

Table S3. Results from Primer 7e 2-way PERMANOVA analysis at a phylum (A), genus (B) and an OTU level (C)

Table 3A – Phylum level: abbreviations Factor 1- NE challenge (Cp) and Factor 2 -probiotic additive (Ad)

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem2
Data type: Similarity
Selection: All
Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 99999

Factors

| Name | Abbrev. | Type | Levels |
|---------|---------|-------|--------|
| Cp | Cp | Fixed | 2 |
| Aditive | Ad | Fixed | 2 |

PERMANOVA table of results

| Source | df | SS | MS | Pseudo-F | P(perm) | Unique perms | P(MC) |
|--------|----|--------|--------|----------|---------|--------------|--------|
| Cp | 1 | 325.9 | 325.9 | 4.7139 | 0.0114 | 95736 | 0.0123 |
| Ad | 1 | 61.781 | 61.781 | 0.89361 | 0.4206 | 95735 | 0.4188 |
| CpxAd | 1 | 240.56 | 240.56 | 3.4795 | 0.0342 | 95740 | 0.0351 |
| Res | 48 | 3318.5 | 69.136 | | | | |
| Total | 51 | 3951.5 | | | | | |

Details of the expected mean squares (EMS) for the model

| Source | EMS |
|--------|----------------------------|
| Cp | 1*v(Res) + 25.923*s(Cp) |
| Ad | 1*v(Res) + 25.923*s(Ad) |
| CpxAd | 1*v(Res) + 12.961*s(CpxAd) |
| Res | 1*v(Res) |

Construction of Pseudo-F ratio(s) from mean squares

| Source | Numerator | Denominator | Num. df | Den. df |
|--------|-----------|-------------|---------|---------|
| Cp | 1*Cp | 1*Res | 1 | 48 |
| Ad | 1*Ad | 1*Res | 1 | 48 |
| CpxAd | 1*CpxAd | 1*Res | 1 | 48 |

Estimates of components of variation

| Source | Estimate | Sq. root |
|----------|----------|----------|
| S(Cp) | 9.905 | 3.1472 |
| S(Ad) | -0.28375 | -0.53269 |
| S(CpxAd) | 13.225 | 3.6367 |
| V(Res) | 69.136 | 8.3148 |

Table 3B – Genus level: abbreviations Factor 1- NE challenge (Cp) and Factor 2 -probiotic additive (Ad)

PERMANOVA

Permutational MANOVA

Resemblance worksheet

Name: Resem1

Data type: Similarity

Selection: All

Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)

Fixed effects sum to zero for mixed terms

Permutation method: Permutation of residuals under a reduced model

Number of permutations: 99999

Factors

| Name | Abbrev. | Type | Levels |
|---------|---------|-------|--------|
| Cp | Cp | Fixed | 2 |
| Aditive | Ad | Fixed | 2 |

PERMANOVA table of results

| Source | df | SS | MS | Pseudo-F | P(perm) | Unique perms | P(MC) |
|--------|----|--------|--------|----------|---------|--------------|--------|
| Cp | 1 | 456.67 | 456.67 | 1.987 | 0.064 | 93875 | 0.071 |
| Ad | 1 | 404.26 | 404.26 | 1.759 | 0.0981 | 93880 | 0.1043 |
| CpxAd | 1 | 316.31 | 316.31 | 1.3763 | 0.1969 | 93966 | 0.2008 |
| Res | 48 | 11032 | 229.83 | | | | |
| Total | 51 | 12202 | | | | | |

Details of the expected mean squares (EMS) for the model

| Source | EMS |
|--------|--|
| Cp | $1 * v(\text{Res}) + 25.923 * S(\text{Cp})$ |
| Ad | $1 * v(\text{Res}) + 25.923 * S(\text{Ad})$ |
| CpxAd | $1 * v(\text{Res}) + 12.961 * S(\text{CpxAd})$ |
| Res | $1 * v(\text{Res})$ |

Construction of Pseudo-F ratio(s) from mean squares

| Source | Numerator | Denominator | Num. df | Den. df |
|--------|-----------|-------------|---------|---------|
| Cp | 1 * Cp | 1 * Res | 1 | 48 |
| Ad | 1 * Ad | 1 * Res | 1 | 48 |
| CpxAd | 1 * CpxAd | 1 * Res | 1 | 48 |

Estimates of components of variation

| Source | Estimate | Sq. root |
|----------|----------|----------|
| S(Cp) | 8.7508 | 2.9582 |
| S(Ad) | 6.729 | 2.594 |
| S(CpxAd) | 6.6719 | 2.583 |
| V(Res) | 229.83 | 15.16 |

Table 3C – an OTU level: abbreviations Factor 1- NE challenge (Cp) and Factor 2 -probiotic additive (Ad)

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem3
Data type: Similarity
Selection: All
Resemblance: S17 Bray-Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 99999

Factors

| Name | Abbrev. | Type | Levels |
|---------|---------|-------|--------|
| Cp | Cp | Fixed | 2 |
| Aditive | Ad | Fixed | 2 |

PERMANOVA table of results

| Source | df | SS | MS | Pseudo-F | P(perm) | Unique perms | P(MC) |
|--------|----|--------|--------|----------|---------|--------------|--------|
| Cp | 1 | 2562.2 | 2562.2 | 1.7576 | 0.0079 | 87709 | 0.023 |
| Ad | 1 | 2012.1 | 2012.1 | 1.3802 | 0.0651 | 87697 | 0.1088 |
| CpxAd | 1 | 2181.3 | 2181.3 | 1.4963 | 0.032 | 87667 | 0.069 |
| Res | 48 | 69976 | 1457.8 | | | | |
| Total | 51 | 76725 | | | | | |

Details of the expected mean squares (EMS) for the model

| Source | EMS |
|--------|--|
| Cp | $1 \cdot V(\text{Res}) + 25.923 \cdot S(\text{Cp})$ |
| Ad | $1 \cdot V(\text{Res}) + 25.923 \cdot S(\text{Ad})$ |
| CpxAd | $1 \cdot V(\text{Res}) + 12.961 \cdot S(\text{CpxAd})$ |
| Res | $1 \cdot V(\text{Res})$ |

Construction of Pseudo-F ratio(s) from mean squares

| Source | Numerator | Denominator | Num. df | Den. df |
|--------|------------------------|----------------------|---------|---------|
| Cp | $1 \cdot \text{Cp}$ | $1 \cdot \text{Res}$ | 1 | 48 |
| Ad | $1 \cdot \text{Ad}$ | $1 \cdot \text{Res}$ | 1 | 48 |
| CpxAd | $1 \cdot \text{CpxAd}$ | $1 \cdot \text{Res}$ | 1 | 48 |

Estimates of components of variation

| Source | Estimate | Sq. root |
|----------|----------|----------|
| S(Cp) | 42.604 | 6.5271 |
| S(Ad) | 21.382 | 4.624 |
| S(CpxAd) | 55.819 | 7.4712 |
| V(Res) | 1457.8 | 38.182 |