Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), are conditions of chronic gastrointestinal inflammation resulting from genetic predisposition and perturbed interactions between gut microorganisms and host immunity. Many studies focus on microbial taxonomic and functional changes during IBD; however, the gut metabolome (comprising diet-, host- and microorganism-derived metabolites) is an equally important contributor to intestinal health.

Certain gut bacteria metabolize dietary fibre into short-chain fatty acids such as butyrate that nourish colonocytes, promote regulatory T-cell expansion and have immunosuppressive functions. Butyrate concentration and butyrate-producing bacteria are depleted in the IBD gut. Other bacteria (for example, Lactobacillus spp., Bacteroides spp. and Clostridium sporogenes) convert tryptophan into indole derivatives that promote healthy intestinal barrier function and immune tolerance. Indole producers are also depleted in IBD. Urease activity, predominantly contributed by Proteobacteria, shifts the microbiome towards the imbalanced state seen in IBD and worsens disease in a murine colitis model. Host-derived metabolites likewise affect microbiota composition: bile acids, enriched in the IBD gut, promote growth of bile acid-metabolizing bacteria and inhibit growth of bile-sensitive bacteria.

Inferring covariations between metabolites and bacteria that are differentially abundant in IBD can functionally implicate gut metabolites and microorganisms in intestinal health. Two recent studies combined microbial metagenomics and untargeted mass spectrometry of metabolites to identify associations between stool bacterial species and metabolites. The first was a cross-sectional study of UC, CD and non-IBD subjects within the Prospective Registry in IBD Study at MGH (PRISM) cohort, and the second was the longitudinal integrative Human Microbiome Project (iHMP). Both concluded that microbial taxonomic changes associated with IBD, such as blooms of facultative anaerobes including Proteobacteria, are accompanied by significant shifts in metabolite composition.

Here, we investigated the effects of intestinal metabolites that are differentially abundant in IBD on the growth of gut bacteria that are also differentially abundant in IBD, finding that metabolites including amines and fatty acids strongly affect bacterial growth. Linoleoyl ethanolamide (LEA), an NAE, impacted growth in ways that reflect altered bacterial abundances in the IBD microbiome. We show that LEA and three structurally related NAEs—palmitoyl ethanolamide (PEA), oleoyl ethanolamide (OEA) and arachidonoyl ethanolamide (AEA)—are enriched in stool from IBD patients and a T-cell transfer model of colitis. These NAEs share common receptors and are part of the endocannabinoid system, although only AEA is considered a true endocannabinoid as it binds the cannabinoid receptors CB1 and CB2. We treated monocultures of bacteria that shift

**Growth effects of N-acylethanolamines on gut bacteria reflect altered bacterial abundances in inflammatory bowel disease**

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Inflammatory bowel diseases (IBD) are associated with alterations in gut microbial abundances and luminal metabolite concentrations, but the effects of specific metabolites on the gut microbiota in health and disease remain largely unknown. Here, we analysed the influences of metabolites that are differentially abundant in IBD on the growth and physiology of gut bacteria that are also differentially abundant in IBD. We found that N-acylethanolamines (NAEs), a class of endogenously produced signalling lipids elevated in the stool of IBD patients and a T-cell transfer model of colitis, stimulated growth of species over-represented in IBD and inhibited that of species depleted in IBD in vitro. Using metagenomic sequencing, we recapitulated the effects of NAEs in complex microbial communities ex vivo, with Proteobacteria blooming and Bacteroidetes declining in the presence of NAEs. Metatranscriptomic analysis of the same communities identified components of the respiratory chain as important for the metabolism of NAEs, and this was verified using a mutant deficient for respiratory complex I. In this study, we identified NAEs as a class of metabolites that are elevated in IBD and have the potential to shift gut microbiota towards an IBD-like composition.
in IBD with these NAEs, demonstrating that NAEs promote the growth of species over-abundant in IBD and restrict the growth of those depleted in IBD. Metagenomic sequencing of complex bacterial communities derived from healthy subjects and treated with NAEs showed taxonomic shifts that mimicked altered abundances observed in IBD patients. Metatranscriptomic analysis of these communities revealed increased expression of respiratory electron transport chain components in Enterobacteriaceae in response to NAEs. These ex vivo results implicate an upregulated host endocannabinoid system in the alteration of microbial communities associated with IBD.

Results

Gut metabolites affect growth of gut bacteria. Previous metabolic profiling of stool from subjects in the PRISM cohort identified 3,829 features with assigned molecular functions, of which 466 (representing 346 unique compounds) were validated using reference standards. From these validated metabolites, we selected 50 spanning 19 molecular classes in the Human Metabolome Database (Supplementary Dataset 1). Thirty-two were differentially abundant between IBD and non-IBD stool (that is, the IBD phenotype coefficient from a log-transformed linear model with Benjamini–Hochberg false discovery rate (FDR) \(q < 0.05\); Supplementary Dataset 2). We quantified their effects on exponential (\(V_{\text{max}}\)) and stationary (\(\text{max OD}_{600\text{nm}}\)) bacterial growth using a microtitre plate screen (Fig. 1a and Supplementary Dataset 3). Each metabolite was tested at a single concentration (Supplementary Table 1) on five gut bacteria: Escherichia coli, Ruminococcus gnavus, Blautia producta, Bacteroides fragilis and Bacteroides cellulosilyticus. In the PRISM cohort, \(E.\) coli, \(R.\) gnavus and \(B.\) producta were more abundant in IBD (FDR \(q < 0.1\)) while \(B.\) cellulosilyticus was depleted (FDR \(q = 0.11\)). While not differentially abundant in the PRISM cohort, we included \(B.\) fragilis due to its well-characterized interactions with host immunity and depletions of \(Bacteroides\) species observed in other IBD cohorts. Many metabolites (\(>50\%\) of those tested) promoted the growth of \(E.\) coli, \(R.\) gnavus and \(B.\) producta, whereas only \(\alpha\)-sphingosine and phytosphingosine inhibited their growth (Fig. 1b). In contrast, \(B.\) cellulosilyticus growth was enhanced by fewer (18\%) of the metabolites tested while its growth was inhibited by \(>50\%\) of the metabolites, most of which are elevated in IBD.

To validate the single-dose growth effects, seven metabolites that inhibited or enhanced growth of at least one species were tested in multiple doses on three strains (Extended Data Fig. 1). The metabolites represented a variety of molecular subclasses: amines (LEA), glycerophosphocholines (C16:0 lysophosphatidylcholine (LPc)), polyols (panthenolic acid), fatty acids (sebacic and dodecanedioic acids), glycerolipids (2-palmitoylglycerol) and linoleic acyls (linolenic acid). Multiple-dose assays confirmed that LEA inhibits \(B.\) cellulosilyticus growth and does not affect \(E.\) coli growth, while \(R.\) gnavus was delayed in its lag phase only by the highest LEA concentration. Growth effects of pantothenic acid and C16:0 LPC were recapitulated at multiple doses. \(B.\) fragilis growth was unaffected by sebacic and dodecanedioic acids at single doses but strongly enhanced in the multiple-dose assay. \(E.\) coli growth was moderately inhibited by sebacic and dodecanedioic acids, although no effects were observed in the single-dose assay. No significant effects were observed with these two metabolites on \(R.\) gnavus growth, similar to the single-dose assay. At higher doses, linolenic acid delayed exponential growth of \(B.\) fragilis, \(R.\) gnavus and \(E.\) coli while enhancing \(B.\) fragilis stationary phase growth. Finally, single-dose growth effects of 2-palmitoylglycerol were captured on \(B.\) fragilis but not on \(R.\) gnavus or \(E.\) coli in the multiple-dose assay. These results highlight the importance of testing a dose range of metabolites on bacterial growth to assess the full extent of metabolite effects.

NAEs are enriched in stool from IBD patients and a murine colitis model. LEA, an NAE enriched in IBD, inhibited the growth of \(B.\) cellulosilyticus and enhanced that of \(E.\) coli, \(R.\) gnavus and \(B.\) producta (Fig. 1b,c) in agreement with altered abundances of these taxa in IBD. Reasoning that structurally related metabolites may have similar effects, we searched the untargeted metabolomics dataset of the PRISM cohort for known NAEs. We identified metabolite features that were enriched in IBD patients corresponding to three additional NAEs: PEA, OEA and AEA (Fig. 2a and Supplementary Dataset 2). We confirmed the presence of AEA in PRISM subject stool using the same liquid chromatography–mass spectrometry (LC–MS) methods that previously validated the presence of PEA, OEA and LEA. While PEA, OEA and LEA were enriched only in CD samples (FDR \(q < 0.05\)), AEA was over-abundant in both CD and UC. We verified NAE abundances in the independent IBD cohort from the iHMP\(^8\) (Fig. 2b and Supplementary Dataset 2). NAE levels were highest in samples with extreme taxonomic differences (active CD/UC) compared to non-IBD samples, supporting a connection between NAEs and altered microbiome composition in IBD.

As the LC–MS methods used do not yield absolute concentrations, we further confirmed elevated NAEs in stool from IBD patients by quantifying NAEs in a subset of samples from the PRISM cohort (Fig. 2c). Average AEA, PEA, OEA and LEA amounts were present in the nanogram per milligram range (0.89–1,503 ng mg\(^{-1}\)) in CD samples. Absolute NAE concentrations highly correlated with relative abundances in the same samples (Extended Data Fig. 2), demonstrating the quantitative quality of the metabolomic methods for detection of these metabolites.

To investigate whether NAEs are elevated during inflammation in a relevant animal model, we measured NAE concentrations in stool collected from \(Rag2^{-/-}\) mice before and after induction of colitis. Transferring naive T cells from wild-type donor mice to \(Rag2^{-/-}\) mice, which are unable to generate mature B and T lymphocytes, induces colitis. We reared \(Rag2^{-/-}\) mice in specific pathogen-free (SPF) conditions and collected stool at week 0, before the transfer of naive T cells obtained from SPF-colonized wild-type mice. After six weeks, colitis developed in \(Rag2^{-/-}\) mice that received T-cell transfusers but not in \(Rag2^{-/-}\) mice that did not receive T-cell transfers. An increase in all four NAEs, at concentrations similar to those found in patients, was detected in stool collected six weeks after T-cell transfer (week 6; Fig. 2d). We additionally performed metagenomic sequencing of DNA from these samples to identify bacterial taxa associated with NAE abundance. Of the species overlapping with those found
in PRISM samples, we observed the same abundance trends in mice with colitis. *Akkermansia, Alistipes* and *Eubacterium* species decreased while *Enterococcus* species increased in relative abundance after T-cell transfer (Supplementary Dataset 4). Thus, both increased NAE abundance and corresponding bacterial community shifts are characteristic features of colitis in mice and humans.
NAEs enhance growth of species enriched in IBD and inhibit growth of species depleted in IBD. To assess growth effects on differentially abundant PRISM species, we tested the four NAEs at two doses on eleven strains: three reference strains used in our initial screen; one reference strain of a species enriched in IBD patients, *Lactobacillus gasseri*; and seven strains isolated from human stool corresponding to three enriched, three depleted and one invariable species in IBD (Extended Data Figs. 3 and 4 and Supplementary Table 2). \( V_{\text{max}} \) and max OD\(_{600\text{mm}} \) were deduced from dose growth curves and plotted as a function of NAE concentration to determine growth effects (Fig. 3a,b and Supplementary Dataset 5). No strain-specific effects were observed with *L. gasseri*, *E. coli* and *R. gnavus*, as strains of the same species responded similarly to NAE treatment. Growth of all IBD-elevated species tested was enhanced following NAE treatment, while growth of IBD-depleted species was inhibited. Growth enhancements were strongest with the addition of OEA and LEA, while inhibitory effects were strongest with LEA and AEA. PEA had minimal impact on growth.

The combination of metagenomic and metabolomic profiling of the PRISM cohort enabled us to identify potential mechanistic associations between bacterial species and metabolites that were differentially abundant in IBD, including the species and NAEs used in this study (Fig. 3c and Supplementary Dataset 6). When comparing human covariation data to our in vitro growth analysis, most strains responded to NAEs as predicted by the association model (Fig. 3b). With the exception of *Streptococcus salivarius* and *Enterococcus faecalis*, while we observed significant correlations in vitro, *S. salivarius* abundances did not vary significantly between IBD and non-IBD subjects. *E. faecalis* did not correlate with NAE abundance in the PRISM cohort.

To decipher molecular mechanisms governing bacterial responses to NAEs, we analysed the transcriptomes of *B. fragilis* after stimulation with LEA and AEA, which had the strongest inhibitory effects (Fig. 3b and Supplementary Dataset 7). We chose *B. fragilis* because it is a well-studied species belonging to a phylum often depleted in IBD.12,19 In both treatments, the most upregulated
genes encode putative membrane-associated proteins functioning in efflux transport (Extended Data Fig. 5a). All upregulated efflux genes are organized in two operons (Extended Data Fig. 5b), suggesting that they may function in a pathway to actively pump NAEs and other fatty amides out of the cell or its periplasm. The most downregulated gene encodes an outer membrane protein homologous to E. coli FadL (BF9343_1803), a long-chain fatty acid (LCFA) importer. Two genes with high similarity to facC (BF9343_1803 and BF9343_3528), encoding the inner membrane-associated fatty acid CoA ligase that catalyses esterification of incoming fatty acids into CoA thioesters, and one gene encoding a FadE homologue (BF9343_3118), an acyl CoA dehydrogenase involved in downstream fatty acid breakdown (log2(fold change) < 1), were also repressed in treated samples, indicating that B. fragilis recognizes both NAEs as LCFA. Equally important in LCFA metabolism is the electron transfer from dehydrogenases to the membrane-bound respiratory chain by electron-transfer flavoproteins. Both electron-transfer flavoprotein subunits (BF9343_3116 and BF9343_3117) were downregulated in response to LEA and AEA. These data suggest that B. fragilis inhibits transport and metabolism of NAEs while promoting their efflux.

NAEs shift ex vivo gut communities towards an IBD-like composition. To determine whether the growth effects of NAEs observed in monoculture are recapitulated in a bacterial community, we obtained samples from two chemostats (A and B), each inoculated with stool from a distinct healthy donor. Samples were treated in duplicate with the four NAEs individually and combined (denoted as NAE-mix) and dimethylsulfoxide (DMSO) as a control. We collected samples before treatment and at 1, 4, 8, 12 and 24 h post-treatment for combined shotgun metagenomic and metatranscriptomic sequencing. Profiling the taxonomic composition of the resulting metagenomes, we identified 66 (chemostat A) and 85 (chemostat B) species before treatment, with an overlap of 28 species and 11 unclassified species from known genera (Supplementary Dataset 8). Several species from our monoculture assays were present: E. coli, R. gnavus, A. shahii, B. fragilis, B. lactis, and R. lactaris. Spearman correlations between species relative abundances and NAEs from the PRISM stool metabolomics and metagenomics dataset (n = 155 samples; with the exception of associations between S. salivarius and the four NAEs, all FDR q < 0.1 based on two-tailed nominal P values; Supplementary Dataset 5). Abundances were first residualized with linear models of subject covariates to limit correlation driven by mutual association with other factors (for example, IBD phenotype). s.e., standard error; NS, not significant.
**Fig. 4 | Effects of NAEs on the composition of a complex microbial community.**

**a.** Taxonomic abundances in chemostat A at the family level. The vertical coloured bars represent the relative abundance of bacterial families in samples 1 and 12 h after addition of DMSO (0.5%), the individual NAEs or NAE-mix. OA, the fatty acid form of OEA, was also tested. Individual NAEs and OA were added to a final concentration of 500 μM. In combination, the PEA/OEA/LEA/AEA ratio was 125:125:125:μM. **b**, The heatmaps show log₂[fold change] in family-level (b) and species-level (c) taxonomic abundances between treated samples and DMSO controls (total n = 66 with per-treatment n ranging from 8 to 10). Species that shifted with statistical significance in response to treatment are shown (*q < 0.20, **q < 0.05, FDR q values derived from nominal two-tailed analyses). Species enriched (red) and depleted (blue) in PRISM CD stool relative to controls (Fig. 1a) are indicated. **d**, Principal coordinate (PCo) analysis on Bray–Curtis dissimilarities between chemostat A (n = 29) and PRISM (n = 155) metagenomes. Times of exposure to AEA, LEA and DMSO control are indicated.

**Alistipes shahii** and **S. salivarius** were found in both chemostats.

**B. fragilis** and **B. producta** were detected in chemostat B.

In chemostat A, Enterobacteriaceae, Clostridiaceae and Veillonellaceae expanded after 12 h of treatment with DMSO (Fig. 4a), a possible consequence of oxygen tension. The most extreme effect after 12 h was seen with AEA, with the community almost entirely comprised of enterobacteria, clostridia and Rikenellaceae (Supplementary Dataset 9). OEA treatment did not impact Enterobacteriaceae abundance but increased relative abundances of Enterococccaceae and Streptococccaceae and decreased those of Bacteroidaceae and Rikenellaceae (Fig. 4b). As a control, we tested oleic acid (OA), the fatty acid form of OEA and a known carbon source for **E. coli**, on samples from chemostat A. Despite its ability to enhance **E. coli** growth in monoculture (Extended Data Fig. 6), OA had no significant effect on Enterobacteriaceae in a microbial community (Fig. 4a,b). However, OA treatment enhanced the growth of Enterococccaceae, Veillonellaceae and Streptococccaceae, suggesting that there may be competition for this molecule within the community. Effects of PEA were not significant.

We observed the greatest species-level effects after treatment with AEA (Fig. 4c), the NAE most significantly enriched in PRISM and iHMP IBD subjects (Fig. 2 and Supplementary Dataset 2). Abundances of **Alistipes** and **Bacteroides** species decreased on treatment with all NAEs except PEA (Fig. 4c), reflecting our monoculture observations (Fig. 3b and Extended Data Figs. 3 and 4) and Bacteroidetes depletion in PRISM and iHMP IBD subjects. **Barnesiella intestinihominis**, also under-represented in PRISM IBD subjects, was inhibited by AEA, LEA and NAE-mix in the complex community. In contrast, **Escherichia** spp. abundances increased...
with AEA, LEA and NAE-mix, again mirroring our observations in monocultures and IBD patients. Although inhibited by NAES in monocolure, *E. faecalis* bloomed in chemostat A, concordant with the positive association between NAES and *E. faecalis* in our model (Fig. 3c). This represents an example of community metabolic interactions changing a microbial growth outcome.

Chemostat B family-level composition remained stable over time with DMSO treatment and followed similar trends to chemostat A in the presence of NAES (Extended Data Fig. 7a,b). At the species level, *B. producta* and *Clostridium clostridioforme* increased in relative abundance with AEA, LEA and NAE-mix. Both species are over-represented in PRISM IBD subjects (Extended Data Fig. 7c). *Klebsiella pneumoniae* and *Proteus mirabilis* also increased under the same conditions, and *K. pneumoniae* is significantly enriched in iHMP IBD subjects. Moreover, these two species correlated with colitis in a mouse model sharing histologic features with human UC. These findings show that LEA, AEA and NAES in combination are sufficient to shift *ex vivo* microbial communities from a healthy to an IBD-like composition. This conclusion was reinforced by combined ordination of species profiles from chemostats and PRISM metagenomes (Fig. 4d and Extended Data Fig. 7d). While the first axis of ordination (PCO1) corresponded with differences between in vivo and *ex vivo* communities, the second (PCO2) aligned with variation from non-IBD to IBD in PRISM subjects and control/early to later time points in chemostat samples.

Transcriptional changes highlight an antioxidant response to NAES. To determine how NAES alter bacterial functions, we quantified Kyoto Encyclopedia of Genes and Genomes (KEGG) orthogroup (KO) abundances from our metatranscriptomes and metagenomes, and then regrouped KO abundances to KEGG modules using KEGG’s structured module definitions (Supplementary Dataset 10). Module RNA relative abundance was divided by DNA relative abundance to produce ‘relative expression’, a measure of community functional activity that can be compared between samples over time. Unlike the preceding analyses of community taxonomy, these functional comparisons were performed on a combination of profiles from the two chemostats (treatment source chemostat as a fixed effect), thus enriching for functional changes consistent across both chemostats (see Methods).

As for the effects on community growth, AEA, LEA and NAE-mix elicited the most significant transcriptional responses (Fig. 5 and Extended Data Fig. 8). Significantly altered KEGG modules included amino acid, cofactor, vitamin, carbohydrate, glycan and lipid metabolism functions (Supplementary Table 3). In the presence of AEA, both the anaerobic, reductive (M00150) and the oxidative (M00010) branches of the citrate cycle were upregulated, an effect driven by Enterobacteriaceae (Extended Data Fig. 9). Concomitant with increased energy metabolism was upregulation of the respiratory electron transport chain following treatment with NAES. Given that complex I (M00144) is required for OA metabolism in *E. coli* under aerobic conditions, we tested whether complex I is important for NAES metabolism under anaerobic conditions. We grew wild-type and complex I-deficient (ΔnuoB) *E. coli* strains in the presence of OA, OEA or LEA (Extended Data Fig. 6). Unlike for the wild type, growth of the ΔnuoB mutant was not enhanced by OA or NAES, highlighting the necessity of a functional respiratory chain for NAES metabolism. Also enhanced in the presence of AEA and LEA was ubiquinone, a membrane-associated coenzyme in *E. coli* that transfers electrons from complex I to a final electron acceptor.

**Fig. 5 | Changes in transcriptional activity in complex microbial communities in response to NAES treatment.** We used a linear model combining profiles of functional activity from both chemostats over time to identify metabolic pathways (KEGG modules) that were consistently differentially expressed under NAES treatment relative to DMSO controls (at the community level; total *n* = 96 ranging from *n* = 15 to 17 per treatment). The top 27 such modules ranked by mean absolute log-scaled fold change in relative expression are shown. Each of these modules was significantly differentially expressed under at least one treatment after correcting for multiple hypothesis testing. Pathway–treatment pairs with open circles had FDR *q* values derived from nominal two-tailed *P* values of the ‘treatment’ coefficient across per-module linear regression analyses. While trends were most significant under AEA treatment, effect sizes trends similarly under LEA and OEA treatment as well.
In aerobic conditions, ubinovatecine prevents oxidative stress resulting from fatty acid metabolism\textsuperscript{22}. Our results suggest that its function is equally important for NAE metabolism in anaerobic conditions.

Assuming that NAEs are metabolized like LCFA\textsubscript{s} after cleavage of the ethanolamine group, we investigated the aerobic beta-oxidation module (M00086) and KEGG orthology groups reported to operate in \textit{E. coli} under anaerobic conditions (K12507, K01782 and K00626)\textsuperscript{24} (Supplementary Dataset 10). In the chemostat communities, anaerobic fatty acid degradation functions were unchanged, and M00086 was repressed in response to OA and NAEs, suggesting that the pathways of interest were insufficiently measured or other mechanisms of fatty acid degradation operate in anaerobic conditions. Indicating unsaturated NAE utilization, Streptococca\textec{e} and Enterococcaceae upregulated an oleate hydratase (K10254) (Extended Data Fig. 10), which hydrates double bonds in fatty acids during an early metabolic step. Additionally, community-level functions involved in ethanolamine utilization (K04024 and K04027) were upregulated in response to AEA, LEA and NAE-mix, pointing to NAE breakdown. Ethanolamine is an abundant nutrient source in the gut and many gut bacteria, especially pathogens, carry enzymes for its utilization\textsuperscript{25}. Lipopolysaccharide (LPS) biosynthesis (M00063 and M00064) was also upregulated in Enterobacteriaceae, indicative of increased replication in response to NAEs (Extended Data Fig. 9). LPS potently activates inflammatory responses and is important in IBD pathogenesis\textsuperscript{39}. The aerobic pathway cytochrome ubiquinol oxidase (M00417) was upregulated in response to AEA; this effect, however, was driven exclusively by Enterobacteriaceae in chemostat A during late exposure times, potentially reflecting oxygen tension. The combined metatranscriptomic analysis of two chemostat communities highlighted the necessity of the respiratory chain for NAE metabolism in Enterobacteriaceae.

**Discussion**

The gut metabolome plays an increasingly recognized role mediating interactions between gut microbiota and the host\textsuperscript{3,12}. In this study, we screened a panel of microorganisms and metabolites that were differentially abundant in IB for growth effects, identifying disease-enriched NAEs (AEA, LEA, OEA and PEA) that shift microbial communities towards an IB-like state. AEA, LEA and a combination of all four NAEs were potent drivers of this shift. NAEs are part of the endocannabinoid system and involved in numerous biological processes including energy homeostasis, inflammation and gut barrier function\textsuperscript{3,5,4,4,5,9,11}. Produced primarily by the host\textsuperscript{9}, NAE production varies with diet\textsuperscript{7,39,39}. Endocannabinoid system imbalances are observed in obesity\textsuperscript{11}, type 2 diabetes\textsuperscript{11} and intestinal inflammation\textsuperscript{9,40}. Although the endocannabinoid system is a promising therapeutic target, few studies linked its dysregulation with altered microbiota composition\textsuperscript{11,41}.

To determine the effects of an upregulated endocannabinoid system on gut bacteria, we uncoupled host molecular activity from the microbiota by treating human microbiotal communities grown ex vivo with NAEs. Metagenomic analysis showed that the combination of NAEs enhanced growth of Proteobacteria at the expense of Bacteroidetes while altering Firmicutes composition, reflecting the microbiome composition in IBD\textsuperscript{5,3,4,5,9,11}. Perhaps the most compelling response to NAEs was Enterobacteriaceae expansion, a hallmark of IBD. The inflamed gut is prone to oxygen gradients that diffuse from the tissue surface to the lumen, favouring expansion of facultative aerobes, such as Enterobacteriaceae, while being detrimental to obligate anaerobes\textsuperscript{11,41–14}. Although our study was designed to mimic the anaerobic conditions of the gut, we cannot exclude effects of oxygen tension, which may be reflected in later chemostat A time points. Nonetheless, the comparison of NAE-treated samples with controls recapitulated perturbed microbial communities observed in IB, implicating excessive NAE production as an important contributor to these taxonomic shifts.

For aEA, the NAEs were detected in human stool in the micromolar range and we used higher concentrations in our ex vivo system over short time periods to measure effects that may be undetectable using lower concentrations.

Growth responses to NAEs were consistent between chemostat communities and monocultures, demonstrating that responses are specific to NAE treatment and not only a consequence of bacterial interactions within a community. LEA, AEA and the combination of NAEs induced community-wide behavioural changes: cellular metabolism was upregulated in families whose growth was enhanced by NAEs, and LPS production increased in Enterobacteriaceae, potentially amplifying inflammatory effects on the host. Elevated AEA production increases gut permeability, which augments plasma LPS levels and perpetuates inflammation\textsuperscript{42}. Not only does this create a feedback loop with LPS exacerbating gut barrier disruption but it also promotes endocannabinoid system imbalance, as LPS stimulates AEA production in immune cells\textsuperscript{43,44}.

How Enterobacteriaceae catabolize NAEs remains unknown. Considering NAE structure, the most likely pathway involves an amidase that separates the amine group from the carbon chain, providing a source of ethanolamine that many gut bacteria can utilize\textsuperscript{42,44}. Although anaerobic beta-oxidation functions remained unchanged in our experiments, we cannot exclude the resulting long-chain aldehyde undergoing beta-oxidation after conversion into a carboxylic acid\textsuperscript{42}. We observed clear distinctions between LEA/AEA and OEA/PEA. The more unsaturated LEA/AEA displayed the strongest effects on bacterial growth and gene expression, suggesting that reductases and/or hydratases (such as the oleate hydratase identified in community transcriptomics) play a role in their metabolism and that organisms carrying these enzymes are favoured in the presence of NAEs. Finally, our results show that the membrane-associated respiratory chain in Enterobacteriaceae is indispensable for NAE metabolism, which may be key for survival on NAEs. The electron transfer chain is critical for \textit{E. coli} growth on non-fermentable carbon sources\textsuperscript{9}, further supporting metabolism of NAEs by Enterobacteriaceae.

Our results show that increased NAE levels result in microbial shifts associated with IB pathogenesis and suggest a pro-inflammatory feedback loop, but precise mechanisms of crosstalk between the host and microbiota remain to be determined\textsuperscript{3,11}. As a consequence of this host–microbial pro-inflammatory loop, NAE concentration could be used as a biomarker in IB. While NAEs are probably one of many factors causing taxonomic shifts in IB, our findings encourage the pursuit of therapies targeting NAE metabolism or receptor binding as a means to prevent deleterious changes in gut microbial composition.

**Methods**

**Bacterial strains and culture conditions.** Strains isolated from human subjects are listed in Supplementary Table 2. The following reference strains were used in this study: \textit{E. coli AIEC NC101}, an adherent–invasive murine isolate that produces colitis in monoassociated \textit{IL10}–/– mice, and colibactin, a genotoxin that promotes development of colorectal cancer\textsuperscript{11,12}; \textit{R. gravis ATCC 29149} and \textit{B. cellulositycicus DSM 14838} originating from human faecal samples; \textit{B. producta DSM 2950} from a case of septicaemia; \textit{B. fragilis ATCC 25285} from an appendix abscess; and \textit{L. gasseri ATCC 20243} from a human sample. \textit{E. coli} and \textit{B. fragilis} strains were grown in BHI medium (37 g\textsuperscript{-1}, Sigma-Aldrich) supplemented with 1% vitamin K\textsubscript{1}–haemin solution (BD Biosciences). All other strains were grown in BHI medium containing: 5% sterile-filtered fetal bovine serum (Sigma-Aldrich), 1% trace mineral supplement (BD Biosciences), 1 g\textsuperscript{-1}–d-(−)-maltose (Sigma-Aldrich), 1 g\textsuperscript{-1}–l-cysteine (Sigma-Aldrich). Media were sterilized using a Corning filter unit (0.22 pm pore diameter). Strains were grown under anaerobic conditions (atmosphere 5% H\textsubscript{2}, 20% CO\textsubscript{2}, 75% N\textsubscript{2}) in a soft-sided vinyl chamber (Coy Laboratory Products). The identity of each strain was confirmed by sequencing the \textit{rrs} gene (16S ribosomal RNA) using the primers 27F (5ʹ-AGAGTTTGGATCCTCAG-3ʹ) and 1492R (5ʹ-GGTTACCTTGTTACGACTT-3ʹ)\textsuperscript{11}. The \textit{E. coli} strains BW25113 and JW8752 (an isogenic mutant in the gene \textit{nuoB})
were obtained from the Coli Genetic Stock Center. Both strains were grown anaerobically in minimal medium comprising 2x M9 salts (Teknova, no. M1901), 4g/l glucose and 1% trace mineral supplement (ATCC). Strains were preserved in cryotubes (Nunc) at ~80 °C in growth medium containing 25% glycerol.

Metabolites and bacterial screen. The 50 metabolites used in this study are described in Supplementary Dataset 1. All metabolites were brought to 100 mM in DMSO (Sigma-Aldrich, D2438-10ML) except metabolites that were insoluble at this concentration (e.g., sphingosine, creatine, C16:0 LPC, t-carnitine, C18:1 CE, 4-guanidinobutanoic acid, C18:1 LPC, ascorbic acid). Purity was assessed by LC-MS (Supplementary Table 1). Twelve metabolites were of unknown purity because they were not detected by LC–MS, five metabolites were below 50% purity (mostly LCFA), 15 metabolites were 80–89% pure and twenty-eight metabolites were 90–100% pure. Assay Ready Plates were prepared on a Labcyte Echo 555 acoustic dispensing instrument using 100 µl dispensed amounts of stock solutions to the bottom of 384-well plates (low evaporation lid, Costar 3800). Once the transfers were completed, the plates were sealed and vacuum packed. A HighRes Biosolutions automation system integrated all the instruments needed for the creation of Assay Ready Plates. Each metabolite was dispensed in four replicates and 54 controls (0.25% DMSO) were included per plate. One metabolite resistant for each molecule was tested in sterile medium to verify metabolite sterility and 24 wells contained medium alone. Overnight bacterial cultures were diluted 100-fold in appropriate media and 40 µl were dispensed per well containing metabolate, DMSO or neither. The plates were shaken to ensure homogeneity and bacterial growth was monitored anaerobically (absorbance at 600 nm) in a microplate reader (PowerWave HT Microplate Spectrophotometer, BioTek) for 41 h at 37 °C without shaking. Values recorded for DMSO controls and metabolate-treated triplicates were averaged. The values recorded for each metabolite in sterile media were subtracted from the treated mean. Growth curves were plotted using GraphPad Prism version 7.0. Growth rates and carrying capacities were estimated by fitting degree-of-smoothness to measurements of absorbance (OD600 nm) using the UnivariateSpline option in Python's scipy.interpolate package (v0.18.0) with a smoothing factor of 0.002. The maximum interpolated value of each smoothing spline was saved as a robust estimate of carrying capacity (max OD600; Supplementary Dataset 3) while the maximum value of the spline’s first derivative was saved as an estimate of maximum growth rate (Vmax; Supplementary Dataset 3). Screen results were validated in dose assays for seven metabolites (Sigma-Aldrich) using different batches from those used in the single-dose screen (Extended Data Fig. 1). Owing to impurities revealed by LC–MS analysis, LC–MS/MS analysis of NAEs from stool. Strains were preserved in cryotubes (Nunc) at ~80 °C in growth medium containing 25% glycerol. SPF-raised C57BL/6 female donors (n = 7) aged eight to twelve weeks received 5 × 107 purified naive CD4+ T cells suspended in 300 µl of cold sterile 1x PBS (Corning Mediatech) injected into the tail vein. Treated and non-treated control mice (n = 4 females; n = 3 males) were housed individually in SPF conditions. Fresh feces were collected and immediately snap-frozen before and six weeks after T-cell transfer. A colitis histology score of 6–8 was confirmed in mice developing inflammation. C57BL/6 mice (n = 4 females) and C57BL/6 Rag2−/− mice (n = 11 females, n = 5 males) were randomly chosen from two and three independent litters, respectively. The numbers were empirically determined on the basis of studies using colitis disease models to achieve statistically significant effects.

Approval for human patient research. Human patient research in the PRISM cohort was reviewed and approved by the Partners Human Research Committee (ref. 2018-P-001067). Stool samples from healthy subjects used in the chemostat experiment were obtained under protocol approved by the institutional review board at MIT (IRB protocol ID no. 1510271617631). Participants under both protocols provided informed consent and all experiments adhered to the regulations of the review boards.

Extraction of NAES from stool. Human (PRISM cohort) and mouse (C57BL/6 Rag2−/−) stool was acquired by 2 days post-colonization. Inactivity and metabolite-treated triplicates were averaged and are represented in Extended Data Fig. 6.

Mice. C57BL/6 and Rag2−/− mice were bred and housed in SPF conditions at the National Gnotobiotic Rodent Resource Center at the University of North Carolina at Chapel Hill (UNC-CH). Mice were fed autoclaved water and a standard rodent chow diet (Teklad, cat. no. TD200). Animal experiments were approved by the UNC-CH Institutional Animal Care and Use Committee (Protocol no. 15-1345).

Adaptive T-cell transfer. Splenic cells were collected from SPF-raised C57BL/6 female donors (n = 4) aged eight to twelve weeks and were mechanically dissociated in RPMI1640 (Gibco/Life Technologies) containing 100 U/ml penicillin–streptomycin (Gibco/Life Technologies). Red blood cells were lysed (Sigma-Aldrich) and naïve T cells (CD4+CD44− T cells) were magnetically purified by negative-sorting with anti-CD8a, CD11b, CD11c, CD19, CD25, B220, CD49b, CD105, MHC class II, Ter-119, TCR-γδ and CD44 microbeads (Miltenyi Biotec). Purity of naïve CD4+ T cells was confirmed to be greater than 98% by flow cytometry using anti-CD3, TCR-β, CD4, CD8, CD44, CD62L and CD45 antibodies; while cell viability was shown to be greater than 94% by exsion. SPF-raised C57BL/6 Rag2−/− female recipient mice (n = 7) aged eight to twelve weeks received 5 × 107 purified naive CD4+ T cells suspended in 300 µl of cold sterile 1x PBS (Corning Mediatech) injected into the tail vein. Treated and non-treated control mice (n = 4 females; n = 3 males) were housed individually in SPF conditions. Fresh feces were collected and immediately snap-frozen before and six weeks after T-cell transfer. A colitis histology score of 6–8 was confirmed in mice developing inflammation. C57BL/6 mice (n = 4 females) and C57BL/6 Rag2−/− mice (n = 11 females, n = 5 males) were randomly chosen from two and three independent litters, respectively. The numbers were empirically determined on the basis of studies using colitis disease models to achieve statistically significant effects.
Sigma–Aldrich). The solution was adjusted to pH 6.7–6.8 and autoclaved. A sterile vitamin mix consisting of 2 mg biotin, 2 mg folic acid, 10 mg pyridoxine, 5 mg thiamine HCl, 5 mg riboflavin, 5 mg nicotinic acid, 5 mg Ca-pantothenate, 0.1 mg vitamin B12, 5 mg p-aminobenzoic acid and 5 mg L-lysine hydrochloride (all chemicals from Sigma–Aldrich) was added to the autoclaved medium.

Complex communities were anaerobically retrieved from the chemostats on day 8 (chemostat A) and day 11 (chemostat B), imported into the anaerobic chamber and diluted fivefold in modified YCFA medium. The diluted communities were divided into 4 ml aliquots in 50-ml Falcon tubes to which DMSO (0.5%), PEA (500 µM), OEA (500 µM), LEA (500 µM), AEA (500 µM) or a combination of all four NAEs (125:125:125:125 µM PEA/OEA/LEA/AEA) was added in duplicate. Samples (150 µl) for metagenomic and metatranscriptomic analyses were collected before treatment and at 1 h, 4 h, 8 h, 12 h and 24 h (chemostat A) alone after treatment and were frozen at −80°C. Total DNA and RNA were extracted using the AllPrep Power Soil kit (Qiagen) after thawing the aliquots on ice, pelleting cells and discarding the supernatant.

Generation of RNA sequencing data. Illumina complementary DNA libraries were generated using a modified version of the RNA-seq protocol57. Briefly, 500 ng of total RNA was fragmented, dephosphorylated and ligated to DNA adapters carrying 5′-AN₃-3′ barcodes of known sequence with a 5′ phosphate and a 3′ blocking group. Barcoded RNAs were pooled and depleting of RNA using the Riboprint rRNA depletion kit (Epitect). Pools of barcoded RNAs were converted to Illumina cDNA libraries in two main steps: SMARTScribe (Takara) reverse transcription of the RNA into cDNA at the constant region of the barcoded adapter and addition of an adapter to the 3′ end of the cDNA by template switching58; and PCR amplification using primers whose 5′ end target the constant regions of the 3′ or 5′ adapters of the cDNA and whose 3′ end contains the full Illumina P5 or P7 sequences. cDNA libraries were sequenced on an Illumina NextSeq for monoculture transcriptomic data and on the Illumina HiSeq 2500 platform to generate paired-end reads for metatranscriptomic data.

Generation of DNA sequencing data. Illumina DNA sequencing libraries were prepared from 100–250 pg of DNA using the Nextera XT DNA Library Preparation Kit (Illumina) according to the manufacturer’s recommended protocol, with reaction volumes scaled accordingly. Equal volumes (200 nl) of each library were pooled and insert sizes and concentrations for each pooled library were determined using an Agilent Bioanalyzer DNA 1000 kit (Agilent Technologies). Metagenomic libraries were sequenced on the HiSeq 2500 platform (Illumina), targeting ~1 Gb per sample with 101-base-pair, paired-end reads.

Analysis of monoculture RNA sequencing data. Sequencing reads from each sample in a pool were demultiplexed on the basis of their associated barcode sequence using custom scripts (https://github.com/broadinstitute/split_merge_pl). Up to one mismatch in the barcode was allowed provided it did not make assignment to the read to a different barcode possible. Barcode sequences were removed from the first read as were terminal Gs from the second read that may have been added by SMARTScribe during template switching. Reads were aligned to the B. fragilis ATCC 25285 genome using the Burrows–Wheeler alignment tool59, and read counts were assigned to genes and other genomic features on the genome using GenomeView61.

Analysis of metagenomic and metatranscriptomic data. We analysed our metagenomes and metatranscriptomes with the bioBakery metaworksflows introduced in McVerrry et al.62. Briefly, we first quality controlled sequencing reads using the KneadData pipeline (v0.7.1), which trims low-quality bases and reads from the metâome using Trimmomatic and then maps remaining high-quality reads to human genome and transcript databases to deplete host contamination (which was expected to be minimal due to the stool being incubated in a chemostat for long periods of time; chemostat A for eight days and chemostat B for 11 days). We profiled community taxonomy from sample metagenomes using MetaPhlAn2 (v2.2.0) analyse and community gene family (UniRef90) abundance using HUMAnN2 (v0.11.1)63. We further profiled community transcript family (UniRef90) abundance from sample metatranscriptomes using HUMAnN2, using the taxonomy profile of each paired genome as a guide.

We regrouped community gene and transcript abundances to KO abundance using HUMAnN2 utility scripts. We then fed these KO abundances back into HUMAnN2 to compute KEGG module abundance using module definitions from January 2019. This process was carried out with HUMAnN2’s ‘inpath’ and ‘gamp’fill’ options disabled to exclude all modules and add robustness to small modules, respectively. Module abundances were normalized to relative abundance units while excluding unincorporated genes and transcript read mass. We then computed module relative expression as a measure of functional activity by taking the log ratio of the module’s RNA relative abundance to its DNA relative abundance (thus adjusting for the tendency of more broadly encoded modules to have higher raw abundance in metatranscriptomes). When computing relative expression, we treated 0/0, x/0 and 0/x ratios as nan, inf and −inf, respectively (for x > 0).

We assessed the differential abundance of taxa between DMSO- and NAE-treated samples within each chemostat separately to compensate for their distinct initial taxonomic compositions. Zero-valued taxa were additionally smoothed within-sample by half the sample’s smallest non-zero taxon abundance. All abundances were then log-transformed to variance stabilize the data before statistical testing. For each taxon exceeding 0.1% (pre-transformed) relative abundance in at least two samples from the same treatment group, we tested differential abundance over treatments using the following linear model evaluated with R’s lm function (v3.2.3): log(relative abundance) ~ treatment + time + replicate

Coefficients of the different NAE treatments were computed relative to the control (DMSO) level. As these coefficients reflect the difference in mean log-transformed abundance under treatment versus control, we interpret them as a summary estimate of log-transformed fold change in abundance after treatment. The statistical significance values (P values) for the treatment coefficients were subjected to Benjamini–Hochberg FDR control within each treatment.

We computed differential relative expression of KEGG modules over a combination of profiles from both chemostats using the above-described log-transformed relative expression values. (Unlike the per-chemostat taxonomic analysis, this analysis therefore enriches for conserved changes in functional activity in distinct taxonomic backgrounds.) To be analysed, a module was required to have finite relative expression in: at least five samples from two different treatment groups; and at least one sample from each replicate. Such modules were then analysed with nan, inf and −inf ratios excluded. Modules were analysed using the following linear model in base R:

log(relative expression) ~ treatment + time + chemostat/replicate

which incorporates a fixed effect for chemostat and for each replicate within each chemostat. Coefficients for treatments and their P values were processed as described above for the models of taxonomic abundances.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Metagenomic, metatranscriptomic and transcriptomic data are available in the NCBI Sequence Read Archive as BioProject PRJNA532456. Tables of processed mouse microbial species, monoculture transcriptomic data and chemostat microbial species are available as Supplementary Datasets 6, 7 and 8, respectively. Source data for Figs. 1 and 2 and Extended Data Figs. 3 and 4 are provided with the paper.

Code availability
Custom scripts used to analyse monoculture transcriptomic data are available at https://github.com/broadinstitute/split_merge_pl. The bioBakery tools (KneadData, MetaPhlAn2 and HUMAnN2) used to process metagenomic data from the two chemostats are available via https://hattenhower.sph.harvard.edu/biobakery as source code and installable packages. Downstream analyses were conducted using custom Python and R scripts. This code (and associated usage notes) is available from the corresponding authors upon request.

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Author contributions
N.F., A.C.T., H.V. and R.J.X. designed the research. N.F. performed bacterial growth and chemostat experiments. E.A.F. and J.B. analysed metagenomic and metatranscriptomic data. J.W.A. performed mass spectrometry analysis. A.O. and R.B.S. provided mouse stool and helped with mass spectrometry data interpretation. J.L.-P. analysed PRISM and iHMP metabolomics data. T.D.A. and A.G. contributed chemostat communities. T.D.A. also provided bacterial isolates. J.A.-P. confirmed the presence of AEA in PRISM and iHMP stool. N.F., H.J.H., J.A.P., C.B.C., C.H., H.V. and R.J.X. supervised the project. R.B.S., C.H. and R.J.X. acquired funding.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Validation of metabolite screen results in dose assays. Growth curves are shown for three strains using seven metabolites in multiple concentrations. Growth was monitored over time in a volume of 40 µL per well in 384-well plates. The final concentration of DMSO per treated and control well was 0.25%. Growth curves representative of three independent tests are shown and error bars in controls represent the standard deviation of the mean of six technical replicates.
Extended Data Fig. 2 | Correlation between absolute and relative NAE abundances in stool from PRISM subjects. NAEs detected in stool from PRISM Crohn’s disease (CD) patients (n=21) in absolute abundances (ng mg⁻¹) are plotted against their respective relative abundances. Pearson correlation coefficients (r) are shown. Progenesis QI (nonlinear DYNAMICS) was used for the extraction of non-targeted LC-MS features and TraceFinder (Thermo Fisher Scientific) was used for the manual peak extraction of known metabolites on basis of their mass to charge ratio (m/z) and retention times determined using authentic standards.
Bacterial species elevated in PRISM IBD subjects

Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Growth effects of NAEs on intestinal bacteria elevated in IBD. Palmitoylethanolamide (PEA), linoleoyl ethanolamide (LEA), oleoyl ethanolamide (OEA) and arachidonoyl ethanolamide (AEA) were added to growing cells (in the range of 10^6 to 10^8 CFU mL⁻¹) in three concentrations (0 µM, 50 µM and 100 µM), and growth was monitored in an absorbance reader in the anaerobic chamber over time. Controls contained 0.4% DMSO. Growth curves representative of two independent experiments are shown and error bars represent the standard deviation of the mean of three technical replicates.
Bacterial species depleted in PRISM IBD subjects

Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Growth effects of NAEs on intestinal bacteria depleted or invariable in IBD. Palmitoylethanolamide (PEA), linoleoyl ethanolamide (LEA), oleoyl ethanolamide (OEA) and arachidonoyl ethanolamide (AEA) were added to growing cells (in the range of $10^6$ to $10^8$ CFU mL$^{-1}$) in three concentrations (0 $\mu$M, 50 $\mu$M and 100 $\mu$M), and growth was monitored in an absorbance reader in the anaerobic chamber over time. Controls contained 0.4% DMSO. Growth curves representative of two independent experiments are shown and error bars represent the standard deviation of the mean of three technical replicates.
Extended Data Fig. 5 | Transcriptional responses of *Bacteroides fragilis* to linoleoyl ethanolamide (LEA) and arachidonoyl ethanolamine (AEA).

**a**, Differential gene expression between three independent exponential cultures treated for 10 minutes with a sub-inhibitory concentration (25 µM) of LEA or AEA and controls (0.04% DMSO). Differential expression was determined with edgeR, and gene functions were defined using InterPro (EMBL), the NCBI Conserved Domain Database (CDD) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Selected significantly differentially expressed genes (|log2 fold-change (treated/control)| > 0.5, FDR < 0.05; Benjamini-Hochberg FDR values were derived from p-values calculated using the likelihood-ratio test) are shown in color.

**b**, Genomic environment of the differentially expressed genes using colors that correspond with (a). Genes in white were not significantly differentially expressed. Coordinate maps refer to the genome of strain *B. fragilis* ATCC 25285. Gene products that have been experimentally shown to be associated with the outer membrane by LC-MS/MS analysis are in bold (Wilson, M. M., Anderson, D. E. & Bernstein, H. D. Analysis of the outer membrane proteome and secretome of *Bacteroides fragilis* reveals a multiplicity of secretion mechanisms. *PLoS ONE* **10**, e0117732 (2015)).
Extended Data Fig. 6 | Oleic acid, oleoyl ethanolamide and linoleoyl ethanolamide do not enhance growth of a complex I mutant. Growth of wild-type (WT) E. coli BW25113 and a derivative deficient for complex I (NADH:quinone oxidoreductase, ΔnuoB) in minimal medium (M9 with glucose 4 g L⁻¹ and 1% trace minerals) supplemented with EtOH 0.05%, 3.5 µM, 7 µM and 14 µM of oleic acid (OA), oleoyl ethanolamide (OEA) or linoleoyl ethanolamide (LEA). Growth curves representative of two independent experiments are shown and error bars represent the standard deviation of the mean of three technical replicates.
Extended Data Fig. 7 | Effects of NAEs on the composition of a complex microbial community. a, Taxonomic abundances in chemostat B at the family level. Vertical colored bars represent the relative abundance of bacterial families in samples 1 and 12 hr after addition of DMSO (0.5%), individual NAEs, or a combination of all four NAEs (denoted as NAE-mix). Individual NAEs were added to a final concentration of 500 µM. In combination, the PEA:OEA:LEA:AEA ratio was 125:125:125:125 µM. Heatmaps show log₂ fold-changes in b, family- and c, species-level taxonomic abundances between treated samples and DMSO controls (total n=41 with per-treatment n ranging from 6 to 7). Species that shifted with statistical significance in response to treatment are shown (* q<0.20, ** q<0.05; FDR q-values derived from nominal two-tailed p-values of the “treatment” coefficient across per-taxon linear regression analyses). Species enriched (red) and depleted (blue) in PRISM CD stool relative to controls (q≤0.1) are indicated. d, Principal coordinate (PCo) analysis on Bray-Curtis dissimilarities between chemostat B (n=21) and PRISM (n=155) metagenomes. Times of exposure to AEA, LEA and DMSO control are indicated.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Changes in transcriptional activity in complex microbial communities in response to NAE treatment. We used a linear model combining profiles of functional activity from both chemostats over time to identify gene families (KEGG orthologies) that were consistently differentially expressed under NAE treatment relative to DMSO controls (at the community level; total n=96 ranging from n=15 to 17 per treatment). The top 100 such orthologies ranked by mean absolute log-scaled fold change in relative expression are shown. Each of these orthologies was significantly differentially expressed under at least one treatment after correcting for multiple hypothesis testing. Pathway–treatment pairs with open circles had FDR $q<0.2$; those with closed circles had FDR $q<0.05$, while an "x" indicates that measurements were insufficient to perform the test (FDR $q$-values derived from nominal two-tailed $p$-values of the ‘treatment’ coefficient across per-orthology linear regression analyses). While trends were most significant under AEA treatment, effect sizes trended similarly under LEA and OEA treatment as well.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Changes in transcriptional activity (KEGG modules) in complex microbial communities in response to NAE treatment. Log$_2$ relative expression is shown for bacterial families contributing to a selection of KEGG modules in samples from chemostats A and B treated with DMSO control, individual NAEs, a combination of all four NAEs (denoted as NAE-mix), or oleic acid (OA). Family-level relative expression values were computed at the species level, averaged over replicates, and then averaged within-family while weighting by species abundance. Unknown (“x”) values represent cases where a function’s DNA and/or RNA abundance were zero for a given stratification (resulting in non-finite log$_2$ relative expression). Col_sort refers to the measure (treatment, time or chemostat) used to order the metadata columns.
Extended Data Fig. 10 | Changes in transcriptional activity (KEGG orthologies) in complex microbial communities in response to NAE treatment. Log$_2$ relative expression is shown for bacterial families contributing to a selection of KEGG orthologies in samples from chemostats A and B treated with DMSO control, individual NAEs, a combination of all four NAEs (denoted as NAE-mix), or oleic acid (OA). Family-level relative expression values were computed at the species level, averaged over replicates, and then averaged within-family while weighting by species abundance. Unknown (“x”) values represent cases where a function’s DNA and/or RNA abundance were zero for a given stratification (resulting in non-finite log$_2$ relative expression). Col_sort refers to the measure (treatment, time or chemostat) used to order the metadata columns.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Gen5 Data Analysis Software (BioTek) was used to collect PowerWave HT Microplate Spectrophotometer (BioTek) reads. FACSDiva software version 6.0 (BD Biosciences) was used to sort mouse T-cells in flow cytometry. Progenesis QI (nonlinear DYNAMICS) was used for the extraction of non-targeted LC-MS features and TraceFinder (Thermo Fisher Scientific) was used for the manual peak extraction of known metabolites on basis of their mass to charge ratio (m/z) and retention times determined using authentic standards. LC-MS/MS data acquisition and analysis were performed in Analyst 1.6.3 (Sciex).

Data analysis

FlowJo software version 10 (FlowJo) was used to analyze mouse T cell populations sorted in flow cytometry. Differential expression analysis of transcriptomic data was conducted with edgeR. Metagenomic and metatranscriptomic data were pre-processed with the free and open biobakery workflow (kneadData v0.7.1 for read-level quality control, MetaPhlAn2 v2.2.0 for taxonomic profiling, and HUMAnN2 v0.11.1 for functional profiling). Statistical analyses were carried out with free and open packages in Python (scipy, matplotlib, statsmodels, and scikit-learn) and R (vegan). Additional software details are provided in the Methods and Code Availability statement.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Metagenomic, metatranscriptomic and transcriptomic data are available in NCBI Sequence Read Archive as BioProject PRJNAS32456.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

No statistical methods were used to predetermine sample size. Absolute concentrations of N-acyl ethanolamines were measured from 16 non-IBD, 15 UC, and 21 CD patient stool samples, as indicated. The T-cell transfer experiment was performed with 7 mice per group, as indicated. Animal numbers were empirically chosen based on studies using colitis disease models (e.g. PMID: 15286805) to achieve statistically significant effects. Chemostat experiments were repeated with 2 independent source samples, as indicated.

**Data exclusions**

No data was excluded.

**Replication**

The metabolite screen was performed once using technical triplicates and multiple dose growth assays were performed in duplicate or triplicate, as indicated. Chemostat experiments were repeated with 2 independent source samples, as indicated. All attempts at replication were successful.

**Randomization**

Human stool samples for measuring N-acyl ethanolamine concentrations were randomly picked across the three study groups (16 non-IBD, 15 UC, and 21 CD). C57BL/6 mice (n=4 females) and C57BL/6 Rag2−/− mice (n=11 females, n=3 males) were randomly chosen from two and three independent litters, respectively. T cell donors and recipients were females as recommended in PMID: 19033538.

**Blinding**

n/a

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐  | Antibodies            |
| ☒  | Eukaryotic cell lines |
| ☒  | Palaeontology         |
| ☒  | Animals and other organisms |
| ☒  | Human research participants |
| ☒  | Clinical data         |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | ChiP-seq              |
| ☒  | Flow cytometry        |
| ☒  | MRI-based neuroimaging|

### Antibodies

Antibodies used: Antibodies used in flow cytometry:

- PE-Cy5-conjugated CD3 (eBioscience; catalog no. 15-0031-81) and isotype IgG (Biolegend; catalog no. 400931);
- AF700-conjugated TCR-β (BD Biosciences; catalog no. 560705) and isotype IgG2 lambda (BD Biosciences; catalog no. 557985);
- APC-conjugated CD4 (Biolegend; catalog no. 100516) and isotype IgG2a (BD Biosciences; catalog no. 554690);
- PE-Cy7-conjugated CD8a (eBioscience; catalog no. 25-0081-81) and isotype IgG2a (Biolegend; catalog no. 400521);
- BV650-conjugated CD44 (BD Biosciences; catalog no. 740455) and isotype IgG2b (BD Biosciences; catalog no. 563233);
- BV605-conjugated CD62L (BD Biosciences; catalog no. 563252) and isotype IgG2a (Biolegend; catalog no. 400539);
- Pacific Orange-conjugated CD45 (Invitrogen; catalog no. MCD4530) and isotype IgG2b (BD Biosciences; catalog no. 553989).

### Validation

Antibody validation can be found on the respective manufacturers websites, including relevant citations using these antibodies.

### Animals and other organisms

Policy information about **studies involving animals**: **ARRIVE guidelines** recommended for reporting animal research.

#### Laboratory animals

|      | Involved in the study |
|------|-----------------------|
| ☒    | C57BL/6 mice (n=4 females, 8-12 weeks old ) |

#### Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals...
| Section                  | Description                                                                                                                                 |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Wild animals             | (were caught and transported and what happened to captive animals after the study [if killed, explain why and describe method; if released, say where and when]) OR state that the study did not involve wild animals. |
| Field-collected samples  | For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field. |
| Ethics oversight         | Animal experiments were approved by the UNC-CH Institutional Animal Care and Use Committee (Protocol# 15-345).                                |

Note that full information on the approval of the study protocol must also be provided in the manuscript.