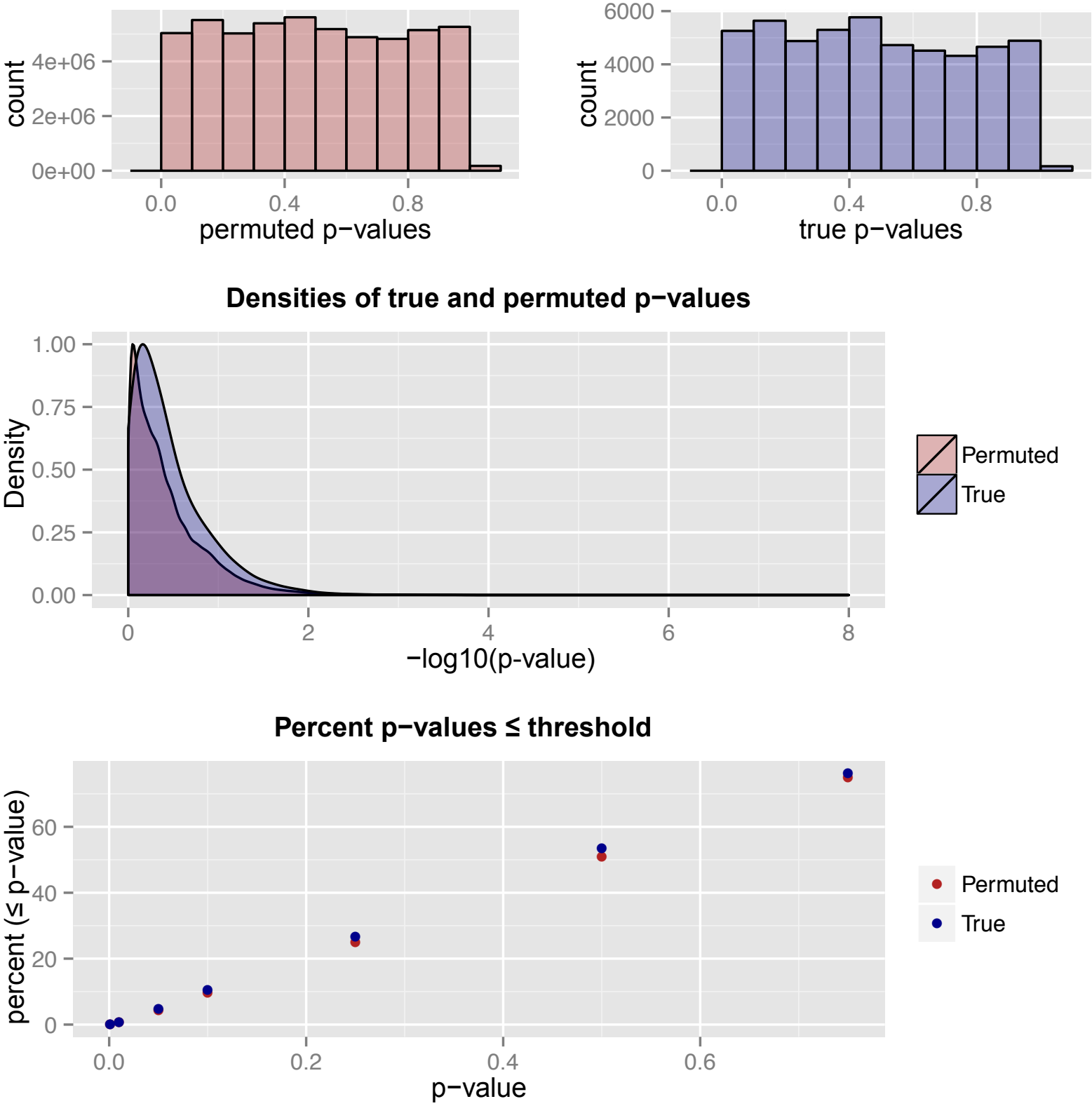

count
4000
2000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

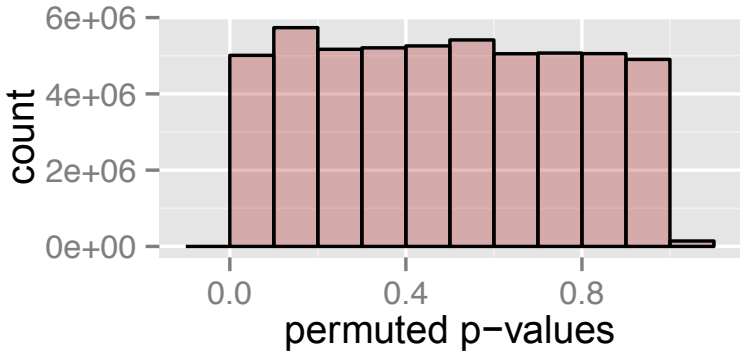
C

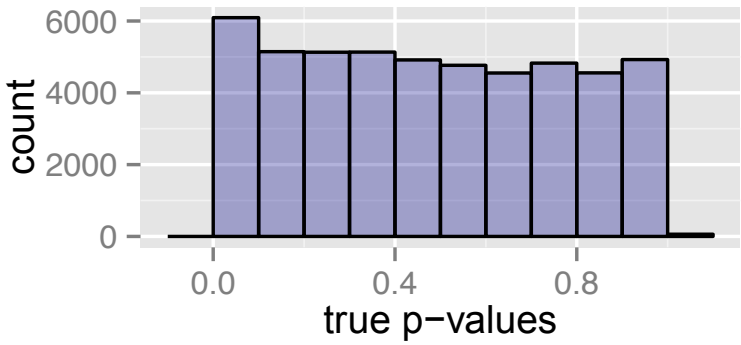
OTU 153137

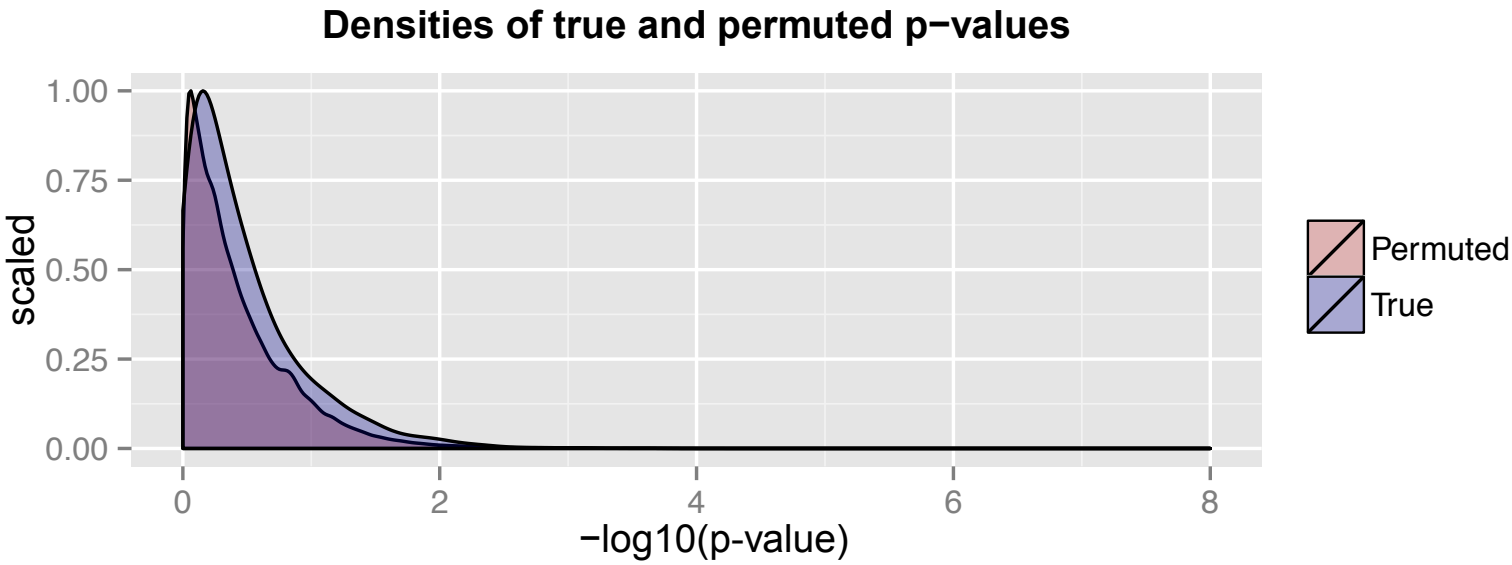

count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values
count
6000
4000
2000
0
0.0
0.4
0.8
true p−values
Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True
Percent p−values ≤ threshold
percent (≤ p−value)
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

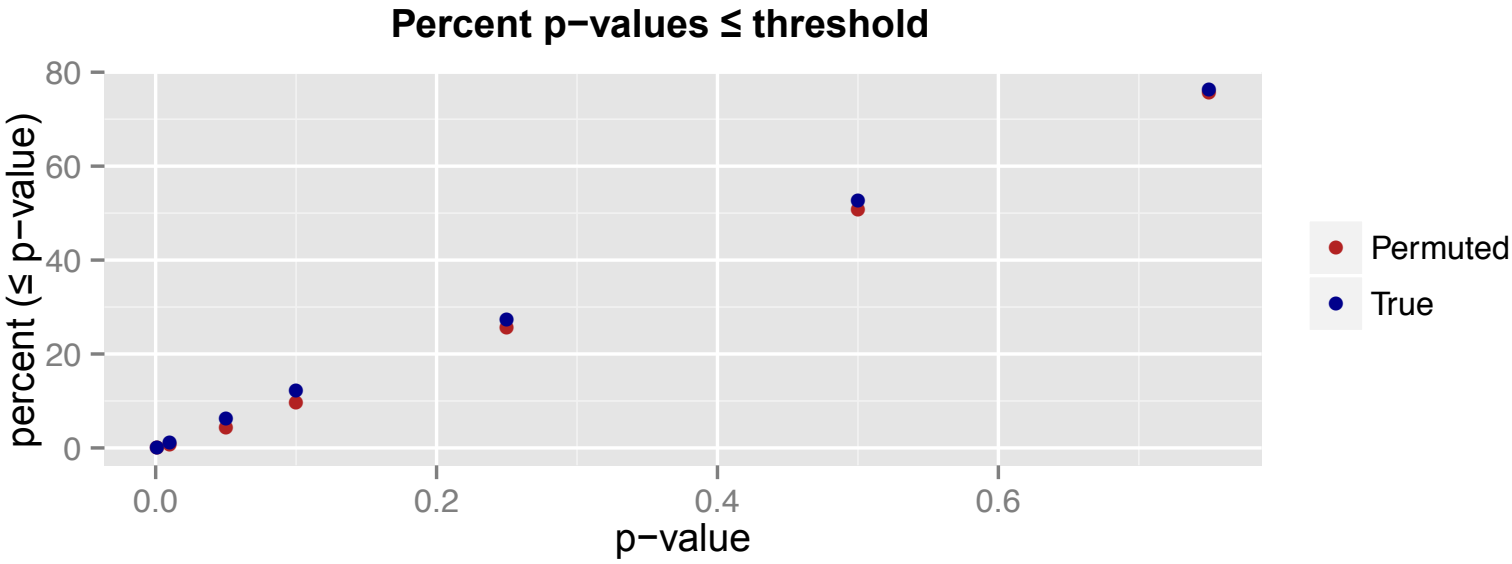
C

OTU 192480


6e+06
4e+06
2e+06
0e+00
count
0.0
0.4
0.8
permuted p−values

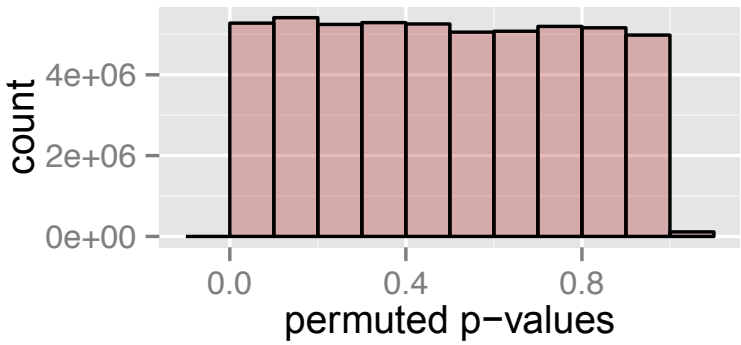

6000
4000
2000
0
count
0.0
0.4
0.8
true p−values

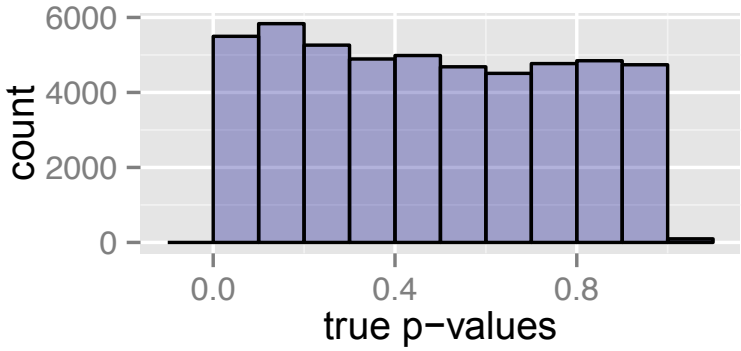

Densities of true and permuted p−values
1.00
0.75
0.50
0.25
0.00
scaled
0
2
4
6
8
−log10(p-value)
Permuted
True

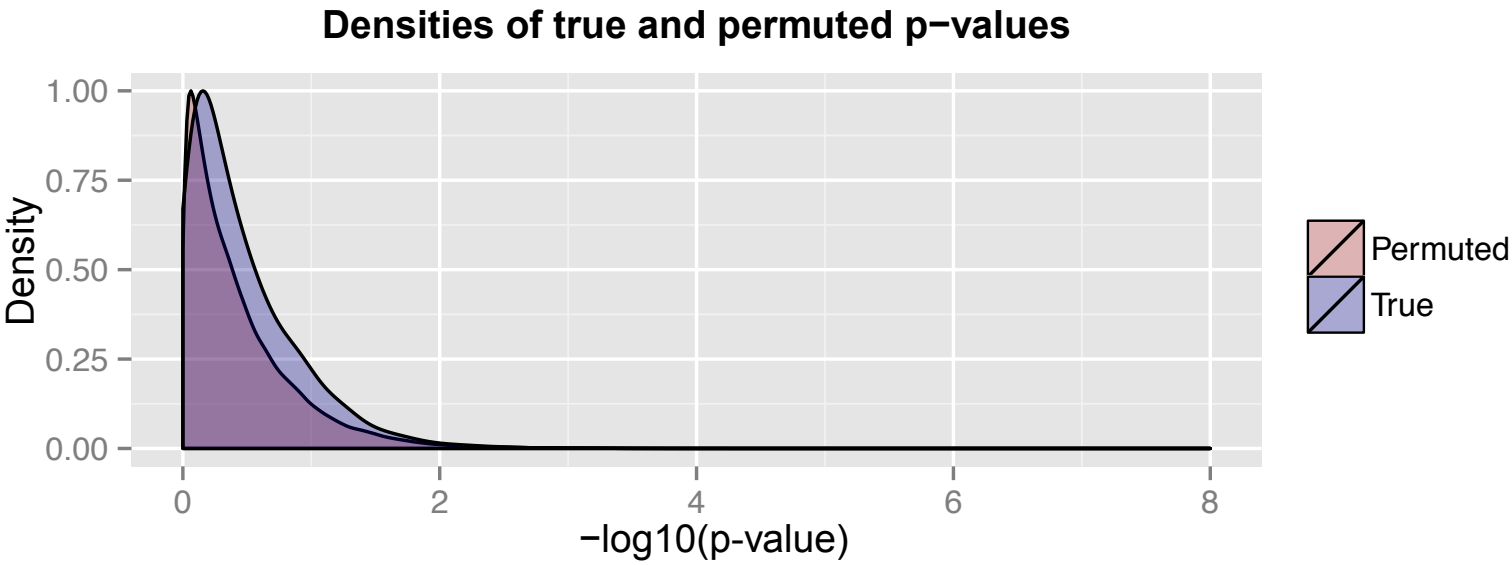

Percent p−values ≤ threshold
80
60
40
20
0
percent (≤ p−value)
0.0
0.2
0.4
0.6
p−value
Permuted
True

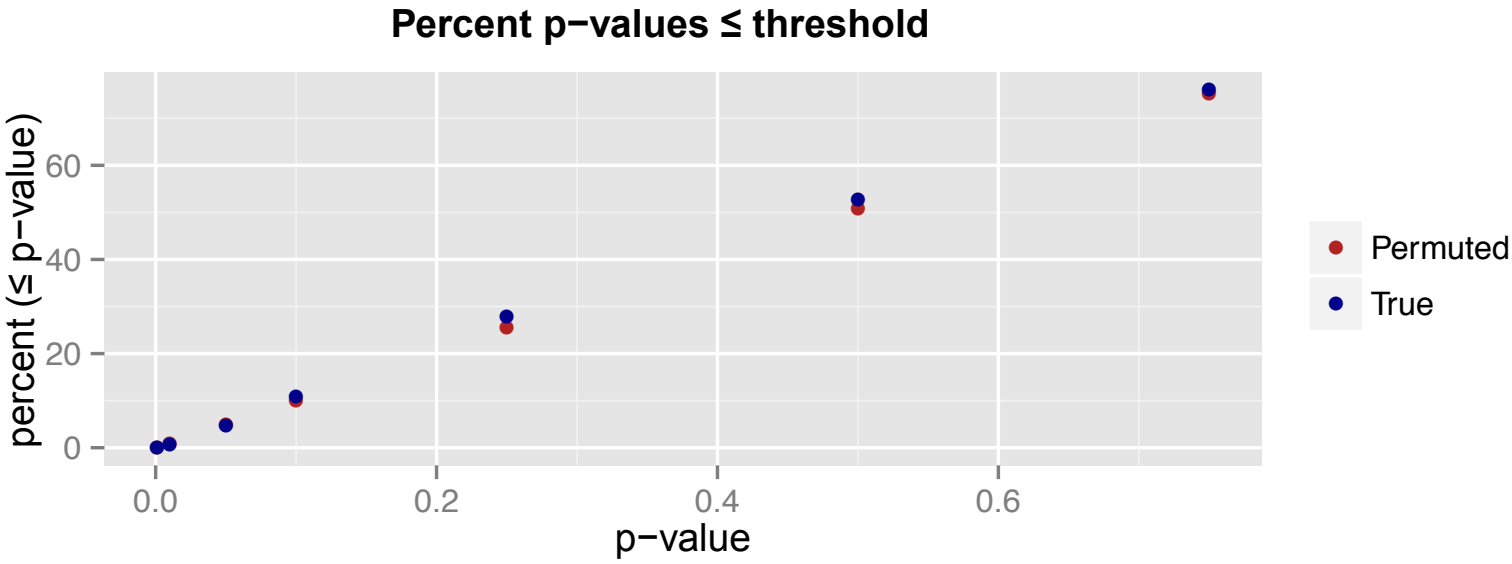
C

OTU 204825


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

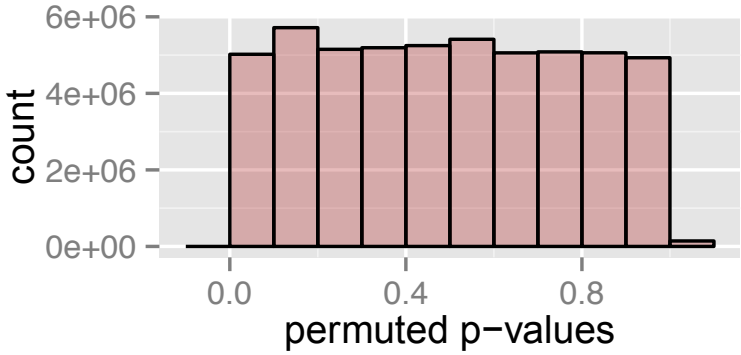

count
6000
4000
2000
0
0.0
0.4
0.8
true p−values

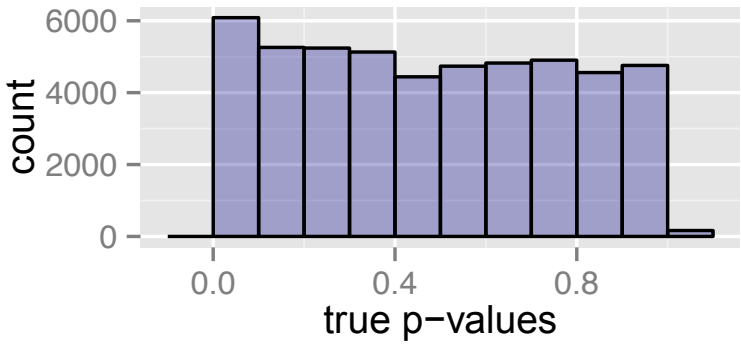

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

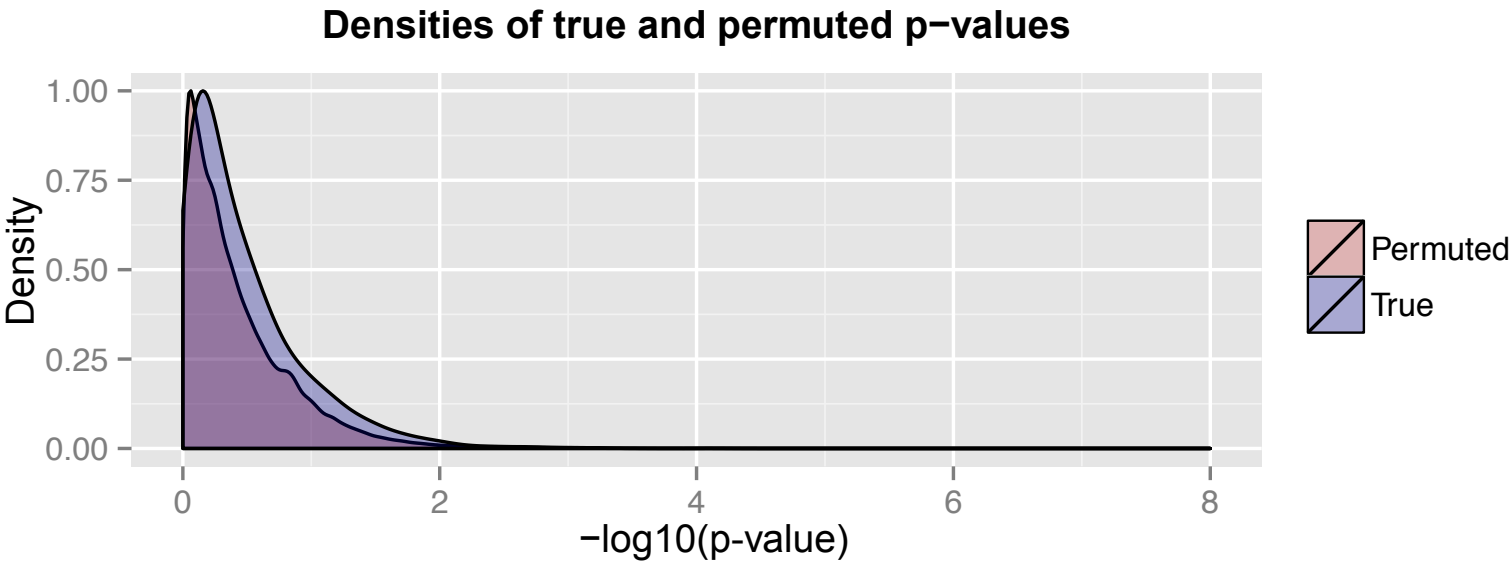

Percent p−values ≤ threshold
percent (≤ p−value)
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

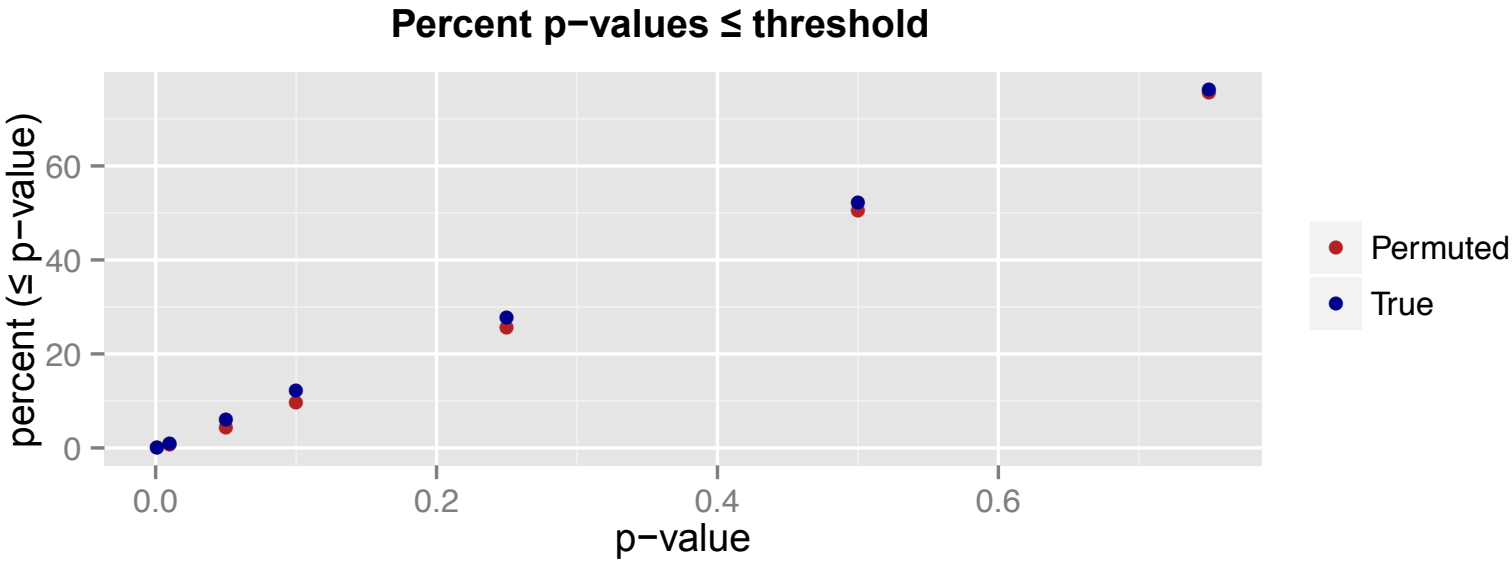
C

OTU 213172


6e+06
4e+06
2e+06
0e+00
count
0.0
0.4
0.8
permuted p−values

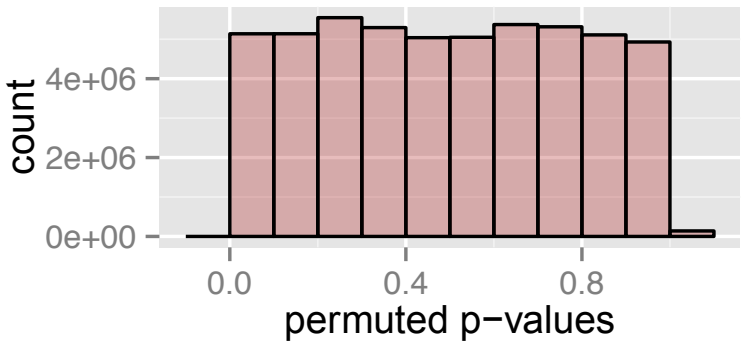

6000
4000
2000
0
count
0.0
0.4
0.8
true p−values

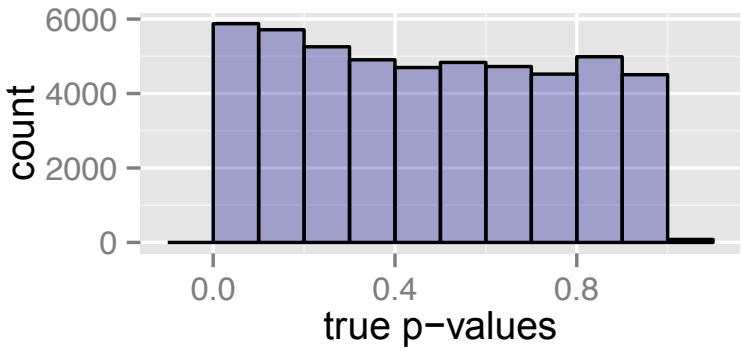

Densities of true and permuted p−values
1.00
0.75
0.50
0.25
0.00
Density
0
2
4
6
8
−log10(p-value)
Permuted
True

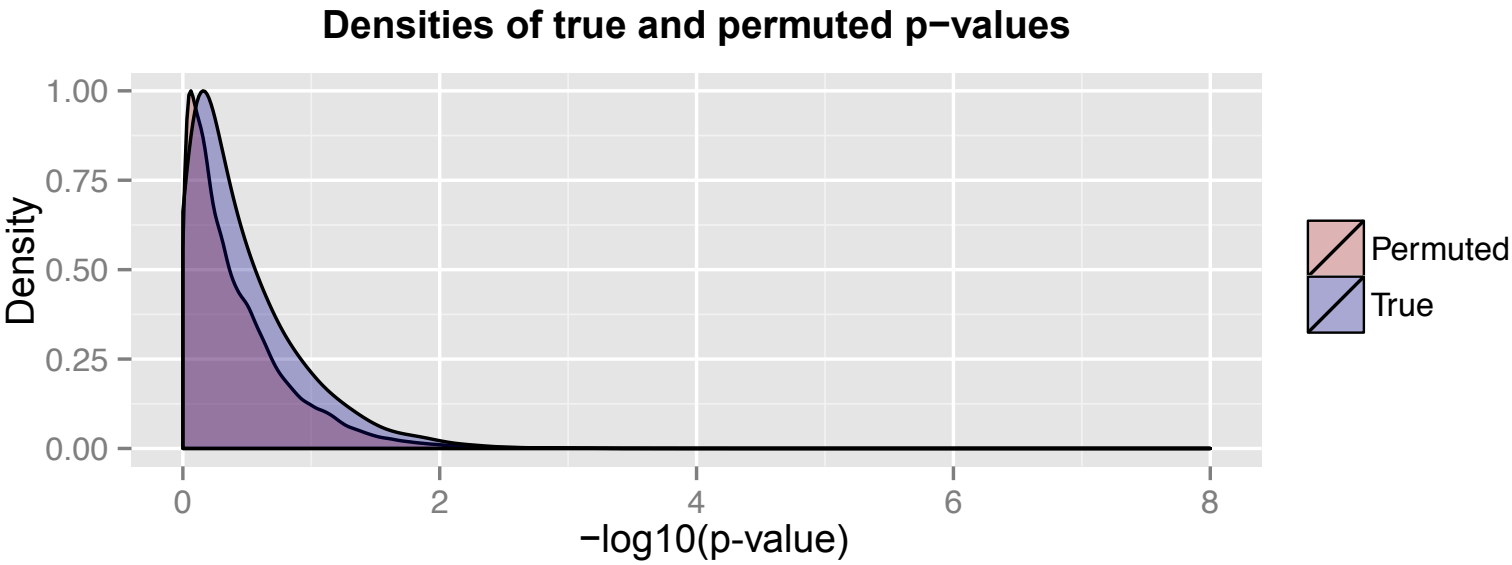

Percent p−values ≤ threshold
60
40
20
0
percent (≤ p−value)
0.0
0.2
0.4
0.6
p−value
Permuted
True

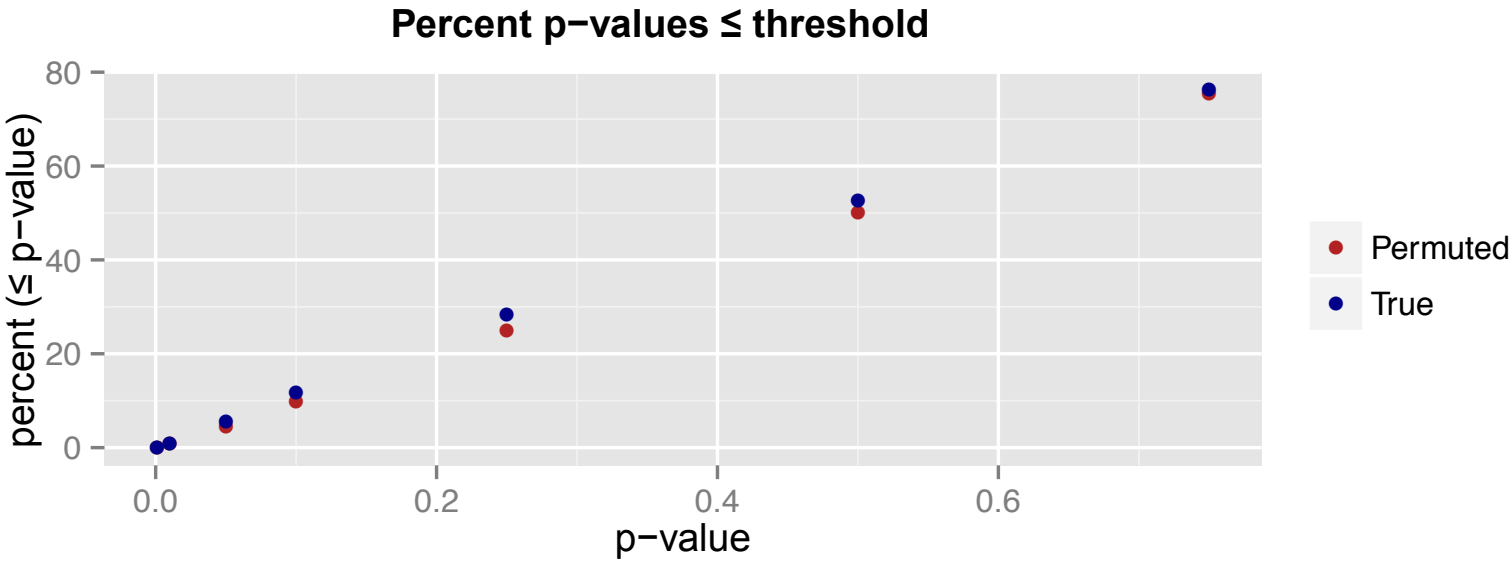
C

OTU 248218


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

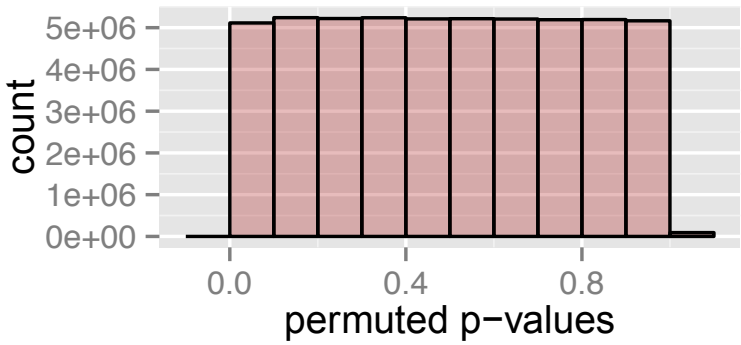

count
6000
4000
2000
0
0.0
0.4
0.8
true p−values

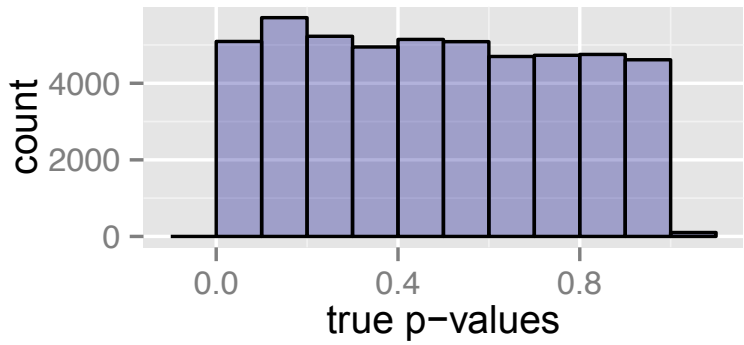

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

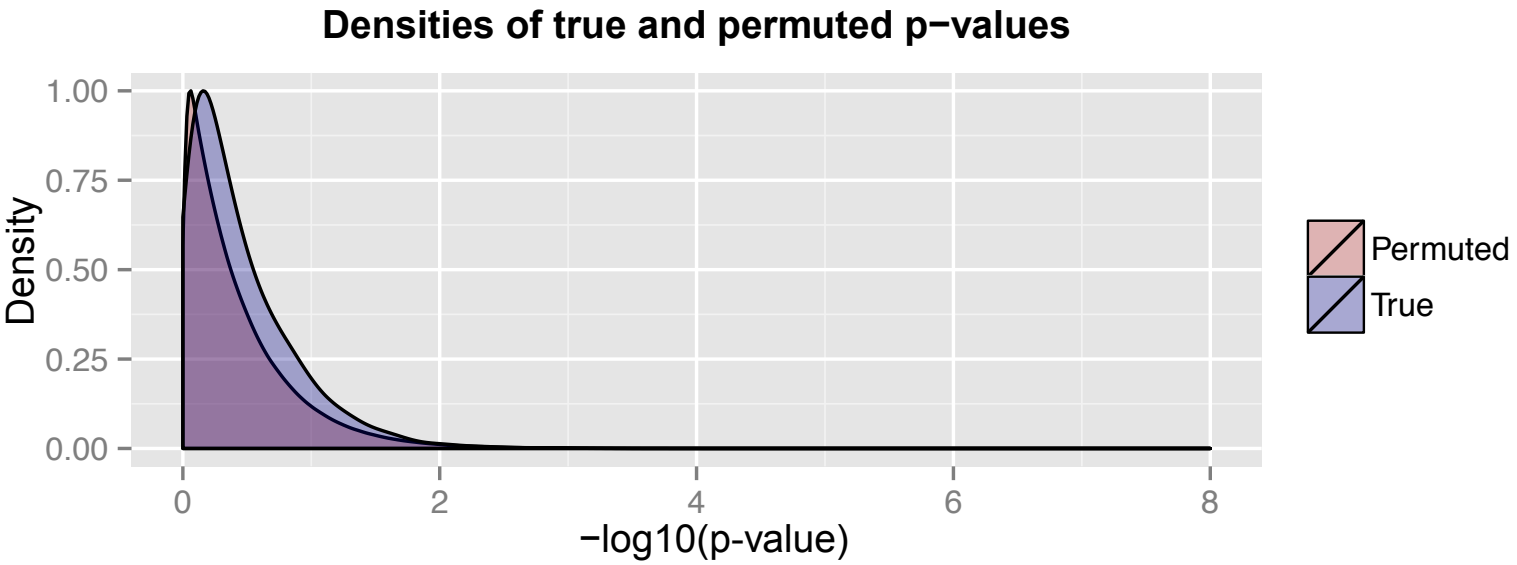

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

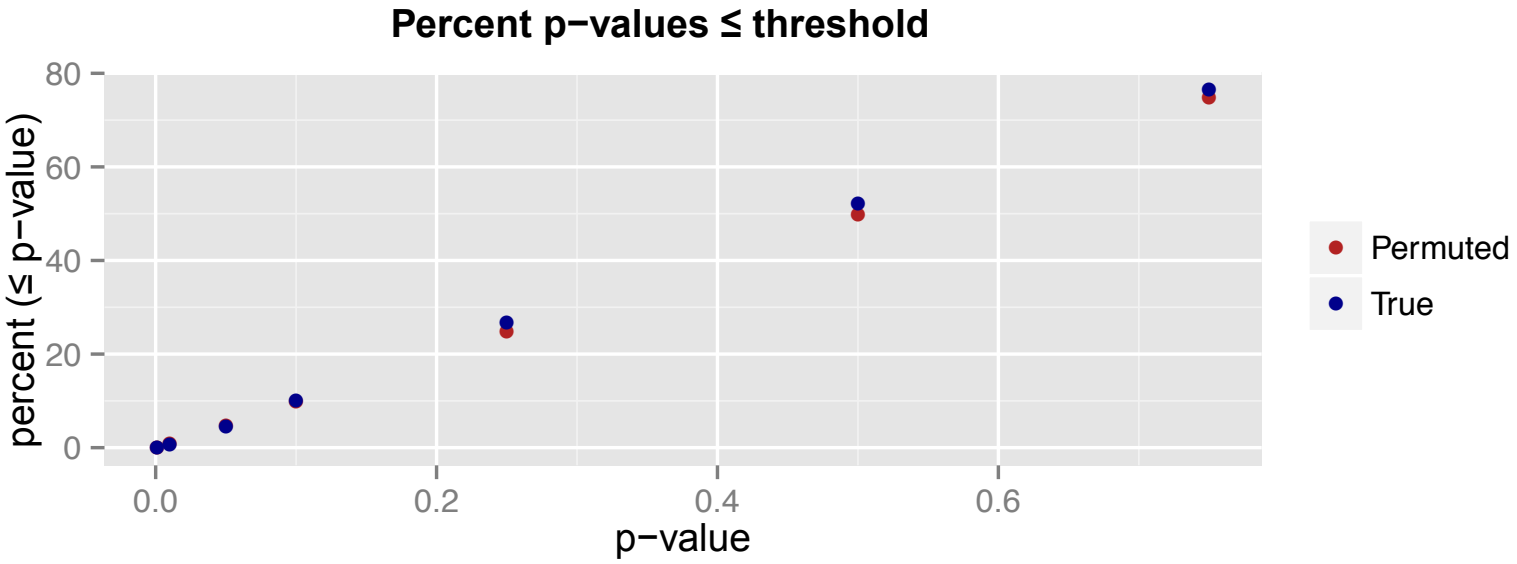
C

OTU 250510


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values

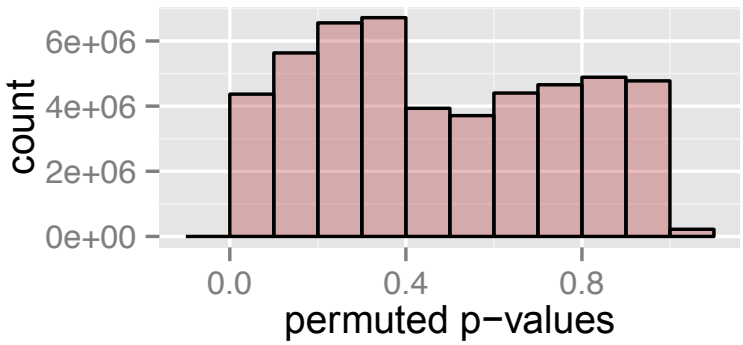

count
4000
2000
0
0.0
0.4
0.8
true p−values

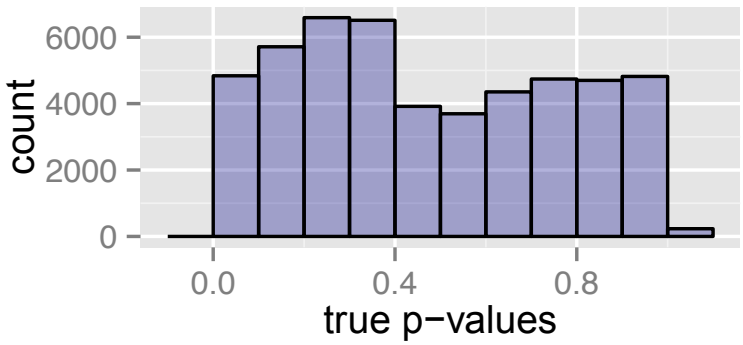

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

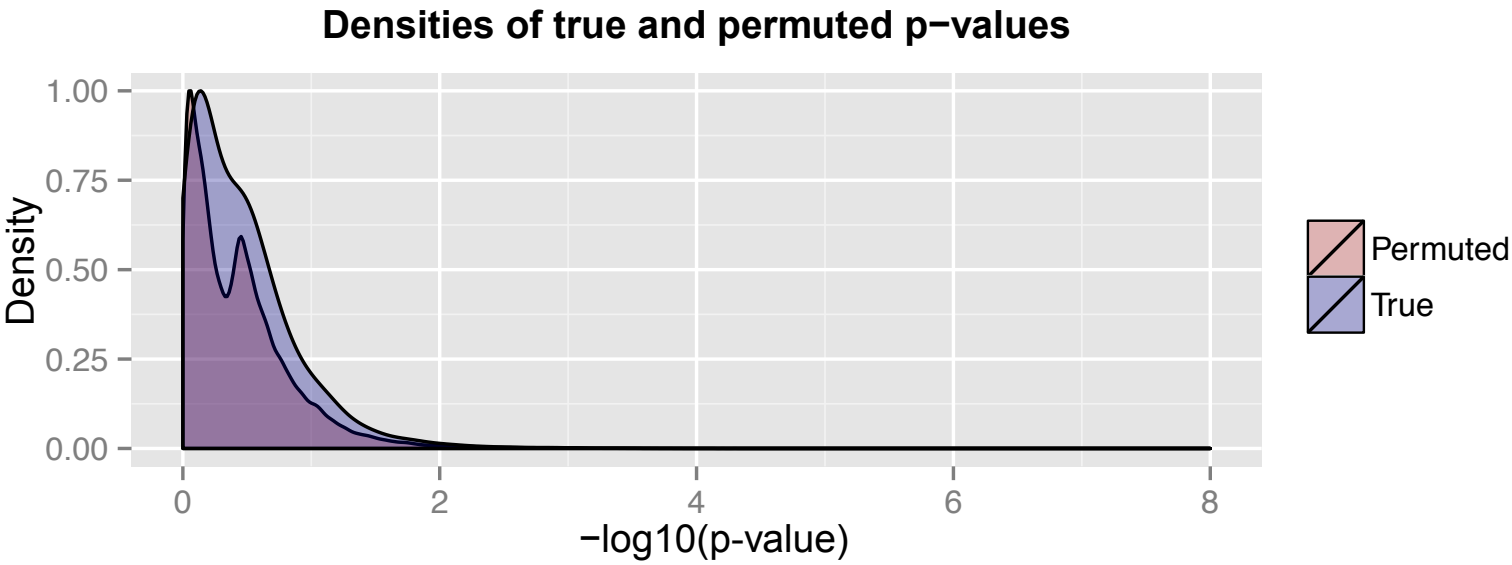

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

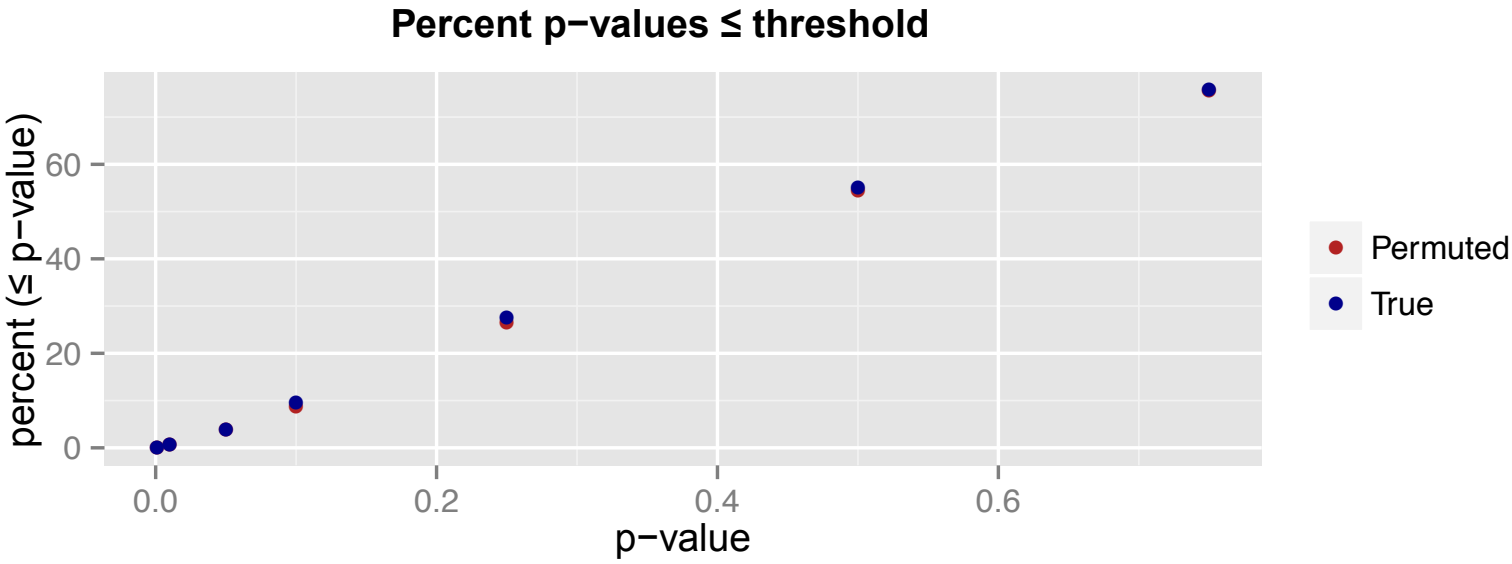
C

OTU 255403


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

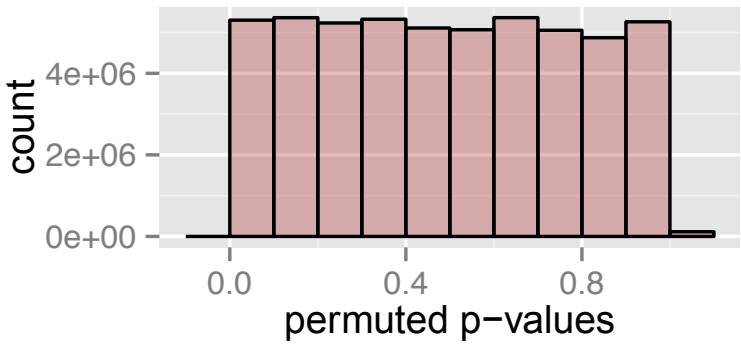

count
6000
4000
2000
0
0.0
0.4
0.8
true p−values

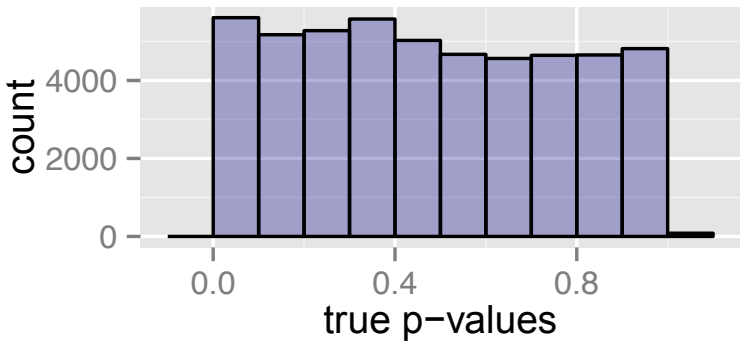

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

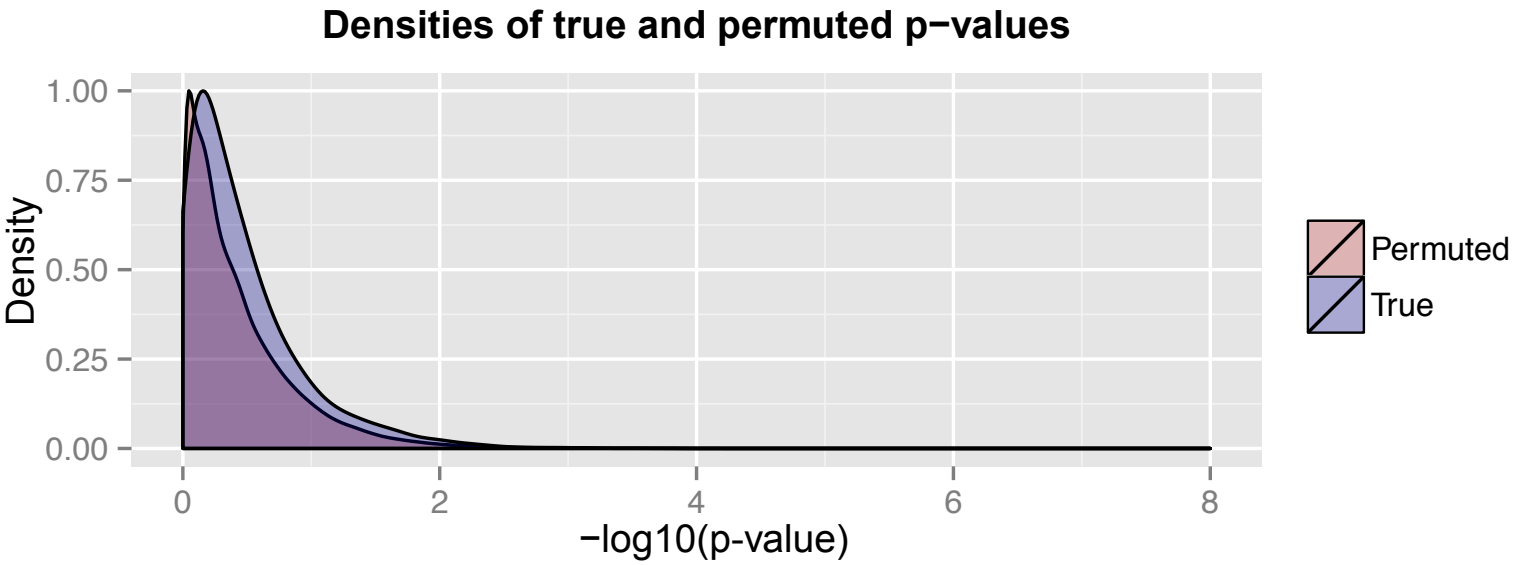

Percent p−values ≤ threshold
percent (≤ p−value)
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

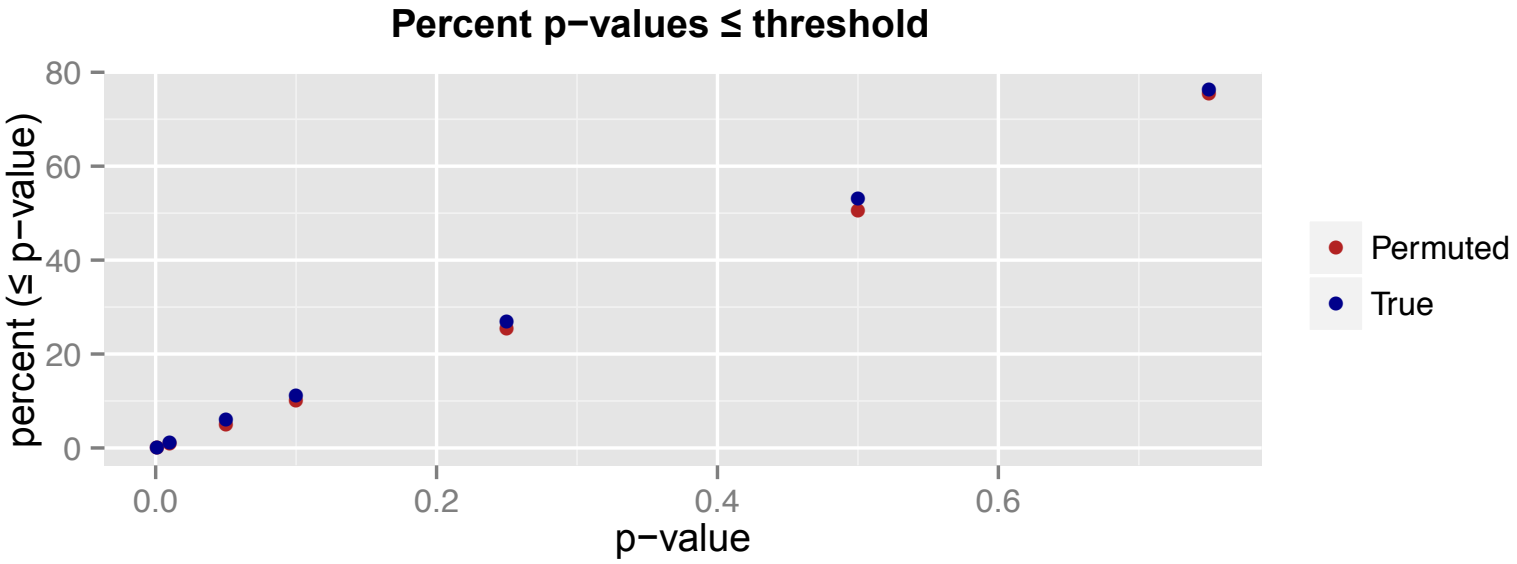
C

OTU 259596


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

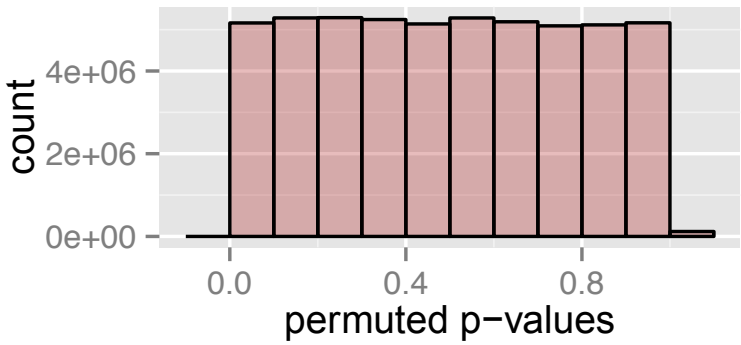

count
4000
2000
0
0.0
0.4
0.8
true p−values

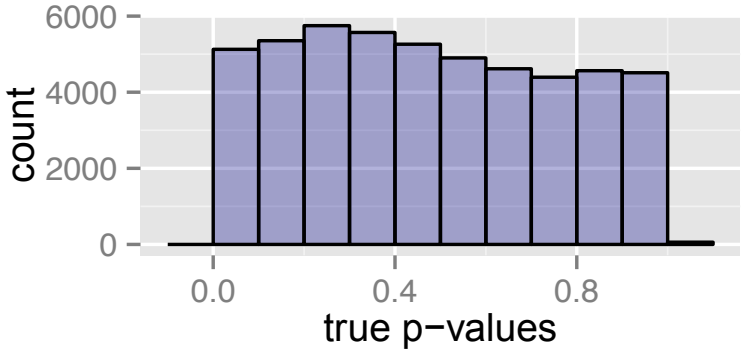

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

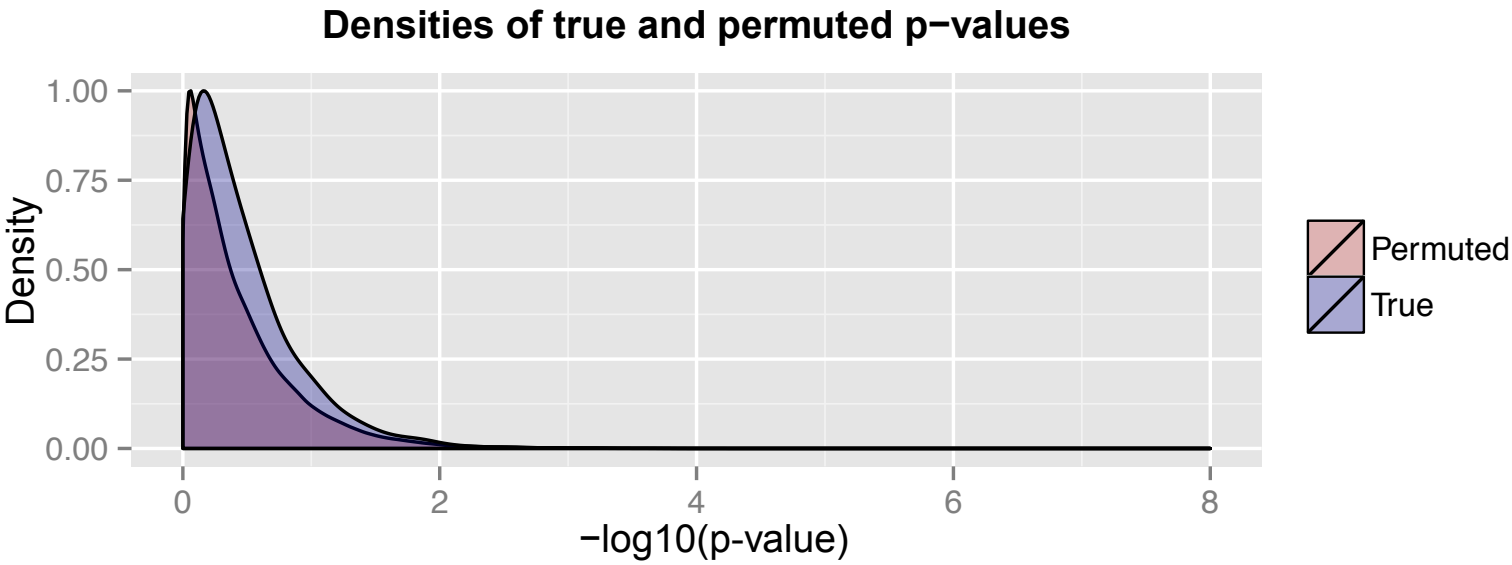

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

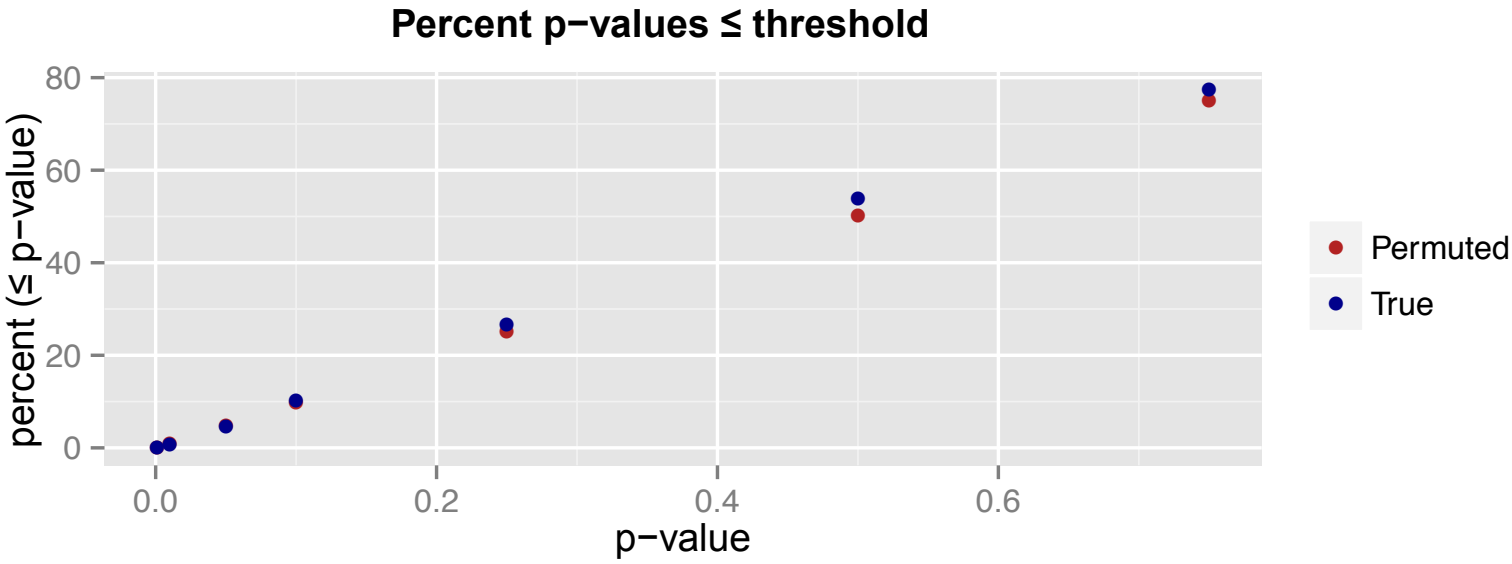
C

OTU 340220


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

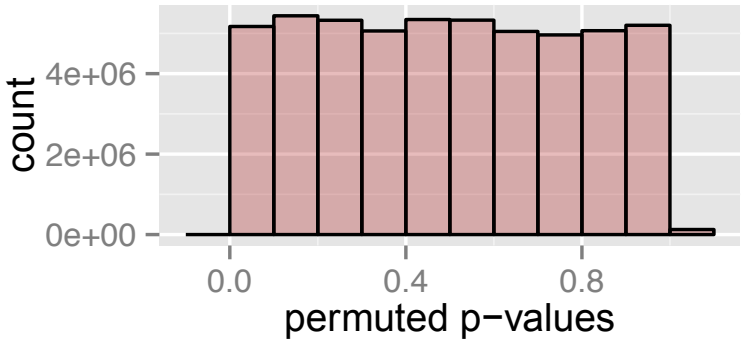

count
6000
4000
2000
0
0.0
0.4
0.8
true p−values

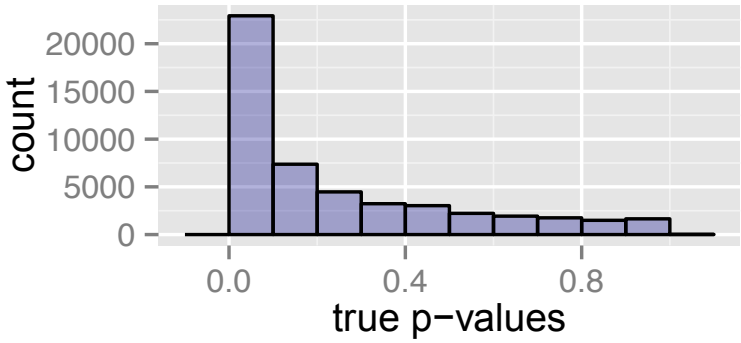

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

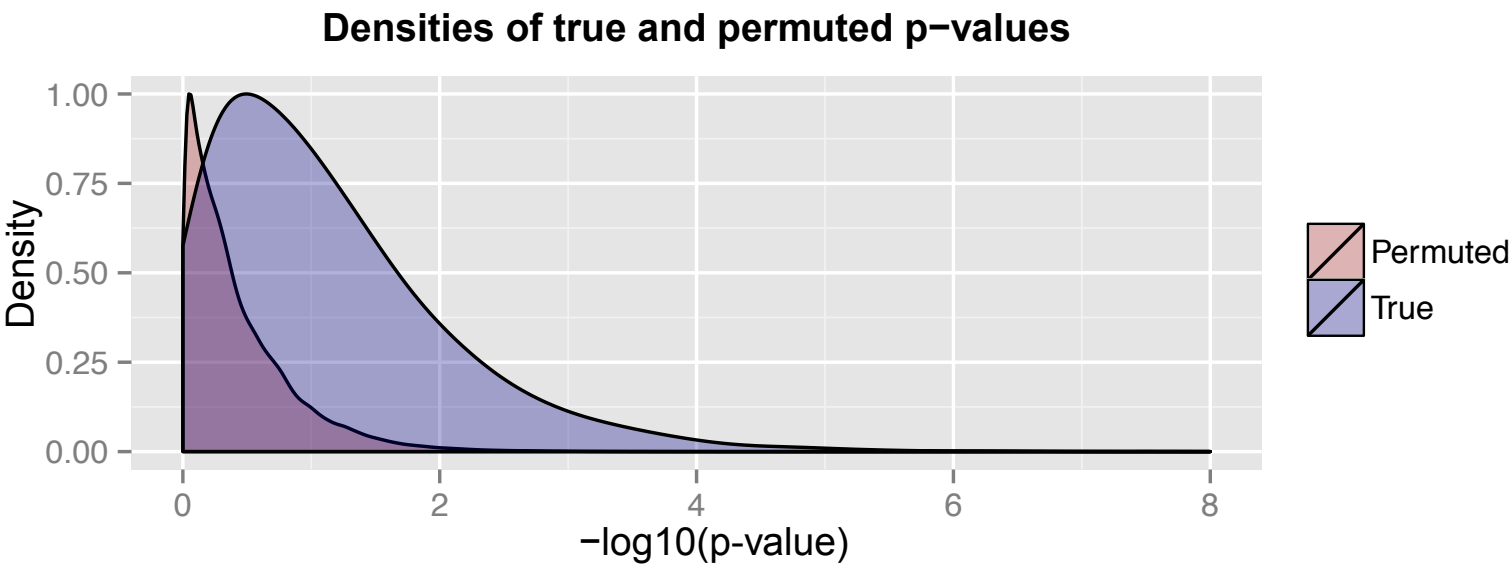

Percent p−values ≤ threshold
percent (≤ p−value)
80
60
40
20
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

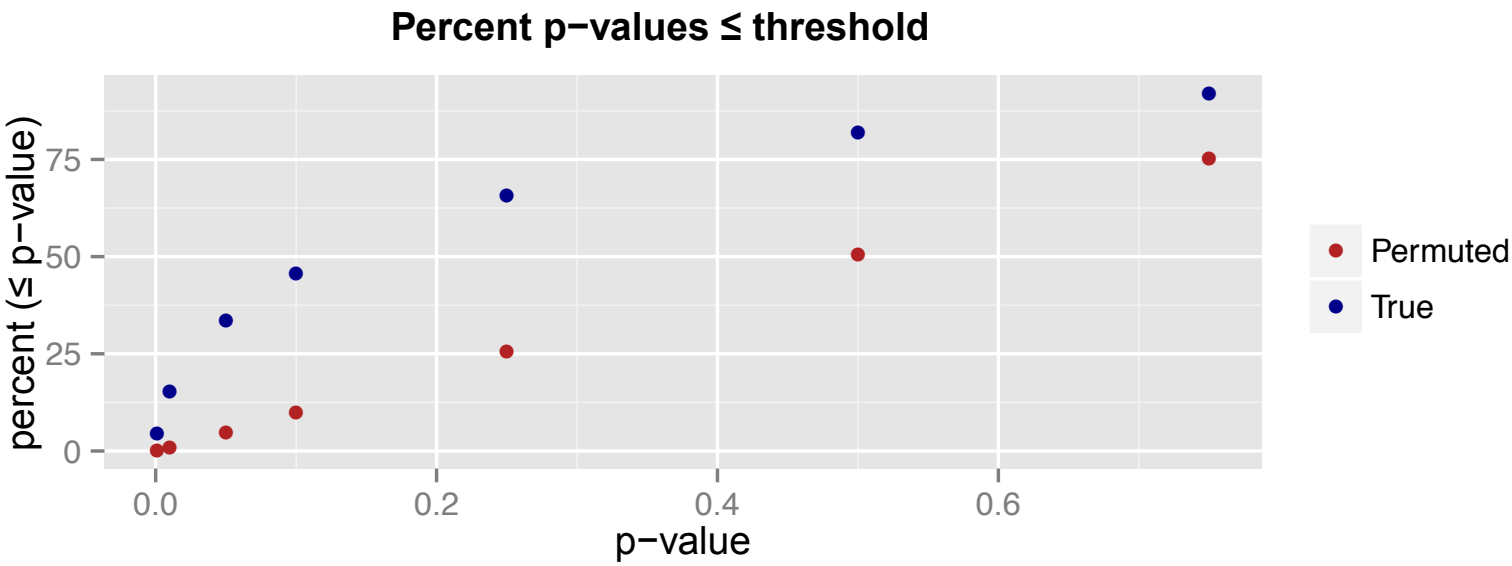
D

OTU 555


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

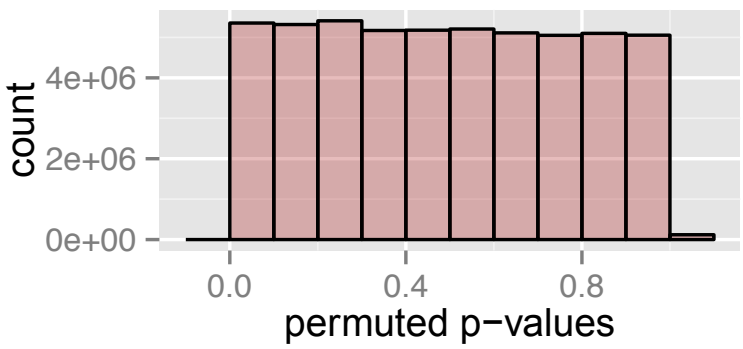

count
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

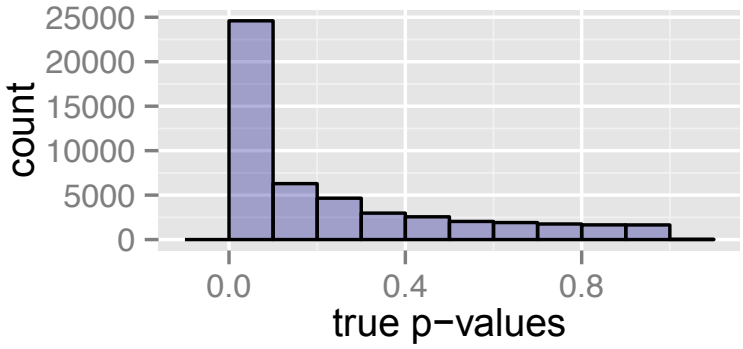

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

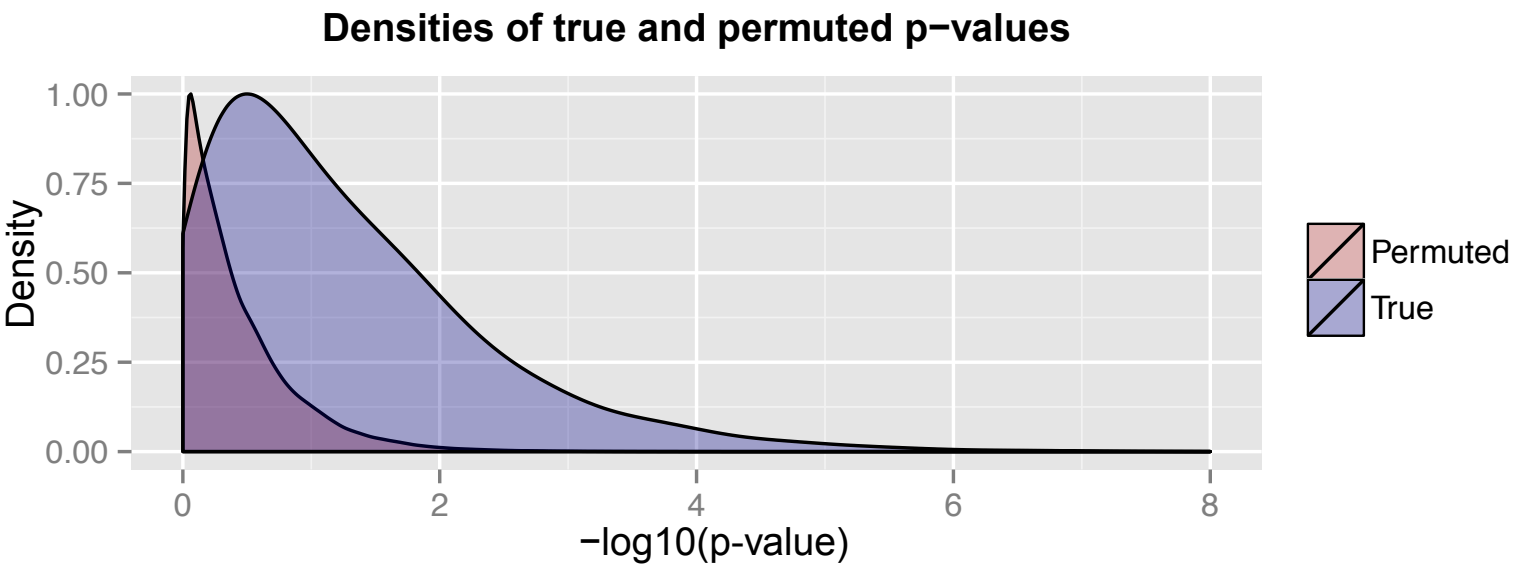

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

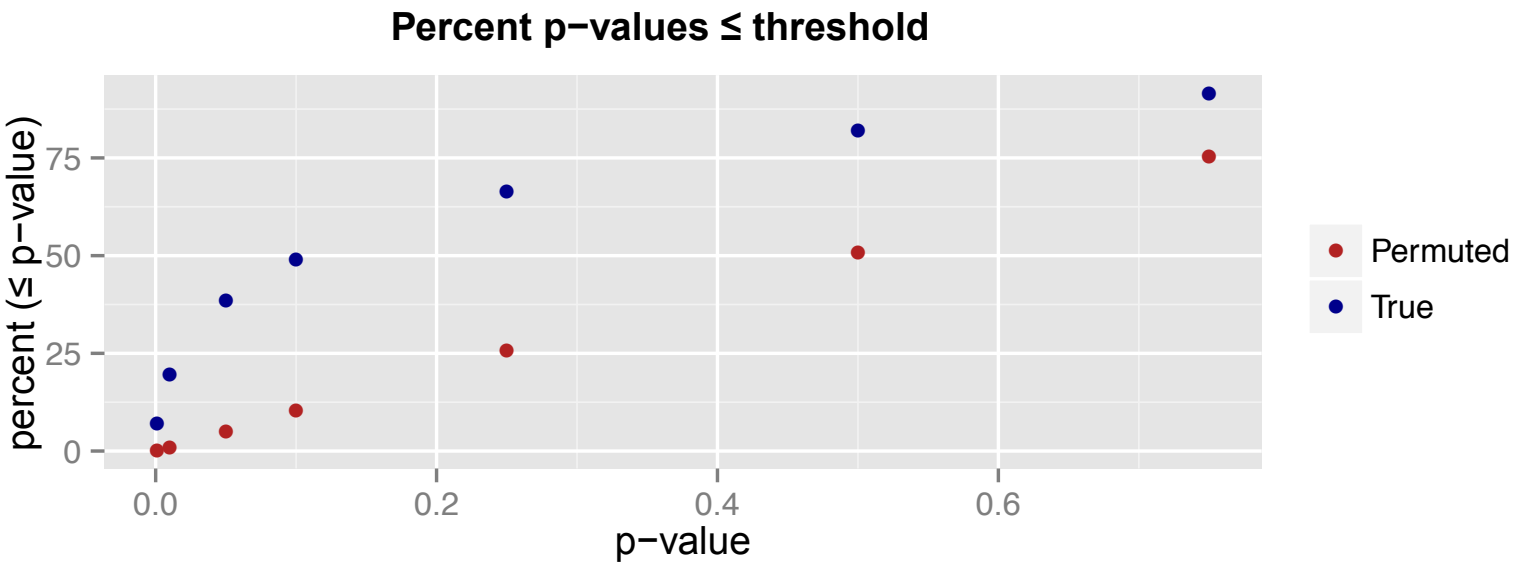
D

OTU 1064


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

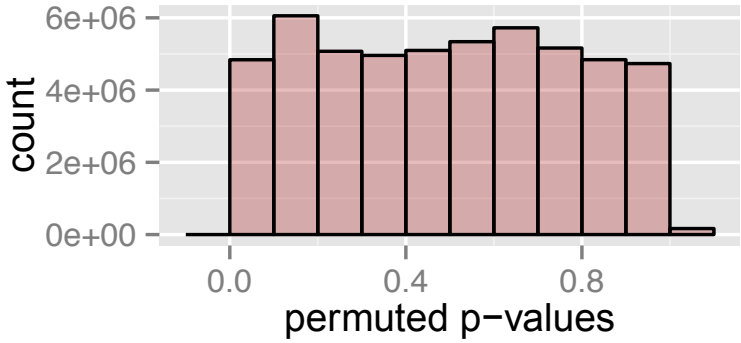

count
25000
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

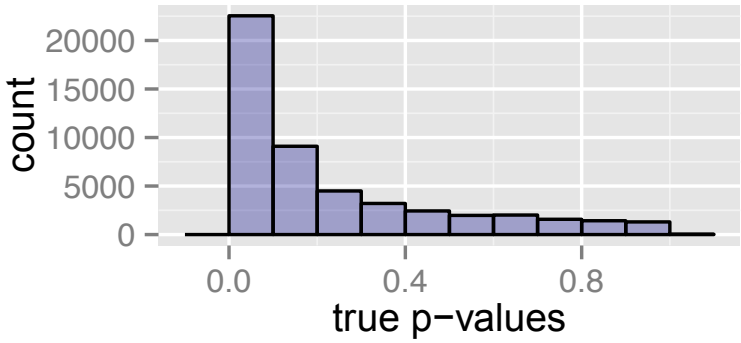

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

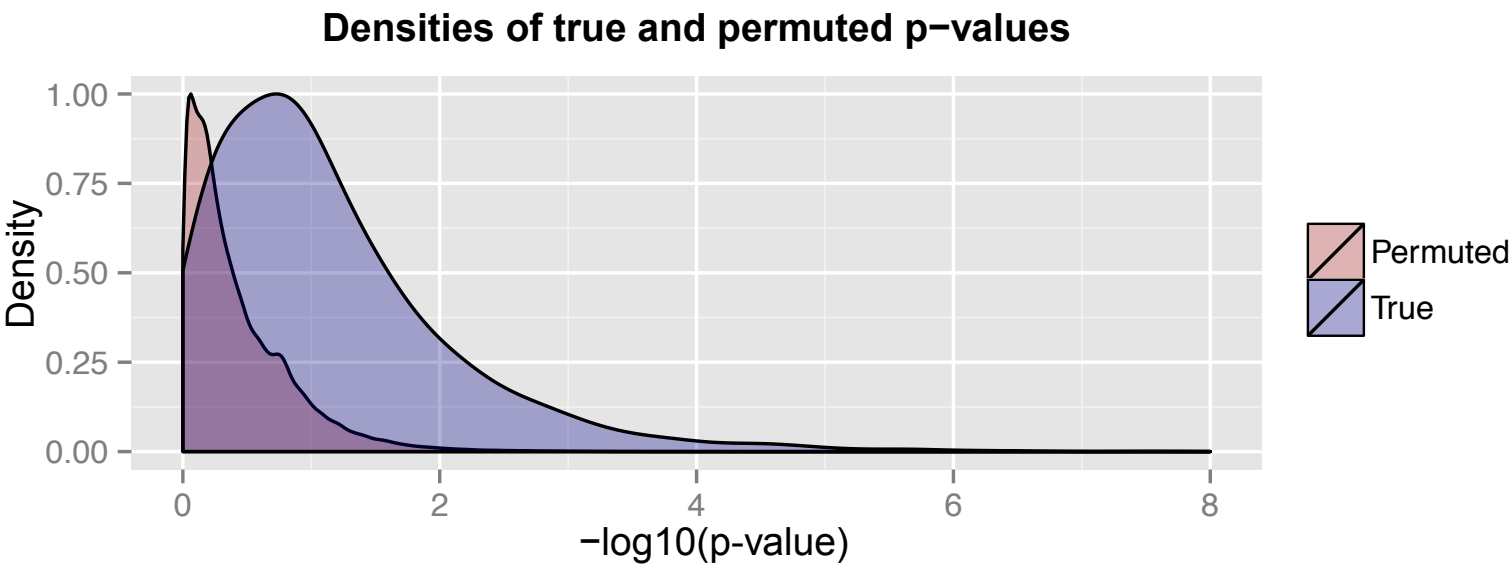

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

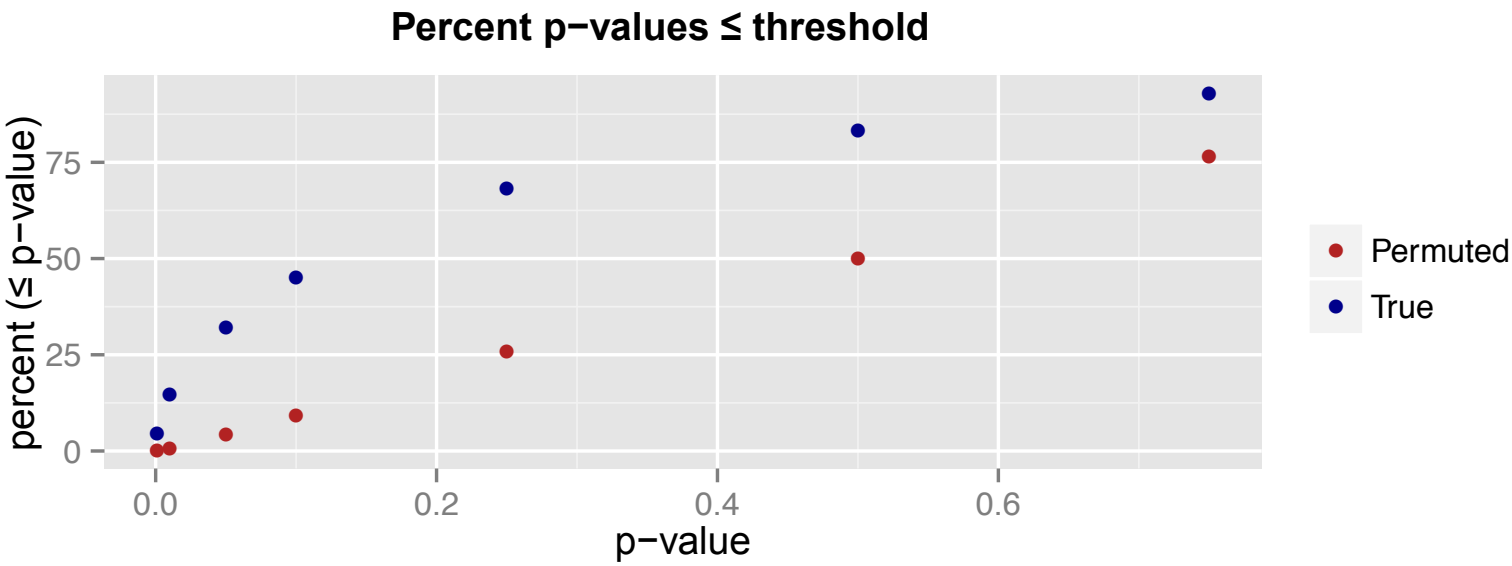
D

OTU 1071


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

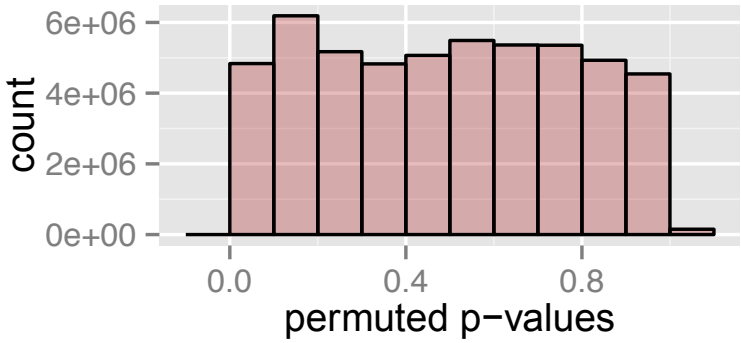

count
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

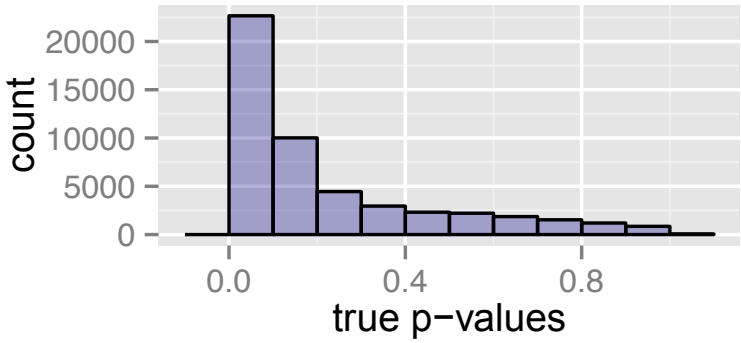

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

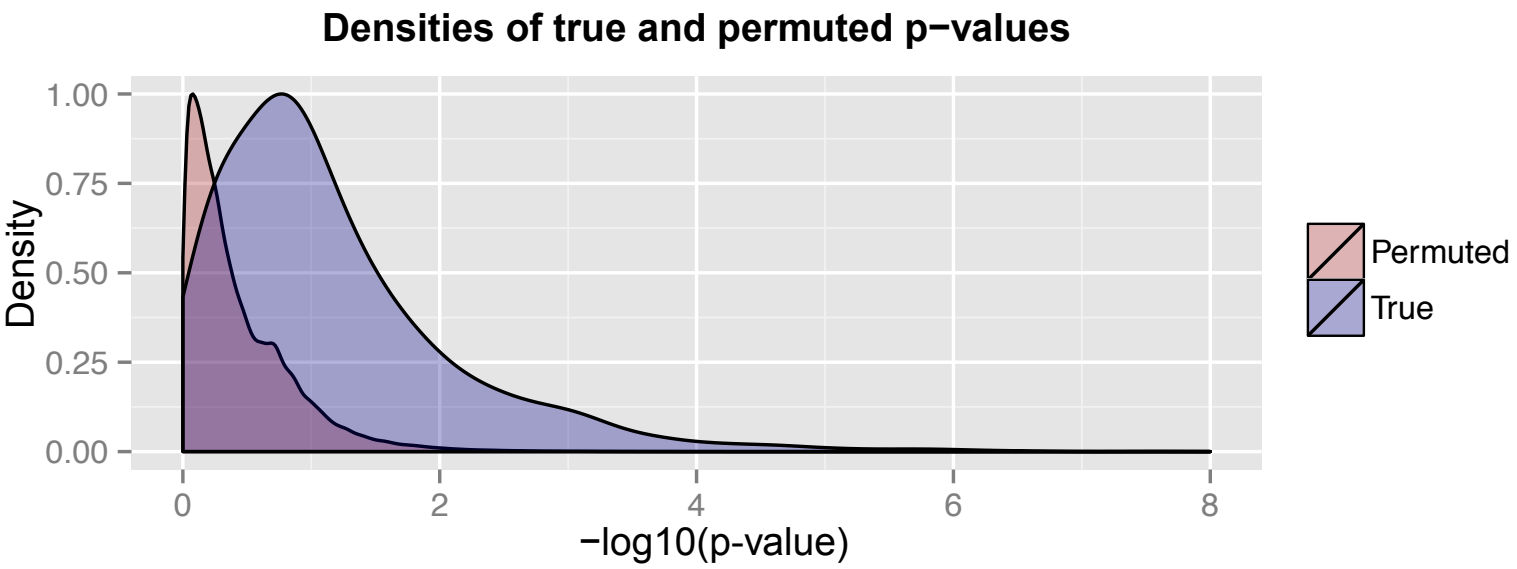

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

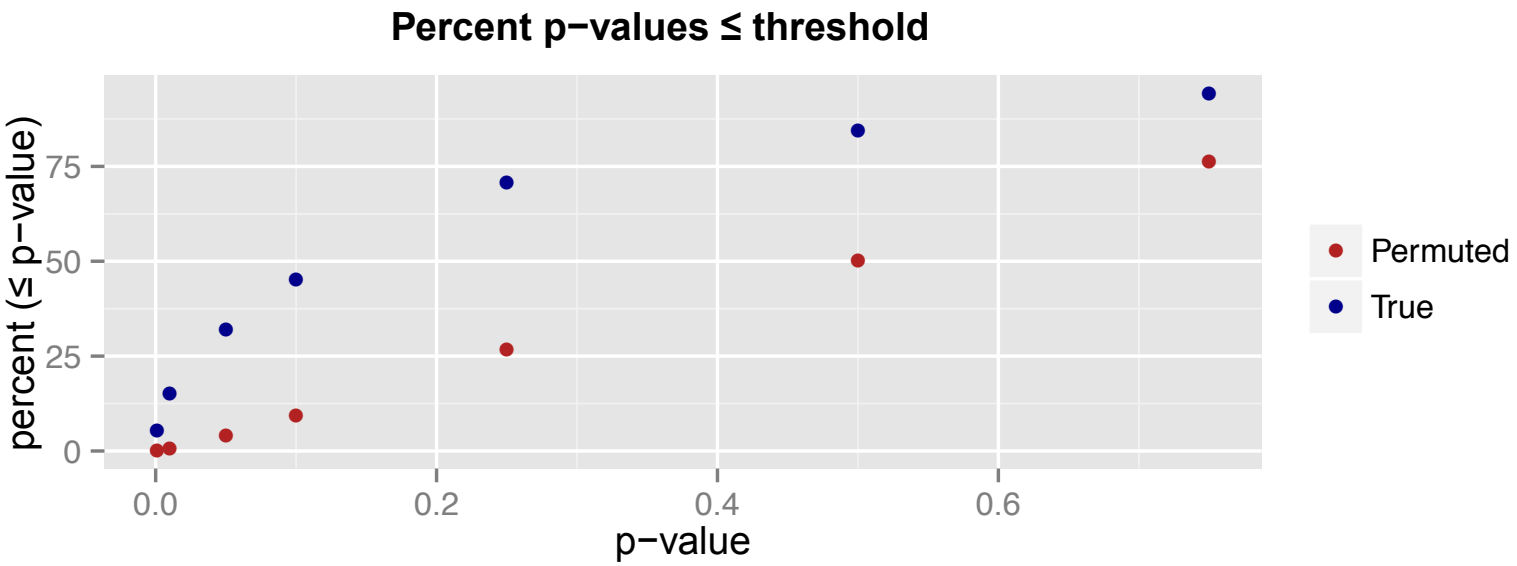
D

OTU 18232


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

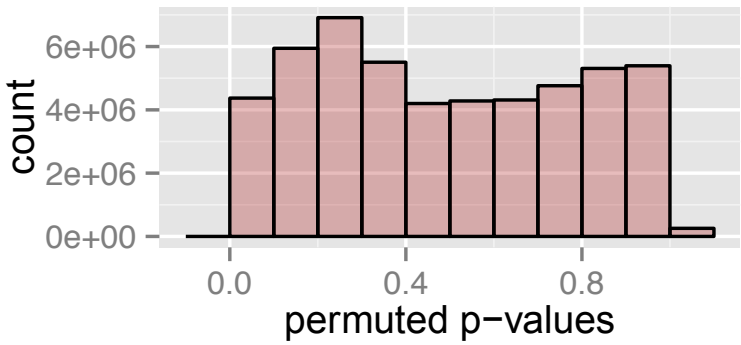

count
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

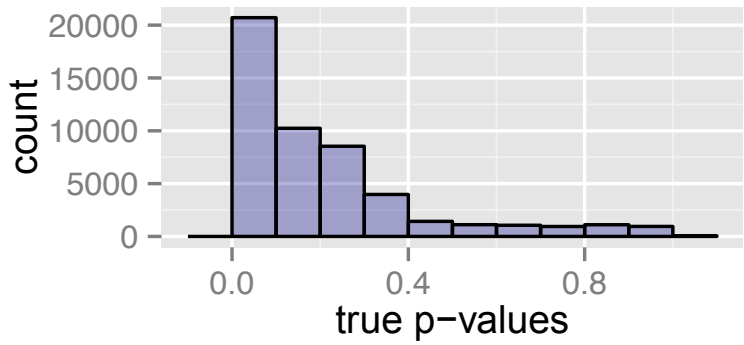

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

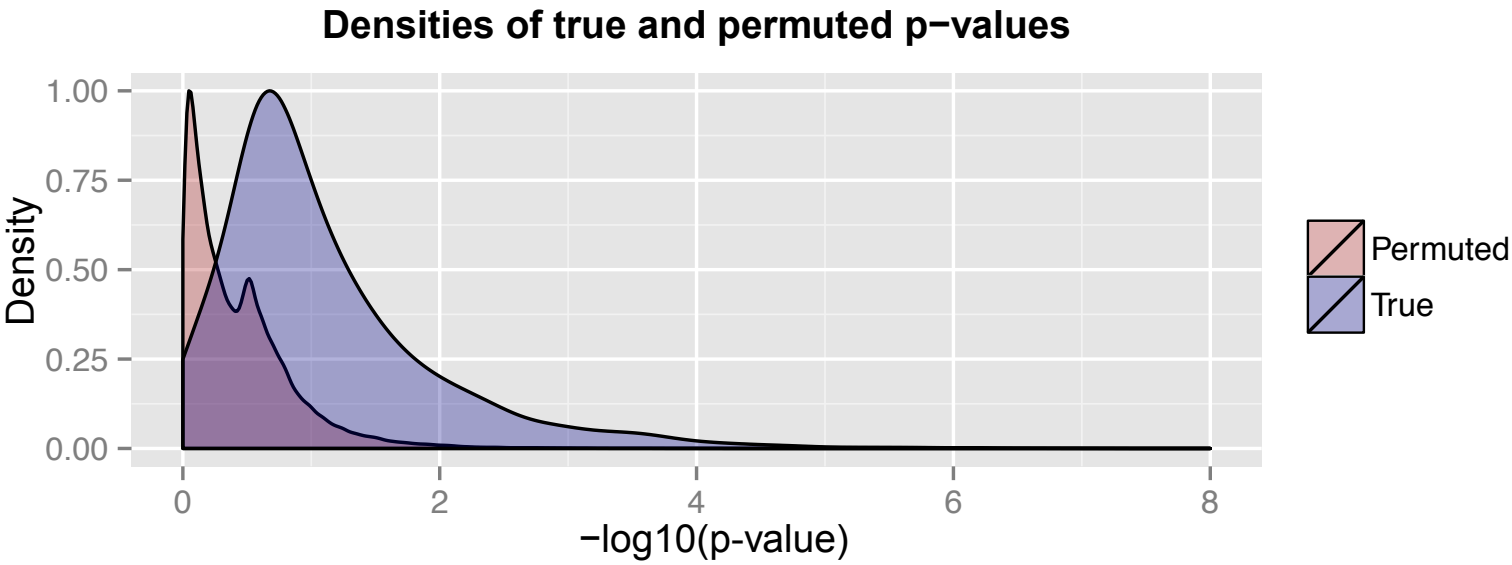

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

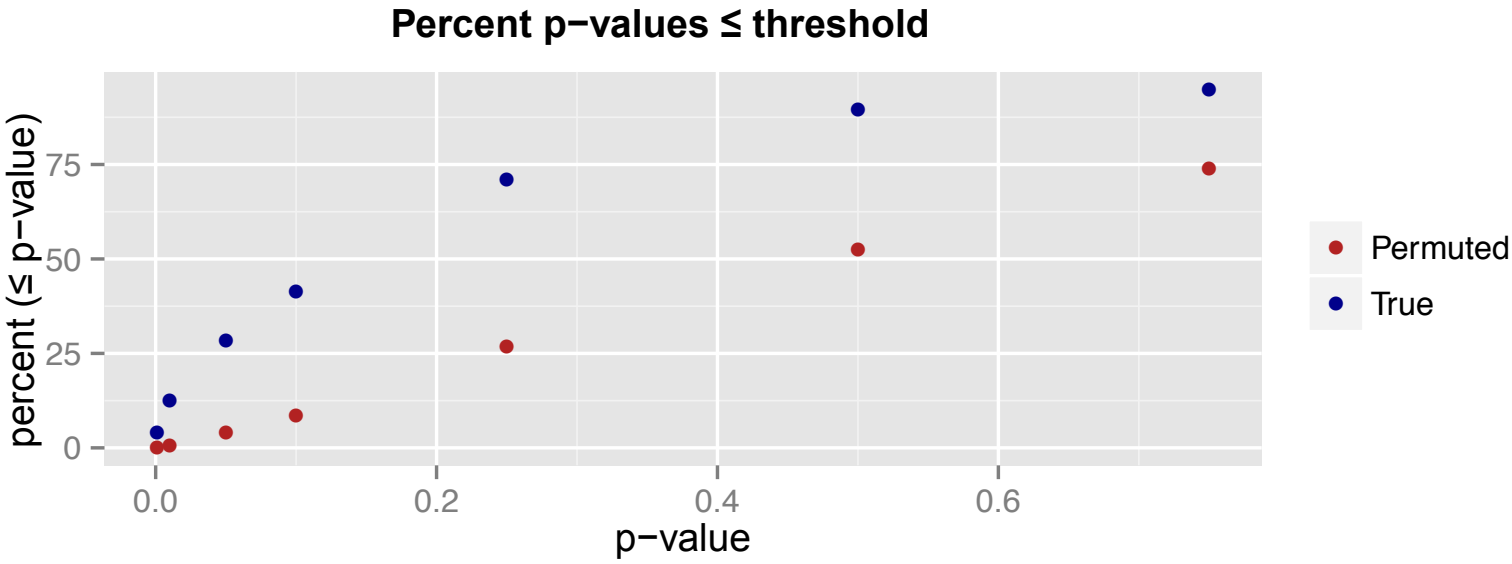
D

OTU 35378


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

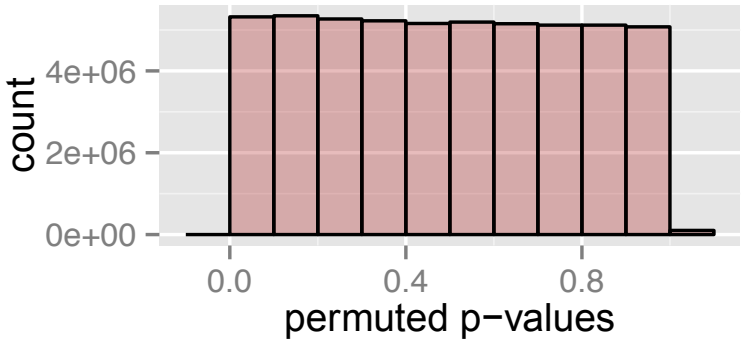

count
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

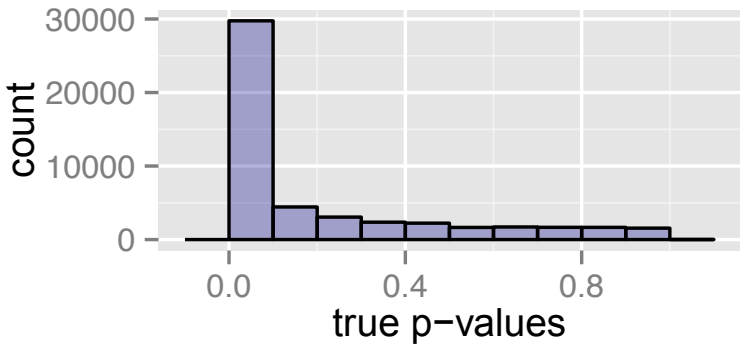

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

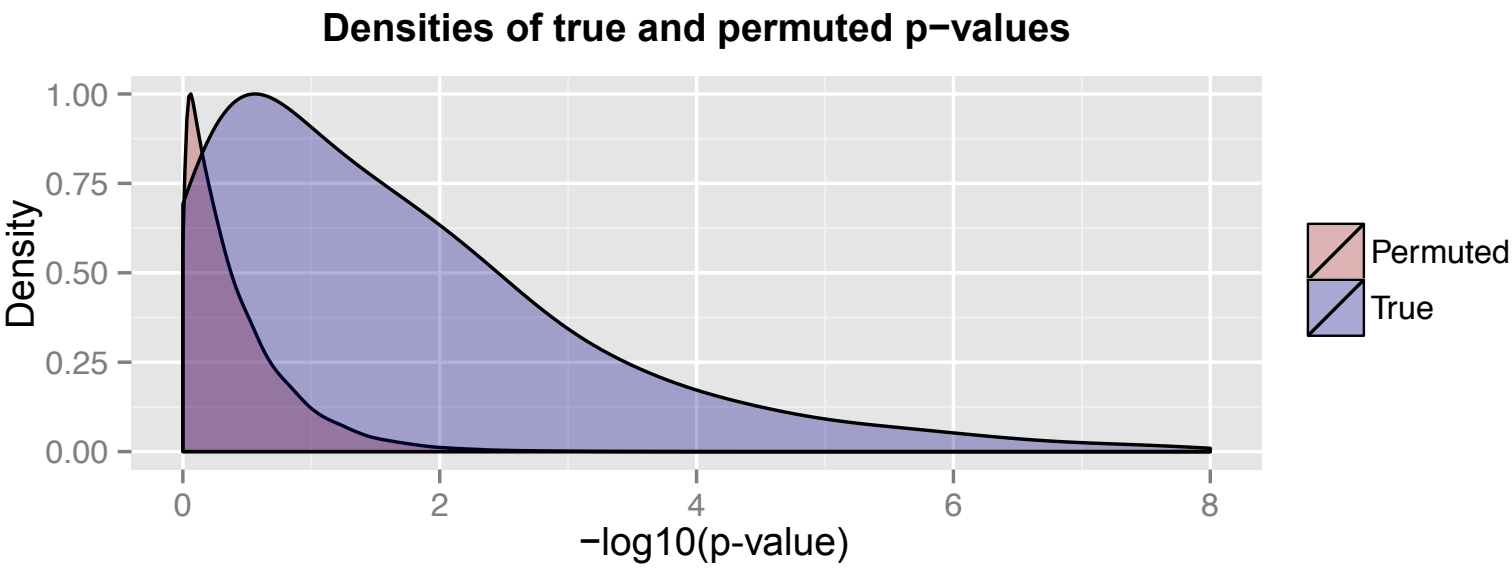

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

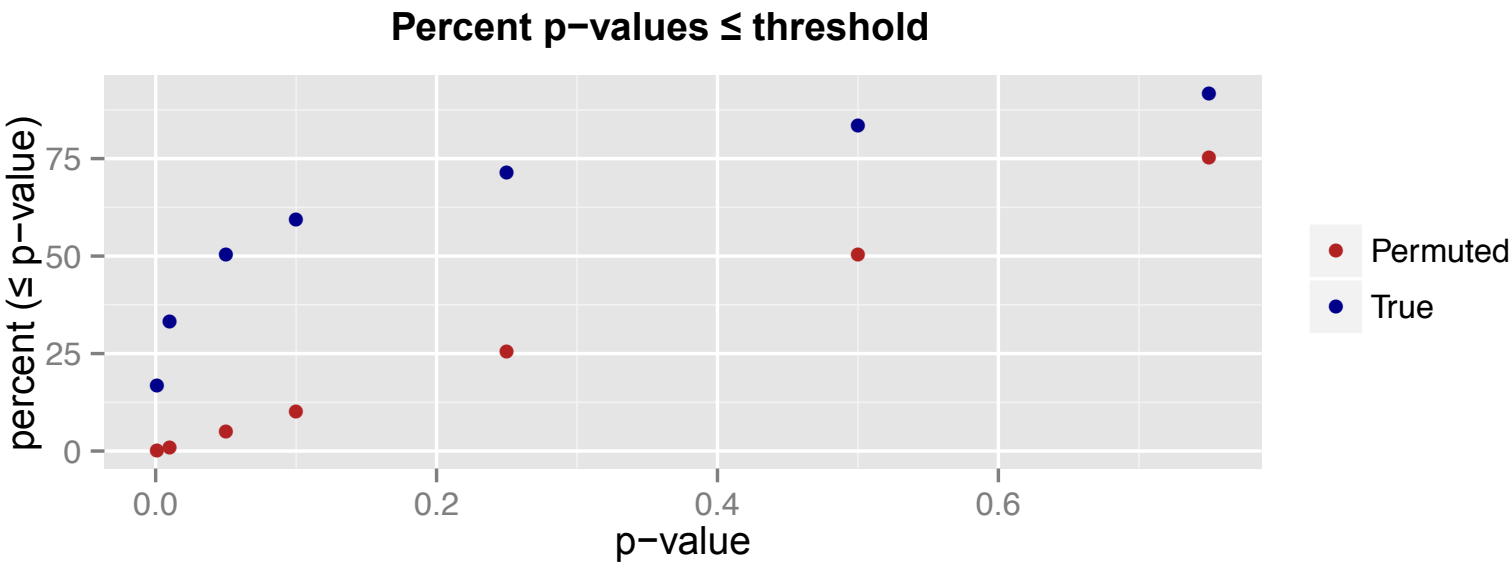
D

OTU 50139


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

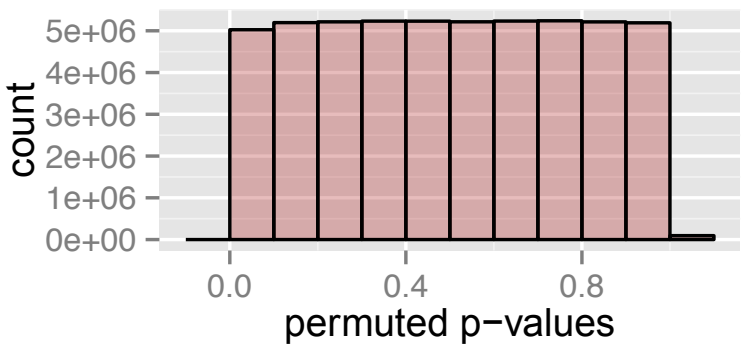

count
30000
20000
10000
0
0.0
0.4
0.8
true p−values

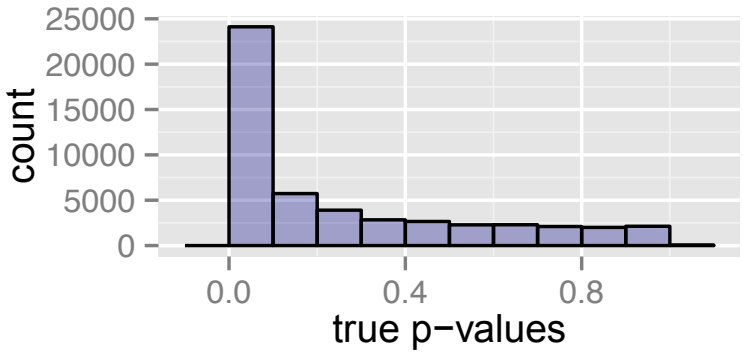

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

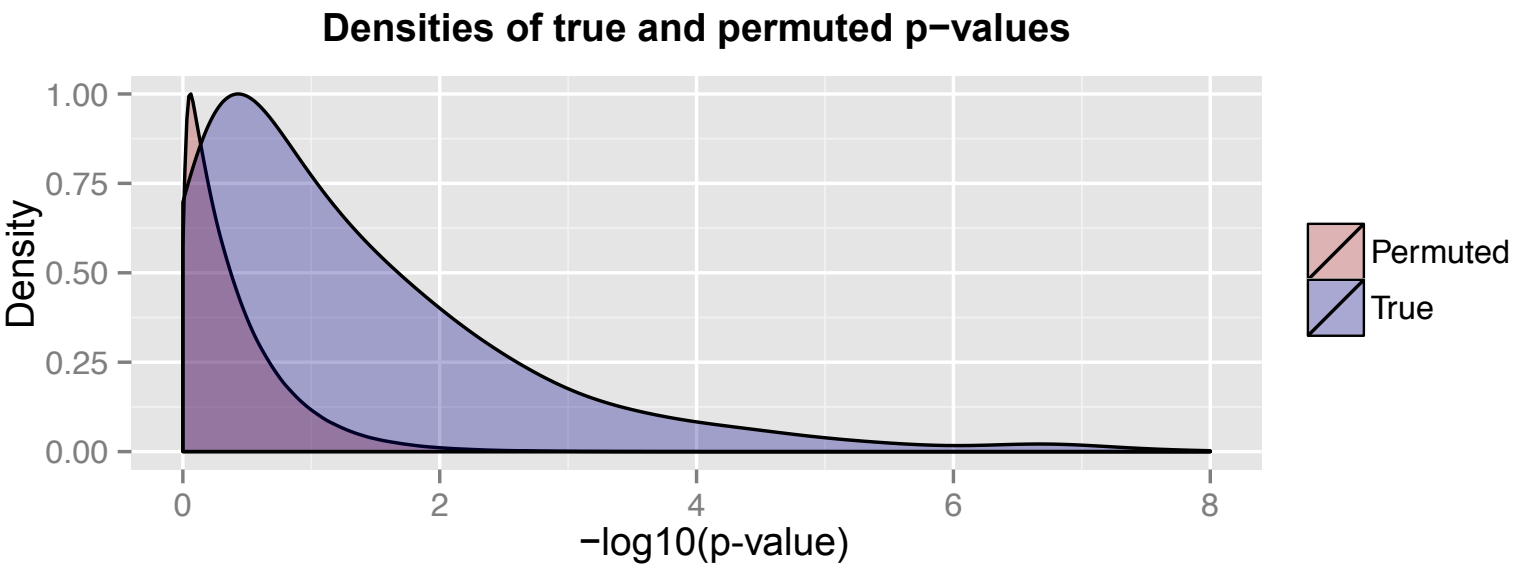

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

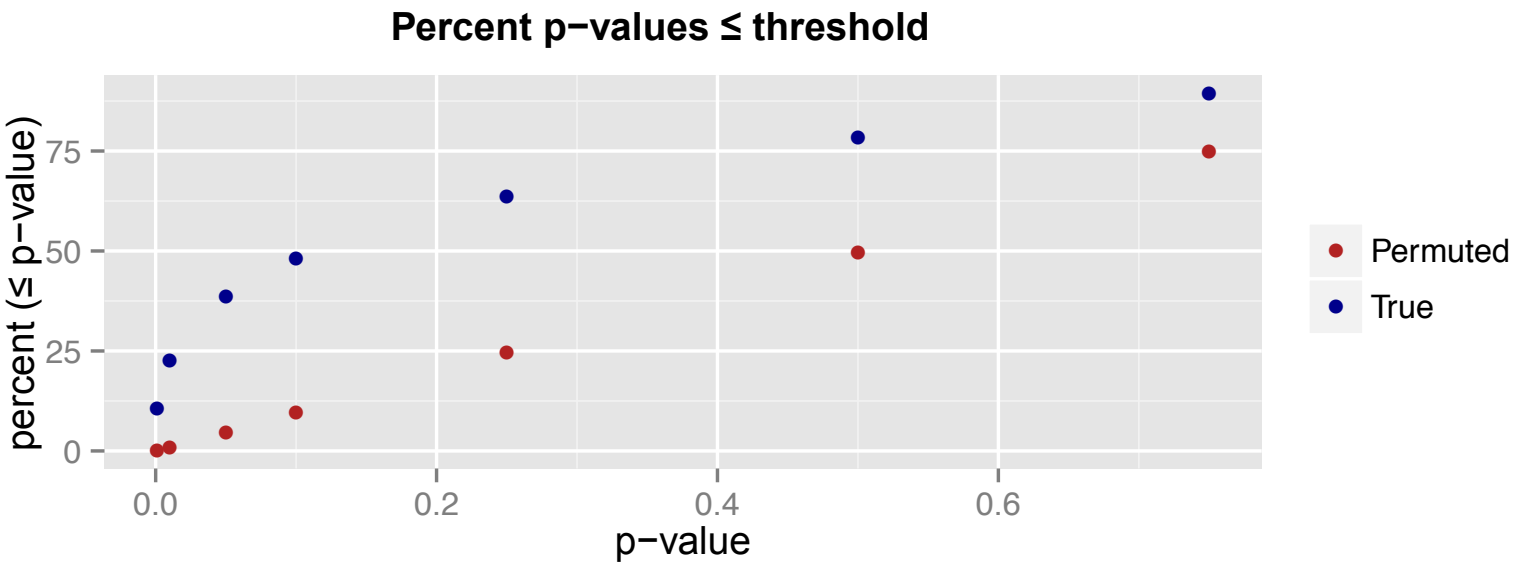
D

OTU 104197


count
5e+06
4e+06
3e+06
2e+06
1e+06
0e+00
0.0
0.4
0.8
permuted p−values

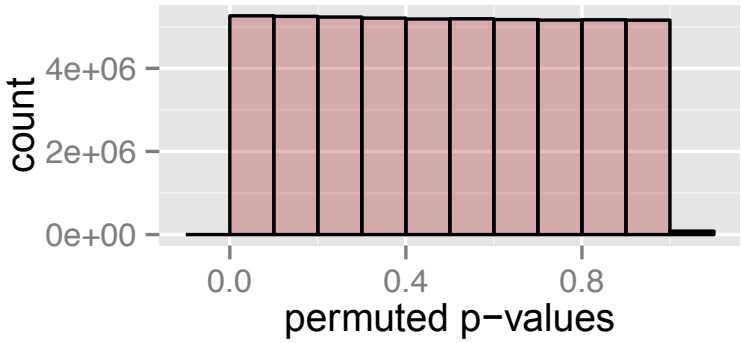

count
25000
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

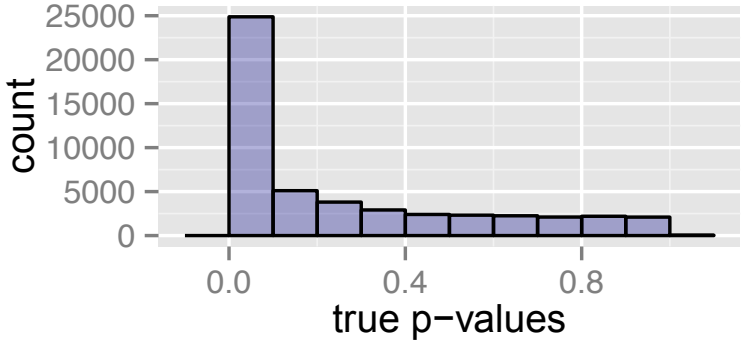

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

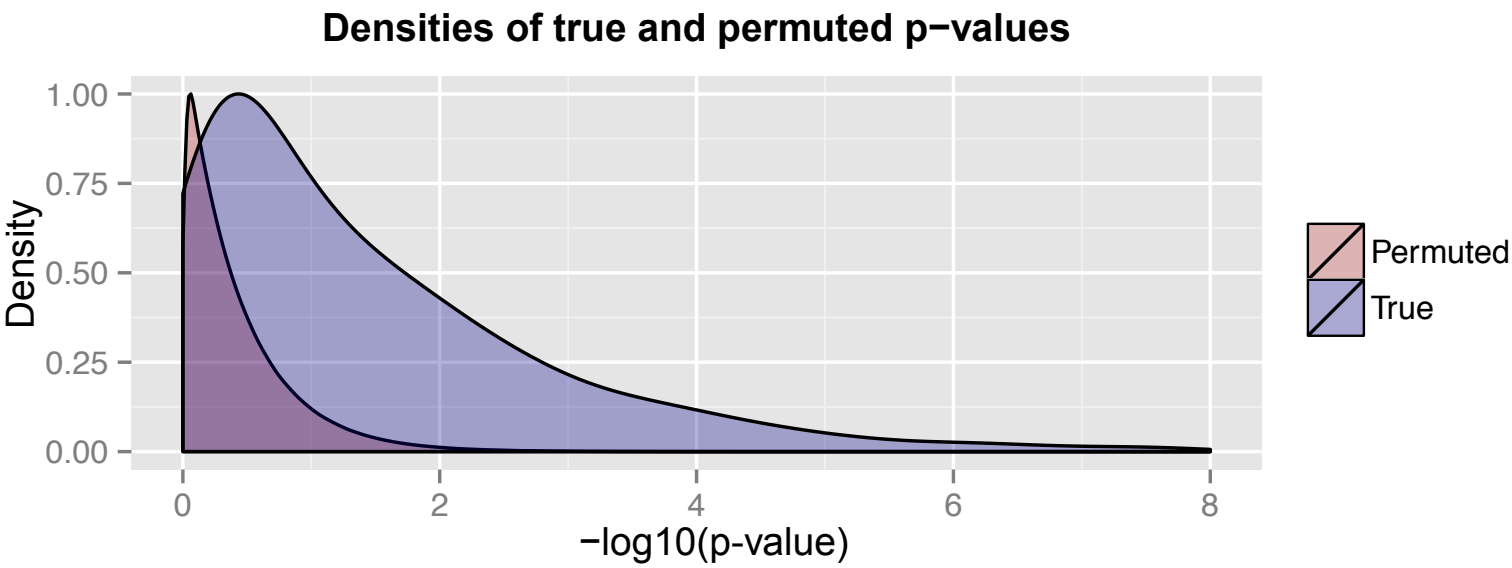

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

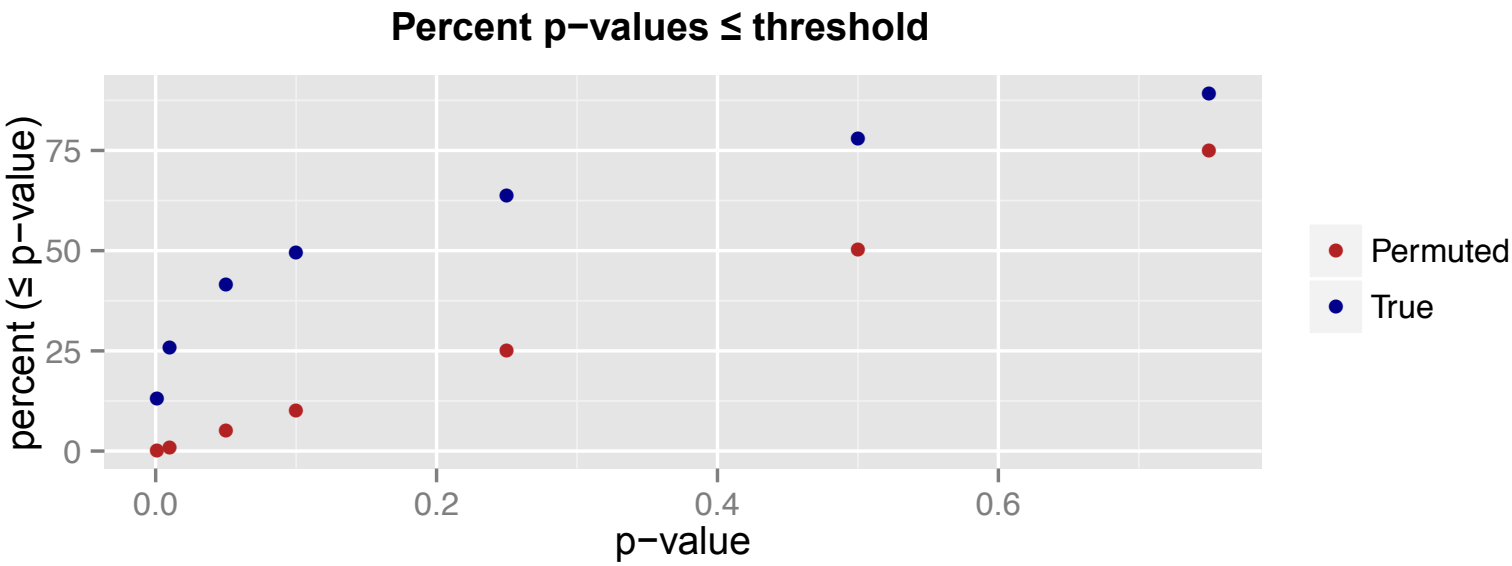
D

OTU 159084


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

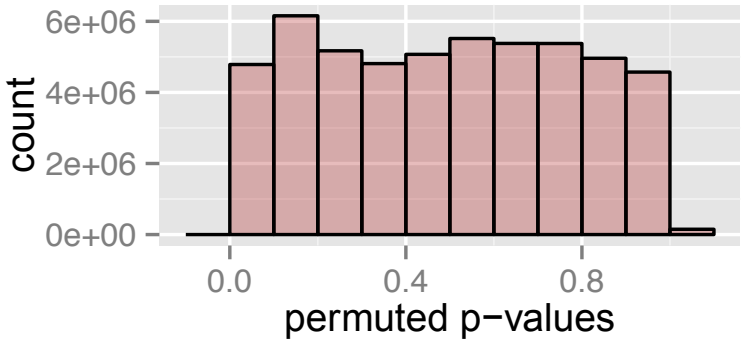

count
25000
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

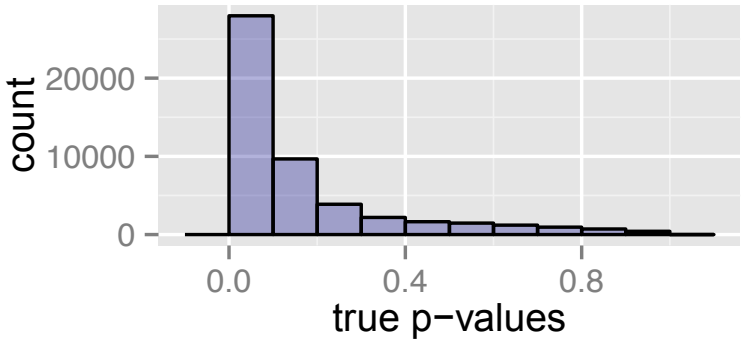

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

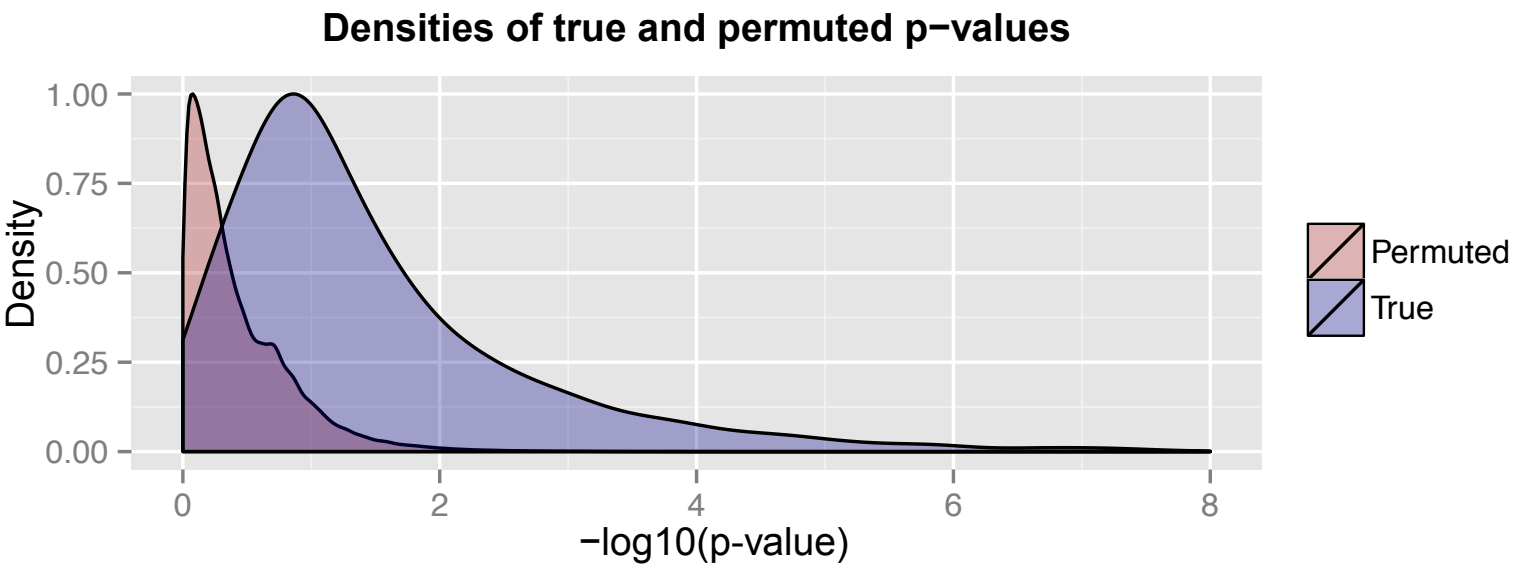

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

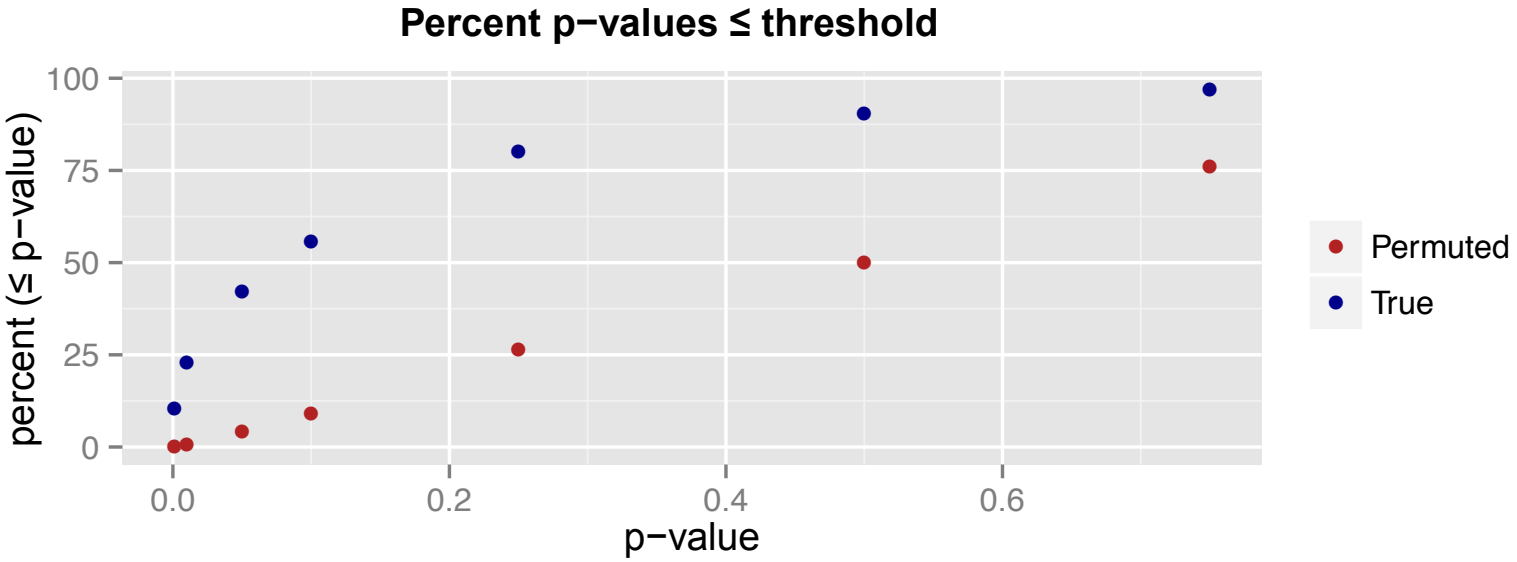
D

OTU 174614


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

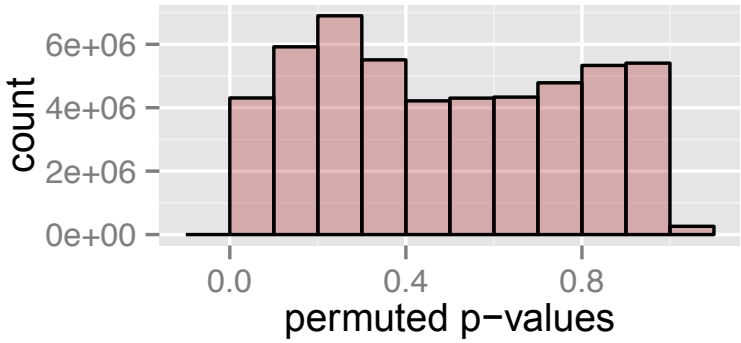

count
20000
10000
0
0.0
0.4
0.8
true p−values

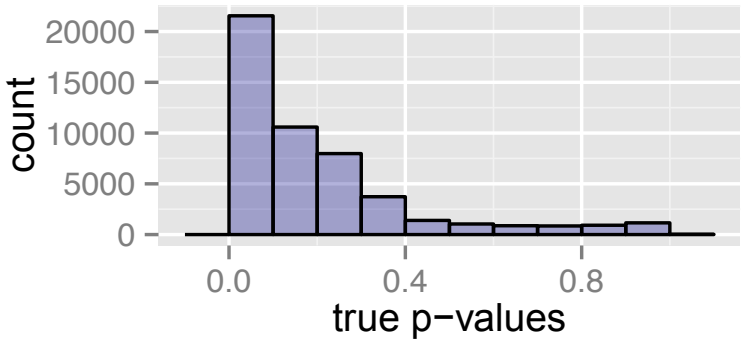

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

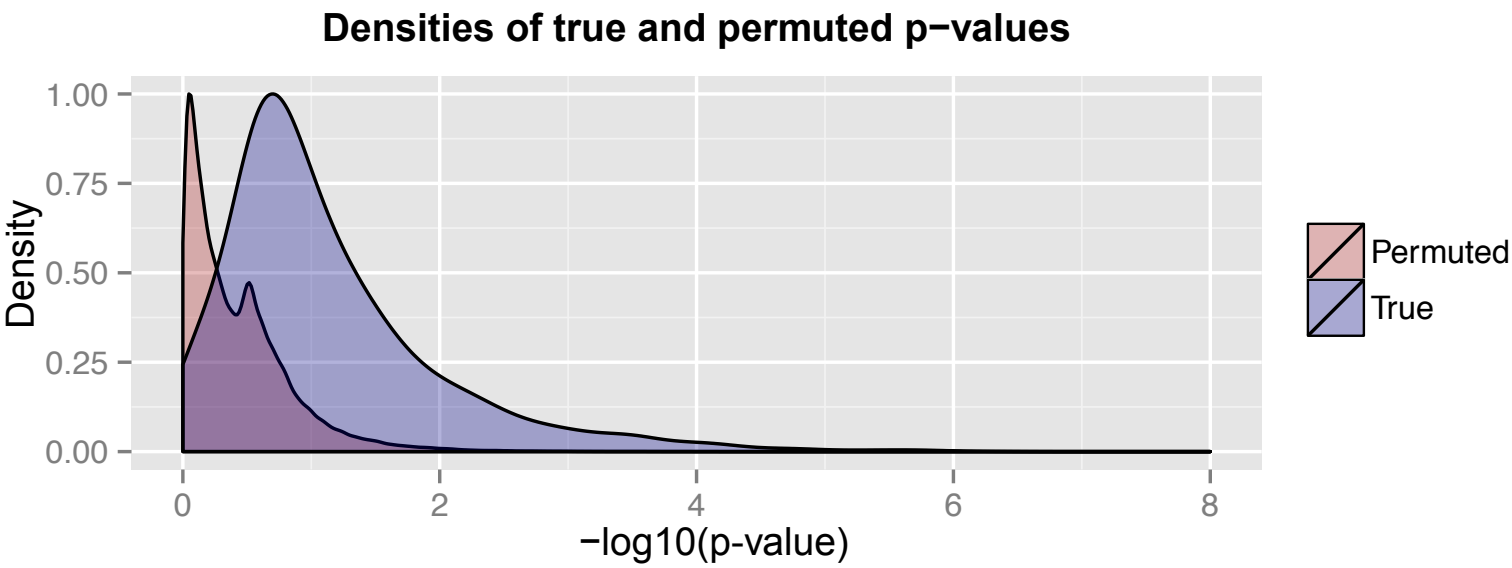

Percent p−values ≤ threshold
percent (≤ p−value)
100
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

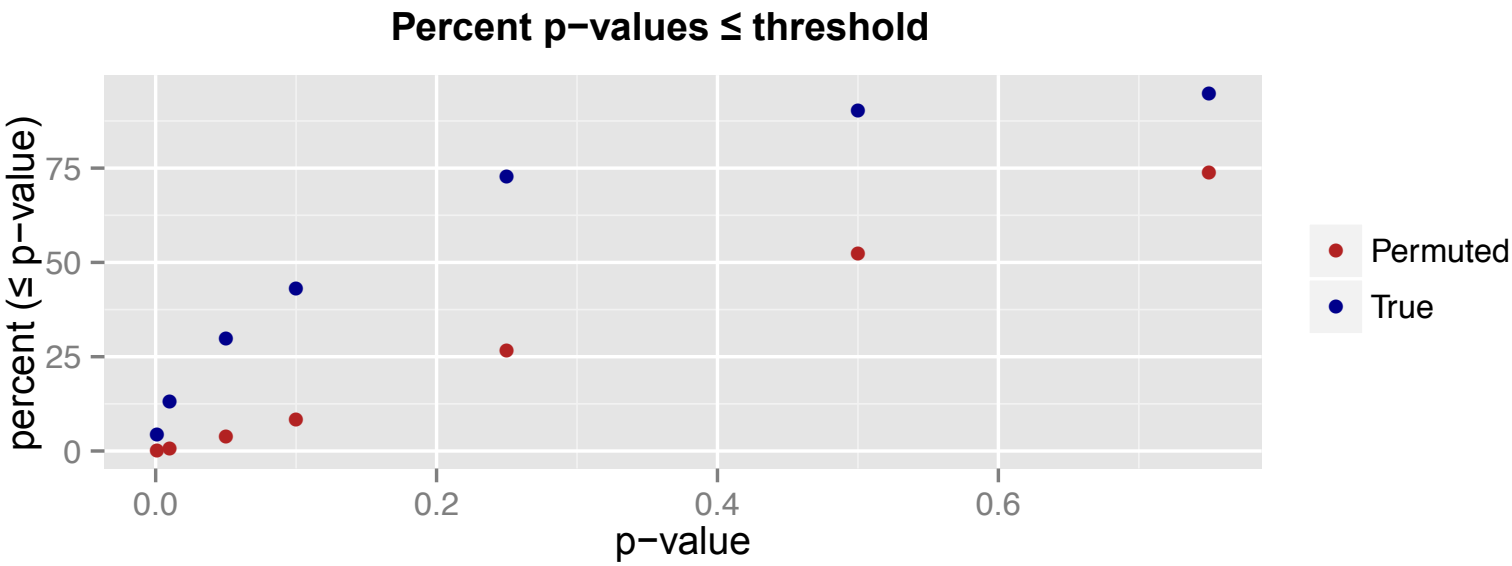
D

OTU 21676


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

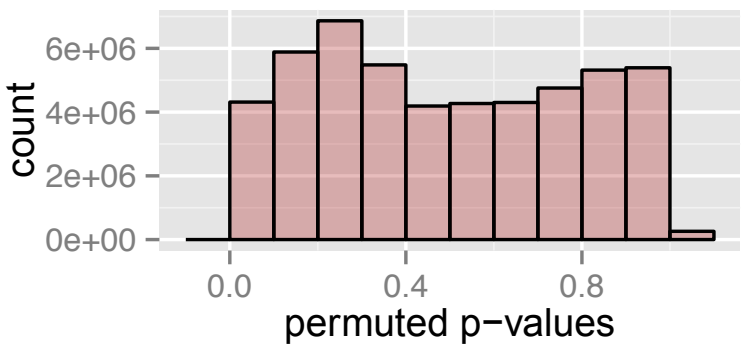

count
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values

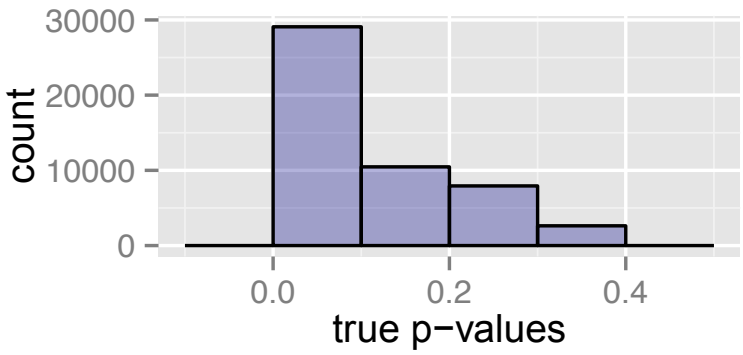

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

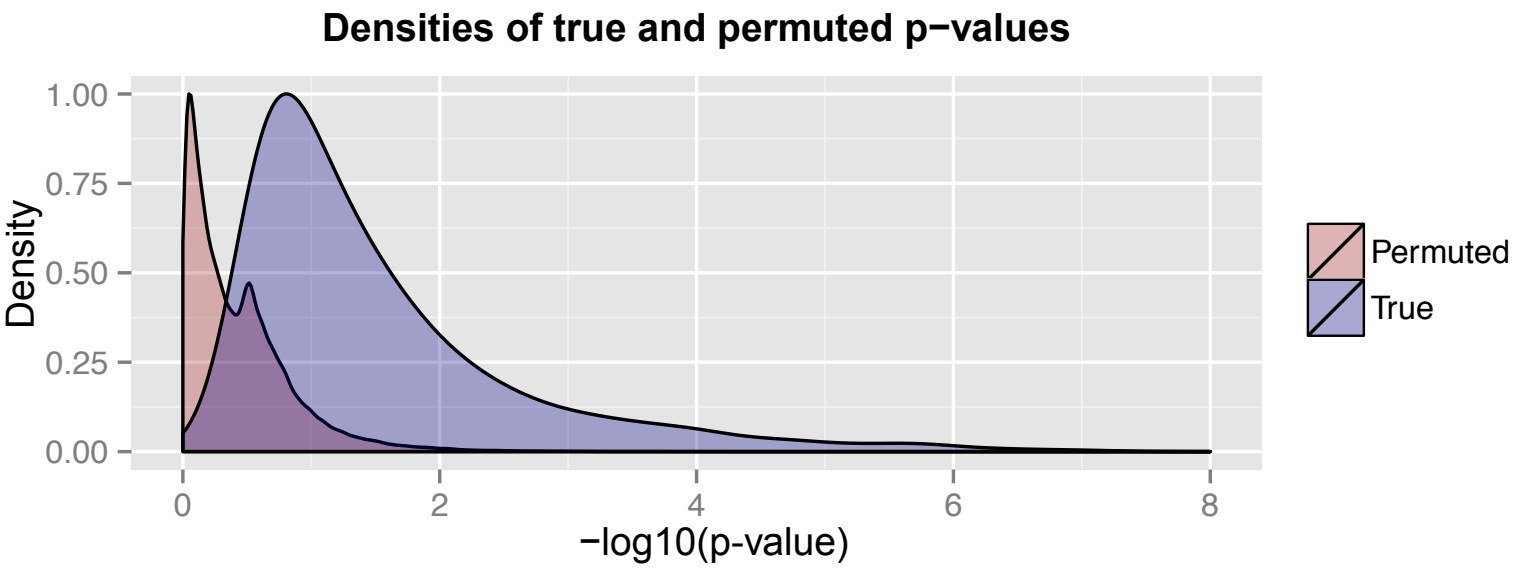

Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

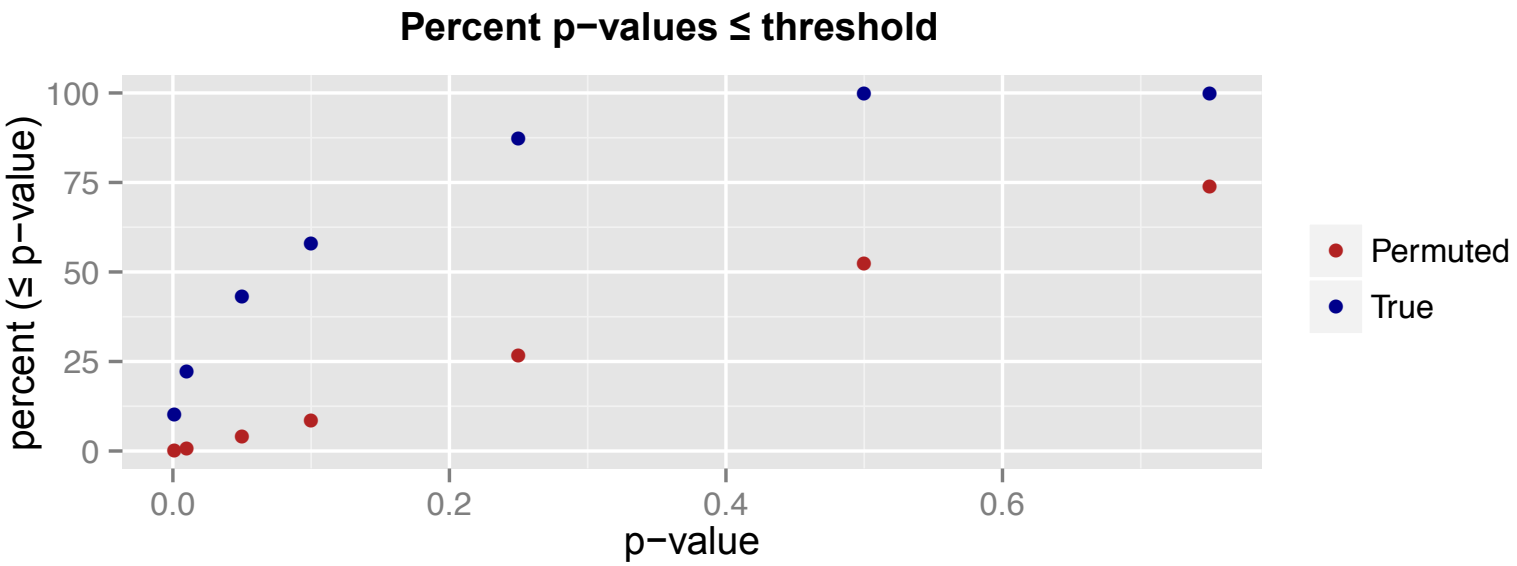
D

OTU 218210


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

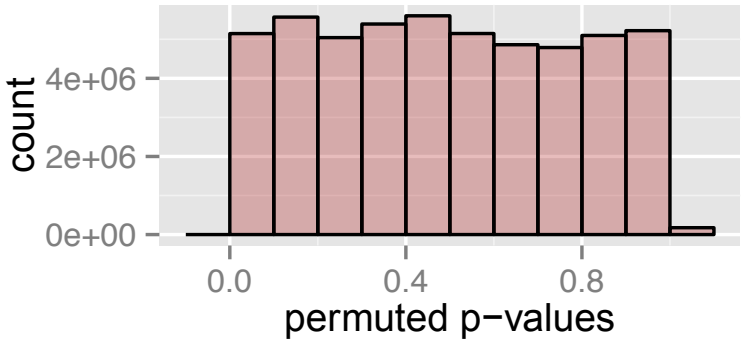

count
30000
20000
10000
0
0.0
0.2
0.4
true p−values

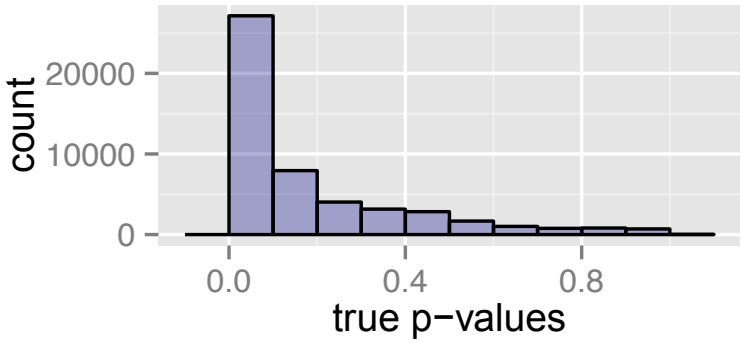

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

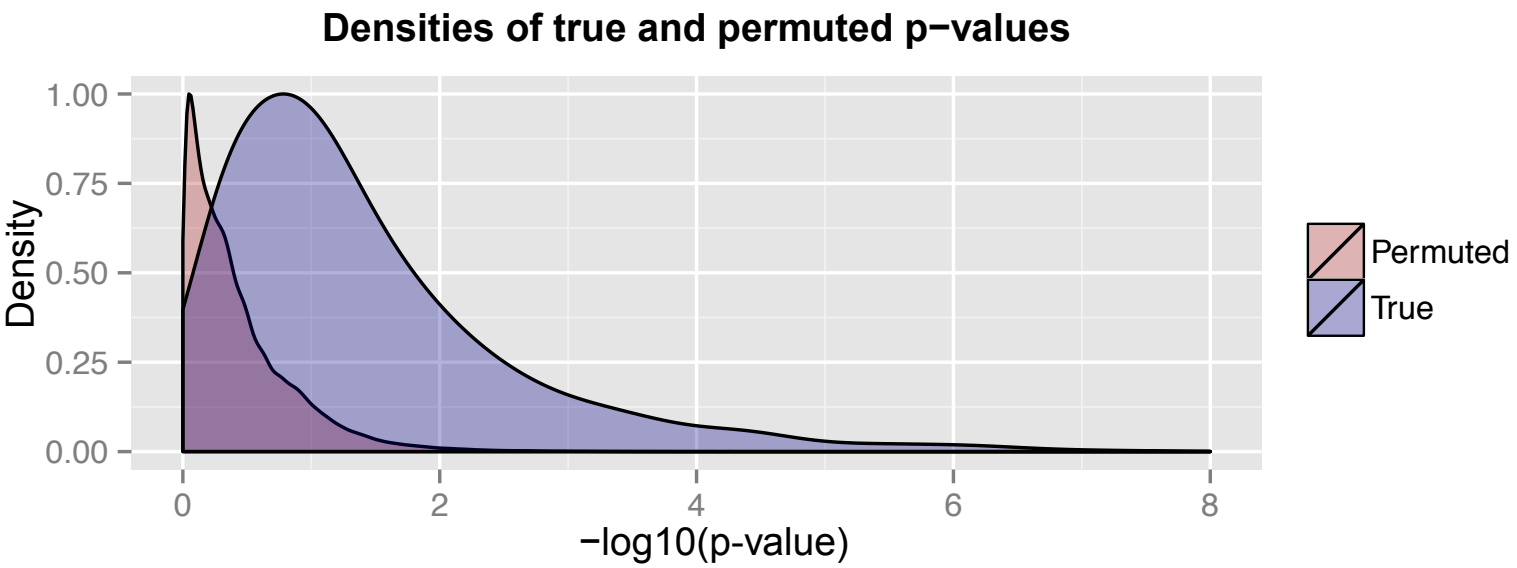

Percent p−values ≤ threshold
percent (≤ p−value)
100
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

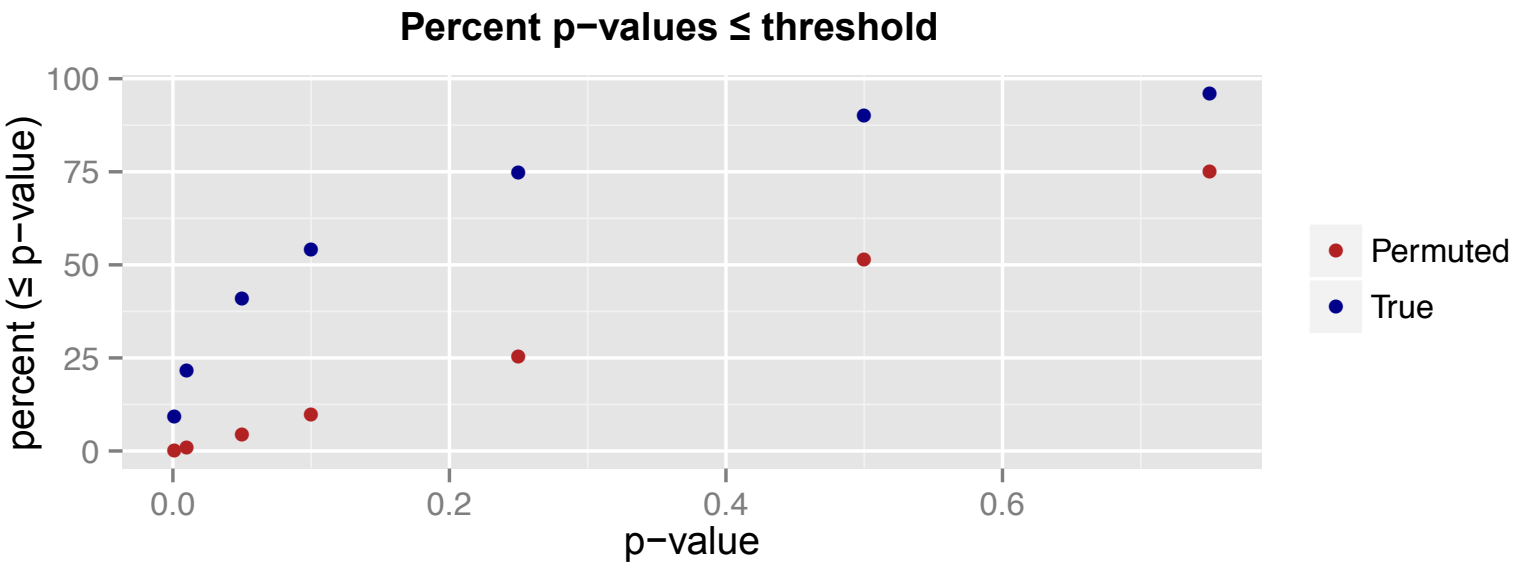
D

OTU 258019


count
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

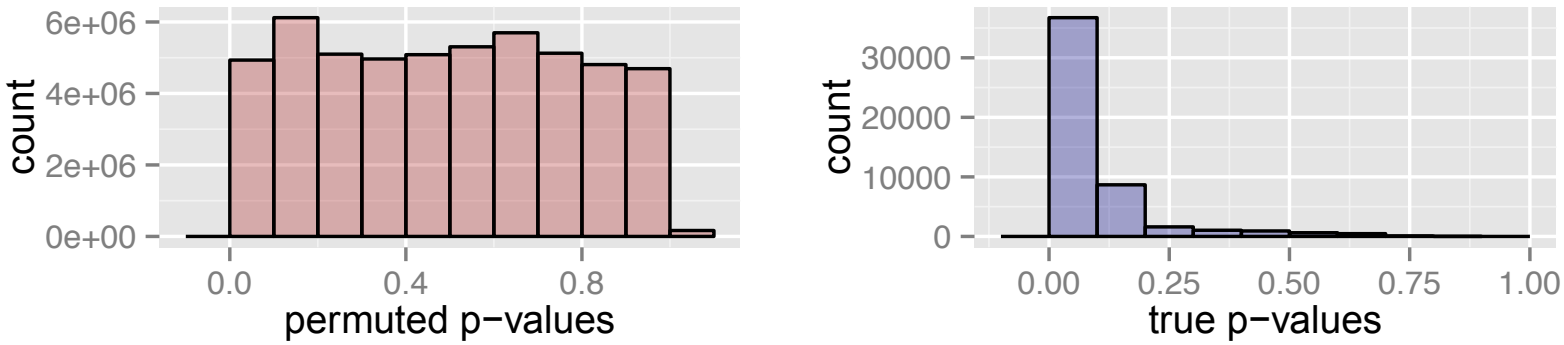

count
20000
10000
0
0.0
0.4
0.8
true p−values

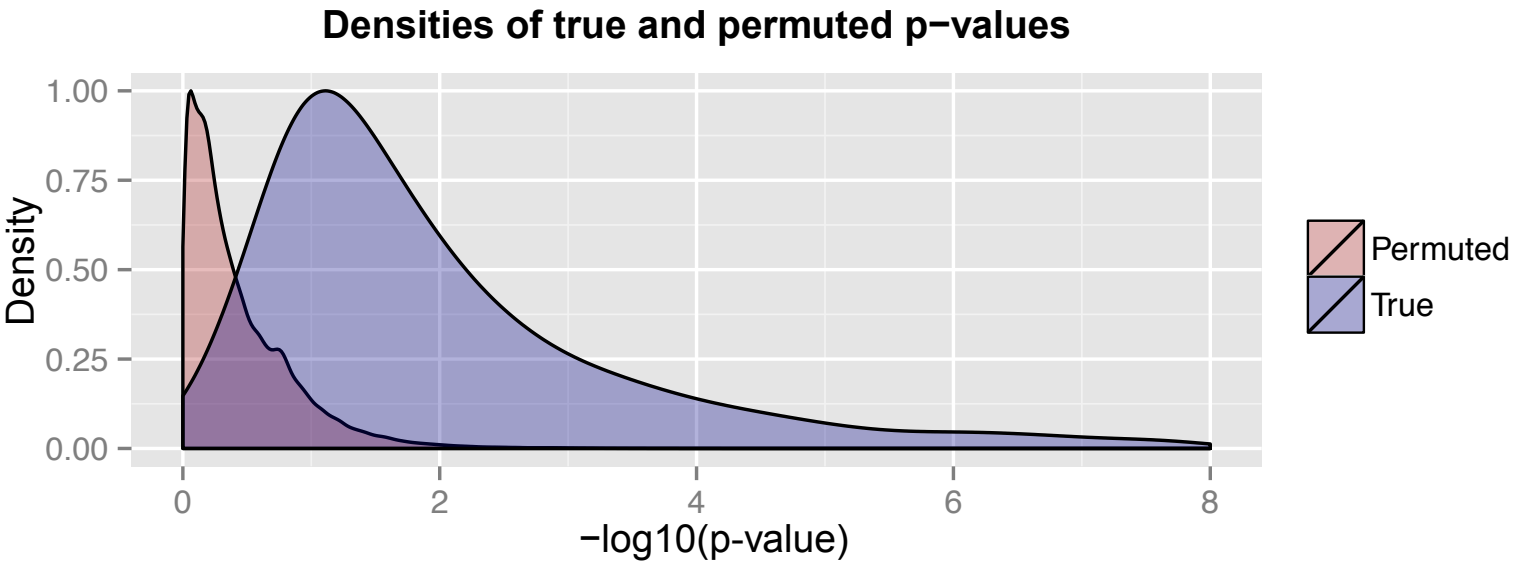

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

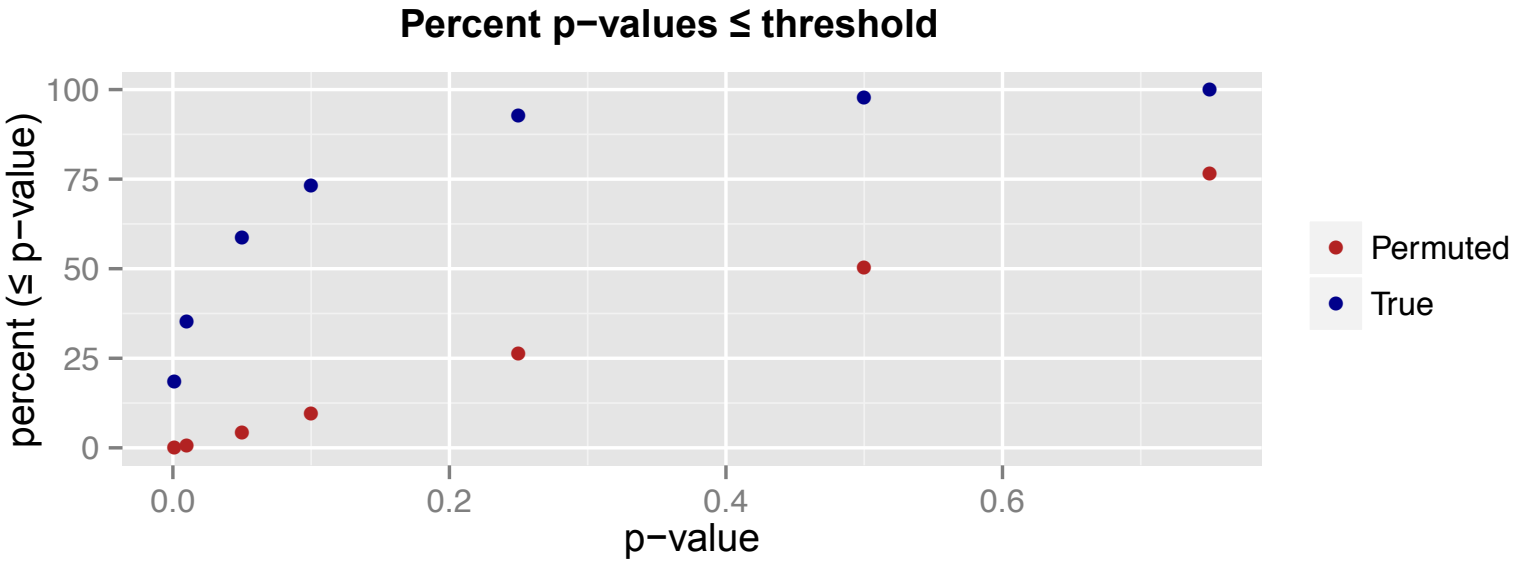

Percent p−values ≤ threshold
percent (≤ p−value)
100
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

D

OTU 275232

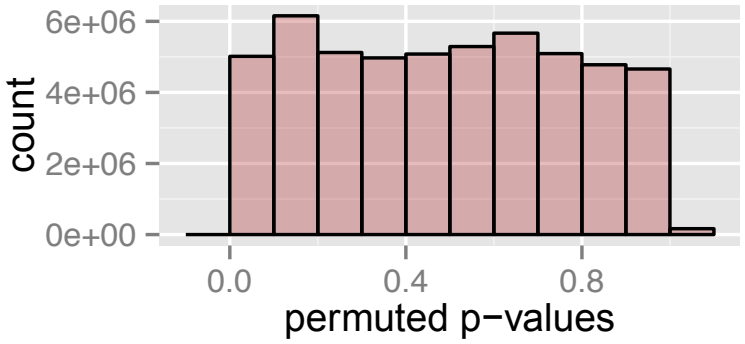

count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values
count
30000
20000
10000
0
0.00
0.25
0.50
0.75
1.00
true p−values

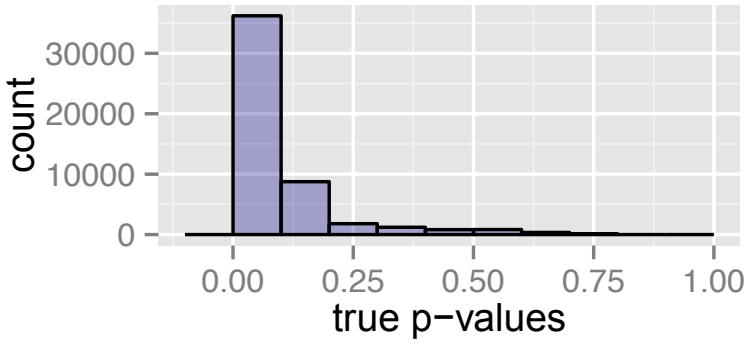

Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True

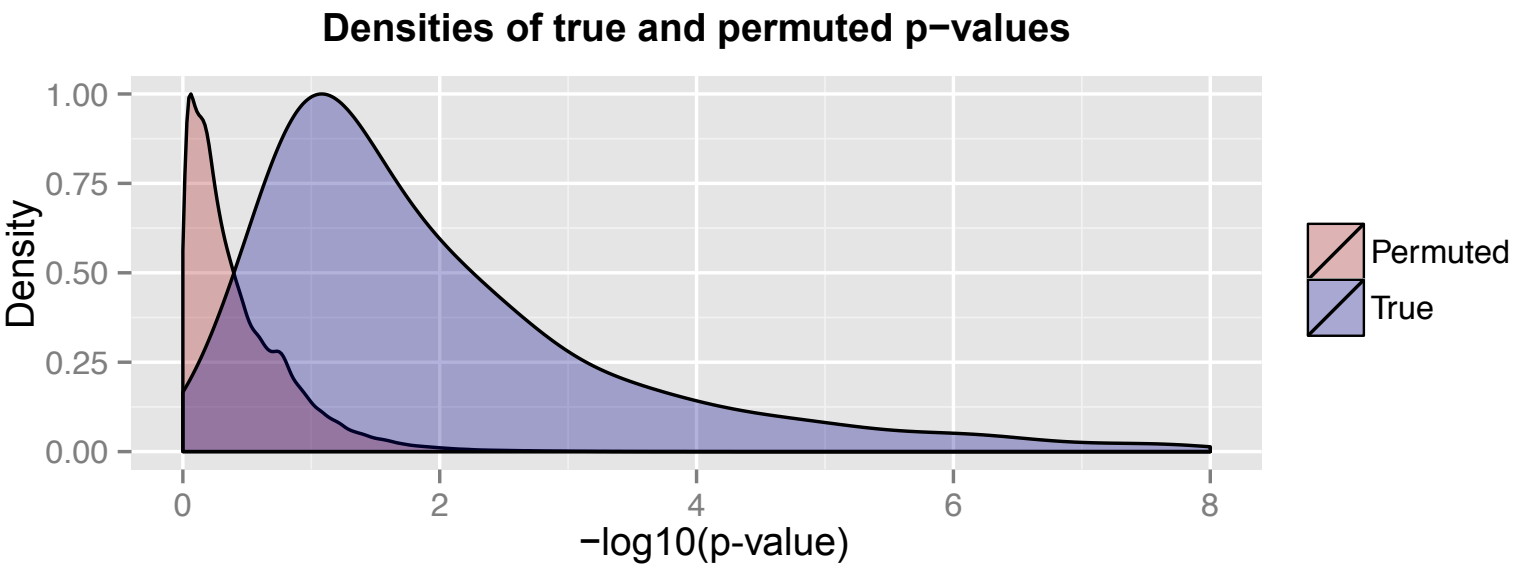

Percent p−values ≤ threshold
percent (≤ p−value)
100
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

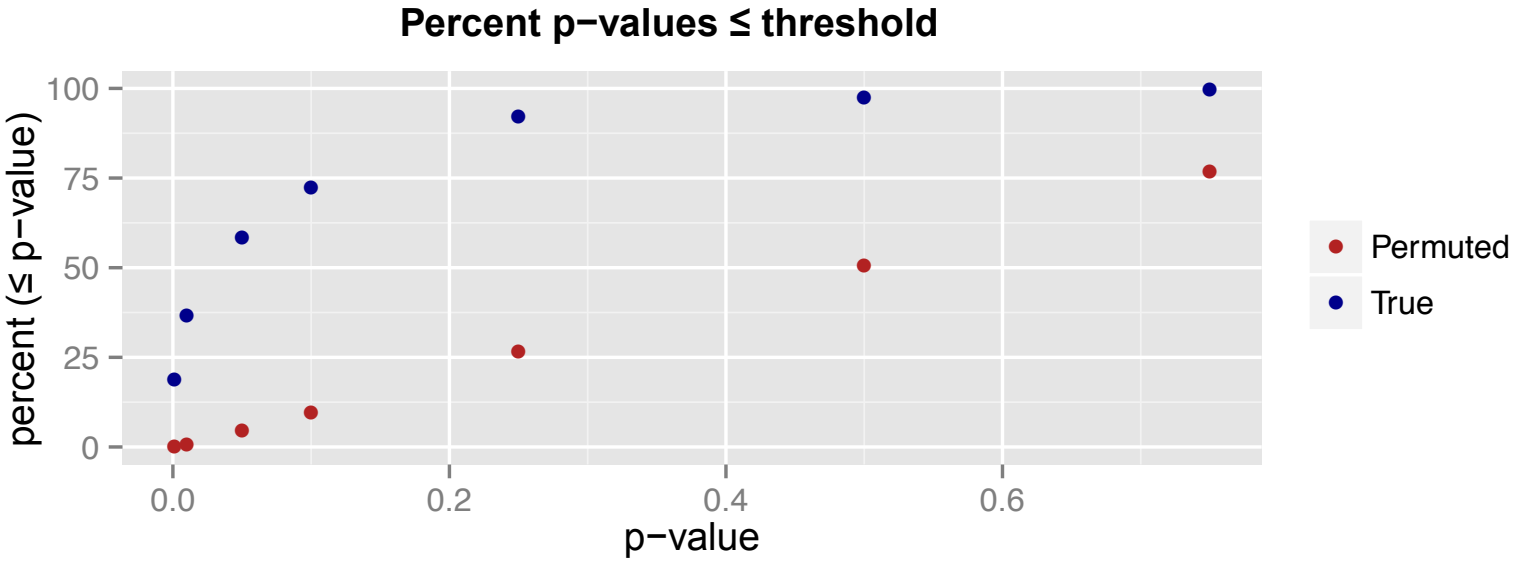
D

OTU 356029


count
6e+06
4e+06
2e+06
0e+00
0.0
0.4
0.8
permuted p−values

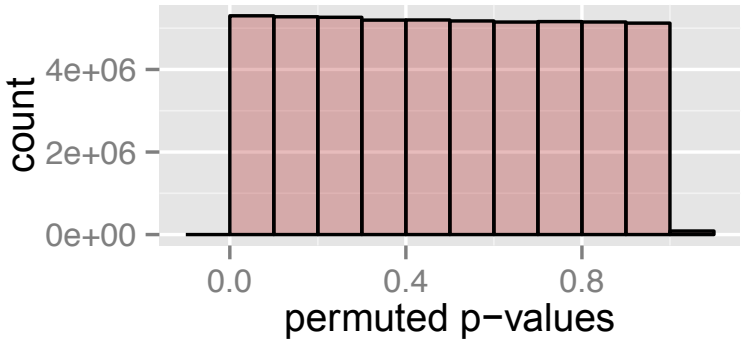

count
30000
20000
10000
0
0.00
0.25
0.50
0.75
1.00
true p−values


Densities of true and permuted p−values
Density
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
100
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

D

OTU 365170


count
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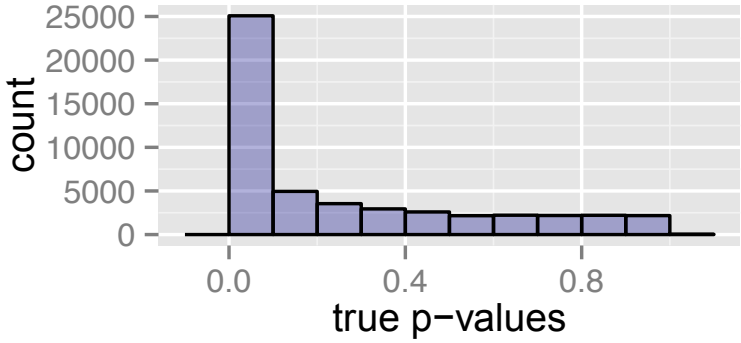
2e+06
0e+00
0.0
0.4
0.8
permuted p−values


count
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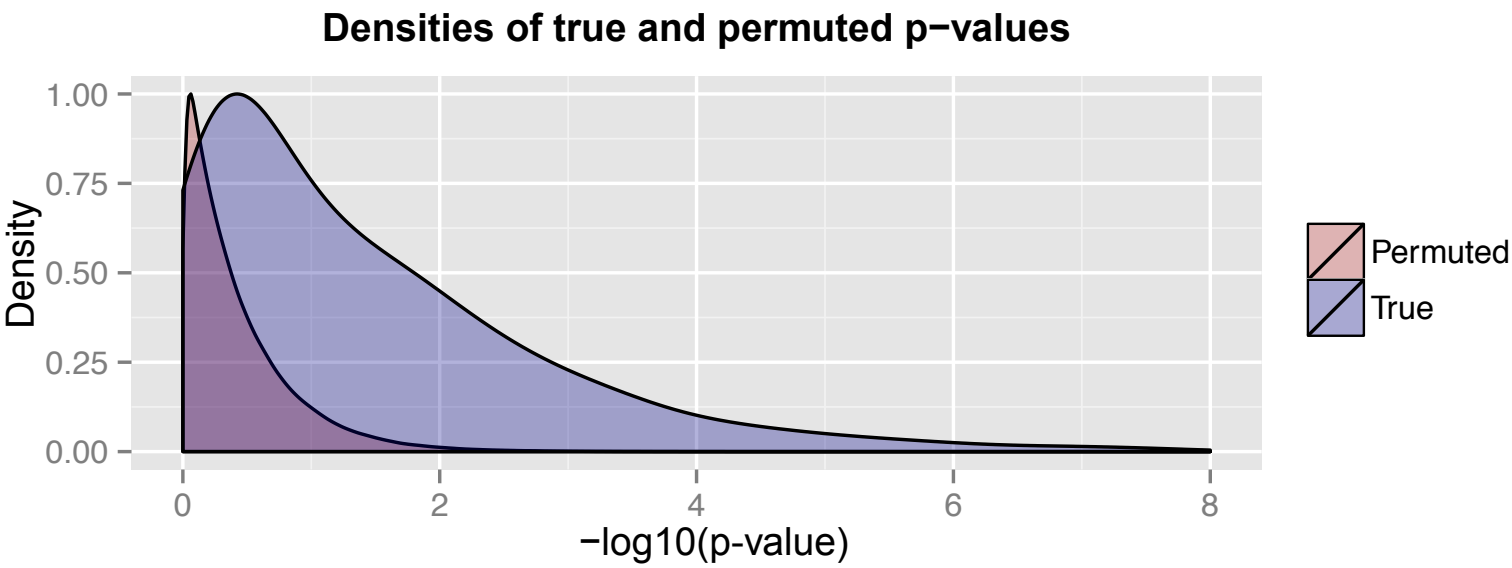
20000
15000
10000
5000
0
0.0
0.4
0.8
true p−values


Densities of true and permuted p−values
Density
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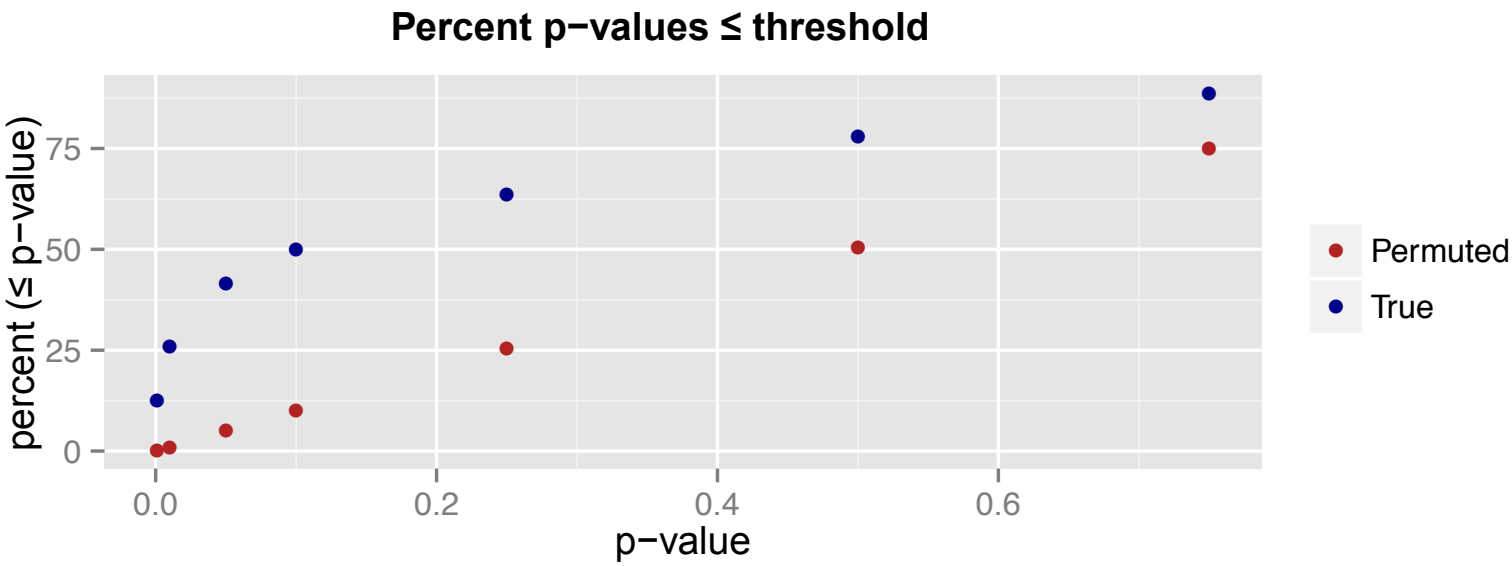
0.75
0.50
0.25
0.00
0
2
4
6
8
−log10(p-value)
Permuted
True


Percent p−values ≤ threshold
percent (≤ p−value)
75
50
25
0
0.0
0.2
0.4
0.6
p−value
Permuted
True

Supplementary Table 1. Meta data of samples used in 16S and metabolite analysis.

16S Amplicons

Lab and BE	Female	Male	Total
UNC	28	29	57
Week 0	28	29	57
BE1 - SPF	15	16	31
BE2 - Barrier	13	13	26
LBNL	114	122	236
Week 2	29	28	57
Week 4	28	30	58
Week 6	28	28	56
Week 8	29	26	55
Week 10, generation 2		10	10
Grand Total	**142**	**151**	**293**

BE and Strain	Female	Male	Total
BE1 - SPF	76	79	155
CC008	5	5	10
CC011	5	6	11
CC013	4	6	10
CC016	4	5	9
CC021	5	4	9
CC026	4	4	8
CC028	5	4	9
CC030	5	5	10
CC032	5	5	10
CC033	5	5	10
CC036	5	6	11
CC038	5	6	11
CC050	5	5	10
CC052	5	5	10
CC057	4	3	7
CC061	5	5	10
BE2 - Barrier	66	72	138
CC001	4	3	7
CC002	5	5	10
CC004	5	5	10
CC010	5	5	10
CC019	5	6	11
CC037	5	6	11
CC039	5	5	10
CC040	5	5	10
CC041	5	6	11
CC042	4	6	10
CC051	4	6	10
CC055	5	4	9
CC059	4	5	9
CC065	5	5	10
Grand Total	**142**	**151**	**293**

Metabolies

Row Labels	Female	Male	Total
UNC	27	28	55
Week 0	27	28	55
BE1 - SPF	15	15	30
BE2 - Barrier	12	13	25
LBNL	26	33	59
Week 8	26	23	49
Week 10		10	10
Grand Total	**53**	**61**	**114**

BE and Strain	Female	Male	Total
BE1 - SPF	30	32	62
CC008	2	2	4
CC011	2	3	5
CC013	2	2	4
CC016	1	2	3
CC021	2	1	3
CC026	2	1	3
CC028	2	2	4
CC030	2	2	4
CC032	2	3	5
CC033	2	2	4
CC036	2	3	5
CC038	2	3	5
CC050	2	2	4
CC052	2	2	4
CC057	1		1
CC061	2	2	4
BE2 - Barrier	23	29	52
CC001	1	2	3
CC002	2	2	4
CC004	2	2	4
CC010	2	2	4
CC019	1	2	3
CC037	2	3	5
CC039	2	2	4
CC040	1	1	2
CC041	1	2	3
CC042	1	3	4
CC051	2	3	5
CC055	2	1	3
CC059	2	2	4
CC065	2	2	4
Grand Total	**53**	**61**	**114**

Supplementary Table 2. Normalized amplicon abundance.
See separate excel sheet.

Supplementary Table 3. Differentially abundant fecal operational taxonomic units (OTUs) between animal facility built environments (BE1 vs BE2).
See separate excel sheet.

Supplementary Table 4. P-values for each genetic locus obtained using Mann-Whitney U test for all OTUs.
See separate excel sheet.

Supplementary Table 5. Joint QTL intervals and candidate genes.
See separate excel sheet.

Supplementary Table 6. Linkage analysis of microbial families.
See separate excel sheet.

Supplementary Table 7. Candidate genes in genetic loci associated with specific microbial families.
See separate excel sheet.

Supplementary Table 8. Human genome-wide association studies of microbiome associated diseases.

PubMed ID	GWAS study
22412388	Crohn's disease
21102463	Crohn's disease
17684544	Crohn's disease
18587394	Crohn's disease
23266558	Crohn's disease
23128233	Crohn's disease
17435756	Crohn's disease
17447842	Crohn's disease
17554261	Crohn's disease
17804789	Crohn's disease
20570966	Crohn's disease
22293688	Crohn's disease
22936669	Crohn's disease
23850713	Crohn's disease
23665963	Crohn's disease
21298027	Crohn's disease and celiac disease
22482804	Crohn's disease and psoriasis
18723019	Crohn's disease and sarcoidosis (combined)
25557950	Crohn's disease-related phenotypes
21699788	Ulcerative colitis or Crohn's disease
23936387	Celiac disease
24999842	Celiac disease
20190752	Celiac disease
17558408	Celiac disease
18311140	Celiac disease
21383967	Celiac disease or Rheumatoid arthritis
20848476	Ulcerative colitis
19915572	Ulcerative colitis
20228799	Ulcerative colitis
19122664	Ulcerative colitis
21297633	Ulcerative colitis
23511034	Ulcerative colitis
18836448	Ulcerative colitis
19915573	Ulcerative colitis
20228798	Ulcerative colitis
24837172	Ulcerative colitis
25082827	Ulcerative colitis
25248455	Irritable bowel syndrome

24797007	Irritable bowel syndrome
24487271	Erythema nodosum in inflammatory bowel disease
17068223	Inflammatory bowel disease
18758464	Inflammatory bowel disease
19915574	Inflammatory bowel disease (early onset)
19268274	Body mass (lean)
23108985	Body mass (lean)
21037115	Body mass in chronic obstructive pulmonary disease
19079260	Body mass index
20935630	Body mass index
20966902	Body mass index
19079261	Body mass index
23563607	Body mass index
22344221	Body mass index
17434869	Body mass index
17903300	Body mass index
18454148	Body mass index
19851299	Body mass index
20397748	Body mass index
21253498	Body mass index
21701565	Body mass index
21701570	Body mass index
22333899	Body mass index
22344219	Body mass index
22446040	Body mass index
22982992	Body mass index
23583978	Body mass index
23669352	Body mass index
24064335	Body mass index
24348519	Body mass index
24827717	Body mass index
24861553	Body mass index
25133637	Body mass index
25378290	Body mass index (change over time)
24281739	Body mass index (education interaction)
23192594	Body mass index (interaction)
22417934	Body mass index and cholesterol (psychopharmacological treatment)
19584900	Body mass index and fat mass
23517042	Body mass index in asthmatics
23300278	Type 2 diabetes
22158537	Type 2 diabetes
22961080	Type 2 diabetes

23945395	Type 2 diabetes
24509480	Type 2 diabetes
20581827	Type 2 diabetes
21874001	Type 2 diabetes
25483131	Type 2 diabetes
22456796	Type 2 diabetes
25102180	Type 2 diabetes
17293876	Type 2 diabetes
17460697	Type 2 diabetes
17463248	Type 2 diabetes
17463249	Type 2 diabetes
17668382	Type 2 diabetes
17846124	Type 2 diabetes
17846125	Type 2 diabetes
17846126	Type 2 diabetes
18372903	Type 2 diabetes
18711366	Type 2 diabetes
18711367	Type 2 diabetes
19056611	Type 2 diabetes
19401414	Type 2 diabetes
20174558	Type 2 diabetes
20418489	Type 2 diabetes
20818381	Type 2 diabetes
20862305	Type 2 diabetes
21490949	Type 2 diabetes
21573907	Type 2 diabetes
21647700	Type 2 diabetes
21799836	Type 2 diabetes
22101970	Type 2 diabetes
22238593	Type 2 diabetes
22693455	Type 2 diabetes
23209189	Type 2 diabetes
23532257	Type 2 diabetes
23937595	Type 2 diabetes
24101674	Type 2 diabetes
24390345	Type 2 diabetes
23386860	Type 2 diabetes (dietary heme iron intake interaction)
24465431	Type 2 diabetes (young onset) and obesity
17848626	Type 2 diabetes and 6 quantitative traits
22179738	Type 2 diabetes and gout
19734900	Type 2 diabetes and other traits
21150874	Type 2 diabetes nephropathy

17653210	Type 2 diabetes nephropathy
19252134	Type 2 diabetes nephropathy
20037589	Metabolite levels
22916037	Metabolite levels
19043545	Metabolite levels
21931564	Metabolite levels
23281178	Metabolite levels
23378610	Metabolite levels
23934736	Metabolite levels (X-11787)
23319000	Metabolite levels (5-HIAA)

Supplementary Table 9. Original intensity of metabolomics data of the detected metabolites from BE1/2 and BE3 mouse chow.

	BE3: LBNL Mouse chow			BE1/2: UNC- Mouse chow		
Metabolite	LBNL_1	LBNL_2	LBNL_3	UNC_A_1	UNC_A_2	UNC_A_3
1-methyl nicotinamide	2126109.5	834369.5	1206885.1	0.0	0.0	0.0
1-methylhydantoin	557566.8	728804.0	622438.9	140175.5	215532.9	316187.9
1-monopalmitin*	6632659.2	5650860.6	6267318.9	1533967.6	1441923.1	1549965.0
2-hydroxyglutaric acid	721951.6	643801.1	802270.0	0.0	0.0	0.0
3,4,5-trihydroxypentanoic acid	990480.9	842008.2	875281.4	121804.7	54112.6	98269.7
3-hydroxycholanic acid*	578125.2	556759.5	504158.5	467551.1	343444.8	267991.7
3-hydroxypyridine	516086.2	435066.1	488055.0	227212.2	231071.2	270617.5
4-guanidinobutyric acid	7322057.7	6068321.7	7592480.8	927115.7	985874.1	1357749.4
4-hydroxy-3-methoxybenzoic acid	452887.0	267793.6	432670.3	129645.6	112442.4	155757.0
aconitic acid	2097816.3	1489856.2	1120709.0	0.0	0.0	0.0
adenine	2522028.5	1756542.5	2298341.7	541575.7	579599.9	627925.6
adenosine	2458398.5	2007179.9	2292650.6	354308.2	367611.4	591184.9
allo-inositol	59855256.6	54103244.7	61595271.1	12621894.7	12288625.9	16417182.1
alpha tocophereol	127322.8	168792.0	192749.4	236637.2	238097.9	290908.8
alphatocoperol acetate*	609262.9	693354.0	1015326.8	472158.8	611265.7	779841.0
arabitol	784016.0	724202.7	822824.6	105693.1	102436.5	199011.9
arachidic acid	1597032.5	1423306.0	1762426.1	732253.1	665101.4	771094.7
behenic acid	1781203.2	1643339.5	2019126.6	490666.1	466727.8	566992.8
beta-alanine	955078.4	911969.5	1052738.3	376457.0	354349.4	409319.7
beta-sitosterol	142237.9	204863.6	225973.7	157630.1	162840.4	197755.5
campesterol	312126.3	409828.2	469957.8	361740.8	368188.7	459213.8
carbonate ion	6435064.7	8299850.2	7650514.5	5228611.7	7854240.5	7813798.4
cellobiose	110993267.0	96891621.0	106829413.3	0.0	0.0	0.0
cholesterol	1415142.1	1712389.6	1868015.8	1967546.7	1947785.9	2353065.3
citric acid	11686397.3	10713775.6	11517466.8	3014890.2	2880607.9	3991187.2
creatinine	2097023.6	3193038.3	1585909.4	400884.1	831720.2	1272215.7
cycloleucine	1136260.5	1778877.6	2004906.1	83997.5	0.0	200809.6
D-glucose	86842391.3	82790234.8	87342842.8	2481503.1	2851729.4	3517230.2
D-malic acid	5450160.2	4615308.4	5424313.4	1099127.6	1111796.4	1536904.5
D-mannitol	15983014.7	13058259.0	16479481.7	1522379.8	1542630.0	2114454.7
D-mannose	11155785.9	9746726.4	12093532.9	630966.5	919106.8	1158483.3
docosahexaenoic acid	1588950.5	1659463.5	2041589.4	1314377.3	1284288.8	1682744.3
D-sorbitol	49681360.1	38199145.3	50729511.2	1124642.6	1299996.1	1713214.5
D-threitol	1434317.5	1140781.4	1443399.4	127706.1	134579.0	169018.3
D-xylulose	1366056.1	1235124.2	1474876.3	101865.7	119560.6	287094.0
ethanolamine*	2095056.6	1793502.7	2230758.6	211726.1	240074.1	478144.0
fructose	62130471.8	55563046.3	59938062.4	6249818.3	7001392.2	8826772.2

fumaric acid	1571650.9	1077810.8	1201089.9	340893.1	155174.6	542529.2
galactinol	4451909.5	6975948.7	7332221.5	860148.8	866737.7	1306845.8
galactonic acid	2423091.5	1847077.2	2324031.9	0.0	0.0	0.0
glucosyclycerol*	3461069.1	2912898.9	3603789.2	2370022.5	3940355.2	4982293.8
glyceric acid	1959202.0	1550837.3	2168663.1	462118.7	539667.2	649371.0
glycerol 3-phosphate	12221545.6	10970701.9	13672833.6	1466105.0	1573183.7	2208515.3
glycine	8066064.0	7328064.0	8720151.0	1477837.0	1506064.0	2231221.0
glycolic acid	840562.7	651374.1	743478.9	323100.5	434110.0	414457.7
heptadecanoic acid	867240.1	840130.9	894224.2	913488.7	1233376.4	1626433.0
hypoxanthine	1130596.2	1192099.3	1403418.4	367204.2	472047.9	692734.3
inosine	1590909.5	1770862.0	2170136.8	319284.1	342885.6	594708.1
L-(+) lactic acid	45583652.4	41941865.1	47482519.9	6165768.0	5412074.9	8080550.2
lactose	110218093.1	121070247.1	110924804.6	0.0	0.0	0.0
L-alanine	5864371.3	7630566.3	7211707.4	2404231.9	2920955.0	3516347.1
L-asparagine	4024455.1	4043611.8	4416636.9	295521.8	454659.2	689615.1
L-glutamic acid	4018705.3	3709658.5	4441862.6	356589.7	356094.7	499735.6
L-glutamine	81618.4	115028.2	127656.0	0.0	0.0	0.0
linoleic acid	85024430.8	91450024.7	100923726.6	44469675.8	41296369.8	48485161.7
L-isoleucine	2074075.6	3484561.7	4360543.6	202682.6	364768.2	455163.9
L-leucine	2692486.2	4636025.7	5467973.4	267550.4	419715.8	611460.4
L-lysine	2727212.7	2782655.1	3314048.5	873066.6	1146700.8	1199541.6
L-methionine	110507312.7	110799844.8	124877475.2	6854454.3	20501838.9	14059374.7
L-methionine sulfoxide	2182177.8	2136539.0	1848413.4	242345.3	448455.8	393968.3
L-ornithine	1151466.9	1173649.4	1356110.4	126318.8	115315.5	188437.9
L-phenylalanine	5024288.6	4795434.2	5332265.1	652338.1	713128.5	1016918.0
L-proline	4404501.5	7410057.6	9347580.7	181546.5	315387.9	466164.1
L-pyroglutamic acid	6686025.5	6232344.6	6556141.6	2609152.8	2836604.1	3646643.3
L-serine	5365866.7	5188074.7	6255800.7	626538.0	835526.6	1072518.5
L-threonic acid*	924453.3	774396.7	961600.6	132154.4	126427.3	168299.5
L-threonine	1618604.6	1567141.6	1845755.1	205436.1	237163.6	349895.4
L-tryptophan	11059540.1	10523443.8	12556155.8	1112436.5	1377941.5	2151207.9
L-tyrosine	3906266.5	3181860.1	4348948.2	709542.7	688845.7	1027830.2
L-valine	5185053.4	4806657.3	5699354.1	347642.9	491842.6	1020567.9
malonic acid	1228214.1	1008522.2	1266489.6	142272.0	165952.6	236273.2
maltose	9187141.3	8000796.5	9500608.0	485049.3	466348.6	698676.9
myo-inositol	14100822.0	11379143.4	14059320.4	1362049.5	1372830.2	1917219.8
myristic acid	5800400.2	4559156.1	6863875.2	3092800.0	2483012.7	2960274.6
N-acetyl-D-glucosamine	358848.1	301697.3	361071.8	0.0	0.0	0.0
nicotinic acid	856608.8	702007.1	2222556.3	226206.1	132075.3	187769.6
oleantile*	3722593.4	3610673.4	3299903.1	6166171.6	5614341.1	5237153.9
oleic acid	62408799.8	64816275.8	75596073.8	34958837.9	32498718.4	33410993.7

palatinose	10706016.7	10016736.8	13103133.8	0.0	0.0	0.0
palmitic acid	73592029.6	73332493.1	86356364.1	35673255.5	31253754.3	35746126.2
palmitoleic acid	574941.2	594742.7	714578.0	385355.6	335926.1	403013.5
pantothenic acid	415823.1	697680.4	503601.3	258901.1	244409.3	266708.9
phosphoric acid	65756631.4	56451983.9	68871679.2	16446307.0	16629347.8	22496900.7
propylene glycol	2584181.1	1856258.9	2125873.9	132246.0	151870.3	446334.1
pyruvic acid	2386638.1	1186755.1	1731189.4	558633.2	488166.7	789795.9
quinic acid	11088802.7	8942723.6	10973879.7	461418.7	514903.9	659114.1
raffinose	11427459.4	10816762.0	12093731.4	959229.0	1029130.8	1406925.7
ribitol	8785584.9	7868729.7	9208095.5	1388410.7	1426888.9	2014298.5
ribonic acid*	1475552.1	1377843.4	1488626.6	161046.6	187792.5	202912.1
salicylic acid	322414.6	297865.4	353517.3	118081.5	179914.8	153112.0
scyllo-inositol	337099.8	281756.9	370270.0	124142.6	131565.0	158742.0
shikimic acid	3427091.0	2686965.9	3316467.8	204529.4	168064.7	232916.4
stearic acid	20862731.4	19285698.6	22642105.2	10802078.1	9241130.2	9977450.8
stearonitrile*	270321.2	255271.4	273518.8	282507.3	235628.1	241512.2
succinic acid	4199743.3	3547581.9	4296277.7	797138.1	863474.1	1227261.2
sucrose	181013494.3	179109364.1	179658321.2	72184886.3	70116182.2	84515764.5
tagatose	10726625.1	8729155.6	9587581.3	487008.0	561048.5	707656.3
thymine	246185.0	230648.9	282921.7	136306.9	181674.4	219581.5
urea	2467256.9	2172084.3	2737484.3	133528.6	160053.8	307040.7
xylitol	1519739.9	1225709.9	1433826.7	347979.9	364995.0	468791.9

Supplementary Table 10. Metabolomics data including original intensity of the detected metabolites from murine feces and their z-scored transformed values in separate tabs.
See separate excel sheet.

Supplementary Table 11. Metabolite profiles in fecal samples of four CC strains maintained on different diets.
See separate excel sheet.

Supplementary Table 12. A list of all metabolites assayed and analyzed in terms of community metabolic potential for each subset of the data, detailing correlations between metabolomics data and community metabolic potential scores and potential taxonomic contributors.
See separate excel sheet.