

Corn Bran Particle Size Modulates the Gut Microbiome Profile and Composition: Results from an *In Vitro* Fermentation Study

Supplementary Material 1

Mothur code

To start cleaning my sequences

```
make.file(inputdir=data/raw/, type=gz, prefix=stability)

make.contigs(file=stability.files, processors=8, inputdir=data/raw, outputdir=data/mothur)

summary.seqs(fasta=data/mothur/stability.trim.contigs.fasta)
screen.seqs(fasta=stability.trim.contigs.fasta, group=stability.contigs.groups, maxambig=0,
maxlength=415, maxhomop=9, inputdir=data/mothur)

get.current()
summary.seqs()

unique.seqs(fasta=stability.trim.contigs.good.fasta)

count.seqs(name=stability.trim.contigs.good.names, group=stability.contigs.good.groups)

summary.seqs(count=stability.trim.contigs.good.count_table)
```

To prepare the reference dataset Silva

```
align.seqs(fasta=data/references/ecoli_v4_v5_blog.fasta,
reference=data/references/silva.seed_v128.align)

summary.seqs(fasta=data/references/ecoli_v4_v5_blog.align)

pcr.seqs(fasta=data/references/silva.seed_v128.align, start=11894, end=28464, keepdots=F)

system(mv data/mothur/silva.seed_v128.pcr.align data/references/silva.v4_v5.fasta)

summary.seqs(fasta=data/references/silva.v4_v5.fasta)
```

To align the cleaned sequences to the reference dataset

```
align.seqs(fasta=stability.trim.contigs.good.unique.fasta,
reference=data/references/silva.v4_v5.fasta, flip=t)

summary.seqs(fasta=stability.trim.contigs.good.unique.align,
count=stability.trim.contigs.good.count_table)

screen.seqs(fasta=stability.trim.contigs.good.unique.align,
count=stability.trim.contigs.good.count_table,
summary=stability.trim.contigs.good.unique.summary, start=1, end=16570)

summary.seqs(fasta=current, count=current)

filter.seqs(fasta=stability.trim.contigs.good.unique.good.align, vertical=T, trump=.)

unique.seqs(fasta=stability.trim.contigs.good.unique.good.filter.fasta,
count=stability.trim.contigs.good.count_table)

summary.seqs(fasta=current, count=current)

pre.cluster(fasta=stability.trim.contigs.good.unique.good.filter.unique.fasta,count=stability.trim.c
ontigs.good.unique.good.filter.count_table, diffs=2)

summary.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.count_table)

chimera.vsearch(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.count_table, dereplicate=t)

remove.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta,
accnos=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.accnos)

summary.seqs(fasta=current, count=current)

classify.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.count
_table, reference=data/references/trainset16_022016.pds.fasta,
taxonomy=data/references/trainset16_022016.pds.tax, cutoff=80)

remove.lineage(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.count
_table,
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.taxono
my, taxon=Chloroplast-Mitochondria-unknown-Archaea-Eukaryota)
```

```
summary.tax(taxonomy=current, count=current)
summary.seqs(fasta=current, count=current)
```

Assessing Error Rate using the Mock Community Sample

```
get.groups(count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch
.pick.pick.count_table,
fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta,
groups=MOCK)
```

```
seq.error(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.fas
ta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.
pick.count_table, reference=data/references/Reference_MockCommunity.fasta, aligned=F)
```

```
dist.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.fas
ta, cutoff=0.03)
```

```
cluster(column=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.di
st,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.
pick.count_table)
```

```
make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.o
pti_mcc.list,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.
pick.count_table, label=0.03)
```

```
rarefaction.single(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pi
ck.pick.opti_mcc.shared)
```

OTU preparation for analysis

```
remove.groups(count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vse
arch.pick.pick.count_table,
fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta,
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.ta
xonomy, groups=MOCK)
```

```
dist.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.fas
ta, cutoff=0.03)
```

```
cluster(column=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.dist,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.count_table)
```

```
make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.o  
pti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.count_table, label=0.03)
```

```
classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.op  
ti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.count_table,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pi  
ck.taxonomy, label=0.03)
```

Phylotype preparation for analysis

```
phylotype(taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.w  
ang.pick.pick.taxonomy)
```

```
make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.  
pick.pick.tx.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.count_table, label=1)
```

```
classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pi  
ck.pick.tx.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.count_table,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pi  
ck.taxonomy, label=1)
```

```
remove.rare(shared=current, nseqs=2)  
rename.file(count=current, shared=current, constaxonomy=current, deleteold=F,  
prefix=data/mothur/phylotype)
```

```
count.groups(shared=current)
```

```
sub.sample(shared=current, size=2874, outputdir=data/mothur)
```

OTU analysis

```

rename.file(count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.
h.pick.pick.pick.count_table,
shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.opti_mcc.s
hared,
constaxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.opt
i_mcc.0.03.cons.taxonomy)

count.groups(shared=stability.opti_mcc.shared)

sub.sample(shared=stability.opti_mcc.shared, size=2874)

```

Alpha diversity measurements

```

rarefaction.single(shared=stability.opti_mcc.shared, calc=sobs, freq=100)

summary.single(shared=stability.opti_mcc.shared, calc=nseqs-coverage-sobs-chao-invsimpson-
simpson-shannon-simpson-even-shannon-even, subsample=2874)

summary.single(shared=stability.opti_mcc.shared, calc=nseqs-coverage-sobs-chao-invsimpson-
simpson-shannon-simpson-even-shannon-even)

```

Beta diversity measurements

```

heatmap.bin(shared=stability.opti_mcc.0.03.subsample.shared, scale=log2, numotu=50)

dist.shared(shared=stability.opti_mcc.shared, calc=thetayc-jclass-braycurtis, subsample=2874)

heatmap.sim(phylip=stability.opti_mcc.thetayc.0.03.lt.ave.dist)
heatmap.sim(phylip=stability.opti_mcc.jclass.0.03.lt.ave.dist)
heatmap.sim(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist)

tree.shared(phylip=stability.opti_mcc.thetayc.0.03.lt.ave.dist)
tree.shared(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist)

```

Parsimony pairwise comparisons

```

parsimony(tree=stability.opti_mcc.thetayc.0.03.lt.ave.tre, group=data/raw/size.design,
groups=all)
parsimony(tree=stability.opti_mcc.thetayc.0.03.lt.ave.tre, group=data/raw/time.design,
groups=all)

```

```
parsimony(tree=stability.opti_mcc.braycurtis.0.03.lt.ave.tre, group=data/raw/size.design, groups=all)
parsimony(tree=stability.opti_mcc.braycurtis.0.03.lt.ave.tre, group=data/raw/time.design, groups=all)
```

Ordination plots to visualize B-diversity distances

```
pcoa(phylip=stability.opti_mcc.thetayc.0.03.lt.ave.dist)
pcoa(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist)

nmds(phylip=stability.opti_mcc.thetayc.0.03.lt.ave.dist)
nmds(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist)

nmds(phylip=stability.opti_mcc.thetayc.0.03.lt.ave.dist, mindim=3, maxdim=3)
nmds(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist, mindim=3, maxdim=3)
```

AMOVA and HOMOVA

```
amova(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size.design)
amova(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/time.design)
amova(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size_time.design)

homova(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size.design)
homova(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/time.design)
homova(phylip=stability.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size_time.design)
```

Correlation with NMDS axes

```
corr.axes(axes=stability.opti_mcc.braycurtis.0.03.lt.ave.nmds.axes,
metadata=data/raw/size_labeled.metadata, method=spearman, numaxes=2)
corr.axes(axes=stability.opti_mcc.braycurtis.0.03.lt.ave.nmds.axes,
metadata=data/raw/time_labeled.metadata, method=spearman, numaxes=2)
corr.axes(axes=stability.opti_mcc.braycurtis.0.03.lt.ave.nmds.axes,
metadata=data/raw/size_time.metadata, method=spearman, numaxes=2)
```

Partitioning into communities

```
get.communitytype(shared=stability.opti_mcc.0.03.subsample.shared)
```

Population level analysis for differentially abundant taxa

```
metastats(shared=stability.opti_mcc.0.03.subsample.shared, design=data/raw/size.design)  
metastats(shared=stability.opti_mcc.0.03.subsample.shared, design=data/raw/time.design)  
metastats(shared=stability.opti_mcc.0.03.subsample.shared, design=data/raw/size_time.design)
```

```
lefse(shared=stability.opti_mcc.0.03.subsample.shared, design=data/raw/size.design)  
lefse(shared=stability.opti_mcc.0.03.subsample.shared, design=data/raw/time.design)  
lefse(shared=stability.opti_mcc.0.03.subsample.shared, design=data/raw/size_time.design)
```

```
lefse(shared=phylotype.tx.1.subsample.shared, design=data/raw/size.design)
```

To repeat the analysis for only corn samples (no Blank and FOS)

```
remove.groups(inputdir=data/mothur, outputdir=data/mothur,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
count_table,  
fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.ta  
xonomy, groups=D1112-D1212-D1312-D1124-D1224-D1324-D1148-D1248-D1348-D2112-  
D2212-D2312-D2124-D2224-D2324-D2148-D2248-D2348-MOCK)
```

Manually renamed files .corn

```
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.corn.fasta  
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pick.corn.ta  
xonomy  
stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.pick.co  
rn.count_table
```

```
dist.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.cor  
n.fasta, cutoff=0.03, processors=8)
```

```
cluster(column=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co  
rn.dist,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.corn.count_table)
```

```
make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.c  
orn.opti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.corn.count_table, label=0.03)
```

```
classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co  
rn.opti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.corn.count_table,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pi  
ck.corn.taxonomy, label=0.03)
```

```
count.groups(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pi  
ck.corn.opti_mcc.shared)
```

```
sub.sample(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick  
.corn.opti_mcc.shared, size=2874)
```

```
rarefaction.single(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pi  
ck.pick.corn.opti_mcc.shared, calc=sobs, freq=100)
```



```
summary.single(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick
.pick.corn.opti_mcc.shared, calc=nseqs-coverage-sobs-chao-invsimpson-simpson-shannon-
simpson-even-shannon-even, subsample=2874)
```

```
dist.shared(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick
.corn.opti_mcc.shared, calc=thetayc-jclass-braycurtis, subsample=2874)
```

```
tree.shared(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick
.corn.opti_mcc.braycurtis.0.03.lt.ave.dist)
```

```
parsimony(tree=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co
rn.opti_mcc.braycurtis.0.03.lt.ave.tre, group=data/raw/size.corn.design, groups=all)
```

```
parsimony(tree=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co
rn.opti_mcc.braycurtis.0.03.lt.ave.tre, group=data/raw/time.corn.design, groups=all)
```

```
parsimony(tree=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co
rn.opti_mcc.braycurtis.0.03.lt.ave.tre, group=data/raw/size_time.corn.design, groups=all)
```

```
nmds(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.corn.
opti_mcc.braycurtis.0.03.lt.ave.dist)
```

```
amova(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co
rn.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size_time.corn.design)
```

```
homova(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co
rn.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size_time.corn.design)
```

```
corr.axes(axes=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.co
rn.opti_mcc.braycurtis.0.03.lt.ave.nmds.axes, metadata=data/raw/size_time.corn.metadata,
method=spearman, numaxes=2)
```

```
get.communitytype(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.
pick.pick.corn.opti_mcc.shared)
```

To repeat the analysis at 24hrs only

```
remove.groups(inputdir=data/mothur, outputdir=data/mothur,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
count_table,  
fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.ta  
xonomy, groups=D1112-D1212-D1312-D1148-D1248-D1348-D2112-D2212-D2312-D2148-  
D2248-D2348-D310-D320-D330-D14112-D14212-D14312-D14148-D14248-D14348-D15112-  
D15212-D15312-D15148-D15248-D15348-D16112-D16212-D16312-D16148-D16248-  
D16348-D17112-D17212-D17312-D17148-D17248-D17348-MOCK)
```

Manually renamed following files .24.

```
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.fasta  
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pick.24.taxon  
omy  
stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.pick.24  
.count_table
```

```
dist.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.  
fasta, cutoff=0.03)
```

```
cluster(column=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24  
.dist,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.24.count_table)
```

```
make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.2  
4.opti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.24.count_table, label=0.03)
```

```
classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24  
.opti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.24.count_table,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pi  
ck.24.taxonomy, label=0.03)
```

```
count.groups(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pi  
ck.24.opti_mcc.shared)
```

```
sub.sample(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick  
.24.opti_mcc.shared, size=3686)
```

```
rarefaction.single(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.shared, calc=sobs, freq=100)
```

```
summary.single(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.shared, calc=nseqs-coverage-sobs-chao-invsimpson-simpson-shannon-simpson-even-shannon-even, subsample=3686)
```

```
dist.shared(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.shared, calc=thetayc-jclass-braycurtis, subsample=3686)
```

```
tree.shared(phylop=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.braycurtis.0.03.lt.ave.dist)
```

```
parsimony(tree=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.braycurtis.0.03.lt.ave.dist, group=data/raw/size.24.design, groups=all)
```

```
nmds(phylop=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.braycurtis.0.03.lt.ave.dist)
```

```
amova(phylop=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size.24.design)
```

```
homova(phylop=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size.24.design)
```

```
get.communitytype(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.0.03.subsample.shared)
```

```
corr.axes(axes=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.24.opti_mcc.braycurtis.0.03.lt.ave.nmds.axes, metadata=data/raw/size.24.metadata, method=spearman, numaxes=2)
```

To repeat the analysis at 48hrs only

```
remove.groups(inputdir=data/mothur, outputdir=data/mothur,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
count_table,  
fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.ta  
xonomy, groups=D1112-D1212-D1312-D1124-D1224-D1324-D2112-D2212-D2312-D2124-  
D2224-D2324-D310-D320-D330-D14112-D14212-D14312-D14124-D14224-D14324-D15112-  
D15212-D15312-D15124-D15224-D15324-D16112-D16212-D16312-D16124-D16224-  
D16324-D17112-D17212-D17312-D17124-D17224-D17324-MOCK)
```

Manually renamed following files .48.

```
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.fasta  
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pick.48.taxon  
omy  
stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.pick.48  
.count_table
```

```
dist.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.  
fasta, cutoff=0.03)
```

```
cluster(column=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48  
.dist,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.48.count_table)
```

```
make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.4  
8.opti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.48.count_table, label=0.03)
```

```
classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48  
.opti_mcc.list,  
count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.  
pick.48.count_table,  
taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.pick.pi  
ck.48.taxonomy, label=0.03)
```

```
count.groups(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pi  
ck.48.opti_mcc.shared)
```

```
sub.sample(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick  
.48.opti_mcc.shared, size=2874)
```

```
rarefaction.single(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.shared, calc=sobs, freq=100)
```

```
summary.single(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.shared, calc=nseqs-coverage-sobs-chao-invsimpson-simpson-shannon-simpsonshannon-shannoneven, subsample=2874)
```

```
dist.shared(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.shared, calc=thetayc-jclass-braycurtis, subsample=2874)
```

```
tree.shared(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.braycurtis.0.03.lt.ave.dist)
```

```
parsimony(tree=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.braycurtis.0.03.lt.ave.tre, group=data/raw/size.48.design, groups=all)
```

```
nmads(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.braycurtis.0.03.lt.ave.dist)
```

```
amova(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size.48.design)
```

```
homova(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.braycurtis.0.03.lt.ave.dist, design=data/raw/size.48.design)
```

```
get.communitytype(shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.0.03.subsample.shared)
```

```
corr.axes(axes=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.48.opti_mcc.braycurtis.0.03.lt.ave.nmads.axes, metadata=data/raw/size.48.metadata, method=spearman, numaxes=2)
```