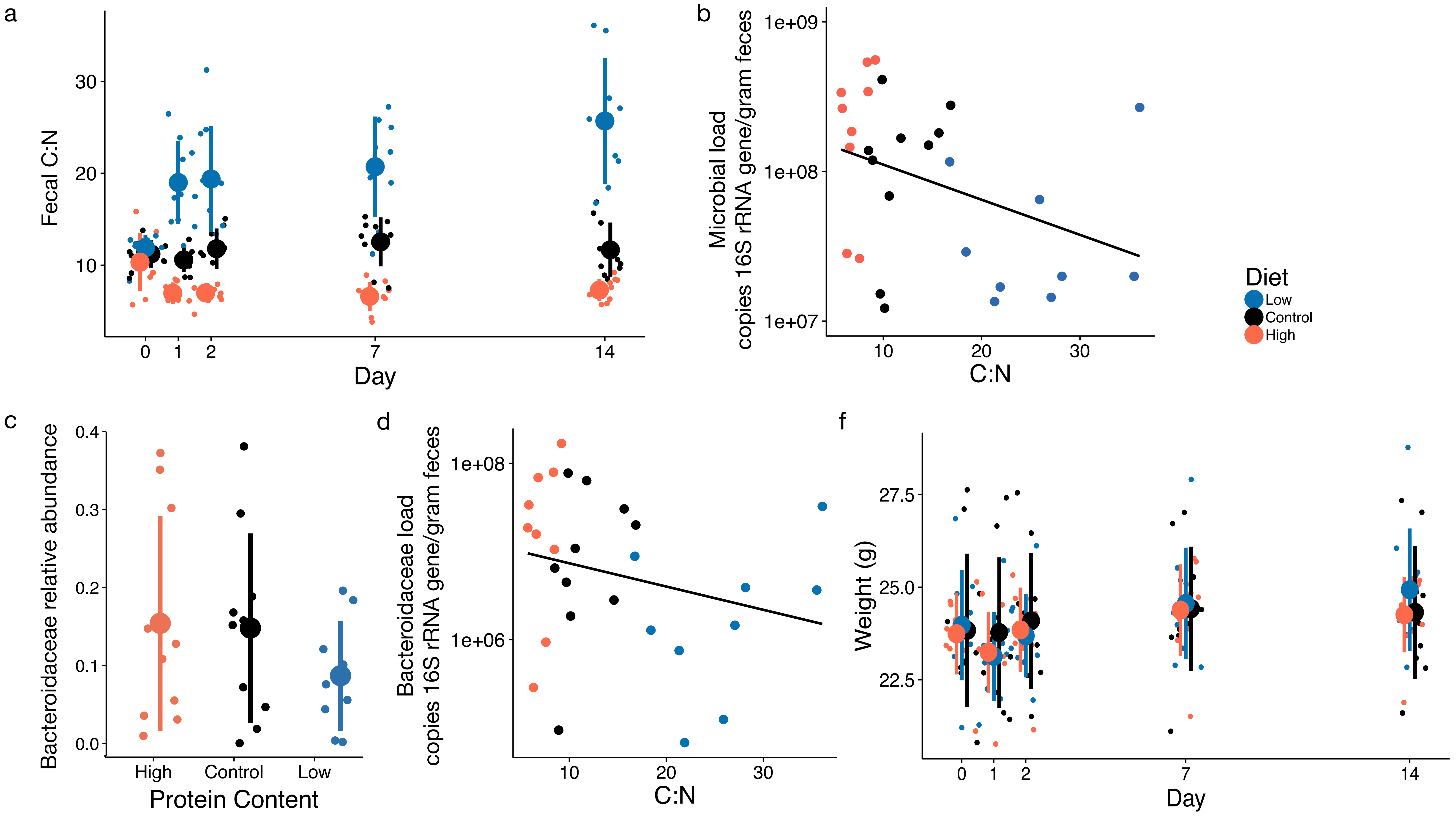
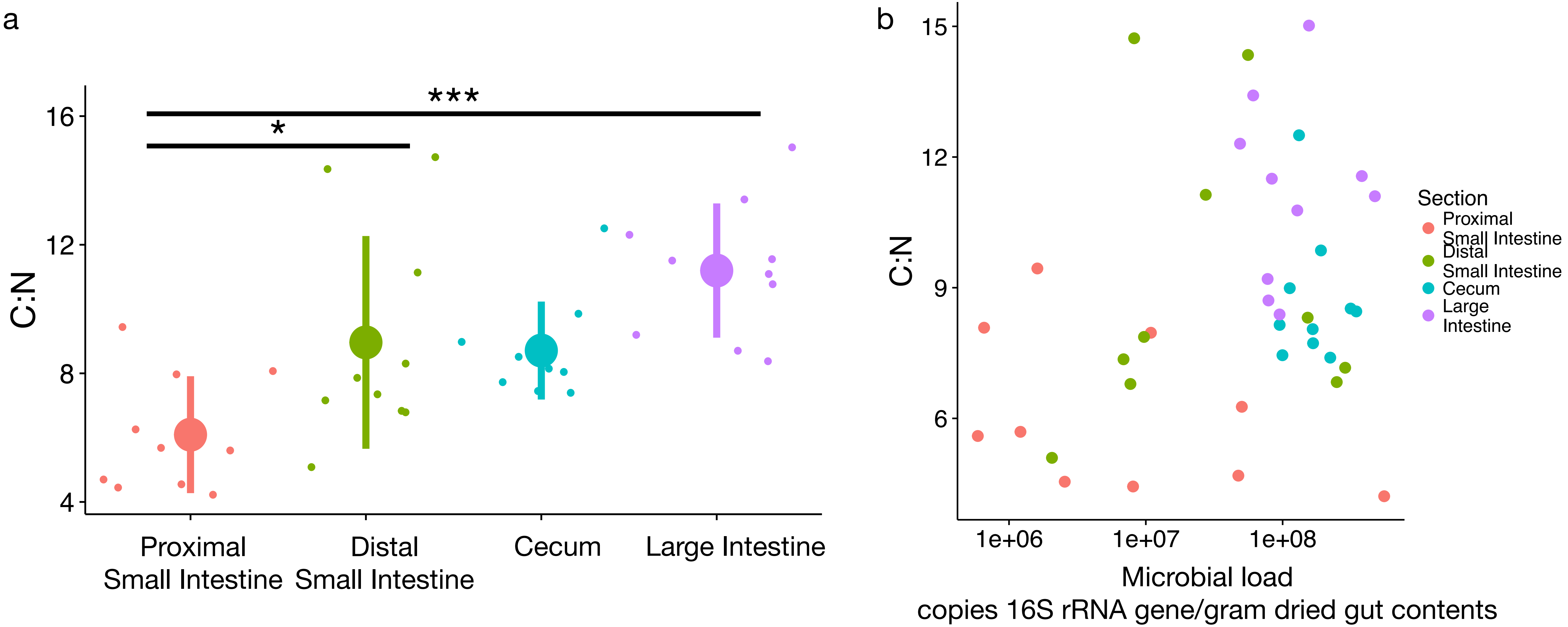
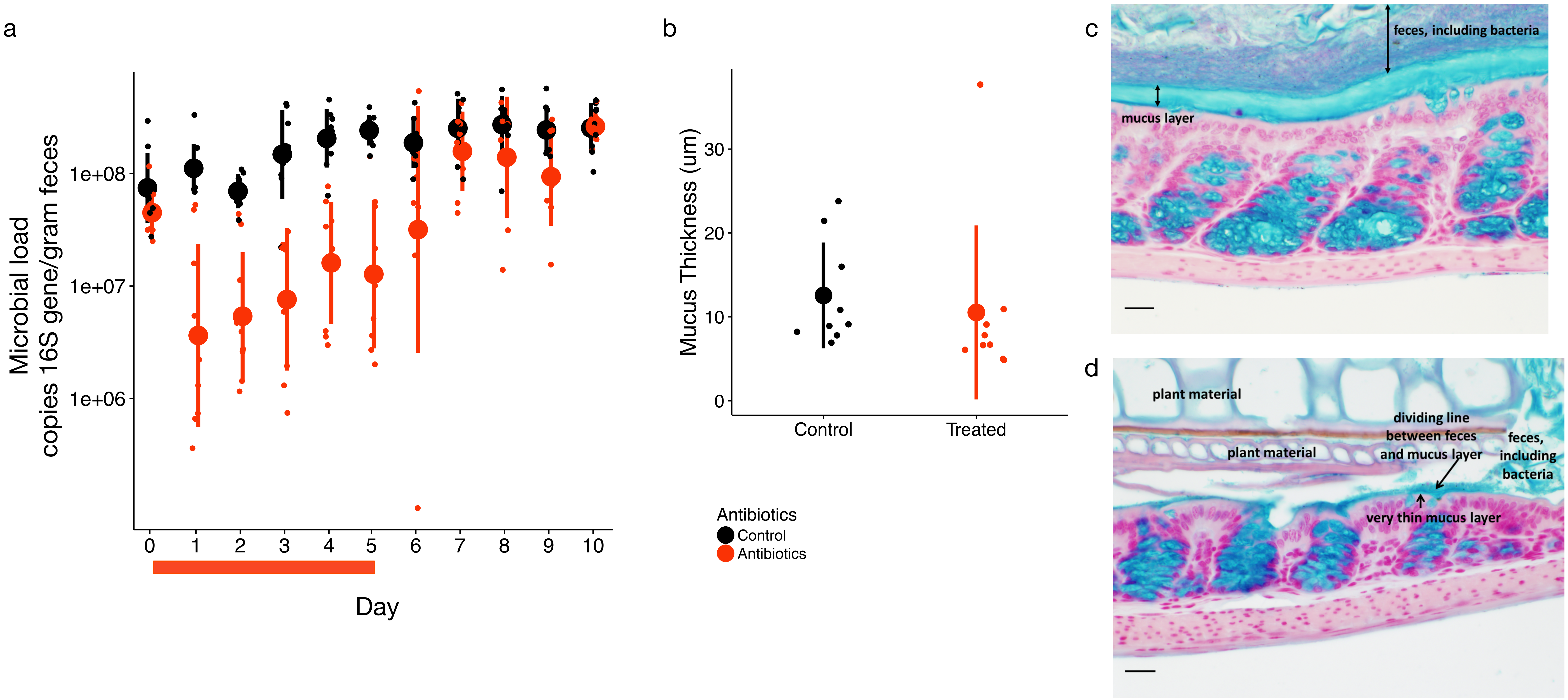


Supplementary Figure 1 | Changing dietary protein content results in changes in C:N associated with some microbial shifts. a, Fecal C:N changed under altered protein diets over 14 days (P< 2.2x10^{-16}, linear mixed effects model likelihood tests; n=9-10 mice per treatment group). **b**, Microbial load, estimated by 16S rRNA gene copy number measured via qPCR, was negatively correlated (linear regression shown) with fecal C:N on the final day of treatment (ρ=-0.4, P=0.03, Spearman correlation; n=28 mice) **c,** Bacteroidaceae relative abundance did not differ between treatment groups (P=0.58, Kruskal-Wallis test; n=9-10 mice per treatment group). **d**, However, Bacteroidaceae absolute abundance (calculated as relateve abundance multiplied by total copies 16S rRNA gene measured via qPCR) trended towards a negatively correlation (linear regression shown) with fecal C:N on the last day of treatment (ρ=-0.29, P=0.1, Spearman correlation; n=28 mice). **f,** Mouse weight did not differ significantly between diet treatments (P=0.93, Kruskal-Wallis test; n=9-10 mice per group). Large circles are means; bars show standard deviations.

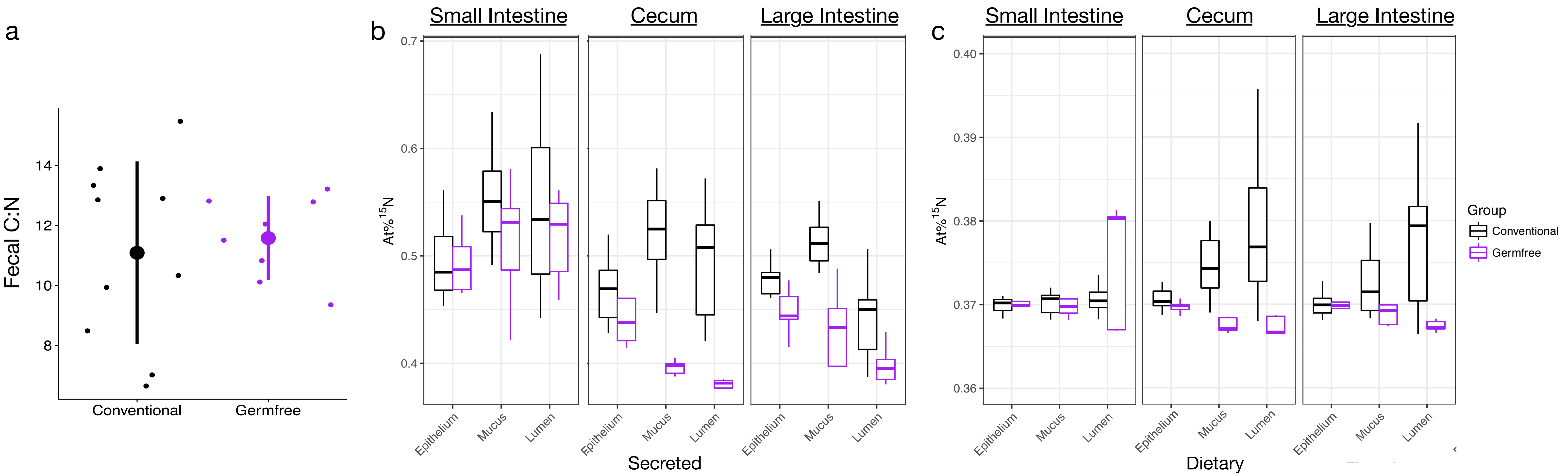


Supplementary Figure 2 | C:N of digesta varies along the length of the gut but is not correlated with total microbial load. a, C:N increased along the gut of mice under normal diets (P=0.05 proximal small intestine compared to distal small intestine, P=0.0001 proximal small intestine compared to large intestine, Tukey's Honestly Significant Difference test; n=10 mice per compartment). Bars indicate groups which differed significantly (*** P<0.001, * P<0.05). **b**, Microbial load, estimated by 16S rRNA gene copy number, was not significantly correlated with gut content C:N (ρ=0.24, P=0.14, Spearman correlation; n=10 per compartment) when analyzed across the entire mouse intestine. Large circles are means; bars show standard deviations.

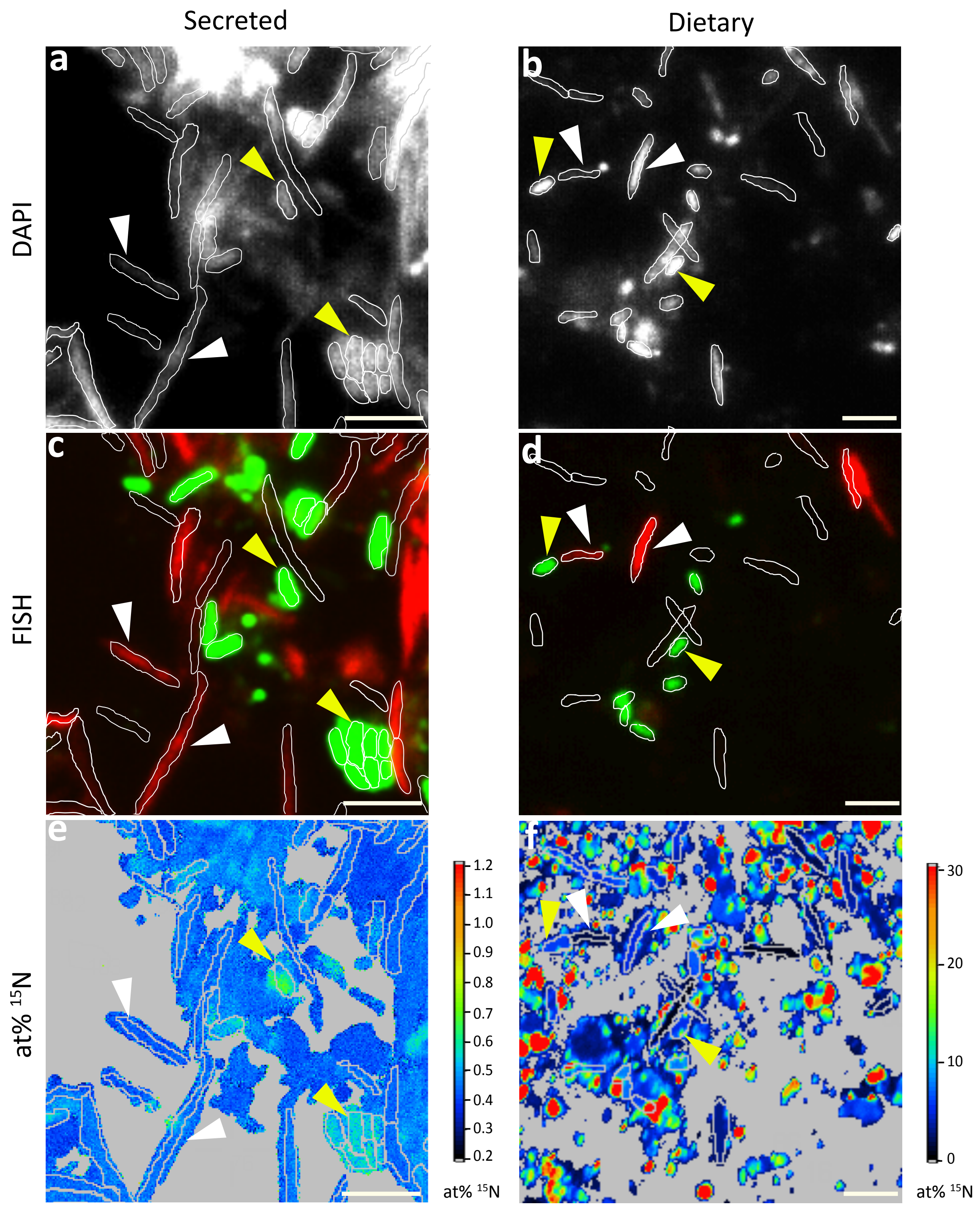


Supplementary Figure 3 | Antibiotic treatment affects gut microbial load and mucus thickness. a, Antibiotics produce a significant decrease in microbial load during the 5 day treatment course (P=9.14x10^{-6}, linear mixed effects model likelihood test; n=9-10 mice per treatment group)), after which microbial load converged between treated and un-treated mice. Microbial load was measured as 16S rRNA gene copy number per gram feces extracted with qPCR detection limits between ~10^4 and ~10^9. **b-d**, Mucus thickness was measured on Carnoy's fixed mouse gut sections stained with Alcian blue after 5 days of treatment. Blinded measurements of an intact AB-stained layer immediately overlying the colonic epithelium, which we believe based on previous literature to represent the inner mucus layer, revealed a trend toward thinner mucus in antibiotics treated mice (P=0.09, Mann-Whitney U test; n=9 mice per treatment group; **b**). However, we did not attempt to measure the thickness of the more amorphous and bacterial-laden outer mucus layer. Representative images for control (**c**) and antibiotic treated (**d**) mice include a 20µm scale bar. Images were prepared for all samples (N=18). See Supplementary Information Table 7 for individual measurements of mucus thickness. Red bar under the x-axis indicate the course of antibiotics. Large circles are means; bars show standard deviations.

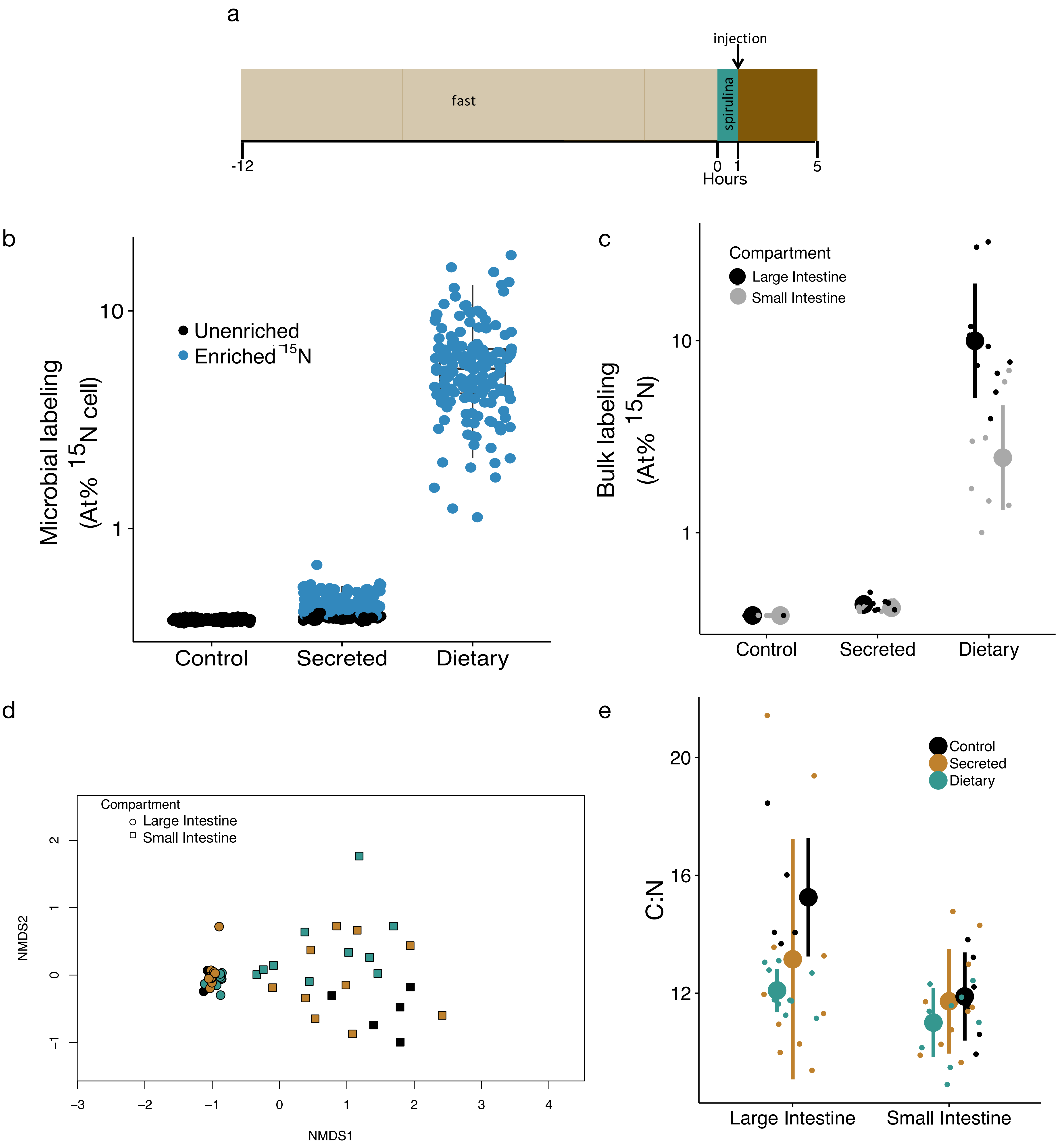
Supplementary Figure 4 | Labeled nitrogen from both host diet and host secretions was delivered to the gut and made available to the microbiota without changing nitrogen availability. a, Isotopic enrichment delivery to the gut was demonstrated throughout the gut after four and six hours. Bars indicate difference between treatments ($P<0.05$, Bonferroni-corrected Mann-Whitney U tests (see Supplementary Information Table 8 for exact p-values); n=6 per treatment group). **b**, C:N did not vary between treatments ($P=0.13$, Bonferroni-corrected Kruskal-Wallis test), indicating the treatments did not change overall nitrogen availability. **c-d**, However, C:N does vary between compartment (small intestine, cecum, large intestine, feces) and among tissue layers within each compartment (epithelium, mucus, lumen) ($P<0.05$, Bonferroni-corrected Kruskal-Wallis tests (see Supplementary Information Table 9 for exact p-values); n=12-18 per section) after 4 (**c**) and 6 (**d**) hours. Large circles are means; bars show standard deviations. Boxplots indicate medians and quartiles; whiskers show 1.5*interquartile range.



Supplementary Figure 5 | Nitrogen allocation patterns in germfree mice. a, Fecal C:N did not differ between convei nal and germfree mice (P=0.9, Mann-Whitney U test; n=8-10 mice per group). **b**-**c**, Atom percent enrichment (*i.e.*, the proportional representation of the heavy isotope times 100) four hours following stable isotope tracer delivery via injection (*i.e.*, host secreted; **b**) or host diet (**c**) differed between germfree and conventional mice (P=0.0007 and P=0.0261, respectively, mixed-model ANOVAs; n=6 per treatment group). Large circles are means; bars indicate standard deviation. Boxplots indicate medians and quartiles; whiskers show 1.5*interquartile range.



Supplementary Figure 6 | Representative NanoSIMS/FISH analysis of large intestine microbiota from mice fed a ^{15}N labeled spirulina diet or injected with ^{15}N/^{13}C labeled threonine. a-d, Large intestine contents were stained with the nucleic acid dye DAPI (gray) and hybridized with the FISH Bac303 (green) and Erec482 (red) probes to visualize bacterial cells of particular taxa (see Supplementary Table 3). **e-f**, NanoSIMS at% ^{15}N distribution maps of the same fields of view for secreted ^{15}N (**e**) and dietary ^{15}N (**f**) are shown. Yellow arrowheads point to higher enrichment levels detected for Bac303 positive cells in comparison with Erec482 positive cells, which are indicated by white arrowheads. All measured bacterial cells are outlined in grey, for easier visualization. Grey background areas (**e-f**) refer to pixels where an unbiased at% visualization is not feasible due to weak C2- and CN- secondary ion signal intensities (ITO surface without biomass). Scale bar = 5 µm. Images were prepared for all samples prepared for NanoSIMS (n=6).



Supplementary Figure 7 | Labeled nitrogen from both host diet and host secretions did not change nitrogen availability or composition but was taken up by the microbiota. a, Experimental setup for single cell studies: mice were fasted overnight and then offered chow with all protein sourced from Spirulina cells (either ^{14}N for control and secreted groups or ^{15}N for dietary group) for one hour before being returned to normal mouse chow for four hours before euthanasia. Control and secreted-group mice received an injection of threonine (^{14}N and ^{15}N respectively) before being returned to normal chow. **b-c,** The diet manipulation produced the greatest overall ^{15}N enrichment of microbial cells ($P<2.2\times10^{-16}$, Bonferroni-corrected Mann-Whitney U tests; n= 2 mice per treatment, n= 78-185 cells per treatment, **b**) likely due to much greater label uptake during feeding, but both interventions resulted in significant overall enrichment relative to controls with no significant differences between intestinal compartments (colors; P=0.16, Mann-Whitney U test, n=5-10 mice per treatment; **c**). **d,** The experimental treatments (colors) did not significantly affect microbial taxonomic composition (P=0.09) as assessed by 16S rRNA gene amplicon sequencing but taxonomic composition did differ significantly between gut compartments (shapes; P=0.001, PERMANOVA; n=5-10 mice per treatment). **e,** The experimental treatments did not affect gut content C:N (P=0.09), but C:N did vary between intestinal compartments (P=0.02, Mann-Whitney U test; n=5-10 mice per treatment). Large circles are means; bars show standard deviations.

Supplementary Table 1: Gut isolate C:N. For isolates with more than one clonal population measured (N>1) C:N reported is the mean.

Species	Phylum	N	Source	C:N
Collinsella aerofaciens	Actinobacteria	1	Human donor	4.1
Eggerthella centa	Actinobacteria	1	Human donor	3.99
Bacteroides massiliensis	Bacteroidetes	1	Human donor	4.58
Bacteroides ovatus	Bacteroidetes	4	Human donor	3.95
Bacteroides thetaiotaomicron	Bacteroidetes	1	Human donor	4.28
Bacteroides uniformis	Bacteroidetes	1	Human donor	4.07
Bacteroides vulgatus	Bacteroidetes	1	Human donor	4.22
Bacillus coccae	Firmicutes	1	Human donor	4.33
Clostridium bartlettii	Firmicutes	1	Human donor	3.85
Clostridium innocuum	Firmicutes	1	Human donor	4.02
Clostridium innocuum	Firmicutes	1	Human donor	3.84
Clostridium sp.	Firmicutes	1	Human donor	4.17
Clostridium sp.	Firmicutes	1	Human donor	4.55
Clostridium sp.	Firmicutes	1	Human donor	3.65
Clostridium sp.	Firmicutes	1	Human donor	4.31
Coprococcus comes	Firmicutes	1	Human donor	3.98
Dorea formicigenens	Firmicutes	1	Human donor	4.1
Dorea formigamerans	Firmicutes	1	Human donor	4.41
Dorea longicatena	Firmicutes	1	ATCC	3.97
Dorea longicatena	Firmicutes	1	Human donor	3.78
Enterococcus faecalis	Firmicutes	4	Human donor	3.94
Enterococcus faecalis	Firmicutes	1	Human donor	4.28
Enterococcus faecalis	Firmicutes	1	Human donor	3.88
Ruminococcus gnavus	Firmicutes	1	Human donor	3.76
Ruminococcus gnavus	Firmicutes	1	Human donor	3.92
Ruminococcus productus	Firmicutes	1	Human donor	4.2
Ruminococcus torques	Firmicutes	1	Human donor	3.62
Streptococcus salivarius	Firmicutes	1	Human donor	3.98
Streptococcus salivarius	Firmicutes	1	Human donor	3.85
Streptococcus salivarius	Firmicutes	1	Human donor	3.97
Enterobacter cloacae	Proteobacteria	1	Human donor	4.02
Raoultella ornithinolytica	Proteobacteria	1	Human donor	4.28
Raoultella ornithinolytica	Proteobacteria	1	Human donor	4.12
Shigella flexneri	Proteobacteria	1	Human donor	3.8
Akkermansia municiphila	Verrucomicrobia	4	ATCC	4.49

Supplementary Table 2: Mammals included in fecal C:N analyses

Common name	**Species**	**N**	**Diet**	**Gut morphology**	**Large intestine length (cm)**	**Length source**	**Collection locale**	**Population**
Dog	*Canis lupus familiaris*	5	Carnivore	simple	68	54	USA	Captive
Aye-aye	*Daubentonia madagascariensis*	4	Carnivore	simple			USA	Captive
White-tailed Mongoose	*Ichneumia albicauda*	1	Carnivore	simple			Kenya	Wild
Aardwolf	*Proteles cristatus*	1	Carnivore	simple	48.81	52	Kenya	Wild
Meerkat	*Suricata suricatta*	9	Carnivore	simple			South Africa	Wild
Impala	*Aepyceros melampus*	10	Herbivore	hindgut			Kenya	Wild
Southern White Rhinocerous	*Ceratotherium simum*	5	Herbivore	hindgut			Kenya	Wild
Eastern Black Rhinocerous	*Diceros bicornis*	7	Herbivore	hindgut	550.73	52	Kenya	Wild
Horse	*Equus ferus caballus*	4	Herbivore	hindgut	747	54	USA	Captive
Grevy's Zebra	*Equus grevyi*	9	Herbivore	hindgut			Kenya	Wild
Plains Zebra	*Equus quagga*	10	Herbivore	hindgut	532.35	52	Kenya	Wild
Crested Porcupine	*Hystrix cristata*	2	Herbivore	hindgut	59.88	52	Kenya	Wild
Snowshoe hare	*Lepus americanus*	15	Herbivore	hindgut	144.03	52	USA	Captive
Elephant	*Loxodonta africana*	10	Herbivore	hindgut	867.9	52	Kenya	Wild
Prairie Vole	*Microtus ochrogaster*	10	Herbivore	hindgut	25.44	52	USA	Captive
Rock Hyrax	*Procavia habessinica*	2	Herbivore	hindgut	74	52	Kenya	Wild
Hippopotamus	*Hippopotamus amphibius*	5	Herbivore	pseudo-ruminant	306.08	52	Kenya	Wild
Cattle	*Bos indicus*	10	Herbivore	ruminant	1106	54	Kenya	Captive
Cow	*Bos taurus*	10	Herbivore	ruminant			USA	Captive
Giraffe	*Giraffa cameloparadalis*	5	Herbivore	ruminant	2383	53	Kenya	Wild
Waterbuck	*Kobus ellipsiprymnus*	1	Herbivore	ruminant			Kenya	Wild
Gunther's Dik-dik	*Madoqua guentheri*	10	Herbivore	ruminant	149.55	52	Kenya	Wild
Sheep	*Ovis aries*	10	Herbivore	ruminant	558.16	52	USA	Captive
Cape Buffalo	*Syncerus caffer*	10	Herbivore	ruminant			Kenya	Wild
Mouse	*Mus musculus*	10	Omnivore	hindgut	7.41	55	USA	Captive
Warthog	*Phacochoerus africanus*	6	Omnivore	hindgut			Kenya	Wild
Vervet Monkey	*Cercopithecus pygerythrus*	1	Omnivore	simple	85.59	52	Kenya	Wild
Human	*Homo sapiens*	5	Omnivore	simple	137.73	52	USA	Wild
Grey Mouse Lemur	*Microcebus murinus*	8	Omnivore	simple			USA	Captive
Yellow Baboon	*Papio cynocephalus*	8	Omnivore	simple	259.39	52	Kenya	Wild

Supplementary Table 3: FISH probes for single cell stable isotope analyses

Probe Name	**Probe sequence (5’→3’)**	**Total hits**[a]	**Major target taxa (coverage %, total hits in taxon)**[a]	**Total non-target hits**[a]	**References**
Erec482	GCT TCT TAG TCA RGT ACC G	95259	Order *Clostridiales* (32.2%, 94787) Family Lachnospiraceae (70.2%, 93102)	2157	71
Bac303	CCA ATG TGG GGG ACC TT	109363	Order *Bacteroidales* (55.9%, 108561) Family *Bacteroidaceae* (91.5%, 56850) Family *Porphyromonadaceae* (17.1%, 8502) Family *Prevotellaceae* (71.1%, 40124)	802	72

[a] According to RDP probe match, performed with database release 11, Update 4 (26 December 2015), containing 3224600 bacterial and archeal 16S rRNA sequences (https://rdp.cme.msu.edu/). Coverage is the percentage of sequences within the RDP target taxon that shows a full match to the probe sequence. The number of nontarget hits indicates the total number of sequences outside the respective RDP taxa that show a full match to the probe sequence.

Supplementary Table 5: Genus level absolute abundance (calculated as relative abundance values multiplied by 16S copy number measured via qPCR) responses to dietary intervention at the end of two weeks. Uncorrected and Bonferroni-corrected p-values are reported for Kruskal-Wallis tests of treatment effects. Only genera with an average abundance of at least 0.5% and at least one sample with greater than 1% abundance were included.

			6% Protein Diet		**20% Protein Diet**		**40% Protein Diet**			
Phylum	**Order**	**Genus**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**p-value**	**adjusted p-value**
Bacteroidetes	Bacteroidales	*Bacteroides*	5.84E+06	1.04E+07	2.18E+07	2.75E+07	4.40E+07	5.44E+07	**0.0550**	1
		Parabacteroides	1.09E+07	1.86E+07	4.18E+07	4.60E+07	5.44E+07	4.05E+07	**0.0090**	0.1710
		Rickenellaceae genus unspecified	1.80E+05	2.99E+05	6.24E+05	5.28E+05	6.50E+05	5.75E+05	**0.0138**	0.2622
		S24-7 genus unspecified	1.11E+07	2.26E+07	1.35E+07	2.97E+07	9.64E+06	2.89E+07	0.1459	1
Deferribacteres	Deferribacterales	Mucispirillum	7.23E+05	1.26E+06	1.25E+06	1.58E+06	4.87E+05	8.29E+05	0.3727	1
Firmicutes	Lactobacillales	*Lactobacillus*	3.21E+05	6.08E+05	5.93E+06	1.50E+07	1.12E+07	1.12E+07	**0.0005**	**0.0095**
		Lactococcus	3.05E+05	2.82E+05	1.41E+06	1.49E+06	6.03E+06	6.32E+06	**0.0243**	0.4617
	Clostridiales	Family unspecified	7.79E+06	9.10E+06	1.40E+07	1.25E+07	1.83E+07	1.53E+07	0.4173	1
		SMB53	1.02E+06	9.50E+05	2.06E+06	5.11E+06	3.77E+06	5.37E+06	0.2105	1
		Lachnospiraceae genus unspecified	1.35E+06	1.12E+06	1.74E+06	1.62E+06	2.55E+06	2.74E+06	0.7240	1
		Dorea	4.17E+05	5.28E+05	5.48E+05	6.52E+05	4.63E+06	6.41E+06	0.0683	1
		rc4-4	7.37E+06	1.17E+07	9.74E+06	8.48E+06	2.09E+07	2.18E+07	0.1101	1
		Ruminococcaceae genus unspecified	2.77E+06	3.11E+06	5.61E+06	5.04E+06	7.36E+06	5.70E+06	0.0841	1
		Oscillospira	2.51E+06	2.29E+06	1.09E+07	1.23E+07	1.13E+07	9.10E+06	0.0616	1
		Ruminococcus	6.50E+05	6.33E+05	2.26E+06	2.54E+06	1.12E+07	2.36E+07	**0.0065**	0.1235
	Erysipelotrichales	Erysipelotrichaceae genus unspecified	3.42E+05	4.53E+05	4.14E+05	2.99E+05	1.55E+06	2.67E+06	0.0659	1
		[*Eubacterium*]	2.00E+05	3.79E+05	5.43E+05	1.35E+06	3.37E+06	9.02E+06	0.1909	1
Tenericutes	RF39	Family unspecified	5.21E+05	9.81E+05	1.08E+06	1.25E+06	1.97E+06	2.53E+06	0.1371	1
Verrucomicrobia	Verrucomicrobiales	*Akkermansia*	4.22E+06	5.80E+06	1.51E+07	2.13E+07	5.13E+07	7.10E+07	0.2481	1

Supplementary Table 6: Select nutrient data for experimental mouse diets including primary protein and carbohydrate sources.

Diet Name	Protein (% by weight)	Casein (g/kg)	Carbohydrate (% by weight)	Sucrose (g/kg)	Cellulose (g/kg)	Fat (% by weight)	Calcium (% by weight)	Phosphorous (% by weight)	Kcal/g
TD.90016	6.1	69.00	75.6	571.80	57.82	5.5	0.7	0.54	3.8
TD.91352	20.3	230.00	61.6	431.70	37.86	5.5	0.7	0.54	3.8
TD.90018	40.0	460.00	41.6	231.82	15.00	5.5	0.7	0.54	3.8

Supplementary Table 7: Mean mucus thickness measurements for antibiotics experiment—data presented in Extended Data 3b.

Mouse	Antibiotics	Thickness (um) ± SD	Measurements (N)
1	N	10.8± 6.4	9
2	N	7.8± 0.1	7
3	N	8.9± 3.7	7
4	N	6.9± 1.7	9
5	N	16.0± 6.5	10
6	N	21.5± 16.1	10
7	N	9.1± 3.2	6
8	N	8.2± 3.0	9
9	N	23.8± 9.3	10
1	Y	37.7± 15.1	6
2	Y	10.9± 4.4	10
3	Y	6.7± 3.7	7
4	Y	9.1± 2.0	6
5	Y	5.0± 1.9	7
6	Y	6.6± 3.2	10
7	Y	7.8± 4.5	10
8	Y	4.9± 1.8	8
9	Y	6.1± 2.8	9

Supplementary Table 8: Bonferroni-corrected p-values for Mann-Whitney U tests to determine effect of labeling route (secreted or dietary) on isotopic enrichment in the gut. Significant effects in bold. Plots of enrichment values are in Supplementary Information Figure 4a.

Hour	**Layer**	**Compartment**	**Treatment effect p-value**
4	Epithelium	Small Intestine	**0.0217**
4	Mucus	Small Intestine	**0.0217**
4	Lumen	Small Intestine	**0.0433**
6	Epithelium	Small Intestine	**0.0217**
6	Lumen	Small Intestine	**0.0217**
6	Mucus	Small Intestine	**0.0217**
4	Epithelium	Cecum	**0.0217**
4	Mucus	Cecum	**0.0217**
4	Lumen	Cecum	1
6	Epithelium	Cecum	**0.0217**
6	Mucus	Cecum	1
6	Lumen	Cecum	1
4	Epithelium	Large Intestine	**0.0217**
4	Mucus	Large Intestine	1
4	Lumen	Large Intestine	1
6	Epithelium	Large Intestine	**0.0217**
6	Mucus	Large Intestine	0.4100
6	Lumen	Large Intestine	1
4		Feces	1
6		Feces	1

Supplementary Table 9: Bonferroni-corrected p-values for Kruskal-Wallis tests to determine effect of gut compartment and layer on C:N during isotopic enrichment studies. Significant effects in bold. Plots of C:N are in Supplementary Information Figure 4c, 4d.

Hour	**Effect**	**p-value**
4	Compartment	0.000006
4	Layer	0.000008
4	Layer (small intestine)	**0.047180**
4	Layer (cecum)	0.750000
4	Layer (large intestine)	**0.005007**
6	Compartment	**0.008304**
6	Layer	**0.000295**
6	Layer (small intestine)	1.000000
6	Layer (cecum)	0.339200
6	Layer (large intestine)	**0.000257**