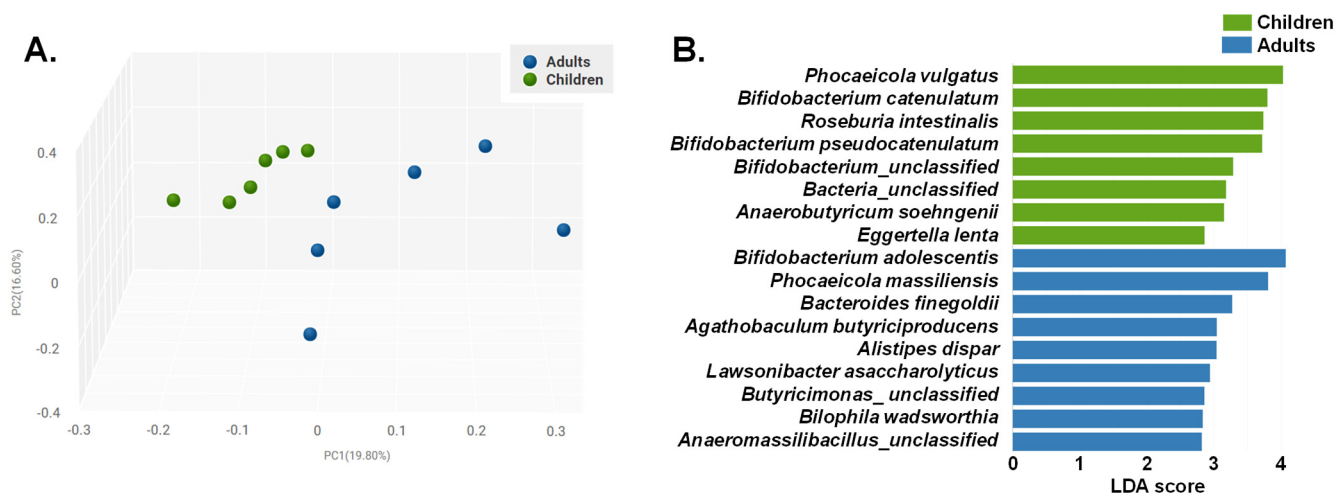
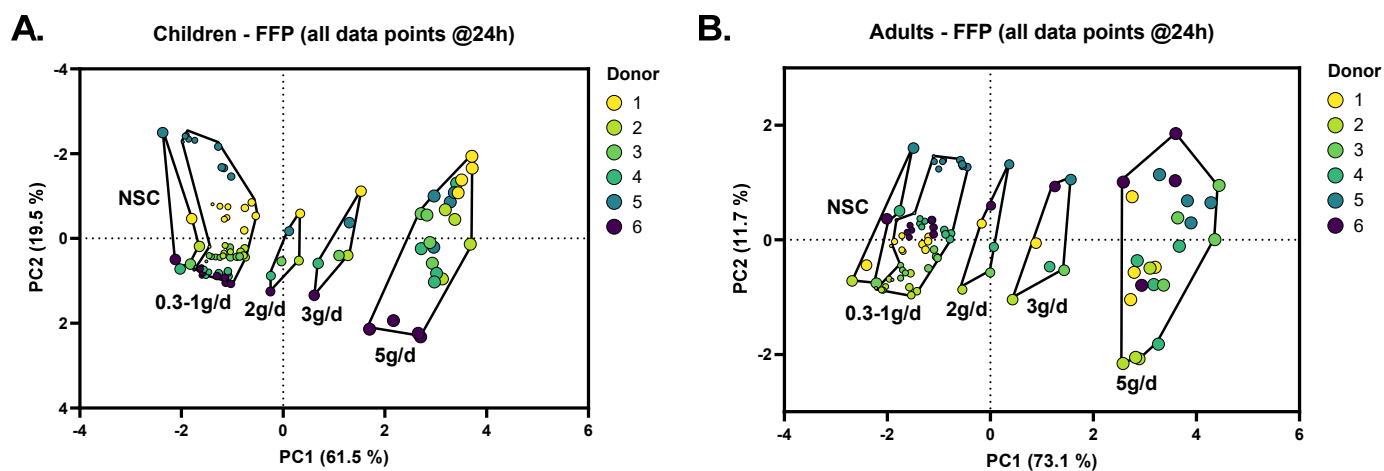


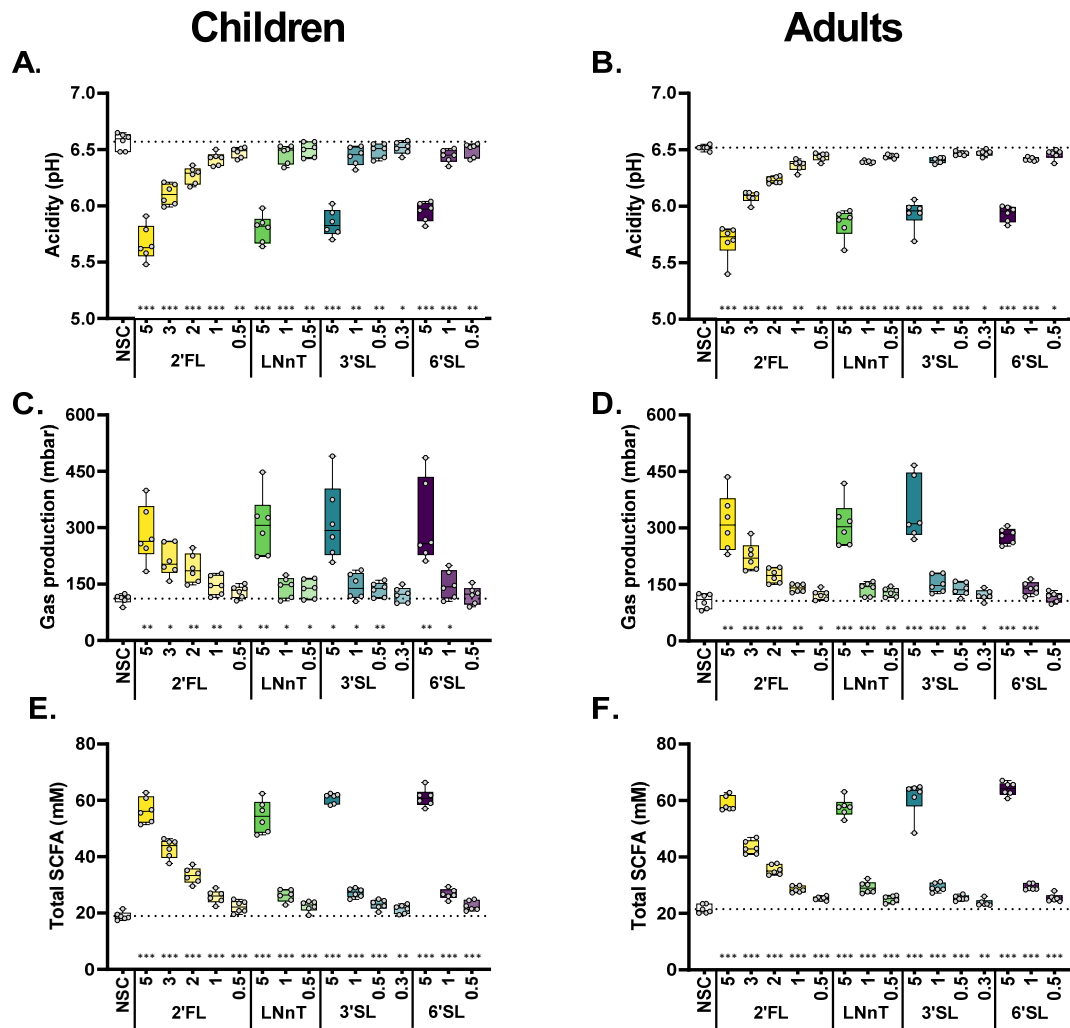
**Figure S1. Marked metabolite production occurred between 0-24h, while the QC samples tightly co-localized.** Principal component analysis (PCA) of level 1-annotated metabolites (LC-MS) for children (A) and adults (B) (n = 6), both at 0h and 24h of treatment with four HMOs (2'FL, LNnT, 3'SL, 6'SL) at doses ranging from equivalent of 0.3 to 5 g/day. QC = quality control sample (= pooled sample of all samples); LC-MS = Liquid chromatography coupled with mass spectrometry.



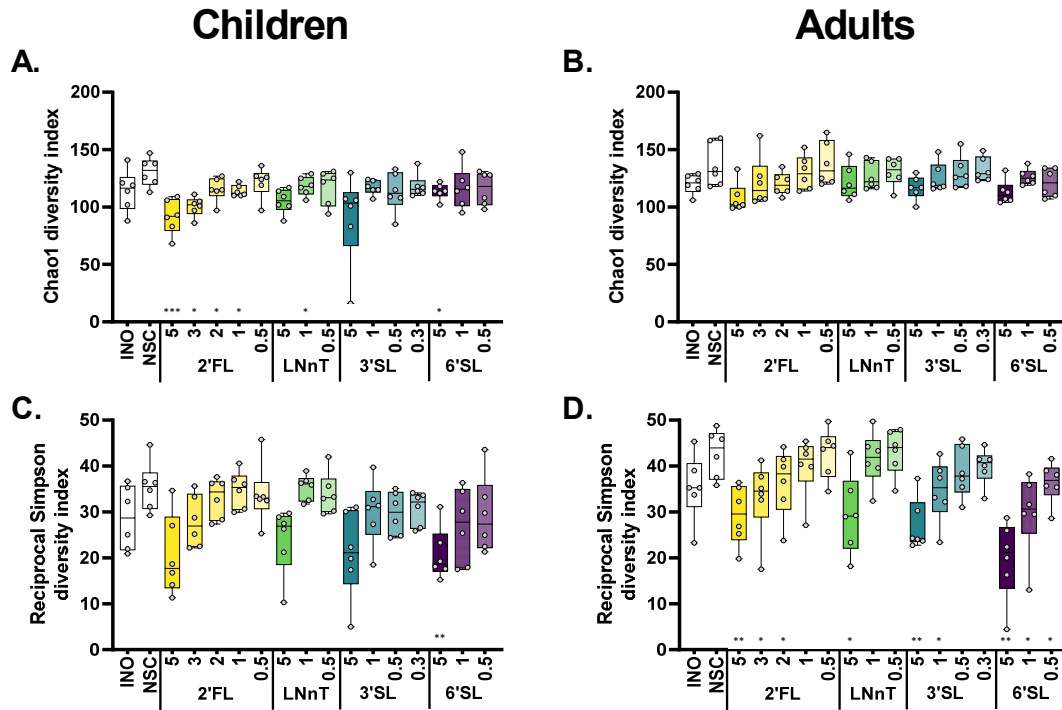
**Figure S2. Fecal microbiota composition of children (6 years old) and adults was fundamentally different.** (A) Principal coordinates analysis (PCoA) based on Bray-Curtis distance for microbial species (%), as quantified via shallow shotgun sequencing (p = 0.024). (B) Linear discriminant analysis effect size (LEfSe) at species level shows the taxa most likely to explain differences between children and adults (LDA threshold = 2).



**Figure S3. HMOs exerted dose-dependent effects on key fermentation parameters from the lowest test dose onwards.** Principal component analysis (PCA) summarizing the levels of fundamental fermentation parameters (pH, SCFA, bCFA and gas production), as averaged across 6 children (A) or human adults (B) at different time points (0, 6, 24 and 48h) in the no substrate control (NSC) and upon treatment with four HMOs (2'FL, LNnT, 3'SL, 6'SL) at doses ranging from equivalent of 0.3 to 5 g/day. **Figure S3 is identical to Figure 2, yet with visualization of the test subjects rather than the treatments.**

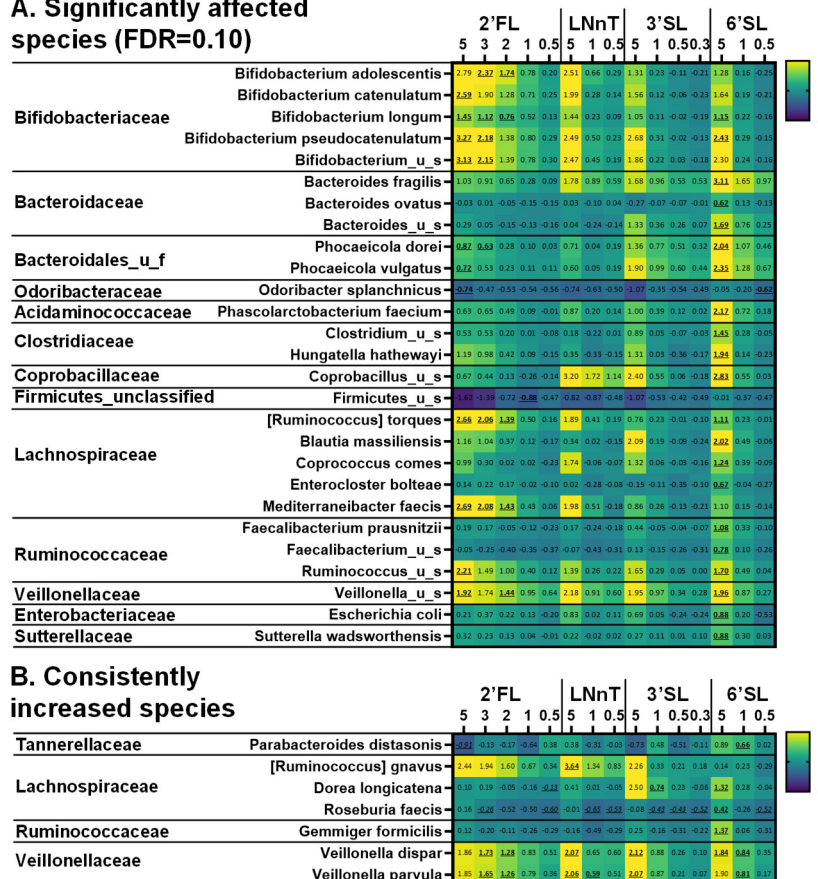


**Figure S4. When administered to the children's and adult microbiota, the four HMOs decreased pH, while increasing gas production and total SCFA levels (significant with only few exceptions). Impact of four HMOs (2'FL, LNnT, 3'SL, 6'SL) at doses ranging from equivalent of 0.3 to 5 g/day on acetate (A, B), propionate (C, D), butyrate (E, F), and bCFA (G, H) levels for simulated gut microbiota of children (A, C, E, G; n = 6) or adults (B, D, F, H; n = 6), at 24h upon initiation of treatment, compared to a no substrate control (NSC), as tested with the *ex vivo* SIFR® technology. Statistical differences between treatments and NSC are indicated with asterisks [\* ( $p_{\text{adjusted}} < 0.05$ ), \*\* ( $p_{\text{adjusted}} < 0.01$ ) or \*\*\* ( $p_{\text{adjusted}} < 0.001$ )].**

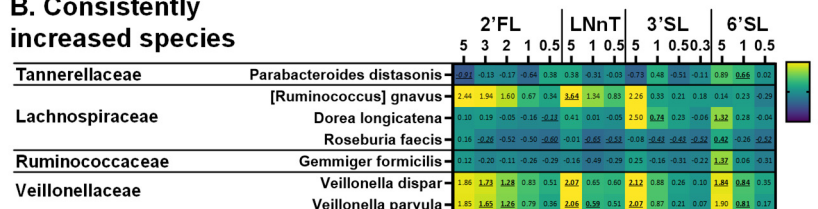


**Figure S5. When administered to the children's and adult microbiota, the four HMOs generally maintained a high microbial diversity.** Impact of four HMOs (2'FL, LNnT, 3'SL, 6'SL) at doses ranging from equivalent of 0.3 to 5 g/day on the Chao1 diversity index (A, B) and the reciprocal Simpson diversity index (C, D) for simulated gut microbiota of children (n = 6) or adults (n = 6), at 24h upon initiation of treatment, compared to a no substrate control (NSC), as tested with the *ex vivo* SIFR® technology. Statistical differences between treatments and NSC are indicated with asterisks [\* (p<sub>adjusted</sub> < 0.05), \*\* (p<sub>adjusted</sub> < 0.01) or \*\*\* (p<sub>adjusted</sub> < 0.001)].

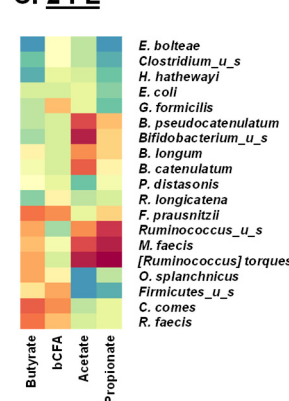
## A. Significantly affected species (FDR=0.10)



## B. Consistently increased species



## C. 2'FL



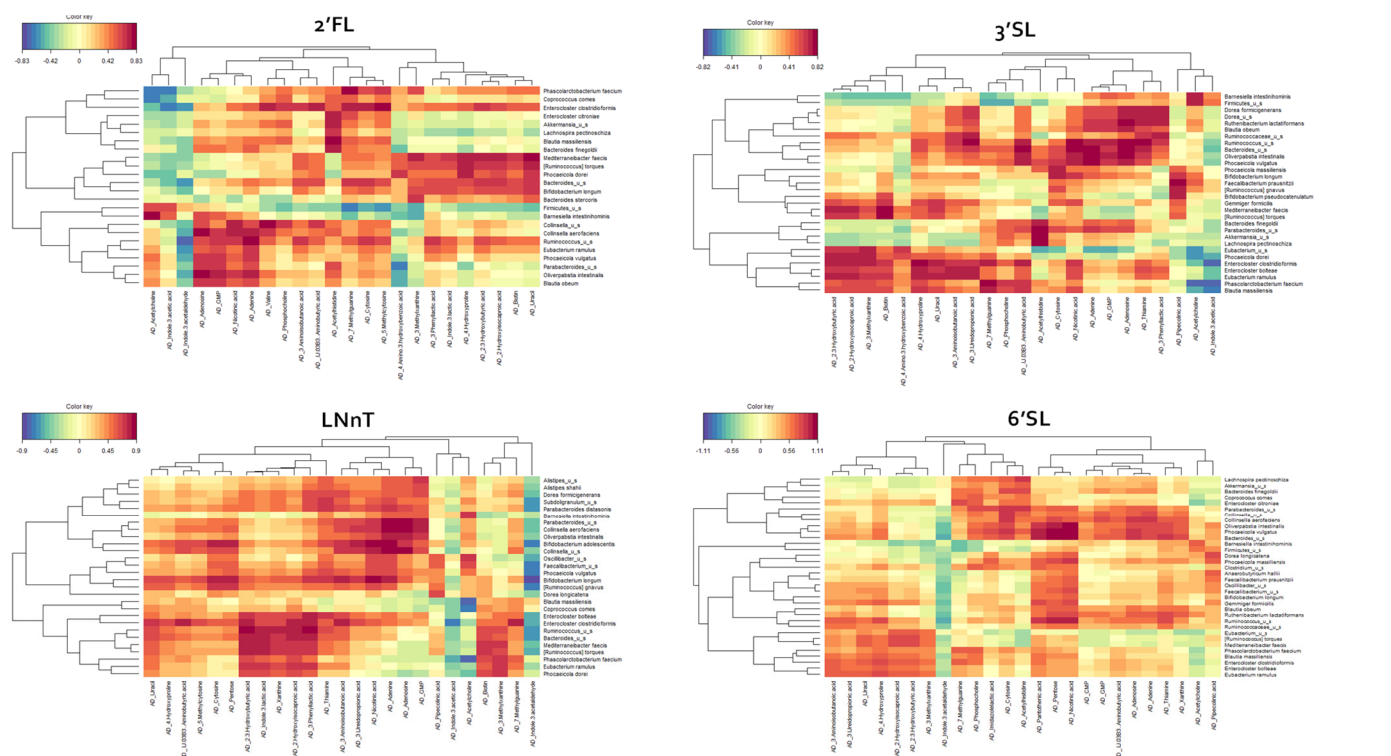
## A. Significantly affected species (FDR = 0.10)

		2'FL					LNnT					3'SL					6'SL				
		5	3	2	1	0.5	5	1	0.5	1	0.5	5	1	0.5	3	1	0.5	1	0.5		
Bifidobacteriaceae	<i>Bifidobacterium adolescentis</i>	<b>2.17</b>	<b>1.64</b>	<b>1.29</b>	<b>0.60</b>	<b>0.25</b>	<b>2.53</b>	<b>0.39</b>	<b>0.51</b>	<b>1.67</b>	<b>0.06</b>	<b>0.00</b>	<b>-0.16</b>	<b>0.42</b>	<b>-0.09</b>	<b>-0.34</b>					
	<i>Bifidobacterium longum</i>	<b>1.69</b>	<b>1.34</b>	<b>1.14</b>	<b>0.58</b>	<b>0.25</b>	<b>2.17</b>	<b>0.53</b>	<b>0.37</b>	<b>1.08</b>	<b>0.25</b>	<b>0.17</b>	<b>-0.13</b>	<b>0.80</b>	<b>-0.01</b>	<b>0.05</b>					
Coriobacteriaceae	<i>Collinsella aerofaciens</i>	<b>2.82</b>	<b>1.97</b>	<b>1.73</b>	<b>1.36</b>	<b>0.29</b>	<b>2.40</b>	<b>0.43</b>	<b>0.44</b>	<b>3.31</b>	<b>0.93</b>	<b>0.54</b>	<b>0.25</b>	<b>1.96</b>	<b>0.56</b>	<b>0.13</b>					
	<i>Collinsella_u_s</i>	<b>3.32</b>	<b>2.06</b>	<b>1.90</b>	<b>1.53</b>	<b>0.39</b>	<b>2.58</b>	<b>0.64</b>	<b>0.56</b>	<b>4.00</b>	<b>1.35</b>	<b>0.77</b>	<b>0.40</b>	<b>1.97</b>	<b>0.82</b>	<b>0.27</b>					
Bacteroidaceae	<i>Bacteroides stercoris</i>	<b>0.62</b>	<b>-0.13</b>	<b>-0.25</b>	<b>-0.51</b>	<b>-0.35</b>	<b>0.47</b>	<b>0.42</b>	<b>-0.30</b>	<b>0.29</b>	<b>-0.31</b>	<b>-0.41</b>	<b>-0.64</b>	<b>0.36</b>	<b>-0.27</b>	<b>-0.65</b>					
	<i>Bacteroides uniformis</i>	<b>0.53</b>	<b>-0.13</b>	<b>-0.24</b>	<b>-0.29</b>	<b>-0.31</b>	<b>0.01</b>	<b>0.50</b>	<b>-0.37</b>	<b>-0.04</b>	<b>-0.37</b>	<b>-0.35</b>	<b>-0.39</b>	<b>0.22</b>	<b>-0.34</b>	<b>-0.40</b>					
	<i>Bacteroides_u_s</i>	<b>0.61</b>	<b>0.45</b>	<b>0.15</b>	<b>-0.34</b>	<b>-0.18</b>	<b>0.67</b>	<b>-0.21</b>	<b>-0.34</b>	<b>1.61</b>	<b>0.52</b>	<b>0.43</b>	<b>-0.03</b>	<b>2.37</b>	<b>0.81</b>	<b>0.31</b>					
Bacteroidales_u_f	<i>Phocaeicola dorei</i>	<b>0.68</b>	<b>0.39</b>	<b>0.25</b>	<b>-0.14</b>	<b>-0.12</b>	<b>0.69</b>	<b>0.00</b>	<b>0.04</b>	<b>0.86</b>	<b>0.47</b>	<b>0.19</b>	<b>0.04</b>	<b>1.80</b>	<b>0.69</b>	<b>0.22</b>					
	<i>Phocaeicola massiliensis</i>	<b>1.27</b>	<b>0.79</b>	<b>0.56</b>	<b>0.12</b>	<b>0.00</b>	<b>1.39</b>	<b>0.57</b>	<b>0.41</b>	<b>1.85</b>	<b>0.98</b>	<b>0.88</b>	<b>0.44</b>	<b>2.60</b>	<b>1.39</b>	<b>0.82</b>					
	<i>Phocaeicola vulgatus</i>	<b>0.85</b>	<b>0.40</b>	<b>0.25</b>	<b>-0.08</b>	<b>-0.15</b>	<b>0.80</b>	<b>0.30</b>	<b>0.04</b>	<b>1.27</b>	<b>0.87</b>	<b>0.85</b>	<b>0.37</b>	<b>2.41</b>	<b>1.16</b>	<b>0.56</b>					
Rikenellaceae	<i>Alistipes shahii</i>	<b>0.25</b>	<b>0.08</b>	<b>0.01</b>	<b>-0.24</b>	<b>-0.36</b>	<b>0.63</b>	<b>-0.39</b>	<b>-0.10</b>	<b>0.32</b>	<b>-0.36</b>	<b>-0.35</b>	<b>-0.46</b>	<b>0.07</b>	<b>-0.29</b>	<b>-0.38</b>					
	<i>Alistipes_u_s</i>	<b>0.38</b>	<b>0.23</b>	<b>0.12</b>	<b>-0.20</b>	<b>-0.38</b>	<b>0.48</b>	<b>-0.43</b>	<b>-0.17</b>	<b>0.28</b>	<b>-0.40</b>	<b>-0.39</b>	<b>-0.43</b>	<b>0.03</b>	<b>-0.33</b>	<b>-0.30</b>					
Tannerellaceae	<i>Parabacteroides distasonis</i>	<b>-0.09</b>	<b>-0.12</b>	<b>-0.34</b>	<b>-0.18</b>	<b>-0.28</b>	<b>0.78</b>	<b>0.03</b>	<b>-0.10</b>	<b>0.44</b>	<b>-0.12</b>	<b>-0.02</b>	<b>0.62</b>	<b>0.67</b>	<b>-0.03</b>	<b>-0.34</b>					
	<i>Parabacteroides_u_s</i>	<b>0.30</b>	<b>0.19</b>	<b>0.07</b>	<b>0.00</b>	<b>-0.11</b>	<b>0.84</b>	<b>0.03</b>	<b>0.13</b>	<b>0.82</b>	<b>0.33</b>	<b>0.06</b>	<b>-0.06</b>	<b>1.09</b>	<b>0.22</b>	<b>-0.07</b>					
Clostridiaceae	<i>Clostridium_u_s</i>	<b>0.20</b>	<b>0.04</b>	<b>0.02</b>	<b>-0.21</b>	<b>-0.27</b>	<b>0.10</b>	<b>-0.36</b>	<b>-0.07</b>	<b>0.31</b>	<b>-0.15</b>	<b>-0.08</b>	<b>-0.21</b>	<b>0.53</b>	<b>-0.05</b>	<b>-0.22</b>					
Eubacteriaceae	<i>Eubacterium ramulus</i>	<b>2.46</b>	<b>1.85</b>	<b>1.20</b>	<b>0.27</b>	<b>0.12</b>	<b>1.20</b>	<b>0.37</b>	<b>0.34</b>	<b>3.39</b>	<b>1.31</b>	<b>0.48</b>	<b>0.21</b>	<b>2.22</b>	<b>1.43</b>	<b>0.41</b>					
	<i>Eubacterium_u_s</i>	<b>0.27</b>	<b>-0.64</b>	<b>-0.55</b>	<b>-0.72</b>	<b>-0.58</b>	<b>0.38</b>	<b>-0.65</b>	<b>-0.45</b>	<b>-0.30</b>	<b>-0.60</b>	<b>-0.60</b>	<b>-0.56</b>	<b>0.00</b>	<b>-0.59</b>	<b>-0.80</b>					
Firmicutes_u_f	<i>Firmicutes_u_s</i>	<b>0.98</b>	<b>-0.64</b>	<b>-0.66</b>	<b>-0.62</b>	<b>-0.45</b>	<b>1.15</b>	<b>0.50</b>	<b>-0.36</b>	<b>-0.28</b>	<b>-0.31</b>	<b>-0.31</b>	<b>-0.37</b>	<b>0.00</b>	<b>0.24</b>	<b>0.34</b>					
Lachnospiraceae	[ <i>Ruminococcus</i> ] <i>torques</i>	<b>3.40</b>	<b>2.53</b>	<b>2.06</b>	<b>0.80</b>	<b>0.39</b>	<b>2.75</b>	<b>0.74</b>	<b>0.39</b>	<b>1.61</b>	<b>0.31</b>	<b>0.11</b>	<b>-0.10</b>	<b>1.47</b>	<b>0.26</b>	<b>0.01</b>					
	<i>Anaerobutyricum hallii</i>	<b>2.17</b>	<b>1.37</b>	<b>0.80</b>	<b>0.05</b>	<b>-0.01</b>	<b>2.23</b>	<b>0.34</b>	<b>0.01</b>	<b>1.53</b>	<b>0.20</b>	<b>-0.08</b>	<b>-0.16</b>	<b>1.31</b>	<b>-0.01</b>	<b>-0.53</b>					
	<i>Blautia massiliensis</i>	<b>2.18</b>	<b>1.78</b>	<b>1.40</b>	<b>0.78</b>	<b>0.20</b>	<b>-0.05</b>	<b>-0.13</b>	<b>0.06</b>	<b>1.76</b>	<b>0.38</b>	<b>0.12</b>	<b>-0.10</b>	<b>1.93</b>	<b>0.34</b>	<b>-0.05</b>					
	<i>Blautia obeum</i>	<b>1.56</b>	<b>0.87</b>	<b>0.46</b>	<b>-0.22</b>	<b>-0.43</b>	<b>-0.52</b>	<b>-0.57</b>	<b>-0.51</b>	<b>2.20</b>	<b>0.22</b>	<b>-0.09</b>	<b>-0.06</b>	<b>2.45</b>	<b>0.42</b>	<b>0.21</b>					
	<i>Coprococcus catus</i>	<b>-0.20</b>	<b>-0.46</b>	<b>-0.52</b>	<b>-0.36</b>	<b>-0.11</b>	<b>0.11</b>	<b>0.22</b>	<b>-0.27</b>	<b>-0.11</b>	<b>0.00</b>	<b>-0.07</b>	<b>0.41</b>	<b>-0.18</b>	<b>-0.36</b>						
	<i>Dorea longicatena</i>	<b>0.82</b>	<b>0.27</b>	<b>0.09</b>	<b>-0.21</b>	<b>-0.22</b>	<b>0.64</b>	<b>-0.27</b>	<b>-0.11</b>	<b>1.83</b>	<b>0.35</b>	<b>0.00</b>	<b>-0.15</b>	<b>0.73</b>	<b>-0.08</b>	<b>-0.29</b>					
	<i>Enterocloster aldensis</i>	<b>0.07</b>	<b>0.45</b>	<b>0.40</b>	<b>-0.25</b>	<b>-0.18</b>	<b>0.59</b>	<b>0.13</b>	<b>0.04</b>	<b>1.25</b>	<b>0.30</b>	<b>-0.13</b>	<b>-0.17</b>	<b>0.40</b>	<b>-0.14</b>	<b>-0.38</b>					
	<i>Enterocloster boltea</i>	<b>0.83</b>	<b>0.36</b>	<b>0.15</b>	<b>-0.22</b>	<b>-0.15</b>	<b>0.86</b>	<b>-0.30</b>	<b>0.03</b>	<b>1.05</b>	<b>-0.05</b>	<b>-0.05</b>	<b>-0.17</b>	<b>0.88</b>	<b>-0.06</b>	<b>-0.36</b>					
	<i>Enterocloster citroniae</i>	<b>-0.22</b>	<b>-0.18</b>	<b>-0.15</b>	<b>-0.24</b>	<b>-0.31</b>	<b>0.02</b>	<b>-0.55</b>	<b>-0.16</b>	<b>0.20</b>	<b>-0.39</b>	<b>-0.23</b>	<b>-0.28</b>	<b>0.27</b>	<b>-0.32</b>	<b>-0.48</b>					
	<i>Enterocloster clostridioformis</i>	<b>1.81</b>	<b>0.40</b>	<b>0.28</b>	<b>0.05</b>	<b>0.00</b>	<b>1.82</b>	<b>-0.14</b>	<b>0.08</b>	<b>1.87</b>	<b>0.02</b>	<b>0.00</b>	<b>-0.05</b>	<b>0.83</b>	<b>0.00</b>	<b>-0.17</b>					
Ruminococcaceae	<i>Lachnospira pectinoschiza</i>	<b>0.50</b>	<b>-0.63</b>	<b>-0.48</b>	<b>-0.91</b>	<b>-0.41</b>	<b>0.40</b>	<b>-0.66</b>	<b>-0.42</b>	<b>-0.36</b>	<b>-0.52</b>	<b>-0.42</b>	<b>-0.36</b>	<b>-0.24</b>	<b>-0.42</b>	<b>-0.71</b>					
	<i>Lachnospiraceae_u_s</i>	<b>1.36</b>	<b>0.86</b>	<b>0.71</b>	<b>0.19</b>	<b>0.06</b>	<b>0.93</b>	<b>-0.06</b>	<b>0.03</b>	<b>0.14</b>	<b>-0.21</b>	<b>-0.15</b>	<b>-0.21</b>	<b>0.37</b>	<b>-0.09</b>	<b>-0.42</b>					
	<i>Oliverpabstia intestinalis</i>	<b>4.18</b>	<b>3.50</b>	<b>2.89</b>	<b>2.11</b>	<b>1.36</b>	<b>2.23</b>	<b>0.79</b>	<b>0.65</b>	<b>4.25</b>	<b>1.94</b>	<b>1.32</b>	<b>0.93</b>	<b>4.67</b>	<b>2.41</b>	<b>1.15</b>					
	<i>Faecalibacterium prausnitzii</i>	<b>0.80</b>	<b>0.07</b>	<b>-0.03</b>	<b>-0.31</b>	<b>-0.37</b>	<b>0.64</b>	<b>-0.23</b>	<b>-0.42</b>	<b>0.75</b>	<b>-0.08</b>	<b>-0.18</b>	<b>-0.40</b>	<b>0.98</b>	<b>-0.02</b>	<b>-0.31</b>					
	<i>Faecalibacterium_u_s</i>	<b>0.48</b>	<b>0.04</b>	<b>-0.07</b>	<b>-0.31</b>	<b>-0.41</b>	<b>0.78</b>	<b>0.34</b>	<b>-0.24</b>	<b>0.75</b>	<b>-0.04</b>	<b>-0.19</b>	<b>-0.38</b>	<b>0.78</b>	<b>0.01</b>	<b>-0.34</b>					
Ruminococcaceae	<i>Gemmiger formicilis</i>	<b>0.95</b>	<b>0.54</b>	<b>0.39</b>	<b>0.05</b>	<b>-0.12</b>	<b>0.93</b>	<b>0.14</b>	<b>-0.01</b>	<b>1.64</b>	<b>0.25</b>	<b>0.15</b>	<b>-0.11</b>	<b>2.04</b>	<b>0.45</b>	<b>0.03</b>					
	<i>Ruminococcus_u_s</i>	<b>2.27</b>	<b>2.53</b>	<b>1.92</b>	<b>0.98</b>	<b>0.39</b>	<b>2.27</b>	<b>0.26</b>	<b>0.11</b>	<b>2.12</b>	<b>0.42</b>	<b>0.13</b>	<b>-0.04</b>	<b>2.38</b>	<b>0.51</b>	<b>0.07</b>					
	<i>Subdoligranulum_u_s</i>	<b>0.97</b>	<b>0.42</b>	<b>0.26</b>	<b>-0.10</b>	<b>-0.28</b>	<b>1.27</b>	<b>0.12</b>	<b>-0.03</b>	<b>1.05</b>	<b>-0.05</b>	<b>-0.07</b>	<b>-0.19</b>	<b>0.84</b>	<b>0.06</b>	<b>-0.25</b>					
Akkermansiaceae	<i>Akkermansia_u_s</i>	<b>0.48</b>	<b>-0.01</b>	<b>-0.12</b>	<b>-0.29</b>	<b>-0.35</b>	<b>0.11</b>	<b>-0.42</b>	<b>-0.07</b>	<b>0.02</b>	<b>-0.14</b>	<b>-0.17</b>	<b>-0.22</b>	<b>0.64</b>	<b>0.42</b>	<b>-0.12</b>					

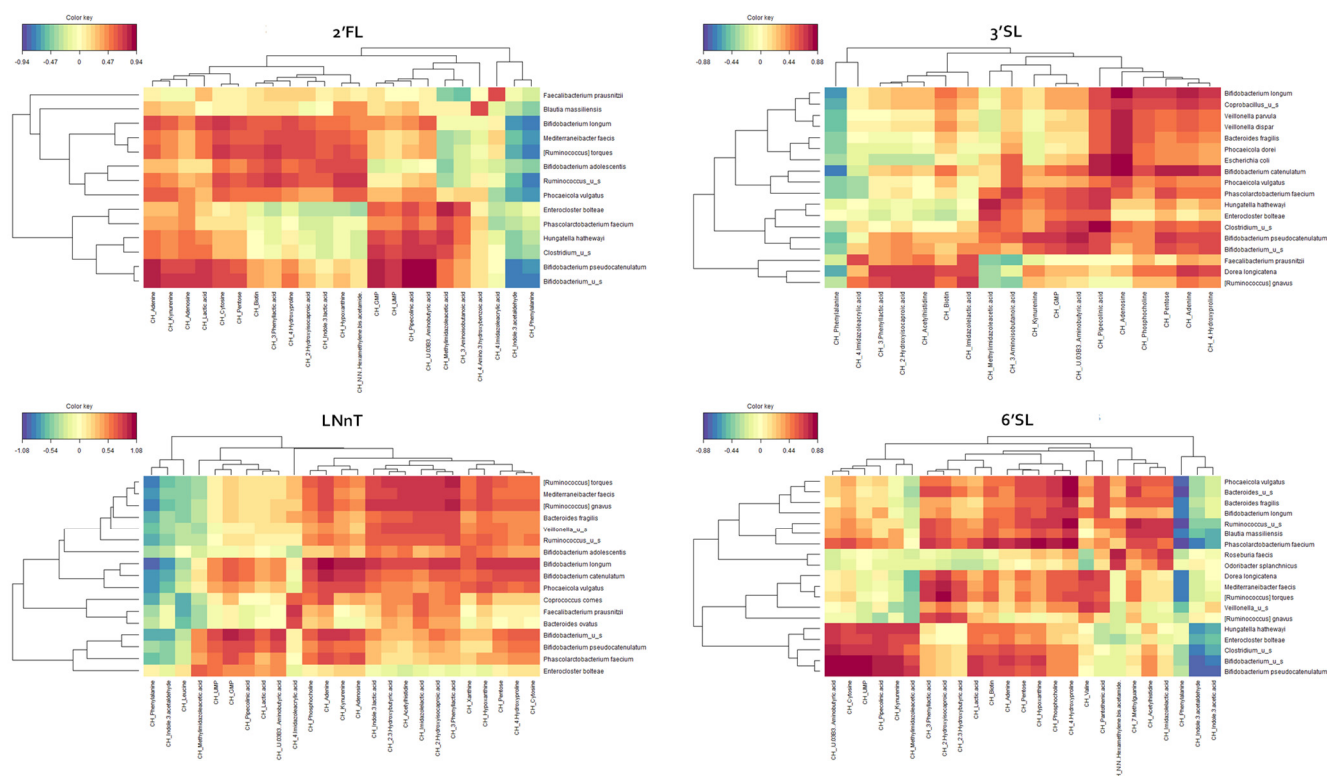
## B. Consistently increased species

increased species		2'FL					LNnT			3'SL			6'SL			
		5	3	2	1	0.5	5	1	0.5	5	1	0.5	3	1	0.5	
Bifidobacteriaceae	Bifidobacterium pseudocatenulatum	1.52	2.38	1.86	0.61	0.34	1.80	0.34	0.36	1.58	0.39	0.08	-0.17	0.94	0.26	0.08
Bacteroidaceae	Bacteroides finegoldii	0.35	0.08	0.00	-0.05	-0.13	0.34	-0.44	-0.14	0.29	-0.33	-0.19	-0.19	0.28	-0.29	-0.21
	Bacteroides fragilis	0.98	1.21	0.75	0.74	0.22	1.36	0.39	0.43	2.71	0.54	0.70	1.47	5.37	3.46	1.25
Barnesiellaceae	Barnesiella intestinihominis	0.48	0.12	0.01	-0.24	-0.22	0.46	-0.31	-0.10	0.22	-0.27	-0.36	-0.20	-0.38	-0.20	-0.24
Acidaminococcaceae	Phascolarctobacterium faecium	0.59	0.43	0.30	-0.04	-0.12	0.53	-0.14	-0.05	1.46	0.44	0.13	-0.04	2.12	0.65	0.04
Lachnospiraceae	[Ruminococcus] gnavus	0.02	0.19	0.71	0.66	0.73	4.80	1.44	1.54	0.54	0.48	0.05	0.05	0.27	0.30	
	Coprococcus comes	0.10	-0.01	-0.17	-0.44	-0.47	0.69	-0.47	-0.24	0.85	-0.17	-0.21	-0.32	0.37	-0.22	-0.25
	Dorea formicigenerans	0.78	0.34	0.08	-0.09	-0.08	0.64	-0.30	-0.03	0.80	0.17	0.04	0.05	0.67	0.16	-0.08
	Dorea_u_s	0.82	0.38	0.30	-0.09	-0.10	0.61	-0.09	0.00	0.83	0.12	0.03	0.04	0.69	0.15	-0.10
	Mediterraneibacter faecis	1.71	2.88	2.83	0.51	0.39	5.43	0.87	0.87	1.58	0.47	0.00	-0.22	1.17	0.19	-0.20
	Roseburia_u_s	0.19	0.25	0.64	1.15	0.84	0.27	1.05	0.46	0.66	0.74	0.90	0.84	0.34	-0.33	0.21
Oscillospiraceae	Oscillibacter_u_s	0.07	0.38	0.38	-0.23	-0.12	1.02	-0.38	-0.05	0.33	-0.27	-0.24	-0.18	0.20	0.05	0.08
Ruminococcaceae	Ruminococcaceae_u_s	0.78	0.21	0.56	-0.05	-0.43	0.75	-0.49	-0.02	0.72	-0.31	-0.09	-0.21	1.11	0.05	0.28
	Ruthenibacterium lactatiformans	0.04	-0.06	-0.40	0.86	0.65	1.63	0.60	0.40	1.34	1.42	1.53	1.18	0.95	2.20	0.41





**Figure S8.** Regularized Canonical Correlation Analysis (rCCA) to highlight correlations between significantly affected metabolites and significantly/consistently affected species, for each of the four HMOs upon administration to the microbiota of children.



**Figure S9.** Regularized Canonical Correlation Analysis (rCCA) to highlight correlations between significantly affected metabolites and significantly/consistently affected species, for each of the four HMOs upon administration to the adult microbiota.