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RESEARCH ARTICLE

Helminth-Induced Human Gastrointestinal Dysbiosis: a Systematic Review and Meta-Analysis Reveals Insights into Altered Taxon Diversity and Microbial Gradient Collapse

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ABSTRACT High-throughput 16S rRNA sequencing has allowed the characterization of helminth-uninfected (HU) and helminth-infected (HI) gut microbiomes, revealing distinct profiles. However, there have been no qualitative or quantitative syntheses of these studies, which show marked variation in participant age, diet, pathogen of interest, and study location. A predefined minimally biased search strategy identified 23 studies in humans. For each of these studies, we qualitatively addressed the effects of helminth infection on within-individual (alpha) and between-individual (beta) fecal microbiome diversity, infection-associated microbial taxa, the effect of helminth clearance on microbiome composition, microbiome composition as a predictor of infection status or treatment outcome, and treatment-specific effects on the fecal microbiome. Concomitantly, we performed a meta-analysis on a subset of 7 of these studies containing raw, paired-end 16S reads and individual-level metadata, comprising 424 pretreatment or untreated HI individuals and 497 HU controls. After reducing the batch effect and adjusting for age, our data demonstrated that intestinal helminth parasites can alter the host gut microbiome by increasing alpha diversity and promoting taxonomic reassortment and gradient collapse. Most strongly influencing the microbiome composition were the helminths found in the large intestine, *Enterobius vermicularis* and *Trichuris trichiura*, suggesting that this influence appears to be specific to soil-transmitted helminths (STH) species and host anatomical niche. In summary, using a large and diverse sample set captured in the meta-analysis, we were able to evaluate the influence of individual helminth species as well as species-species interactions, each of which explained a significant portion of the variation in the microbiome.

IMPORTANCE The gut microbiome has established importance in regulating many aspects of human health, including nutrition and immunity. While many internal and environmental factors are known to influence the microbiome, less is known about the effects of intestinal helminth parasites (worms), which together affect one-sixth of the world's population. Through a comprehensive qualitative systematic review and quantitative meta-analysis of existing literature, we provide strong evidence that helminth infection dynamically shifts the intestinal microbiome structure. Moreover, we demonstrated that such influence seems to be specific to helminth species and host anatomical niche. Our findings suggest that the gut microbiome may underlie some of the pathology associated with intestinal worm infection and support future work to understand the precise nature of the helminth-microbiome relationship.

KEYWORDS helminth, intestinal bacteria, intestinal parasites, microbiome, nematodes, soil-transmitted helminth

Helminth parasites belong to a diverse taxonomic group of complex metazoans with differences in their biological life cycles along with marked variation in tissue tropism and habitat (1). Helminth infections are among the most common communicable

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diseases worldwide, affecting more than one-sixth of the world's population, predominantly in low- and middle-income countries (2). Among these parasites are those termed soil-transmitted helminths (STH), which collectively include roundworms (*Ascaris lumbricoides* and *Strongyloides stercoralis*), hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworms (*Trichuris trichiura*), pinworms (*Enterobius vermicularis*) and others. The STH are marked by long-standing chronic intestinal infections in adults and infections of variable duration in children, punctuated by annual or biannual anthelmintic treatment. Clinical expressions of STH infections range from asymptomatic (or subclinical) infections to more serious conditions that include intestinal occlusion, malnutrition, rectal prolapse, and delayed development in children (3–5).

The gut microbiome includes all bacteria, fungi, protozoa, archaea, and viruses that reside in and along the gastrointestinal tract and may exist parasitically or commensally (6). The gut microbiome plays important roles in metabolism, gut epithelial health, and innate and adaptive immune responses (7, 8). As the STH and microbes have coevolved throughout evolutionary history in the gastrointestinal niche, it is hypothesized that they have adapted to exert dynamic influences on each other (9). The growing feasibility of large-scale, high-throughput sequencing (HTS) of 16S rRNA has allowed large-scale testing of normal and dysbiotic microbiomes, including those from helminth-infected (HI) and helminth-uninfected (HU) individuals across a wide range of ages and backgrounds. Such comparisons reveal differential microbiota signatures in HU and HI guts.

However, significant environmental variation (diet, sanitation, worm burden, etc.) and the batch effect introduced by sample collection and processing make it difficult to reach a consensus on the effects of helminths on the gut microbiome and vice versa. A summary review of the literature reveals inconsistent associations between helminth infection and parameters (e.g., species richness and diversity and taxonomic abundance) commonly used to examine the microbiome (10, 11). These inconsistencies suggest that host genetics, nutrition, anthelmintic treatment history (12), and coinfections may influence the effects of helminths on the microbiome; this reinforces the need for meta-analyses to draw cross-study and cross-population conclusions.

Here, we provide a comprehensive systematic review and meta-analysis using human 16S rRNA sequence data sets from the human fecal microbiome linked to individual-level metadata to investigate the relationship between intestinal helminths and the fecal microbiome. By defining a search strategy *a priori* to minimize selection bias and examining studies from diverse populations and age groups, we suggest, through a minimally biased synthesis of available scientific literature on the microbiome-helminth interface, that intestinal helminth parasites can alter the host gut microbiome by increasing alpha diversity and promoting taxonomic reassortment and gradient collapse, findings that appear to be STH species and host anatomical niche specific. We found that *Trichuris trichiura* and *Enterobius vermicularis*, found in the large intestine, appear to most strongly influence the microbiome composition compared with other helminth species captured in the study (*Ascaris*, hookworm, *Strongyloides*, and *Haplorchis taichui*), all of which dwell in the small intestine.

RESULTS

Study selection. Keyword searches of PubMed and CENTRAL databases yielded 629 unique articles, from which 153 reviews were excluded. Of the remaining 476 full-length articles assessed for eligibility, 453 were excluded as they failed to meet the inclusion criteria. Twenty-three full-length articles were used for the qualitative analyses; a subset of 7 studies were included in the quantitative synthesis (Fig. 1). Publication years ranged from 2013 to 2020, and the studies comprised individuals from the Americas, Africa, Europe, and Asia (Fig. 2). The studies included anthelmintic ("deworming") treatment arms and noninterventional observational studies. The most common helminth infection in these studies was *Trichuris trichiura* (n = 581; 22% cross-study prevalence), followed by hookworm species (n = 402; 15%) and *Ascaris* spp. (n = 401; 15%), while many individuals were

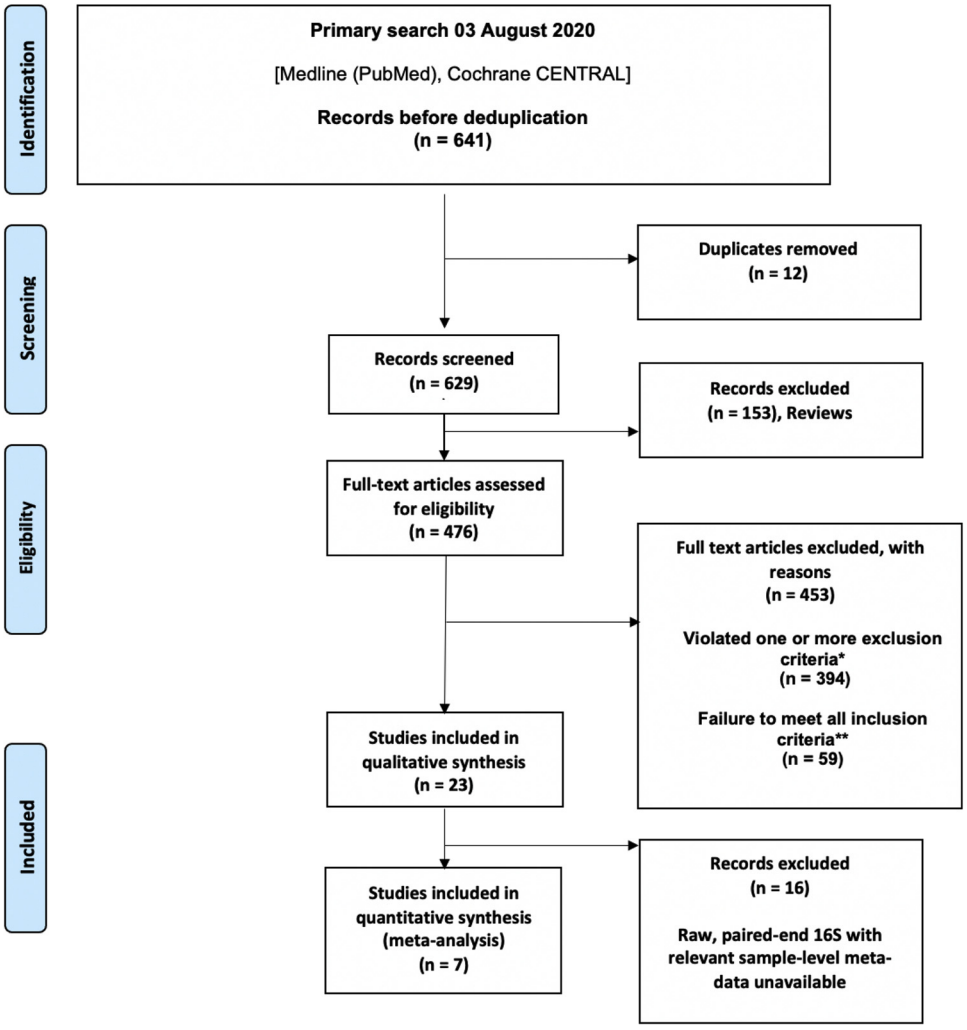

FIG 1 PRISMA flow diagram of the study selection process. Study selection was performed according to the most recent Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. *, Exclusion criteria included nonhuman host, nonintestinal helminth, intestinal comorbidity, focus exclusively on helminth's microbiome, and no experimentation or data collection. **, Inclusion criteria included publication between 1 January 1975 and 3 August 2020, helminth infection with intestinal involvement, and human studies with ≥10 participants.

infected with multiple helminths. Individual helminth infection numbers are likely higher than we have summarized here, as some studies did not specify beyond infected or uninfected status. Of 23 selected human studies, 21 contained unique data sets while 2 (13, 14) provided new analyses of data sets redundant with other selected studies. One study did not provide individual-level helminth species identification for HI individuals (15), and another reported two individuals infected with unspecified helminth larvae (16).

Alpha and beta diversity are altered in human helminth infection. Of the 21 selected studies with unique sample data sets, 17 (81%) assessed changes in within-individual fecal microbiome diversity (alpha diversity). Six of 17 (35%) studies that evaluated alpha diversity found no significant differences when comparing HI and HU individuals (17–22); 7 (41%) found increased alpha diversity in HI individuals (15, 16, 23–27), and 4 (24%) found greater alpha diversity in HU individuals (28–31). On the other hand, 14 studies assessed the beta (between-individual) diversity (16–22, 24–29, 32). In summary, the majority of these studies ($N = 8$) found that beta diversity was significantly influenced by helminth infection (16, 20, 21, 24–27, 29) (Table 1).

Fecal microbiota operational taxonomic units (OTUs) from 16S high-throughput sequencing were analyzed in 20 of the selected studies. Multivariate analysis of

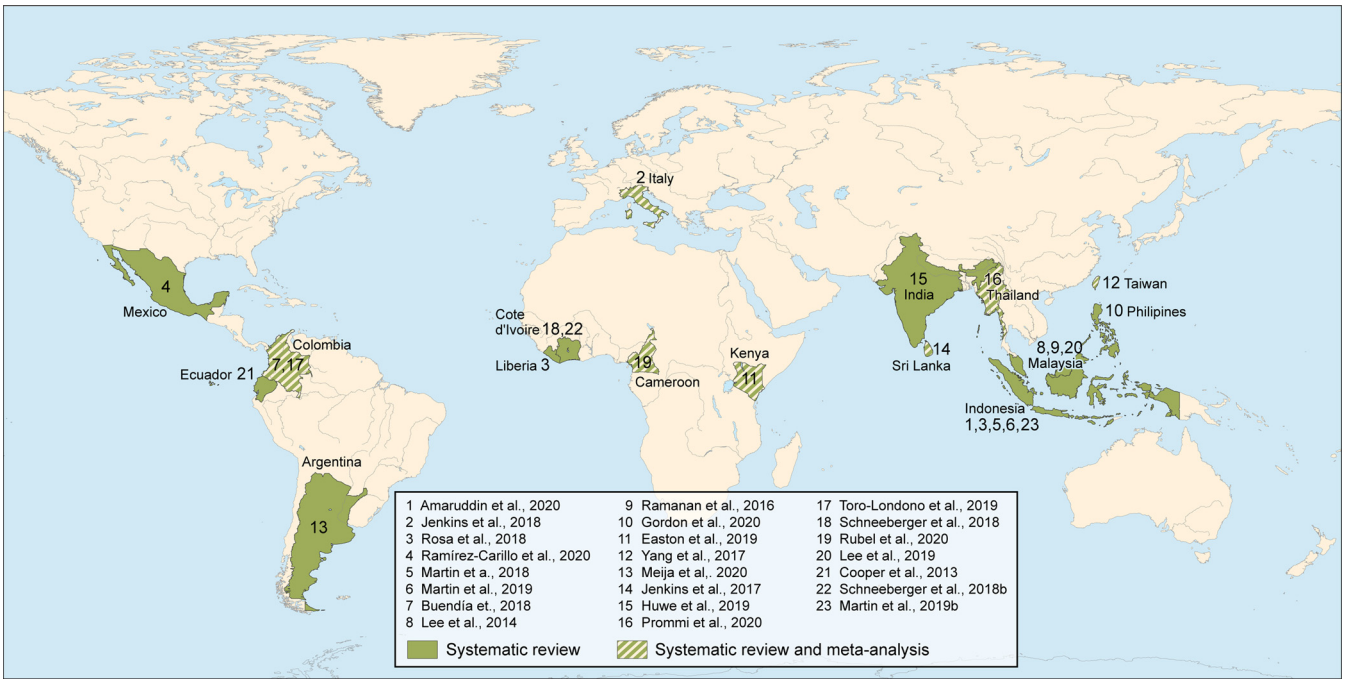

FIG 2 Study population. World map highlighting the countries where the 23 studies selected for the systematic review and 7 studies selected for the meta-analysis obtained their data. Buendía et al., 2018 (32) was used just for the systematic review.

sequencing data and helminth infection, including principal coordinates analysis (PCoA) and canonical correspondence analysis (CCA) found that the overall microbiome composition significantly differed based on infection status in a slight majority (13/21 [62%]) of studies with unique 16S rRNA gene sequencing data sets. Seventeen of these studies (85%), regardless of whether they found significant overall compositional differences, described at least one OTU which was differentially abundant in the HI and HU groups. The most cited infection-associated fecal microbiome taxa were in the order *Bacteroidales* (including the genera *Bacteroidetes* and *Prevotella*) or the family *Ruminococcaceae*. *Ruminococcaceae* were almost exclusively positively associated with infection (27, 29, 33) (negatively associated in one study [25]), even after controlling for environmental factors such as diet, and were further shown to positively correlate with worm burden, as measured by eggs per gram of stool (33). The direction of association between *Bacteroidales* and helminth infection was less consistent, with findings of positive (16, 25) and negative (20, 24, 26, 34) associations, bacterial species-specific associations (28), and a possible influence of anthelmintic treatment history (20) (Table S2).

Meta-analysis of the helminth-influenced fecal microbiome. A total of 7 studies containing raw, paired-end 16S reads and individual-level metadata (including age, sex, and helminth infection and treatment status) were included in our meta-analyses. Meta-analysis focused exclusively on individuals without documented recent anthel-

TABLE 1 Helminth infection impacts within-individual (alpha) and between-individual (beta) gut microbiome taxa diversity[a]

	Studies showing:		
Diversity	Higher diversity in infected individuals	Higher diversity in uninfected individuals	No difference
Alpha	15, 16, 23–26,[b] 27	28–31	17–22
Beta[d]	20, 21,[c] 26[b]	24	17–19, 22, 28

[a]Diversities were averages from all 16S OTUs at baseline.
[b]Significant only for the Liberia cohort.
[c]Significant for *Necator* only.
[d]Four studies reported beta diversity significantly changed but the direction was unclear: 16, 25, 27, 29.

TABLE 2 Characteristics of the individuals selected from studies included in the meta-analysis[a]

	Value in reference:							
Parameter	**19**	**20**	**24**	**29**	**25**	**28**	**27**	**Total**
Individuals	139	20	29	28	574	22	109	921
Age								
Infancy (1–3 yr)	11	0	0	0	0	14	0	25
Childhood (4–11 yr)	63	0	0	0	47	8	109	227
Adolescence (12–20 yr)	29	17	0	2	58	0	0	106
Adulthood (21–40 yr)	15	2	0	2	195	0	0	214
Middle age (41–69 yr)	16	0	14	22	233	0	0	285
Advanced age (70+ yr)	5	1	15	2	41	0	0	64
Infection type								
Uninfected (HU)	71	14	11	14	341	16	30	497
Helminth singly infected	68	6	18	14	87	1	79	273
Helminth multi-infected	0	0	0	0	146	5	0	151
Helminth								
Ascaris	36	6	0	0	11	1	0	54
Enterobius	0	0	0	0	0	0	79	79
Haplorchis	0	0	0	14	0	0	0	14
Hookworm NA	0	0	0	0	1	0	0	1
Hookworm N	32	0	0	0	0	0	0	32
Hookworm A	0	0	0	0	7	0	0	7
Strongyloides	0	0	18	0	12	0	0	30
Trichuris	0	0	0	0	56	0	0	56
Various	0	0	0	0	146	5	0	151

[a]Samples included those from untreated or pretreatment individuals where raw, paired-end 16S rRNA reads and relevant associated metadata were available. Hookworm N, *Necator* sp.; Hookworm A, *Ancylostoma* sp.; Hookworm NA, either *Ancylostoma* or *Necator* sp.

mintic treatment history in order minimize treatment effects on the fecal microbiome, which may be significant (19). After filtration for sampling depth, the analyzed cohort contained 921 pretreatment or untreated individuals distributed among the age categories: childhood or adolescence (n = 333 [36%]), adulthood or middle age (n = 499 [54%]) and smaller numbers of infants (n = 25 [2.7%]) and advanced-aged individuals (n = 64 [6.9%]). Sixty-two percent of individuals were female. Four hundred twenty-four individuals (46%) were infected with one or more helminth species, most commonly *Enterobius vermicularis* (n = 79), *Trichuris trichiura* (n = 56), or *Ascaris lumbricoides* (n = 54), while 151 individuals were infected with multiple helminth species (Table 2). After filtering, we were left with 40,918,808 16S rRNA reads (mean reads per sample = 81,220; standard deviation [SD] = 798 reads) comprising 547 taxa. *Prevotella* spp. were the most abundant taxa at 13.1% of all reads; *Lactonifactor* spp. were the least abundant at 5.1×10^{-7}% of all reads.

Heterogeneity statistical analysis revealed a homogenous distribution of study results and minimal reporting bias (Fig. S2A and B). Read depth was largely consistent between samples, and plateauing of rarefaction curves indicated that samples sufficiently represented the diversity of the microbiomes explored (data not shown). Correction reduced (permutational multivariate analysis of variance [PERMANOVA] R^2, 11.6% to 10.5%; analysis of similarity [ANOSIM] R, 14.1% to 12.7%) but did not eliminate the batch effect (Fig. S3). After batch adjustment, microbial community composition was most attributed to (R, ANOSIM) country (49%), helminth infection (12.7%), and age (9.6%) (Table 3). Due to the large effects of age on the microbiome, downstream ANOVA was performed using a model stratified for age category.

Alpha diversity is increased with multispecies helminth infections. ANOVA revealed a significant influence of age on the alpha diversity of HU individuals (Shannon $P = 1.5 \times 10^{-9}$; inverted Simpson index [InvSimpson] $P = 3.7 \times 10^{-9}$); consequently, alpha

TABLE 3 Results of the batch effect analysis

Effect	Before batch adjustment		After batch adjustment	
	PERMANOVA R^2 (%)	ANOSIM R (%)	PERMANOVA R^2 (%)	ANOSIM R (%)
Study ID/country	18	55	14.1	39.9
Infection status (infected vs. uninfected)	0.8	2.6	1.1	4.0
Infection type (single vs. multiple)	3.7	4.5	3.99	4.1
Gender	0.3	2.5	0.38	1.5
Age category	4.7	10.2	4.8	8.6
Helminth species	13.2	14.1	10.3	10.7

diversity analyses were performed with non-age-adjusted and age-adjusted tests. Across all samples, alpha diversity did not significantly differ between HU and HI microbiomes (Shannon $P = 0.673$; InvSimpson $P = 0.553$). Comparing only the multispecies-HI and HU individuals showed significantly higher Shannon (3.275 versus 3.146; $P = 0.0031$) and InvSimpson (15.67 versus 14.12; $P = 0.0035$) diversity with helminth infection. Alpha diversity differences were most pronounced between multiple- and single-helminth-species-infected individuals (Shannon index, 3.275 versus 3.132, $P = 0.0001$; InvSimpson index, 15.67 versus 13.55, $P = 0.0006$) (Fig. 3A and B). Species evenness was homogenous across the cohorts, while species richness was significantly lower in those with helminth infection (Chao1 value, 79.69 versus 85.47; $P = 0.0038$) (Fig. 3C). Interestingly, multispecies-HI individuals presented with markedly increased species richness compared to HU individuals (Chao1 value, 91 versus 85.47; $P = 0.027$) (Fig. 3D). After accounting for the influence of age on the microbiome, alpha diversity was found to be significantly different between HI and uninfected samples (Shannon $P = 9 \times 10^{-15}$; InvSimpson $P = 3.3 \times 10^{-10}$), suggesting that infection status influences the alpha diversity of the microbiome. Age-nested ANOVA reaffirmed the differences between single- and multiple-helminth-species-infected microbiomes (Shannon $P = 2.8 \times 10^{-11}$; InvSimpson $P = 3.5 \times 10^{-8}$). Alpha diversity indices for individual helminth species categories that were ANOVA stratified per age category were both significant (Shannon $F = 2.122$, $P = 7.6 \times 10^{-11}$; InvSimpson $F = 1.99$, $P = 1.1 \times 10^{-8}$), indicating that helminth species identity has a significant effect on the alpha diversity of the microbiome (Fig. 3E and Fig. S4). To confirm these results, the cumulative groups were removed and the test rerun, confirming a helminth species-specific effect on the alpha diversity of the microbiome (Shannon $P = 6.5 \times 10^{-7}$; InvSimpson $P = 5.3 \times 10^{-4}$). Sex was not found to significantly influence the alpha diversity when nesting for age (Shannon $P = 0.34$; InvSimpson $P = 0.30$).

Beta diversity analysis reveals a dysbiotic gradient with helminth infection. Analysis of beta diversity of HU microbiomes showed a diverse and variable structure with no clustering observed based on age (PERMANOVA $P = 0.11$; ANOVA $P = 0.09$) and a minor influence of sex (4.2% contribution; PERMANOVA $P = 0.021$; ANOSIM $P = 0.007$). Study ID-adjusted PERMANOVA and ANOSIM tests showed significant variation based on infection status (HI versus HU; ANOSIM $R = 0.37$; $P = 0.001$) and infection type (single versus multiple; ANOSIM $R = 0.34$; $P = 0.001$). Helminth species identity contributed to 33% of the beta diversity variation (ANOSIM $R = 0.33$; $P = 0.001$) (Fig. 4A and B). For a more detailed pairwise PERMANOVA exploration of the effect of individual and multiple helminth species infections on beta diversity, see Table S3.

Often, microbial populations do not manifest as discrete clusters but rather as continuously variable community configurations (gradients) characterized by a trade-off dominant taxon in response to disease, treatment, or environmental conditions (35, 36). HU individuals across studies presented with a natural gradient produced by shifting of species within the class *Bacteroidia*. Conversely, HI individuals' microbiomes were defined by a collapsed gradient dominated by *Firmicutes* and *Clostridia*, including *Lachnospiraceae*, *Oscillospiracea*, and *Christensenellaceae*. Permutational analysis of

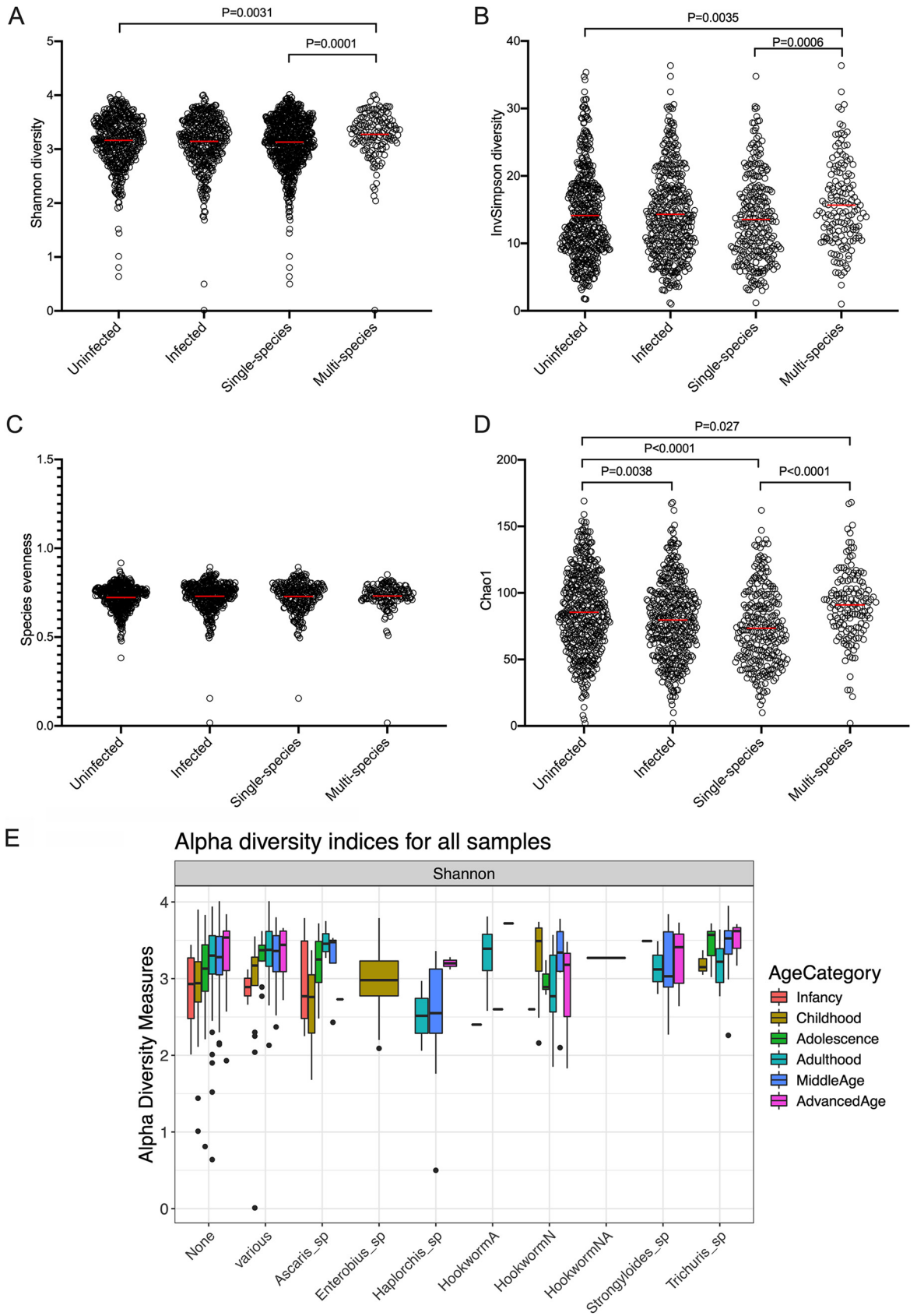

FIG 3 Effects of helminth infection on measures of microbiome diversity, richness, and evenness. (A to D) Measures across all age groups. (A) Shannon alpha diversity. (B) InvSimpson alpha diversity. (C) Species evenness. (D) Species richness (Chao1). (E) Shannon alpha diversity nested by age and helminth infection status. HookwormA, *Ancylostoma* sp.; HookwormN, *Necator* sp.; HookwormNA, either *Ancylostoma* or *Necator* sp.

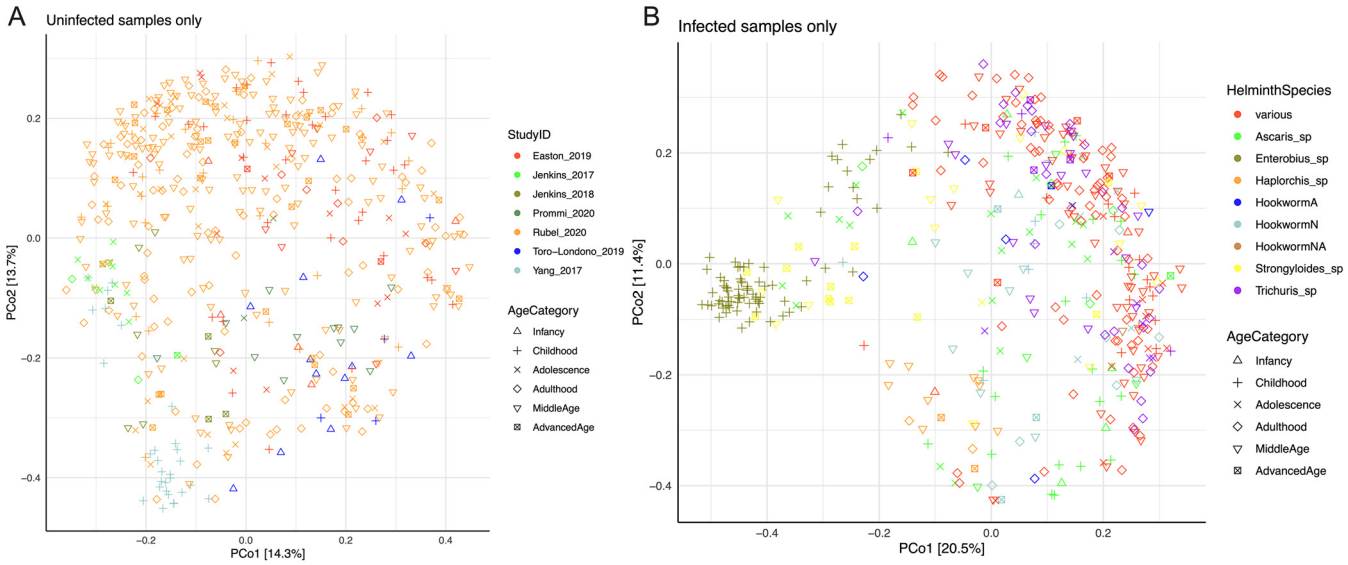

FIG 4 Beta diversity of HU and HI cohorts. PCoA plots of the (A) HU and (B) HI gut microbiomes. The relative contribution (Eigenvalue) of each axis to the total inertia of the data is indicated in the percent values on the axes. HookwormA, *Ancylostoma* sp.; HookwormN, *Necator* sp.; HookwormNA, either *Ancylostoma* or *Necator* sp.

multivariate dispersions (PERMDISP) testing for homogeneity of dispersion indicated a similar level of dispersion of sampled HU microbiomes across studies. Alternatively, HI microbiomes presented with more contrast within the same distribution space, reflected by a high F value ($F = 65.8$; $P = 0.001$), suggesting that infected samples are distinguishable based on their dispersion (Fig. 5). Dispersion tests between single-species- and multispecies-infected cohorts presented an even higher difference in dispersion ($F = 158.2$; $P = 0.001$), clearly demonstrating a significant difference in beta diversity variability between these cohorts. Dispersion caused by study ID alone (indicative of a batch effect) was present but showed minimal influence ($F = 4.2$; $P = 0.001$).

Helminth species identity influences taxonomic abundance. Examination of microbial profiles showed an overall trend toward increase of the 35 most abundant microbiome taxa in HI individuals, while in these individuals the 512 least abundant taxa tended to decrease across different age groups (Fig. 6). While overall profiles of the most prevalent taxa did not drastically differ between HI and HU cohorts, there were visible associations between the dominant taxa and sample age and helminth infection status. Among the taxa identified as most significantly associated with helminth infection in the differential abundance (DESeq2) analysis were *Catenibacterium* sp. ($P_{adj} = 8.48 \times 10^{-46}$), *Enterococcus* sp. ($P_{adj} = 2.67 \times 10^{-17}$), *Lachnospiraceae* UCG-001 ($P_{adj} = 1.21 \times 10^{-23}$), *Bacteroides togonis* ($P_{adj} = 7.91 \times 10^{-70}$), *Bacteroidales* RF16 group sp. ($P_{adj} = 5.22 \times 10^{-68}$), and *Akkermansia* sp. ($P_{adj} = 4.33 \times 10^{-17}$) (see Fig. S4 and S5 for the normalized log abundance graphs of the top 16 most significantly differentiating taxa).

Large intestine-dwelling helminths exert the largest effects on microbiome composition. The helminth species that had the most significant effect on the fecal microbiome composition were *Enterobius* and *Trichuris*, both helminths that reside in the large intestine (both $|r| > 0.5$; $P < 0.05$) (Fig. 7). The presence of *Enterobius* showed a strong positive association with *Megamonas* sp., *Halomonas* sp., *Phascolarctobacterium faecium*, and *Flavonifractor plautii* and was negatively associated with *Prevotellaceae* sp., *Alloprevotella* sp., and *Succinivibrio* sp. *Trichuris* infection was associated positively with *Treponema* sp., *Anaerovibrio* sp., *Rikenellaceae RC9 gut group*, and multiple *Prevotellaceae* species; *Enterobius* was negatively associated with *Fusicatenibacter saccharivorans*. Notably, while *Enterobius* primarily affected younger individuals, *Trichuris*-infected individuals showed a diverse range of ages (range, 3 to 78 years; mean, 27.3 years; SD = 18.2 years), suggesting that age alone does not explain these disproportionately large effects.

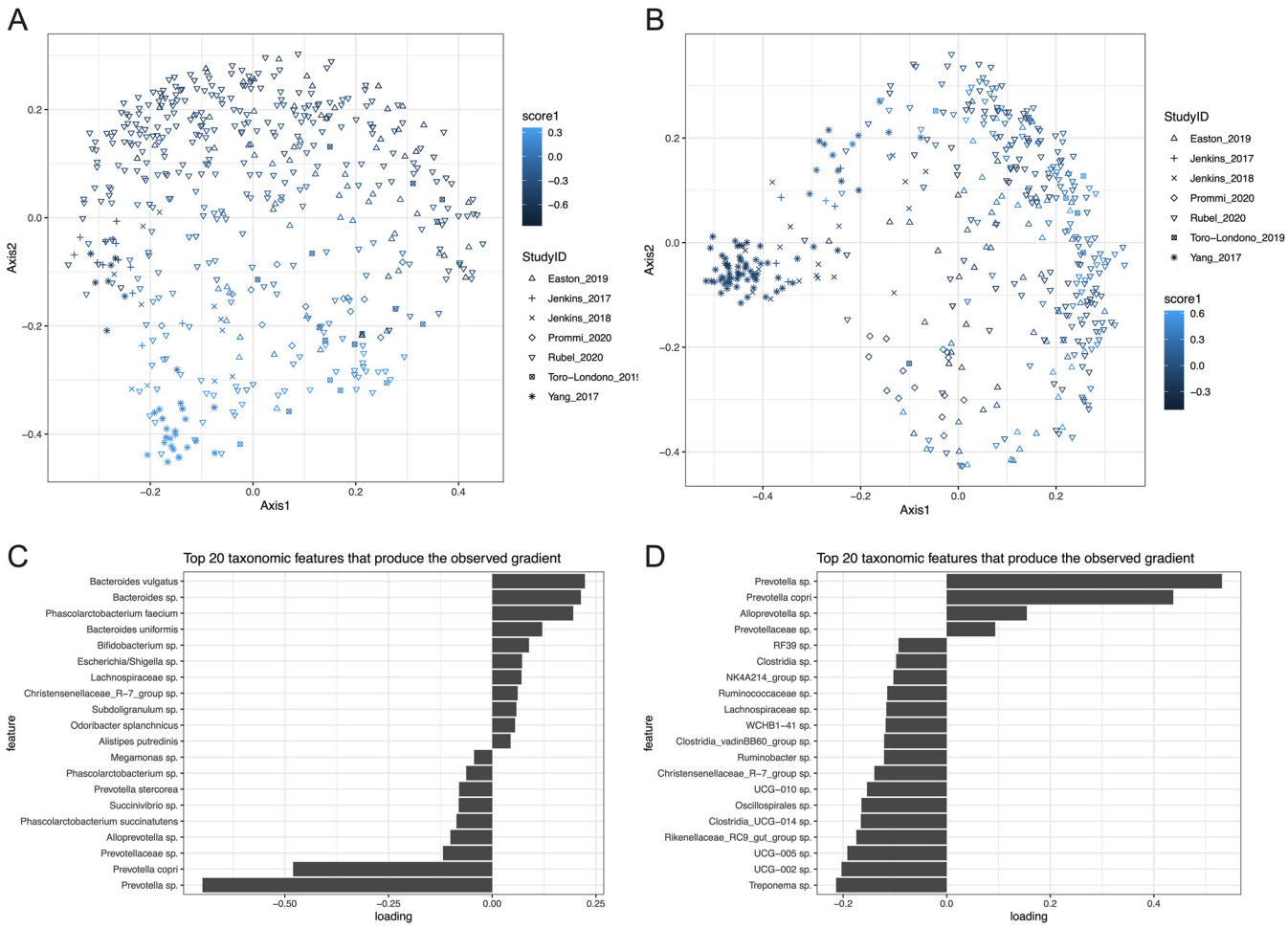

FIG 5 Microbiome gradient analysis. (A and B) Continuously variable community configurations (gradients) visualized through PCoA plots of HU and HI microbiomes, respectively. (C and D) Bar plots of the most significant taxonomic trade-offs defining the gradients of HU and HI microbiomes, respectively.

Anthelmintic treatment exerts large effects on the human gut microbiome but does not solely explain helminth-associated changes. It is difficult to discriminate between the influence of deworming (the clearance effect) and anthelmintic drugs themselves (the treatment effect) in most field studies. Most insightful, but relatively rare, are treatment arms with matched, uninfected controls. There was evidence of treatment-specific effects among the selected studies, such as findings of treatment-associated taxa, including *Sphingobacteriaceae* and *Flavobacteriaceae* (26); increased and decreased *Actinobacteria* and *Bacteroidetes*, respectively, after placebo comparison (18); mebendazole treatment-induced changes in the diversity and abundance of *Collinsella* and *Blautia* (27); and, most strikingly, significant overall reassortment of the microbiome seen in PCoA comparing uninfected controls at baseline and posttreatment (19). From the selected studies meeting an additional criterion of treated placebo controls, the consensus appears to be that treatment effects, if present, are generally small, transient, and largely drug-specific (12, 34). Where present, treatment effects explained only a portion of microbiome variation in helminth-cured and HI individuals (26). In several cases, microbiome composition was found to change significantly only in treated subjects who remained infected posttreatment (13, 18), suggesting an interaction between anthelmintics and helminth infection. Deworming effects were investigated in a further (9) of the selected human studies. Only 2 studies failed to find significant differentially abundant taxa or overall compositional differences when comparing infected and dewormed individuals (18, 30), while the remainder found significant

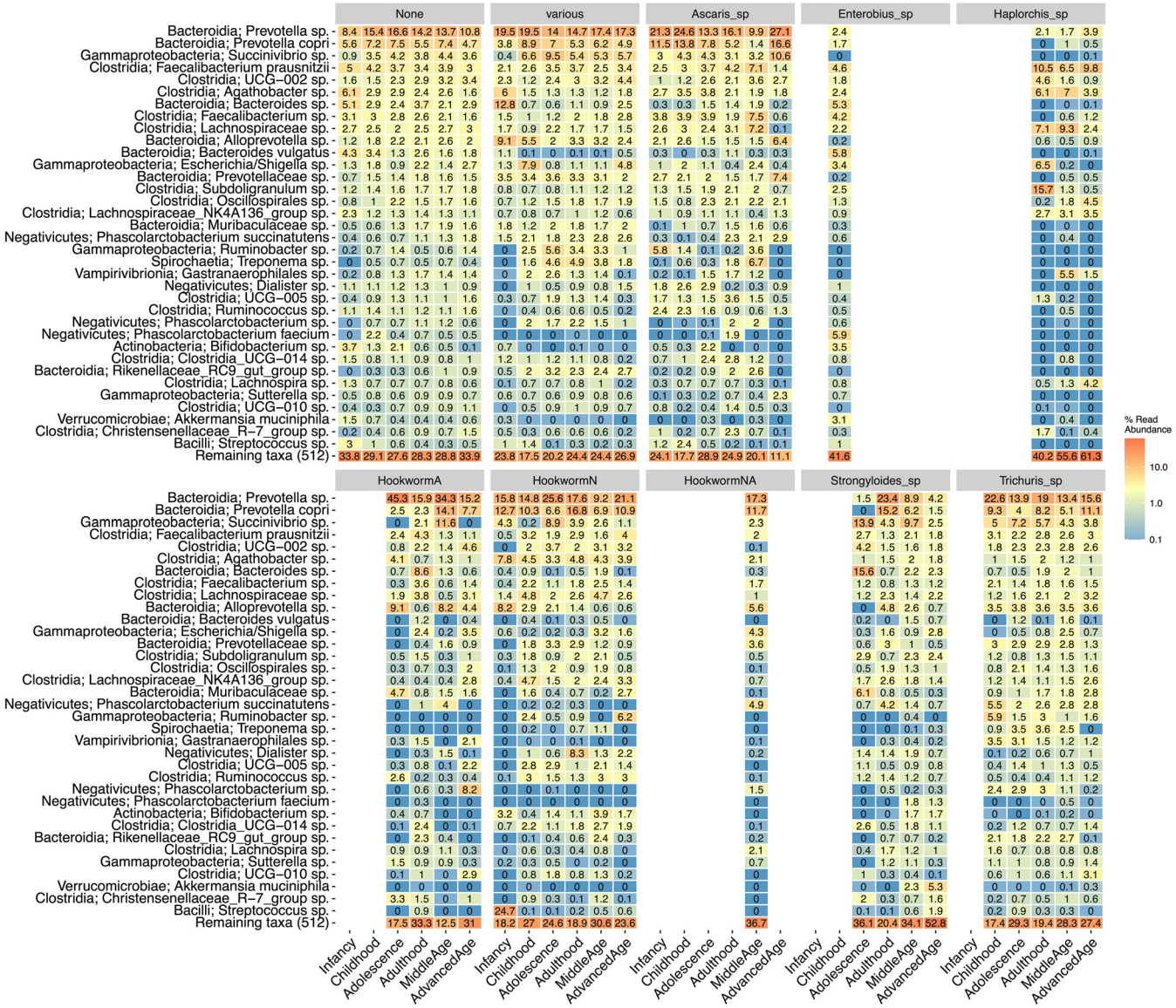

FIG 6 Profiles of the dominant microbial communities in HU and HI cohorts. Microbial profile heatmap representing the 35 most prevalence taxonomies (presented at the species level) within all samples per age category, segregated by individual helminth species. Red indicates greater abundance, while blue indicates lower abundance. Numbers indicate the percentage of 16S rRNA reads made up of the various taxa. HookwormA, *Ancylostoma* sp.; HookwormN, *Necator* sp.; HookwormNA, either *Ancylostoma* or *Necator* sp.

differences, although these varied from minor, taxon-specific changes to significant reassortment of the overall microbiome composition (19, 20, 22, 24, 26, 27, 34). Consistent with their association with helminth infection, several studies found increased abundance of *Bacteroidales* bacteria posttreatment (12, 20, 34). Commonly associated with clearance were bacteria of the order *Clostridiales* (considering individual genus- and order-wide associations), which were largely shown to decrease (19, 27, 34) posttreatment.

Microbiome composition predicts anthelmintic treatment success and disease burden. While anthelmintics are highly effective for treating most intestinal helminth parasites, a subset of patients fails to completely clear infection or otherwise quickly becomes reinfected following treatment (37). Five of the 23 studies selected for systematic review correlated treatment success or severity of infection with microbiome composition or taxa abundance and found specific taxa or overall compositional changes which were associated with treatment outcome (complete or incomplete parasite clearance) or disease burden (18, 22, 26, 29, 34). Associated with incomplete

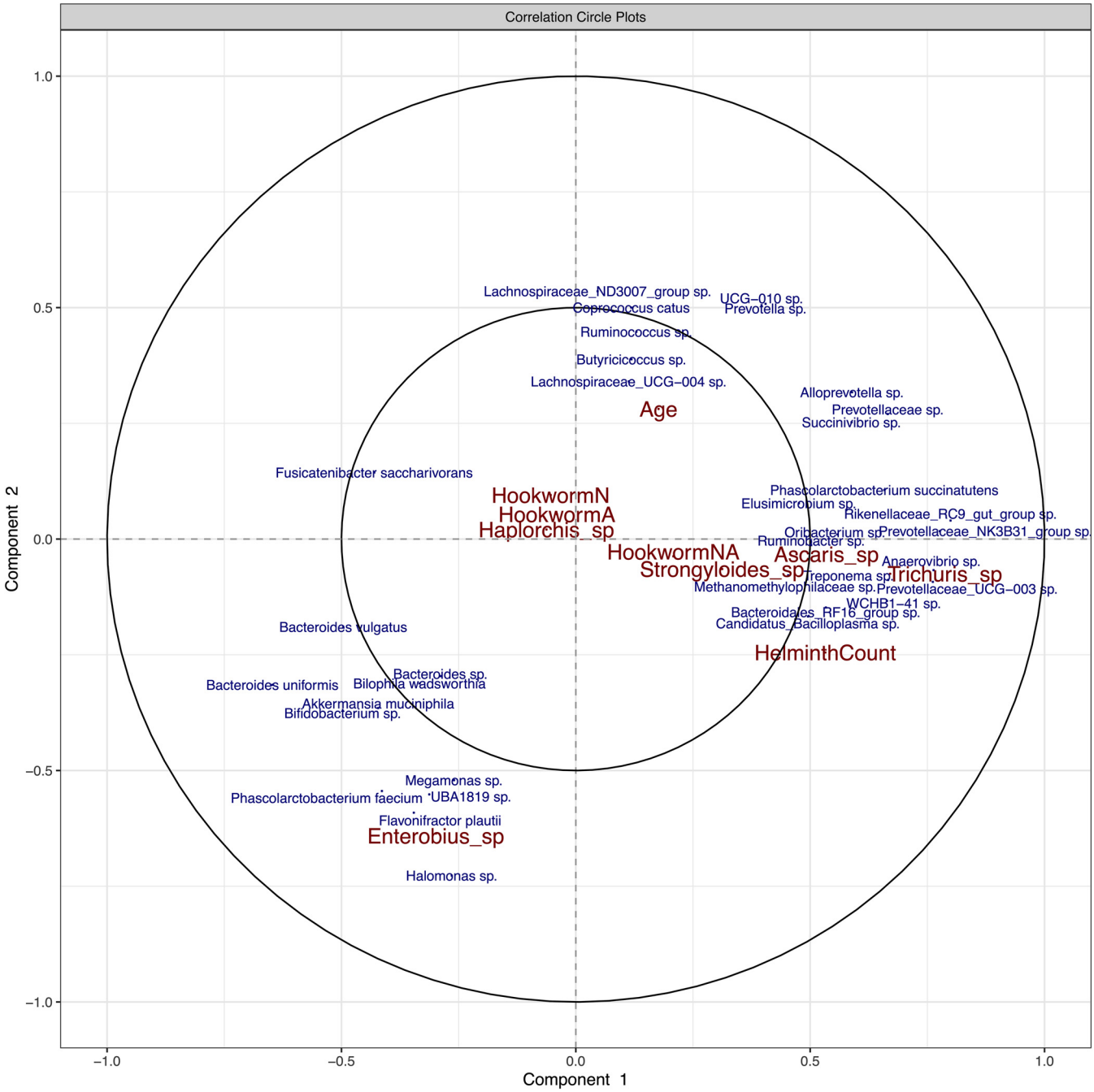

FIG 7 Correlation circle plot of helminth-microbiome interactions. Angles represent the strength and direction of correlation, and distance from the center represents significance of the correlation. The top 20 most significant bacterial taxonomies (blue) and associated factors of interest (red; age, number of helminths, and helminth species) are included. Negative cosine interactions are negative correlations; cosine (0) (90° angle) indicates no interaction. The inner circle represents a mark for significance (0.05 threshold), where points outside are significant. HookwormA, *Ancylostoma* sp.; HookwormN, *Necator* sp.; HookwormNA, either *Ancylostoma* or *Necator* sp.

parasite clearance were the taxa *Dialister* (26), *Clostridium* XIVa (26), *Bacteroides* (18), *Weissella* (22), and *Bacilli* (22). Higher abundances of *Subdoligranulum* (26), *Fusobacteriales* (22), *Rickettsiales* (22), and *Neisseriales* (22) were associated with improved treatment outcomes. There was also evidence that compositional changes may generally be associated with successful parasite clearance; in one study, such changes were significant only in patients whose parasite egg counts in stool decreased with treatment (34) but not in treated patients who showed no decreased egg burden. Concurrently, it was found that a higher decrease rate in *Fusobacterium* bacteria is

associated with a more favorable treatment outcome for *Schistosoma mansoni*-infected children, providing evidence for a potential driver of the increased compositional change in helminth-cleared individuals (22).

DISCUSSION

While qualitative review of the microbiome-helminth literature reveals general trends, our selection revealed a variety of (and also inconsistent) effects of helminth infection on measures of alpha and beta diversity, species richness and evenness, and infection-associated taxa. Among the studies that found a significant association between helminth infection and alpha diversity, there was a tendency toward increased alpha diversity and beta diversity with helminth infection. Our meta-analysis reaffirmed the qualitative synthesis as we found significantly higher alpha diversity among the global (Shannon) and core (InvSimpson) microbial communities in multiple-helminth-species-infected but not single-species-infected individuals, indicating a possible helminth species-species interaction. Such an effect is also suggested by the species richness indicator Chao1, which was increased with multispecies but decreased with single-species infection. We found that age dramatically influenced alpha diversity, visible in the positive linear trend of alpha diversity (Fig. 3E), consistent with multiple studies (38, 39). After isolating the effect of helminth infection from natural age-related variation (by nesting for age category), we found significant effects of helminth infection status and helminth species identity on alpha diversity, providing strong evidence that helminths uniquely influence the microbiome composition within an individual.

We found that variation in beta diversity of the microbiome was largely explained by helminth infection status (37%) and species identity (33%). Helminth infection was associated with a collapsed beta diversity gradient characterized by a shift from *Bacteroidetes* to *Firmicutes* and *Clostridia*, consistent with findings in *Trichuris*-infected individuals (40), indicating a shift from type 1 (*Bacteroides*-enriched) to type 3 (*Firmicutes*-enriched) enterotype (41). The *Firmicutes*/*Bacteroides* ratio has been shown to be biologically relevant, suggesting that the helminth-induced enterotype may have implications for host health (42, 43). Microbiome-helminth research has given special attention to microbial species which produce short-chain fatty acids (SCFAs), nondigestible carbohydrates implicated in metabolic and immune functioning (44) including butyrate, a compound associated with anti-inflammatory effects beneficial to patients with irritable bowel disorder (45, 46). Among the top differentially abundant taxa associating with helminth infection were the butyrate-producing *Lachnospiraceae* and lactic acid-producing *Akkermansia* sp., which differed significantly in abundance depending on helminth species, supporting a role of helminths in modulating immunity and gut health via altered SCFA production profiles (47–50).

Interestingly, while it has been shown that helminth parasites alter the composition of intestinal bacterial communities, leading to more microbial-derived SCFAs that regulate the expansion of regulatory T cells (Treg cells) (51) and stimulate the production of interleukin 10 (IL-10) by effector cells (41), it has been well documented that the expansion of regulatory T cells and elevated IL-10 levels, hallmarks of long-standing helminth chronic infection (1, 52, 53), downregulate the immune response against the helminth parasites and also to bystander pathogens, vaccine products, and allergens. These observations gave rise to a new hypothesis suggesting a potential link between the changes in microbial composition/abundance/diversity with the helminth-driven hyporesponsiveness against themselves as well as with the suppression of the allergic inflammation (49) and modulation of the severity of inflammatory autoimmune disease (54). As findings on the immunological effects of the helminth-influenced microbiome have been largely observational, future experiments are needed confirm whether taxa that are differentially abundant in HI individuals actually mediate immunomodulation.

While the heterogeneity (e.g., age, geography, and gender) of sample populations contained in our study allowed us to draw generalizable conclusions on the effects of

helminths on the microbiome, noise introduced by environmental and batch variation may obscure the influence of rarer but biologically relevant taxa, a limitation of our study. Furthermore, we identified gross taxonomic changes (decreased *Bacteroidetes* and increased *Firmicutes*) consistently associated with helminth infection in humans and other mammals; such cross-species comparisons may provide further validity for the genetics- and environment-independent influence of helminths on the gut microbiome landscape (55).

A benefit of such a large sample size of HI individuals is that we were able to determine helminth species-specific effects and the role of species-species interactions on the microbiome. For example, we could elucidate not only the influence of *Trichuris* infection alone but also how the microbiome of *Trichuris*- and *Ascaris*-coinfected individuals differed from that of HU controls (Table S3). Interactions between STH infections and nonintestinal parasites have been shown to produce distinct microbiome signatures (56). Studies on the microbiome impacts of parasitic coinfections ("polyparasitism"), which were found in over one-third of the HI cohort in this study and have been found at a much higher prevalence in certain populations, are sparse (23). In examining infections with multiple helminth species, we found that *Enterobius* and *Trichuris* infection had the largest impact on microbiome composition. Interestingly, these two parasites reside for the majority their life cycle within the large intestine (colon), while the other helminth species captured in the study (*Ascaris*, hookworm, *Strongyloides*, and *H. taichui*) are all small intestine dwelling (43, 57). Fecal samples, the source of 16S data in all selected studies, are known to accurately capture the colonic microbiome while underrepresenting the contents of the small intestine, particularly the rarer taxa (58, 59). In addition to the colon being a richer, denser, and more diverse site of bacterial colonization, the overall microbiota composition of these sites may differ dramatically (58, 60, 61). Thus, the outsized effect of large-intestinal helminths on fecal 16S profiles may result from either their access to a richer microbiota or overrepresentation of their effects (and underrepresentation of the small-intestinal helminths' effects) produced by current sampling methods, or a combination of these factors. While our meta-analysis surrounding *Trichuris*' outsized effect on the fecal microbiome is based on a large and diverse population of individuals infected with this helminth, *Enterobius*-infected individuals derived almost entirely from a single study (27). Furthermore, the gold standard for detection of *Enterobius* infection, the Scotch tape test, was used only in reference 27, so our selected meta-analysis subset may contain undiagnosed infections (62). Additional work is therefore needed before generalizing *Enterobius*' effects on the fecal microbiome. Another possibility which we were not able to evaluate was the influence of worm burden on the microbiome, as increased burden in *Trichuris*- and *Enterobius*-infected individuals could partially explain these findings. Worm burden has been found to significantly correlate with microbiome measures such as species richness (29). Moreover, Ramanan et al. (34), utilizing data from Orang Asli in Malaysia before and after deworming treatment with albendazole, showed that *Dialister* and *Coprococcus* (two members of the order *Clostridiales*) were positively associated with changes in *Trichuris trichiura* egg burden, whereas the *Bacteroidales* genus *Prevotella* and another OTU were negatively associated, independently of age and gender.

Finally, it is important to consider the effects of factors such as diet and intestinal protozoan infections (e.g., giardiasis), which may preferentially associate with HI or HU populations and influence the gut microbiome independently. Rural and urban populations which show distinct microbiome signatures due to environmental factors may also face unequal risk of acquiring helminth infection, potentially confounding the helminth-specific effects on the microbiome (63). With the exception of a single study (16), endemic controls and HI individuals derived from the same general population. Notably, helminth infection explained 10% of the fecal microbiome variation independent of study ID (Table 3), suggesting that helminth infection alone strongly influences the microbiome. Previous work suggests that helminths exert a stronger impact on the fecal microbiome than diet (33). Further analysis is needed to deconvolute the effects of intestinal protozoan infection which may associate with helminthiasis.

Collectively, the results of these analyses lend credence to the multiple studies describing differential gut microbiota assemblages in individuals infected with intestinal helminths, while underlining the importance of studying species- and site-specific effects. As the microbiome-helminth literature continues to expand and encompass diverse populations and parasite species, these types of comprehensive syntheses will be important to draw cross-population conclusions on the dynamic parasite-microbe interactions.

MATERIALS AND METHODS

Literature search and research study selection. The study selection protocol was designed and executed in strict compliance with the most recent Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (64). We performed a comprehensive literature search of PubMed and the Cochrane Central Register of Controlled Trials (CENTRAL) for articles containing the keywords: (microbiom* OR micro-biom* OR microbiot* OR micro-biot*) AND (helminth OR helminth parasite OR nematode OR trematode OR cestode OR soil-transmitted helminth OR geohelminth OR Ascaris OR whipworm OR hookworm OR roundworm OR intestinal roundworm). Included articles were published between 1 January 1975 and 31 July 2020. All data were downloaded into EndNote 20.

Eligibility criteria. Articles were assessed for eligibility based on predefined inclusion and exclusion criteria (Table S1). Commentary articles, perspectives, review articles, and surveillance reports were excluded.

Data extraction and assessment of bias. All articles identified through the keyword search from the online databases were downloaded into EndNote 20 (Clarivate Analytics, Philadelphia, PA, USA). Citations were deduplicated, and reviews were excluded. Studies were screened using the abstract and, where necessary, the body of the manuscript. Bias in the meta-analysis was determined through heterogeneity statistics analysis with a random/mixed-effects modeling of the effect size (R^2 statistic of PERMANOVA) for each factor of interest and its accompanying standard error.

Data processing and statistical analysis. All selected human studies were included in the qualitative synthesis. From the manuscript bodies and supplemental materials, we populated a single database for the selected studies in response to our predefined questions: study description (participants [number, geographic location, and age], helminth infections [treatment and controls], and 16S sequencing platform), effects of helminth infection on alpha and beta diversity, infection-associated taxa, the effect of helminth clearance on microbiome composition (the deworming effect), microbiome composition as a predictor of infection status or treatment outcome, genetic content and metabolic pathways represented by the helminth-associated microbiome, and treatment-specific effects on the gut microbiome (Data Set S1). Helminth-associated taxa and nature of association were derived manually from close reading of the selected articles as well as from the Microbiome Helminth Interactions Database (MICHELINdb), where available (65).

Meta-analysis assessment. For the meta-analysis, we included only selected human studies containing raw, paired-end Fastq format 16S data from high-throughput sequencing technology. From these data sets, we selected individuals with no documentation of recent anthelmintic treatment to isolate the effects of helminth infection alone. Sample IDs were downloaded from the Short Read Archive (SRA) or European Nucleotide Archive (ENA) and collated with associated metadata, including 16S rRNA sequencing primers, helminth infection status, age, sex, and country. Age categories were assigned as follows: infancy (1 to 3 years), childhood (4 to 11 years), adolescence (12 to 20 years), adult (21 to 40 years), middle age (41 to 69 years), and advanced age (≥70 years). See Data Set S2 for the complete human metadata database. Infection status was classified as "none" (uninfected), "various" (multiple helminths) or "single infection." Samples with a sequencing depth of fewer than 400 reads and taxa which appeared in fewer than 3 samples and with fewer than 2 reads in a sample were removed. Amplicon sequence variants (ASVs) were determined and taxonomy assigned to them using the DADA2 native algorithm and the SILVA 16S database v138. Each ASV was assigned a taxonomy down to the lowest classifiable level. The produced ASV count in each taxonomy for each sample was summated based on the produced lineage (all taxonomic ranks available for that ASV, concatenated). Thus, ASV counts were converted into taxonomic counts for each sample, then collated into one taxonomic counts table representing all samples, and used for downstream analysis. The batch effect, variation derived from technical differences in sample processing and sequencing, was quantified using the R^2 statistic of PERMANOVA applied on a Bray-Curtis distance matrix, as well as the R value of ANOSIM. The R package MMUPHin (66) was used to reduce the batch effect while maintaining the magnitude of biological variation, and any downstream analysis was performed on batch-adjusted feature counts. PCoA ordination plots were used to visualize the relative contribution of study ID to the total differentiation of the samples.

Alpha diversity measurement. Alpha diversity (the diversity of species within an individual) was represented by the Shannon index, a measure of the number of taxa in a sample as related to their even distribution throughout the community, and the inverted Simpson index (InvSimpson), which preferentially weighs more dominant species within a community, thus capturing the diversity of the sample's core community. Alpha diversity (Shannon and InvSimpson) was calculated for helminth-uninfected (HU), helminth-infected (HI), and single- and multiple-infection ("various") cohorts, and significance was calculated using the Mann-Whitney test (two-tailed, nonparametric t test); graphing and statistical analysis were performed using PRISM 8 software (GraphPad, San Diego, CA, USA). Species evenness and richness (Chao1) were calculated similarly. ANOVA was performed for both Shannon and InvSimpson

diversity statistics with and without nesting for age category to isolate the age-dependent and -independent effects of helminth infection status and helminth species.

Beta diversity measurement. Beta diversity is a measure of the similarity or dissimilarity of the taxonomic content between samples. Beta diversity was calculated using PERMANOVA and ANOSIM for infection status, infection type, helminth species, and age and gender. A Bray-Curtis dissimilarity matrix was calculated and plotted in a PCoA ordination to represent the variation between samples. All tests were nested for study ID to minimize the batch effect. Taxonomies that were not present at more than 0.01% relative abundance in any sample were removed prior to PCoA.

To assess which taxa significantly responded to the presence of each helminth species, differential abundance analysis (DESeq2) was performed by use of negative binomial generalized linear models, which estimates the dispersion and logarithmic fold changes in taxonomic abundances. Criteria to distinguish such taxa were as follows: (i) taxonomic abundance must have a 2-fold or greater change between compared samples and (ii) the adjusted *P* value (P_{adj}) must be less than 10^{-6}. To produce the DESeq2 plots, the log relative normalization was performed on the batch-adjusted abundance for each cohort. Due to the sparsity of the collated taxonomic data, samples with zero values for the presented taxonomies were removed from the visualization of the DESeq2 results. Finally, taxonomic correlation analysis based on sparse partial least square regression was performed with the R package mixOmics (67) to determine taxa consistently correlated with helminth infection across samples; briefly, a binary abundance matrix was created to represent the presence or absence of a helminth species in a sample. This pseudomatrix was then correlated with the microbiome abundance matrix to reveal associations between specific microbiome taxa and helminth species. The log-transformed abundance matrix was used as a means of batch reduction and normalization of the microbial abundances. The top 20 most significant interactions were then visualized on a correlation circle plot including age and number of helminth species to depict their effect on the explored communities. The complete code for our meta-analysis is available in the GitHub repository (http://github.com/angelovaag/helminth_2021).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

DATA SET S1, XLSX file, 0.02 MB.

DATA SET S2, XLSX file, 0.1 MB.

FIG S1, TIF file, 1.4 MB.

FIG S2, TIF file, 1.1 MB.

FIG S3, TIF file, 1.2 MB.

FIG S4, TIF file, 1.4 MB.

FIG S5, TIF file, 1.5 MB.

TABLE S1, DOCX file, 0.01 MB.

TABLE S2, DOCX file, 0.01 MB.

TABLE S3, DOCX file, 0.1 MB.

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