# Metabolic phenotypes and the gut microbiota in response to dietary resistant starch type 2 in normal-weight subjects: a randomized crossover trial

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## Supplementary Information

**Table S1.** Nutrients intake at baseline and after 4-week CS or RS supplementation.

| Variable | CS | | RS | | *p* value |
|---|---|---|---|---|---|
| | 0 week | 4 weeks | 0 week | 4 weeks | |
| Total energy (kcal) | 1578.09±33.51 | 1560.02±32.39 | 1548.09±33.51 | 1536.51±33.88 | NS |
| Protein (g) | 63.36±2.08 | 64.43±2.08 | 65.46±2.08 | 64.35±1.94 | NS |
| Fat (g) | 41.14±1.53 | 42.21±1.31 | 43.11±1.51 | 42.68±0.92 | NS |
| Cholesterol (mg) | 354.29±22.50 | 348.86±24.53 | 358.39±23.50 | 339.81±13.36 | NS |
| Fiber (g) | 9.77±0.48 | 10.04±0.57 | 9.84±0.58 | 54.99±6.62***$^{###}$ | <0.001 |
| Carbohydrates (g) | 223.29±5.78 | 223.59±5.27 | 223.34±6.08 | 221.52±5.21 | NS |

Data are presented as mean ± SEM. Significance was determined by GEE model. *p* value was statistical significance between CS effect and RS effect; *** $p < 0.001$, RS 0 week vs. RS 4 weeks, $^{###}$ $p < 0.001$, RS 4 weeks vs. CS 4 weeks.

**Table S2.** Summary of serum metabolites of subjects before and after 4-week CS or RS supplementation.

| Variable | CS | | RS | | *p* value |
|---|---|---|---|---|---|
| | 0 week | 4 weeks | 0 week | 4 weeks | |
| Isobutyrate (nM) | 0.45(0.42,0.59) | 0.46(0.31,0.55) | 0.63(0.43,0.88) | 0.45(0.38,0.56) | NS |
| Valerate (nM) | 0.46(0.40,0.54) | 0.43(0.35,0.48) | 0.48(0.43,0.63) | 0.43(0.38,0.47) | NS |
| Isovalerate (nM) | 0.44(0.37,0.71) | 0.41(0.32,0.44) | 0.56(0.4,0.68) | 0.37(0.29,0.47) | NS |
| Hexanoate (nM) | 1.44(1.27,1.60) | 1.15(1.07,1.37) | 1.5(1.26,1.67) | 1.35(1.21,1.50) | NS |
| Carnitine C2:0 | 0.90(0.68,1.27) | 0.85(0.74,1.13) | 0.97(0.72,1.11) | 0.87(0.80,1.03) | NS |
| Carnitine C3:0 | 1.01(0.88,1.18) | 1.07(0.82,1.21) | 0.95(0.82,1.18) | 0.92(0.77,1.09) | NS |
| Carnitine C4:0 | 1.04(0.80,1.25) | 1.09(0.86,1.40) | 0.97(0.80,1.50) | 1.03(0.87,1.34) | NS |
| Carnitine C5:0 | 0.98(0.81,1.40) | 1.08(0.81,1.31) | 1.07(0.81,1.26) | 1.11(0.84,1.24) | NS |
| Carnitine C10:0 | 1.22(0.84,1.36) | 1.20(0.84,1.46) | 0.97(0.77,1.44) | 1.17(0.87,1.44) | NS |
| Carnitine C10:1 | 0.99(0.86,1.29) | 1.22(0.82,1.44) | 0.97(0.78,1.28) | 1.11(0.91,1.42) | NS |
| Carnitine C12:0 | 1.15(0.90,1.33) | 1.12(0.83,1.35) | 0.96(0.77,1.45) | 1.11(0.99,1.31) | NS |
| Carnitine C12:1 | 1.02(0.85,1.19) | 0.95(0.87,1.19) | 1.08(0.90,1.16) | 1.05(0.91,1.24) | NS |
| Carnitine C14:0 | 1.15(0.90,1.39) | 0.98(0.81,1.30) | 1.00(0.86,1.16) | 1.09(0.99,1.30) | NS |
| Carnitine C14:1 | 1.10(0.86,1.24) | 1.05(0.77,1.34) | 0.90(0.60,1.34) | 1.01(0.83,1.32) | NS |
| Carnitine C18:2 | 1.05(0.73,1.4) | 0.88(0.77,1.53) | 0.93(0.71,1.13) | 1.01(0.94,1.18) | NS |
| PC 1-acyl 32:0 | 1.04(0.92,1.25) | 1.03(0.90,1.10) | 1.08(0.91,1.17) | 1.17(1.03,1.26) | NS |
| PC 1-acyl 32:1 | 0.96(0.86,1.29) | 0.89(0.80,1.06) | 1.04(0.78,1.22) | 1.07(0.87,1.27) | NS |
| PC 1-acyl 34:0 | 1.13(0.77,1.34) | 0.94(0.80,1.20) | 1.22(0.93,1.38) | 1.09(1.02,1.25) | NS |
| PC 1-alk 34:1 | 1.16(0.91,1.47) | 1.17(0.87,1.29) | 1.09(0.93,1.31) | 1.05(0.97,1.24) | NS |
| PC 1-alk 34:2 | 1.09(0.87,1.31) | 1.05(0.92,1.18) | 1.15(1.04,1.28) | 1.22(1.06,1.33) | NS |
| Indoxyl sulfate | 1.22(1.02,1.74) | 0.83(0.59,1.38) | 1.07(0.86,1.57) | 1.29(1.05,1.77) | NS |
| Indolepropionate | 0.22(0.13,0.33) | 0.52(0.31,0.75) | 0.27(0.17,0.35) | 0.22(0.17,0.33) | NS |
| p-Cresol sulfate | 0.91(0.75,1.52) | 0.82(0.39,1.79) | 0.67(0.35,1.14) | 1.06(0.62,1.65) | NS |
| Phenylacetylglutamine | 1.03(0.87,1.33) | 1.13(0.44,1.73) | 1.02(0.83,1.71) | 1.33(0.94,1.65) | NS |
| Methionine | 1.07(0.96,1.35) | 1.10(0.96,1.15) | 1.08(0.96,1.22) | 1.09(1.02,1.19) | NS |
| 5-oxoproline | 1.01(0.89,1.10) | 1.06(0.98,1.13) | 1.00(0.76,1.12) | 1.04(0.96,1.15) | NS |
| Palmitic acid | 1.09(0.72,1.55) | 1.01(0.88,1.31) | 1.27(1.00,1.55) | 0.97(0.80,1.29) | NS |
| Phenylalanine | 0.99(0.82,1.13) | 0.94(0.86,1.12) | 1.07(0.89,1.19) | 1.07(0.98,1.15) | NS |

Data are presented as median (interquartile range), and the data are log transformed before analysis; Significance was determined by GEE model. *p* value was statistical significance between CS effect and RS effect.

**Table S3.** Glucose metabolism before and after 4 weeks' RS or CS supplementation in normal-weight subjects.

| Variable | CS | | RS | | *p* value |
|---|---|---|---|---|---|
| | 0 week | 4 weeks | 0 week | 4 weeks | |
| Blood glucose 0 min (mmol/l) | 4.86±0.09 | 4.84±0.10 | 4.78±0.07 | 4.85±0.10 | NS |
| Blood glucose 30 min (mmol/l) | 7.58±0.19 | 7.69±0.23 | 7.49±0.21 | 7.80±0.22 | NS |
| Blood glucose 60 min (mmol/l) | 6.88±0.33 | 6.57±0.28 | 7.04±0.34 | 6.79±0.32 | NS |
| Blood glucose 120 min (mmol/l) | 5.84±0.25 | 5.54±0.16 | 5.64±0.21 | 6.03±0.28 | NS |
| Insulin 0min (μU/ml) | 8.33±0.95 | 8.13±1.03 | 8.43±1.10 | 8.31±0.76 | NS |
| Insulin 30 min (μU/ml) | 56.11±6.17 | 56.98±7.57 | 55.29±6.82 | 69.7±6.90*$^{\#}$ | 0.050 |
| Insulin 60 min (μU/ml) | 49.21±4.46 | 48.27±6.87 | 47.21±4.57 | 59.50±6.86 | NS |
| Insulin 120 min (μU/ml) | 31.70±2.49 | 28.98±2.62 | 30.16±2.57 | 38.48±4.54 | NS |
| C peptide 0min (ng/ml) | 1.67±0.11 | 1.79±0.15 | 1.69±0.12 | 1.78±0.09 | NS |
| C peptide 30min (ng/ml) | 5.56±0.44 | 5.72±0.48 | 5.50±0.47 | 6.59±0.45***$^{\#\#}$ | 0.008 |
| C peptide 60min (ng/ml) | 6.59±0.42 | 6.56±0.50 | 6.50±0.42 | 7.01±0.38 | NS |
| C peptide 120min (ng/ml) | 5.74±0.40 | 5.56±0.49 | 5.64±0.41 | 6.44±0.49 | NS |
| GIR (mg*kg-1*min-1) | 7.97±0.58 | 8.42±0.72 | 8.27±0.51 | 8.09±0.51 | NS |
| HOMA-IR | 1.75±0.20 | 1.69±0.20 | 1.81±0.24 | 1.81±0.18 | NS |
| HOMAβ (%) | 119.18±8.32 | 117.69±9.34 | 132.67±17.71 | 127.00±11.40 | NS |

Data are presented as mean ± SEM. Significance was determined by GEE model. p value was statistical significance between CS effect and RS effect; * $p < 0.05$, *** $p < 0.001$, RS 0 week vs. RS 4 weeks, $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, RS 4 weeks vs. CS 4 weeks..

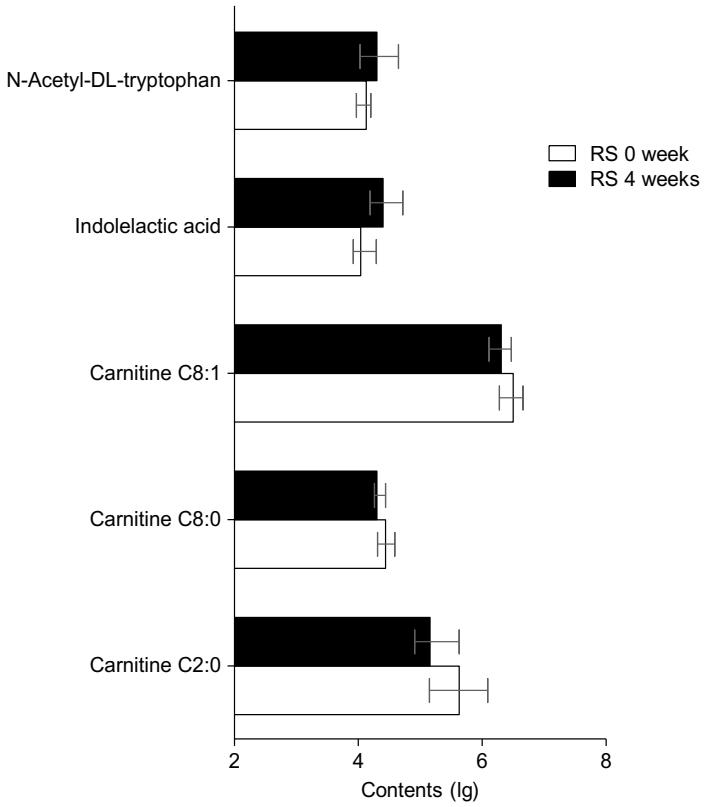


**Fig. S1.** Urine metabolomics before and after 4-week RS supplementation in normal-weight subjects. Urine metabolomics before and after 4-week RS supplementation were analyzed. Only the urine metabolomics whose content were significantly changed before and after 4-week RS supplementation in individuals with normal weight were displayed (n=19). Data represent median (interquartile range), and the data are log transformed before analysis; Significance was determined by GEE model. All were $p < 0.05$.

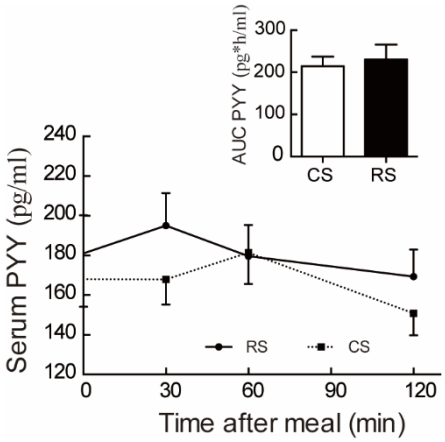


**Fig. S2.** Serum PYY levels and AUC after a standardized meal in participates after 4-week RS or CS supplementation. Data are presented as mean ± SEM.

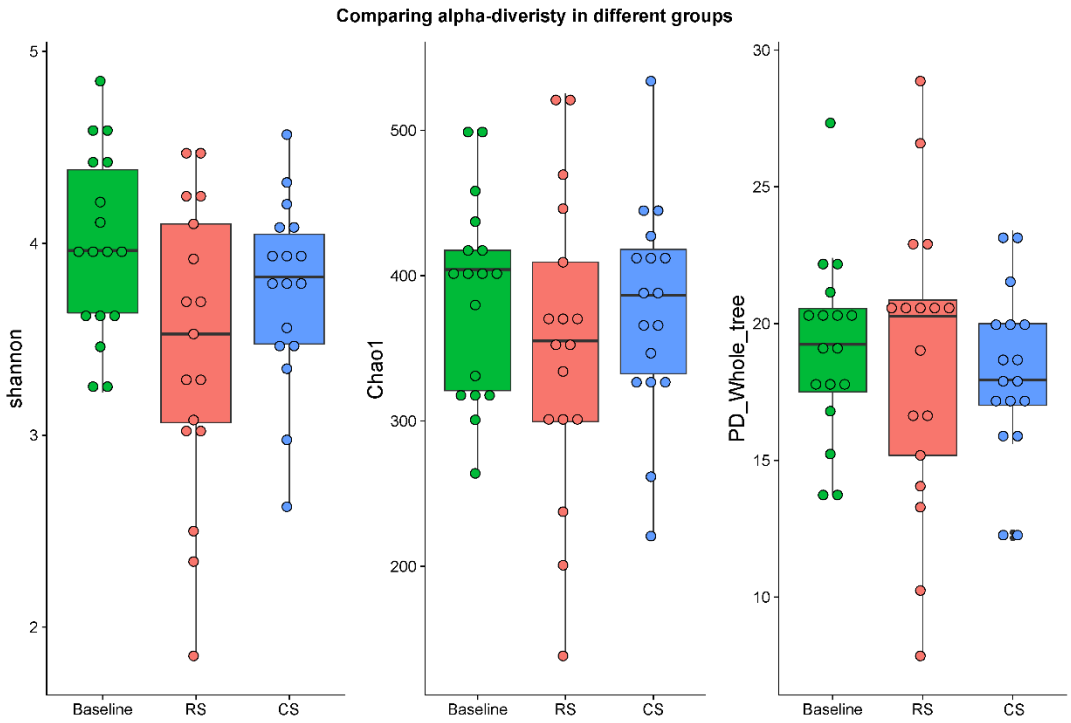


**Fig. S3.** Alpha diversity at baseline, after 4-week RS supplementation and after 4-week CS supplementation were analyzed (n=17). Data represent median (interquartile range); Significance was determined by Mann-Whitney test between groups.

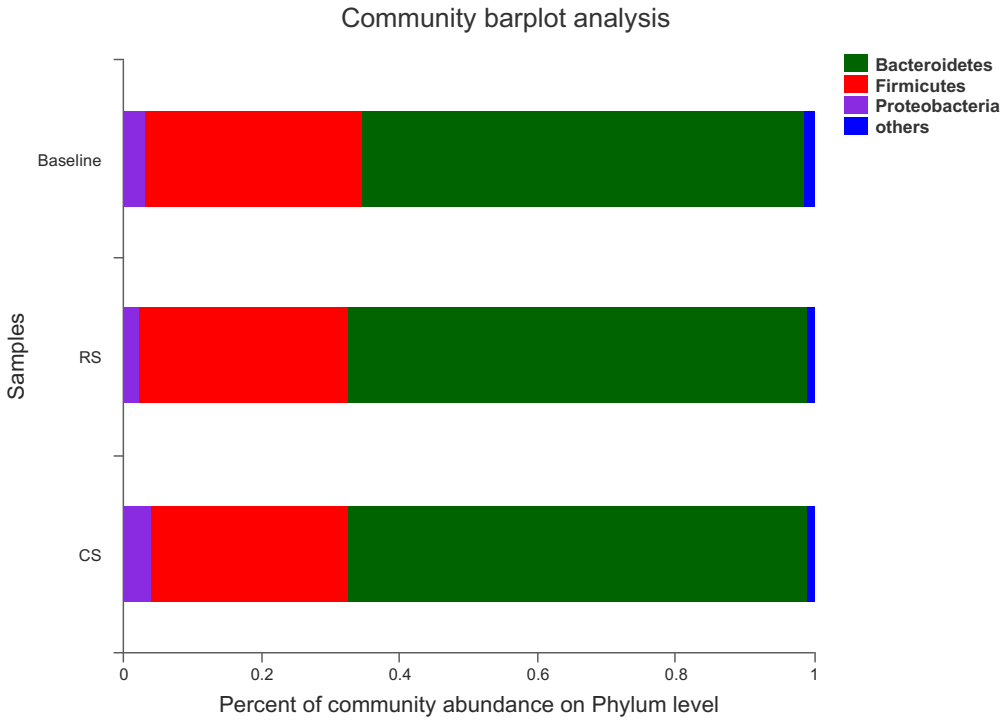


**Fig. S4.** Percent of community abundance on phylum level at baseline, after 4-week RS supplementation and after 4-week CS supplementation.

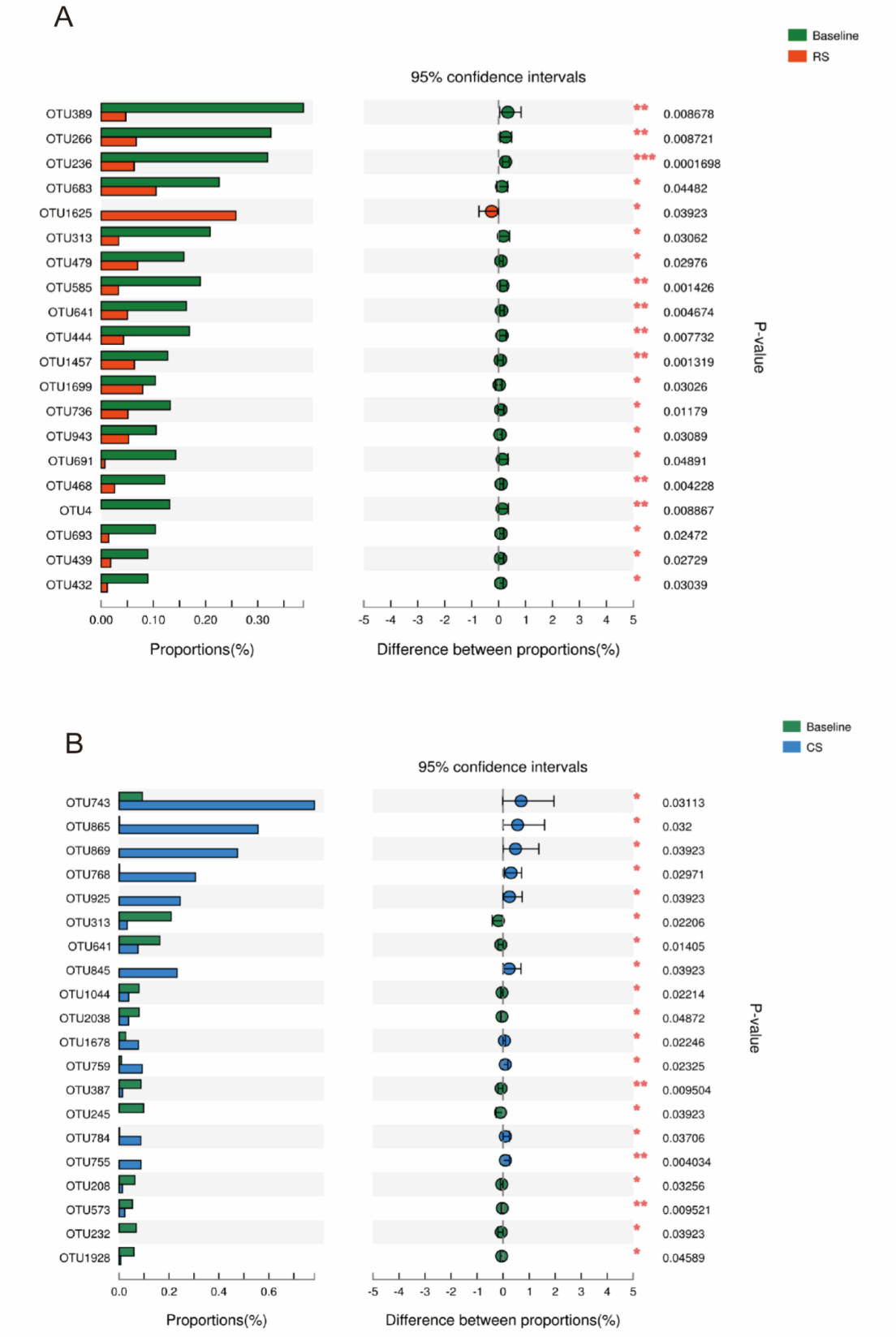


**Fig. S5.** The change of gut microbial composition after RS supplementation at the OTU level. The differences of bacterial abundances at the OTU-level before and after 4-week RS (A) or CS (B) intake in normal-weight individuals were analyzed, only the top 20 OTU whose abundances were significantly changed (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Wilcoxon signed-rank test) were displayed (n=17). Mean abundances of all samples at baseline or after supplementation were used.

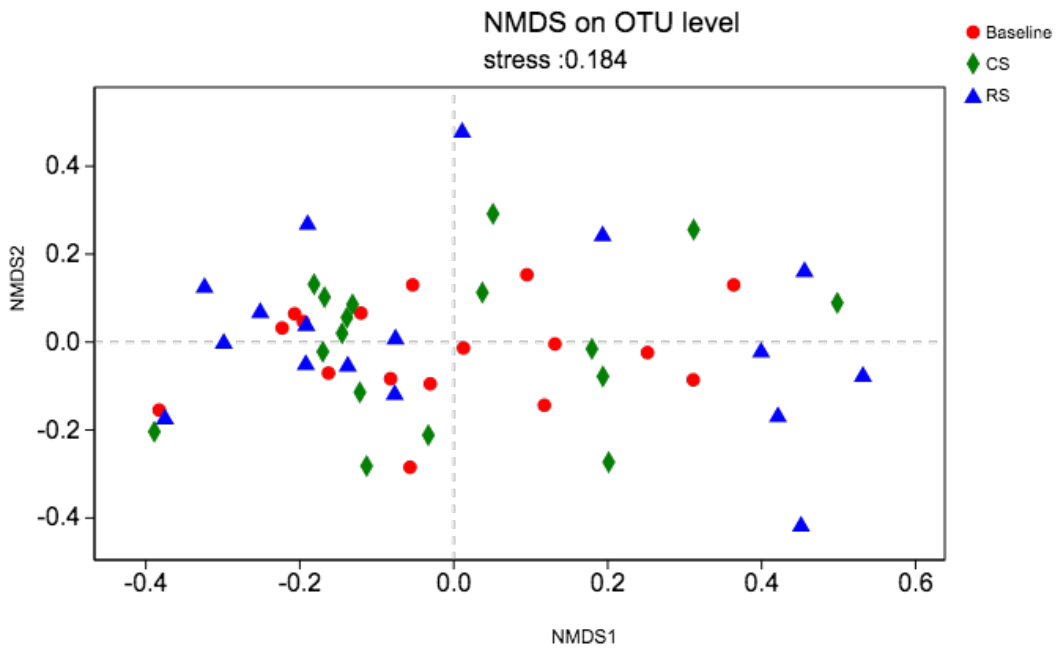


**Fig. S6.** Non-metric multidimensional scaling (NMDS) ordination plot based on OTU-level microbial beta diversity (UniFrac distances).