

# Gut Microbiome Changes Associated With HIV Infection and Sexual Orientation

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Zhou J, Zhang Y, Cui P, Luo L, Chen H, Liang B, Jiang J, Ning C, Tian L, Zhong X, Ye L, Liang H and Huang J (2020) Gut Microbiome Changes Associated With HIV Infection and Sexual Orientation. Front. Cell. Infect. Microbiol. 10:434. doi: 10.3389/fcimb.2020.00434 **Background:** Many studies have explored changes in the gut microbiome associated with HIV infection, but the consistent pattern of changes has not been clarified. Men who have sex with men (MSM) are very likely to be an independent influencing factor of the gut microbiome, but relevant research is still lacking.

**Methods:** We conducted a meta-analysis by screening 12 published studies of 16S rRNA gene amplicon sequencing of gut microbiomes related to HIV/AIDS (six of these studies contain data that is relevant and available to MSM) from NCBI and EBI databases. The analysis of gut microbiomes related to HIV infection status and MSM status included 1,288 samples (HIV-positive (HIV+) individuals, n = 744; HIV-negative (HIV-) individuals, n = 544) and 632 samples (MSM, n = 328; non-MSM, n = 304), respectively. The alpha diversity indexes, beta diversity indexes, differentially enriched genera, differentially enriched species, and differentially enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) functional pathways related to gut microbiomes were calculated. Finally, the overall trend of the above indicators was evaluated.

**Results:** Our results indicate that HIV+ status is associated with decreased alpha diversity of the gut microbiome. MSM status is an important factor that affects the study of HIV-related gut microbiomes; that is, MSM are associated with alpha diversity changes in the gut microbiome regardless of HIV infection, and the changes in the gut microbiome regardless of HIV infection, and the changes in the gut microbiome regardless of HIV infection, and the changes in the gut microbiome composition of MSM are more significant than those of HIV+ individuals. A consistent change in *Bacteroides caccae*, *Bacteroides ovatus*, *Bacteroides uniformis*, and *Prevotella stercorea* was found in HIV+ individuals and MSM. The differential expression of the gut microbiome may be accompanied by changes in functional pathways of carbohydrate metabolism, amino acid metabolism, and lipid Metabolism.

**Conclusions:** This study shows that the changes in the gut microbiome are related to HIV and MSM status. Importantly, MSM status may have a far greater impact on the gut microbiome than HIV status.

Keywords: HIV, AIDS, sexual orientation, gut microbiome, 16S rRNA gene amplicon sequencing

1

# INTRODUCTION

Early studies have shown that the intestinal mucosa is the primary site of early HIV-1 reproduction, irrespective of the way in which HIV-1 invades the body, whether by sexual contact or blood transfusion (Mehandru et al., 2004). HIV-1, which enters the intestinal mucosa at the very early stages of infection, can cause the Th17 CD4<sup>+</sup>T cells of the intestine to be destroyed and depleted and the integrity of the intestinal mucosa to be impaired (Epple et al., 2010; Hirao et al., 2014). In addition, gut microbiome translocation can occur, and the gut microbiome and its products can enter the systemic blood circulation (Balagopal et al., 2008), eventually leading to activation of the immune system and spread of the HIV-1 infection (Brenchley et al., 2006).

In recent years, exploration of the role and mechanism of the gut microbiome in the development of HIV infection has gradually become a popular topic of academic research. However, there is still inconsistent evidence about the alpha diversity and composition of the gut microbiome after HIV infection. Most current studies suggest that HIV+ status is related to the downregulation of alpha diversity in the gut microbiome (Mutlu et al., 2014; Yu et al., 2014; Nowak et al., 2015; Dubourg et al., 2016; Noguera-Julian et al., 2016; Pinto-Cardoso et al., 2017; Vesterbacka et al., 2017; Villanueva-Millan et al., 2017). Some researchers (McHardy et al., 2013; Dinh et al., 2015; Nowak et al., 2017) also compared the alpha diversity of the gut microbiome in HIV+ and HIV- individuals, but no significant difference was found. The study by Lozupone et al. (2013) showed that the alpha diversity of the gut microbiome in HIV+ individuals who did not receive antiretroviral therapy (ART) was significantly higher than that of HIV- individuals. Moreover, in many studies, there are inconsistent results regarding the change in the composition of the gut microbiome after HIV infection. Some studies have shown that the abundance of Prevotella increases significantly and the abundance of Bacteroides decreases significantly in HIV+ individuals compared to HIV- individuals (Vujkovic-Cvijin et al., 2013; Dillon et al., 2014; Mutlu et al., 2014; Vázquez-Castellanos et al., 2015; Sun et al., 2016; Yang et al., 2016; Armstrong et al., 2018; Neff et al., 2018). However, a study by Noguera-Julian et al. (2016) showed that the increase in the Prevotella/Bacteroides ratio is associated with MSM status rather than HIV status, which has since been corroborated by several other studies (Armstrong et al., 2018; Neff et al., 2018; Li et al., 2019). Although many studies have explored the changes in the gut microbiome associated with HIV infection, the pattern of these changes has not been elucidated. MSM status is very likely an independent influencing factor of the gut microbiome, but there is still a lack of relevant research to explore it.

In addition, HIV infection can cause dysregulation of multiple functional pathways in the human body (Vázquez-Castellanos et al., 2015, 2018). On the one hand, HIV-related gut microbiomes are well-adapted to inflammatory environments, such as the high expression of the anti-oxidative stress response pathway and the low expression of the anti-inflammatory response process. On the other hand, the gut microbiome can promote the occurrence and development of intestinal

inflammation. Therefore, exploration of the functional changes related to HIV infection based on the gene expression profile of the gut microbiome can increase our understanding of the interaction between the gut microbiome and the human body.

To clarify the diversity of the gut microbiome related to HIV infection, to determine whether MSM status is an independent factor influencing the gut microbiome, and to explore the consistent change in the gut microbiome and functional pathways in HIV+ individuals and MSM, we screened 12 published studies of 16S rRNA gene amplicon sequencing of the gut microbiome related to HIV/AIDS (six of these studies contain data that is relevant and available to MSM) from NCBI and EBI databases. The alpha diversity indexes, beta diversity indexes, genera, species, and KEGG functional pathways related to the gut microbiome were calculated. Finally, the overall trend in the above indicators was evaluated.

# MATERIALS AND METHODS

### **Research Strategy**

Studies of human fecal flora related to HIV/AIDS by 16S rRNA gene amplicon sequencing before October 2019 were retrieved from the NCBI and EBI databases. Studies were screened according to the following inclusion and exclusion criteria: (1) cross-sectional studies, (2) each sample should give the HIV status of the corresponding subject, (3) the sample types of the sequences should be stool or rectal swabs, and (4) the sequencing method should be 16S rRNA gene amplicon sequencing. Studies with sample sizes of HIV+ or HIV- individuals of <5 were excluded. The technical route of the study is shown in **Figure 1**.

## Processing of Raw Data

The raw sequences were processed using QIIME version 1.9 (Caporaso et al., 2010), and the general process included FLASH software (Magoc and Salzberg, 2011) used for splicing pairedend 16S rRNA gene reads. After splicing, Cutadapt was used for removing primers from the sequences, and low-quality sequences were removed. Based on the chimera database of UCHIME (Edgar et al., 2011), Usearch version 6.1.554 was used to identify and remove chimeras in the sequences, and open reference operational taxonomic unit (OTU) picking was performed with UCLUST (Edgar, 2010) against the Greengenes database (DeSantis et al., 2006), version 13.8, with a similarity of at least 97% (Rideout et al., 2014). The analysis after clustering used the platforms of MicrobiomeAnalyst (Dhariwal et al., 2017), R 3.5.1, Galaxy (Goecks et al., 2013), and REVMAN 5.3.

## **Data Filtering**

Data filtering is done to remove low quality or uninformative features to improve downstream statistical analysis. The minimum count and prevalence in the samples (%) were filtered according to the characteristics of each dataset, which may be caused by sequencing errors or low levels of contamination in the sample. At the same time, for the features that were close to constant throughout the experiment, which are conditions that are not likely to be associated with the conditions under study,



we used the interquartile range (IQR) to detect their variances and filters.

## **Data Normalization**

To address the variability in the sampling depth and the sparsity of the data in order to enable more biologically meaningful comparisons, we used total sum scaling (TSS) to bring all samples to the same scale by dividing the samples by a scaling factor.

## **Data Analysis**

At the OTU level, to assess alpha diversity, richness (Observed, Chao1, and ACE) and diversity (Shannon, Simpson, Fisher, and Invsimpson) indexes were calculated. Differences between two groups were analyzed via the Student's *t*-test and the Mann–Whitney *U*-test. For principal coordinates analysis

(PCoA), distance matrices were calculated using the Bray–Curtis, Jensen–Shannon divergence, and Jaccard ecological dissimilarity indexes. The permutational multivariate analysis of variance (PERMANOVA) test was performed on this distance matrix.

The function of the gut microbiome was inferred using a phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) (Langille et al., 2013) in the Greengenes database. In brief, the general process corrected the OTU table for multiple 16S copy numbers. Then, the normalized phylotype abundance was multiplied by the respective set of gene abundances, represented by the KEGG, to identify estimates for each taxon. The accuracy of the KEGG prediction results was evaluated by the nearest sequenced taxon index (NSTI). For the identification of different genera, species, and KEGG functional pathways, we used the linear discriminant analysis effect size

(LEfSe) method to perform the identification (LDA score was  $\geq$ 2) and the DESeq2 and Random Forests methods to verify the results of LEfSe.

REVMAN 5.3 software was used to build the forest plots based on the alpha diversity indexes, and  $Chi^2$  and  $I^2$  were used for heterogeneity testing for each study. If p > 0.1 or  $I^2 < 50\%$ , the constructed model is not heterogeneous. Sensitivity analysis removes the study with the largest sample size and non-European/non-U.S. studies and converts the fixed effects model (FEM) to the random effects model (REM). All *p*-values were corrected for multiple comparisons through the false discovery rate (FDR) technique. All tests were two-sided, and an FDR p < 0.05 was considered statistically significant.

## RESULTS

### **Study Description**

A total of 36 studies related to HIV infection were retrieved from the NCBI and EBI databases. Twelve studies were finally selected for subsequent analysis. The total number of samples included in the overall analysis was 1,288 (HIV+ individuals, n = 744; HIV- individuals, n = 544). Six of the 12 studies contain data that is relevant and available to MSM status, including 632 samples (MSM, n = 328; non-MSM, n = 304). The metadata variables were HIV status, age, gender, body mass index (BMI), MSM status, ART use, CD4<sup>+</sup>T cell count, and HIV viral load (VL). A summary of the included studies is presented in **Table 1**. The unfiltered sequencing quality results are presented in **Supplementary Table 1**.

### Richness and Diversity of the Gut Microbiome Based on HIV Status

The calculation results of the alpha diversity indexes of the OTU level of 12 studies are shown in **Table 2**. Before controlling for other confounding factors, the alpha diversity of the HIV+ individuals was significantly lower than that of the HIV- individuals, including the ACE (Z = 2.92, FDR p = 0.009), Shannon (Z = 3.44, FDR p = 0.004), Simpson (Z = 2.37, FDR p = 0.028), and Insimpson indexes (Z = 2.85, FDR p = 0.009) of the FEM. The results after the sensitivity analysis remained consistent. The forest maps (**Figure 2a**, **Supplementary Figure 1**) and boxplots (**Figure 3**) of the 12 studies included also showed this trend.

# Restricting the Analysis to Gender, Sexual Orientation, Age, and BMI

The subgroup analysis controlled for gender, including 472 men and 175 women. In men, there was no significant difference in alpha diversity between HIV+ and HIV- individuals (**Supplementary Figure 2**). Among women, HIV+ status was associated with a significant decrease in alpha diversity, including Observed (Z = 2.78, FDR p = 0.035) and ACE (Z =2.52, FDR p = 0.035) (**Figure 2b**, **Supplementary Figure 3**). In MSM (n = 316), there was no significant difference in alpha diversity between HIV+ and HIV- individuals (**Supplementary Figure 4**). In non-MSM (n = 288), HIV+ status was associated with a significant decrease in alpha diversity, including Observed (Z = 3.32, FDR p = 0.006), Chao1 (Z = 2.69, FDR p = 0.010), ACE (Z = 2.89, FDR p = 0.010), Shannon (Z = 2.89, FDR p = 0.010), and Simpson (Z = 2.81, FDR p = 0.009) (**Figure 2c, Supplementary Figure 5**). When age (age <45 years, n = 409; age  $\geq 45$  years, n = 288) and BMI (BMI = 18.5–23.9, n = 206; BMI = 24–27.9, n = 201; BMI > 28, n = 133) (**Supplementary Figures 6–10**) were controlled, there was no significant difference in alpha diversity between HIV+ and HIV– individuals. The results after the sensitivity analysis remained consistent.

# Restricting the Analysis to CD4<sup>+</sup>T Cell Count, ART, and HIV Viral Load

The subgroup analysis controlled for the CD4<sup>+</sup>T cell count, including 146 HIV+ individuals with a CD4<sup>+</sup>T cell count of <500, 272 HIV+ individuals with a CD4<sup>+</sup>T cell count of  $\geq$ 500, and 217 HIV– individuals. HIV+ individuals with a CD4<sup>+</sup>T cell count of <500 have significantly lower alpha diversity than HIVindividuals (Shannon [Z = 2.78, FDR p = 0.035]). However, there is no significant difference in the alpha diversity of HIV+ individuals with a CD4<sup>+</sup>T cell count of  $\geq$ 500 compared to HIV- individuals (Figure 2d, Supplementary Figures 11, 12). When controlled for the ART, including 188 HIV+ non-ART users, 240 HIV+ ART users, and 294 HIV- individuals. The results showed that the alpha diversity of HIV+ non-ART users was significantly lower than that of HIV- individuals (Shannon [Z = 2.91, FDR p = 0.021]), and the difference was not found in HIV+ ART users and HIV- individuals (Figure 2e, Supplementary Figures 13, 14). In the stratified analyses examining the HIV VL (VL  $\leq$  200, n = 244; VL > 200, n = 174; and HIV- individuals, n = 217) was not significantly associated with alpha diversity (Supplementary Figures 15, 16). The results after the sensitivity analysis remained consistent.

## Richness and Diversity of the Gut Microbiome Based on MSM Status

The calculation results of the alpha diversity indexes of the OTU level of six studies are shown in Table 3. Before controlling for other confounding factors, the alpha diversity of the MSM individuals was significantly lower than that of the non-MSM individuals, including the Simpson (Z = 3.32, FDR p = 0.001) and Invsimpson (Z = 3.49, FDR p = 0.001) of the FEM. The forest maps (Figure 4a, Supplementary Figure 17) and boxplots (Figure 5) of the six studies included also show this trend. The analysis was restricted to HIV status (HIV+ individuals, n =406; HIV- individuals, n = 210), age (age <45 years, n = 334; age  $\geq$ 45 years, *n* = 230), and BMI (BMI = 18.5–23.9, *n* = 164; BMI = 24–27.9, n = 161; BMI > 28, n = 96). In the HIV+ (Fisher [Z = 6.01, FDR p < 0.000]), age  $\geq 45$  years (Fisher [Z =6, FDR p < 0.000]), and BMI = 24–27.9 (Fisher [Z = 3.84, FDR p = 0.001]) (Figures 4b,e,f, Supplementary Figures 18, 21–24) individuals, MSM status was associated with increasing alpha diversity. Among HIV– (Invsimpson [Z = 4.61, FDR p < 0.000]) and age <45 years individuals (Invsimpson [Z = 3.05, FDR p =0.005]), MSM status was associated with a significant decrease in alpha diversity (Figures 4c,d, Supplementary Figures 19, 20). The results after the sensitivity analysis remained consistent.

#### TABLE 1 | Summary of the included studies.

References	Title	Bioproject accession	San siz	nple ze		Age	CD4 <sup>+</sup> T ce count	II HIV viral load	d Ma	n/Woman	MSM	/non-MSM	Treatment	16S rRNA variable region/Sequencing	Country
		number	HIV+	HIV-	HIV+	HIV-			HIV+	HIV-	HIV+	HIV-		platform	
Lozupone et al. (2013)	Alterations in the Gut Microbiota Associated with HIV-1 Infection	PRJEB4335	30	22	29 (34.7) 1 unknown	22 (37.5)	30 (584.9)	30 (45594.6)	Unknown	Unknown	Ur	nknown	16 ART users 14 non-ART users	V4/Illumina Miseq	USA
Dillon et al. (2014)	An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia	PRJNA227062	18	14	18 (32.5)*	14 (31)	18 (425)	18 (51350)	13/5	9/5	Ur	nknown	18 non-ART users	V4/Illumina Miseq	USA
Dinh et al. (2015)	Intestinal Microbiota, Microbial Translocation, and Systemic Inflammation in Chronic HIV Infection	PRJNA233597	21	15	21 (50.2)	15 (44.0)	21 (741)	19 (574) 2 unknown	17/4	11/4	12/4	0/4	21 ART users	V3-V5/Roche GS FL	X USA
Vázquez-Castellanos et al. (2015)	<ul> <li>Altered metabolism of gut microbiota contributes to chroni immune activation in HIV+ individuals</li> </ul>	PRJEB5185 c	9	12	ι	Jnknown	Unknown	Unknown	Unknown	Unknown	U	nknown	9 ART users	V1, V2, and V3/Roche GS FLX	Spain
Dubourg et al. (2016	) Gut microbiota associated with HIV infection is significantly enriched in bacteria tolerant to oxygen	PRJEB10578	56	50	ι	Jnknown	Unknown	Unknown	Unknown	Unknown	Ur	nknown	Unknown	V3-V4/Illumina MiSec	q France
Noguera-Julian et al. (2016)	Gut Microbiota Linked to Sexual Preference and HIV Infection	PRJNA307231	206	34	206 (42.8)	34 (40.0)	205 (613) 1 unknown	123 (92848) 82 undetectable 1 unknown	147/59	29/5	96/110	23/11	58 non-ART users 71 ART users 77 unknown	V3-V4/Illumina MiSec	q Spain/ Sweden
Vesterbacka et al. (2017)	Richer gut microbiota with distinct metabolic profile in HIV infected Elite Controllers	PRJNA354863	47	15	47 (46)	15 (49.9)	Unknown	Unknown	24/23	7/8	12/34 1 unknown	3/12	15 ART users 32 non-ART users	V3-V4/Illumina MiSeo	q Sweden
Armstrong et al. (2018)	An exploration of <i>Prevotella</i> -rich microbiomes in HIV and men who have sex with men	PRJEB28485	112	105	112 (43.6)	105 (34.8)	112 (674)	112 (58713)	92/19 1 FTM	64/41	90/22	35/70	67 ART users 45 non-ART users	V4/Illumina MiSeq	USA
Cook et al. (2019)	Effects of HIV Viremia on the Gastrointestinal Microbiome of Young Men who have Sex with Men	PRJNA422134	183	200		383 (31)	183 (625)	Unknown	3	83 male	38	33 MSM	Unknown	V4/Illumina MiSeq	USA
Lee et al. (2018)	Enrichment of gut-derived <i>Fusobacterium</i> is associated with suboptimal immune recovery in HIV+ individuals	PRJNA489590	26	20	26 (42.6)	20 (37.2)	26 (639)	Undetectable	26 male	20 male	14/12	11/9	26 ART users	V4/Illumina MiSeq	Malaysia
Neff et al. (2018)	Fecal Microbiota Composition Drives Immune Activation in HIV+ individuals	PRJEB25418	24	21	24 (46.6)	21 (37.7)	24 (593)	23 (57654) 1 unknown	17/7	16/5	Uı	nknown	15 ART users 9 non-ART users	V2/Illumina MiSeq	USA
Li et al. (2019)	Gut microbiota from high-risk men who have sex with men drive immune activation in gnotobiotic mice and <i>in vitro</i> HIV infection	PRJEB31328	12	36	ι	Jnknown	Unknown	Unknown	48/0	NA	12/0	20/16	12 non-ART users	V2/Illumina MiSeq	USA

MSM, men who have sex with men; ART users, HIV+ ART (antiretroviral therapy) users received suppressive ART for > 12 months; non-ART users, HIV+ non-ART users were no ART exposure / < 10 days of ART at any time prior to entry / not been on treatment for 47 days in the preceding 6 months / at least 6 months in the absence of ART; FTM, Female to male transgender. \*Sample size (Mean).

References	Observed	Chao1	ACE	Shannon	Simpson	Fisher	Invsimpson
	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p
Lozupone et al. (2013)	0.041*	0.049*	0.041*	0.750	0.750	0.580	0.640
Dillon et al. (2014)	0.078	0.078	0.078	0.078	0.078	0.078	0.078
Dinh et al. (2015)	0.970	0.970	0.970	0.770	0.970	0.770	0.800
Vázquez-Castellanos et al. (2015)	0.020*	0.027*	0.020*	0.027*	0.160	0.020*	0.014*
Dubourg et al. (2016)	0.000***	0.000***	0.000***	0.000***	0.003**	0.320	0.000***
Noguera-Julian et al. (2016)	0.003**	0.003**	0.003**	0.021*	0.092	0.003**	0.272
Vesterbacka et al. (2017)	0.000***	0.000***	0.000***	0.001**	0.012*	0.007**	0.028*
Armstrong et al. (2018)	0.990	0.990	0.990	0.990	0.550	0.000***	0.680
Cook et al. (2019)	0.850	0.850	0.850	0.850	0.850	0.850	0.850
Lee et al. (2018)	0.550	0.550	0.550	0.690	0.550	0.550	0.550
Neff et al. (2018)	0.970	0.970	0.970	0.770	0.770	0.770	0.770
Li et al. (2019)	0.990	0.990	0.990	0.990	0.990	0.990	0.990

\*FDR p < 0.05, \*\*FDR p < 0.01, and \*\*\*FDR p < 0.001.

# Composition of the Gut Microbiome Associated With HIV+ and MSM Status

We explored the potential influence of HIV and MSM status on the composition of the gut microbiome, according to the PERMANOVA test of ecological distances. PCoA ordination plots of Bray–Curtis showed that samples from works by Lozupone et al. (2013) ( $R^2 = 0.154$ , FDR p < 0.001), Dubourg et al. (2016) ( $R^2 = 0.080$ , FDR p < 0.001), and Armstrong et al. (2018) ( $R^2 = 0.059$ , FDR p < 0.001) were significantly clustered according to HIV status (**Figure 6, Table 4**). The samples from works by Noguera-Julian et al. (2016) ( $R^2 = 0.122$ , FDR p <0.001), Vesterbacka et al. (2017) ( $R^2 = 0.081$ , FDR p < 0.001), Armstrong et al. (2018) ( $R^2 = 0.125$ , FDR p < 0.001), and Li et al. (2019) ( $R^2 = 0.115$ , FDR p < 0.001) showed better clustering according to MSM status rather than HIV status (**Figure 7, Table 5**).

We identified the genus and species that cause changes in the composition of the gut microbiome of HIV+ individuals and MSM. In at least three studies, the genus of Bacteroides, Coprococcus, Faecalibacterium, and SMB53 and the species of Bifidobacterium adolescentis, Bacteroides caccae, Coprococcus catus, Parabacteroides distasonis, Akkermansia muciniphila, Blautia obeum, Bacteroides ovatus, Faecalibacterium prausnitzii, and Bacteroides uniformis were significantly reduced in HIV+ individuals. The species of Prevotella stercorea was significantly increased in HIV+ individuals (Figure 8). In MSM, the genus of Catenibacterium, Eubacterium, Mitsuokella, Phascolarctobacterium, Prevotella, and Slackia and the species of Eubacterium biforme, Prevotella copri, and Prevotella stercorea were significantly increased, and the genus of Adlercreutzia, Bacteroides, Bifidobacterium, Bilophila, Holdemania, Odoribacter, Parabacteroides and the species of Bacteroides caccae, Parabacteroides distasonis, Bacteroides ovatus, Ruminococcus torques, and Bacteroides uniformis were significantly reduced (Figure 9).

# Differential KEGG Functional Pathway Analysis

We analyzed the differential functional pathways of KEGG related to HIV and MSM status. For HIV status, there

were 91 differential KEGG III functional pathways that co-exist in multiple studies ( $\geq 3$  studies). For MSM status, there were 97 differential KEGG functional pathways that co-exist in multiple studies (>3 studies). Among HIV+ individuals and MSM, most of the KEGG III pathways under metabolism were downregulated, and most of the KEGG III pathways under genetic information processing were downregulated (Figures 10, 11). For example, in HIV+ individuals and MSM, carbohydrate-metabolism-related pathways of galactose metabolism, pyruvate metabolism, and the pentose phosphate pathway, were downregulated; amino-acid-metabolism-related pathways of histidine metabolism, arginine and proline metabolism, and valine, leucine, and isoleucine biosynthesis, were downregulated; and lipid-metabolism-related pathways of linoleic acid metabolism, primary bile acid biosynthesis, and secondary bile acid biosynthesis, were downregulated. The prediction accuracy of different PICRUSt studies related to HIV and MSM status are shown in Supplementary Table 2 and Table 3.

# DISCUSSION

In this study, we collected 12 studies to evaluate the relationship between the gut microbiome and the HIV and MSM status. In the overall assessment of 12 datasets and by restricting the analysis to woman and non-MSM individuals, HIV+ status was associated with decreased alpha diversity, consistent with the results of a recent meta-analysis (Tuddenham et al., 2020). Importantly, when controlling for a CD4<sup>+</sup>T cell count of <500 and non-ART, HIV+ status was also significantly associated with decreased alpha diversity. The assessment of the overall effect of six datasets related to MSM status showed that MSM status was associated with decreased alpha diversity, but the results of the subgroup analysis (restricting the analysis to HIV status, age <45 years, age >45 years, BMI = 24-27.9) were inconsistent. The analysis of the microbiome composition showed that in multiple studies the sample clustered in different areas of the PCoA coordinate axis according to HIV and MSM status. This clustering phenomenon is more significant between MSM and non-MSM. We also found

		UNA			1111/			Stid Maan Difference	Std Maan Difference
Study or Subaroup	Mean	SD	Total	Mean		Total	Weight	IV Fixed 95% Cl	IV Fixed 95% Cl
13.1.1 a. All-simpson	mean	00	Total	Wear	00	Total	Weight	IV. 1 1XCU, 5570 OI	
Armstrong et al. (2018)	0 8875522	0 113183	112	0 90590088	0 07513888	105	10.5%	-0 19 [-0 46 0 08]	
Cook et al. (2019)	0.9159005	0.05803462	183	0.91402454	0.06961112	200	18.6%	0.03 [-0.17, 0.23]	-
Dillon et al. (2014)	0.65524692	0.1482913	18	0.62953464	0.2017705	14	1.5%	0.14 [-0.55, 0.84]	
Dinh et al. (2015)	0.90544267	0.08624238	21	0.91483824	0.06948011	15	1.7%	-0.12 [-0.78, 0.55]	
Dubourg et al. (2016)	0.77464168	0.15295301	56	0.85984979	0.12561432	50	4.9%	-0.60 [-0.99, -0.21]	
Lee et al. (2018)	0.89121627	0.03049065	26	0.8753665	0.04964237	20	2.2%	0.39 [-0.20, 0.98]	+
Li et al. (2019)	0.87089454	0.0697832	12	0.88756978	0.08656145	36	1.7%	-0.20 [-0.85, 0.46]	
Lozupone et al. (2013)	0.8841443	0.10745952	30	0.86922436	0.19992167	22	2.5%	0.10 [-0.45, 0.65]	
Neff et al. (2018)	0.86170117	0.11534349	24	0.8986127	0.06251112	21	2.1%	-0.38 [-0.98, 0.21]	
Noguera-Julian et al. (2016)	0.91982865	0.05998578	206	0.93389985	0.03818292	34	5.7%	-0.24 [-0.61, 0.12]	
Vesterbacka et al. (2017)	0.92080144	0.06904631	47	0.95381426	0.02589046	15	2.2%	-0.53 [-1.12, 0.06]	
Vazquez-Castellanos et al. (2015) Subtotal (95% CI)	0.89476898	0.13555248	9 744	0.96960296	0.01063082	12 544	0.9% 54.5%	-0.81 [-1.72, 0.09] -0.14 [-0.26, -0.02]	•
Heterogeneity: $Chi^2 = 17.47$ , df = 11 Test for overall effect: Z = 2.37 (P = $10^{-1}$	(P = 0.09); I <sup>2</sup> = 0.02)	37%							
13.1.2 h Restricting the analysis t	o woman-obse	arved							
Armstrong et al. (2018)	191 4210526	79 18074667	10	216 0243902	59 0235071	41	2 5%	-0.37 [-0.92 0.18]	
Dinh et al. (2015)	127 75	67 21296998	4	120.25	23 14267343	4	0.4%	0 13 [-1 26 1 52]	
Neff et al. (2018)	216 1428571	87 7770444	7	296.6	104 6126187	5	0.5%	-0 78 [-1 99 0 43]	
Noquera-Julian et al. (2016)	319.4576271	89.50964541	59	362.2	65.24722216	5	0.9%	-0.48 [-1.40, 0.44]	
Vesterbacka et al. (2017)	343.6086957	77.39258781	23	423,125	36.55695165	8	1.0%	-1.11 [-1.97, -0.25]	
Subtotal (95% CI)			112			63	5.3%	-0.53 [-0.91, -0.16]	◆
Heterogeneity: $Chi^2 = 3.14$ , $df = 4$ (P Test for overall effect: Z = 2.78 (P =	= 0.53); l <sup>2</sup> = 0% 0.005)	6							
13.1.3 c. Restricting the analysis t	o non-MSM-sir	mnson							
Armstrong et al. (2018)	0 87103/1	0 13174712	22	0 02504571	0.04804511	70	3 10/	0.60[1.18_0.20]	
Dinh et al. (2015)	0.93831144	0.03337379	4	0.91892595	0.05368107	4	0.1%	0.38 [-1.03 1.79]	
Lee et al. (2018)	0.89427758	0.02389707	12	0.8838636	0.04103578	9	1.0%	0.31 [-0.56, 1.18]	
Noquera-Julian et al. (2016)	0.92214671	0.0626714	110	0.94727261	0.02781261	11	1.9%	-0.41 [-1.03, 0.21]	
Vesterbacka et al. (2017)	0.92738885	0.04901866	34	0.95581271	0.02898466	12	1.7%	-0.62 [-1.29, 0.05]	
Subtotal (95% CI)			182			106	8.1%	-0.44 [-0.74, -0.13]	•
Heterogeneity: Chi <sup>2</sup> = 5.41, df = 4 (P Test for overall effect: Z = 2.81 (P =	= 0.25); l <sup>2</sup> = 26 0.005)	%							
13.1.4 d Restricting the analysis t	o CD4+T cell c	out<500-shar	non						
Armstrong et al. (2018)	3 28/350/6	0 72055675	32	3 40107807	0 57556078	105	1 8%	0 10 [ 0 50 0 21]	
Dinb et al. (2015)	3 26122523	0.72033073	32	3 57022355	0.57550978	105	4.0 %	-0.19 [-0.59, 0.21]	
Lee et al. (2018)	2 77454735	0.20603418	11	2 75272515	0.34047523	20	1.4%	0.07 [-0.67 0.81]	
Lozupone et al. (2013)	3.07009518	0.54678562	10	3.22130299	0.85441446	22	1.3%	-0.19 [-0.94, 0.56]	
Neff et al. (2018)	3.01410463	0.65969255	11	3.3491159	0.61180982	21	1.4%	-0.52 [-1.26, 0.22]	
Noguera-Julian et al. (2016)	3.60037173	0.49915549	75	3.84837146	0.39487328	34	4.4%	-0.52 [-0.94, -0.11]	
Subtotal (95% CI)			146			217	14.1%	-0.33 [-0.56, -0.10]	◆
Heterogeneity: $Chi^2 = 3.14$ , df = 5 (P Test for overall effect: Z = 2.78 (P =	= 0.68); l <sup>2</sup> = 0% 0.006)	6							
13.1.5 e. Restricting the analysis to	o non-ART-sh	annon							
Armstrong et al. (2018)	3.34718766	0.73991471	45	3.40197897	0.57282242	105	6.1%	-0.09 [-0.44, 0.26]	
Dillon et al. (2014)	2.87514176	0.53783811	18	3.22590879	0.41169951	14	1.4%	-0.70 [-1.42, 0.02]	
Li et al. (2019)	3.12740573	0.56255602	12	3.36342294	0.61333953	36	1.7%	-0.39 [-1.04, 0.27]	
Lozupone et al. (2013)	3.44248075	1.2931113	14	3.22130299	0.83477012	22	1.7%	0.21 [-0.46, 0.88]	
Neff et al. (2018)	2.82529908	0.55799129	9	3.3491159	0.59706525	21	1.1%	-0.87 [-1.69, -0.05]	
Noguera-Julian et al. (2016)	3.71310074	0.50776154	58	3.84837146	0.38902298	34	4.1%	-0.29 [-0.71, 0.14]	+
Vesterbacka et al. (2017) Subtotal (95% CI)	3.70157361	0.57309825	32 188	4.07986109	0.16600442	15 247	1.9%	-0.77 [-1.40, -0.13]	
Heterogeneity: Chi <sup>2</sup> = 8.85, df = 6 (P	= 0.18); l <sup>2</sup> = 32	!%	100			241	10.076	-0.00 [-0.01, -0.10]	•
Test for overall effect: Z = 2.91 (P =	0.004)								
Total (95% CI)			1372			1177	100.0%	-0.24 [-0.33, -0.15]	◆
Heterogeneity: Chi <sup>2</sup> = 45.53, df = 34	(P = 0.09); I <sup>2</sup> =	25%						-	-2 -1 0 1 2
Test for overall effect: Z = 5.47 (P <	0.00001)								Enriched in HIV- Enriched in HIV+
rest for subaroup differences: Chi <sup>2</sup> =	= 1.52. dt = 4 (P	$r = 0.11$ ). $I^2 = 46$	.8%						

**FIGURE 2** Forest plots comparing HIV+ to HIV- individuals. The fixed effects models (FEMs) with a 95% CI above or below zero were considered statistically significant. The heterogeneity analysis included estimates of  $Chi^2$  and  $l^2$ . Before controlling for other confounding factors, the alpha diversity of the HIV+ individuals was significantly lower than that of the HIV- individuals (**a**, Simpson index). When restricting the analysis to women (**b**, Observed index), non-MSM individuals (**c**, Simpson index), individuals with CD4<sup>+</sup>T cell count of <500 (**d**, Shannon index), and non-ART individuals (**e**, Shannon index), HIV+ status was associated with a significant decrease in alpha diversity.

that HIV+ and MSM status were related to consistent changes in the specific genera, species, and KEGG functional pathways.

In recent years, consistent alpha diversity of the gut microbiome associated with HIV infection has not been clarified (Lozupone et al., 2013; Mutlu et al., 2014; Dinh et al., 2015; Dubourg et al., 2016; Nowak et al., 2017). Therefore, we assembled the largest dataset to date to evaluate the alpha diversity of the gut microbiome related to HIV status. Whether in the overall effect analysis based on 12 studies or in the subgroup analysis, our results indicated that HIV+ status was associated with decreased alpha diversity of the gut microbiome, which was consistent with most current research results (Mutlu et al., 2014; Nowak et al., 2015; Dubourg et al., 2016; Noguera-Julian et al., 2016; Vesterbacka et al., 2017; Tuddenham et al., 2020). In our



FIGURE 3 | Boxplots showing the alpha diversity in terms of the Simpson index by study and HIV status (red, HIV– individuals; blue, HIV+ individuals). Most studies showed decreased alpha diversity in HIV+ individuals compared to HIV– individuals.

#### TABLE 3 | The alpha diversity results of MSM in OTU level.

D-f	Ob a survey of	011	405	01	0:	<b>C</b> :-1	I
References	Observed	Chaol	AGE	Snannon	Simpson	Fisher	Invsimpson
	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p	FDR p
Dinh et al. (2015)	0.660	0.750	0.660	0.630	0.630	0.660	0.630
Noguera-Julian et al. (2016)	0.000***	0.000***	0.000***	0.190	0.480	0.000***	0.110
Vesterbacka et al. (2017)	0.630	0.630	0.630	0.630	0.630	0.630	0.630
Armstrong et al. (2018)	0.880	0.700	0.700	0.190	0.098	0.015*	0.300
Lee et al. (2018)	0.590	0.590	0.590	0.930	0.630	0.590	0.840
Li et al. (2019)	0.340	0.840	0.840	0.000***	0.000***	0.460	0.000***

\*FDR p < 0.05, \*\*FDR p < 0.01, and \*\*\*FDR p < 0.001.

### TABLE 4 | The beta diversity results of HIV in OTU level.

References	E	Bray-Curtis	Index	Jenser	n-Shannon I	Divergence		Jaccard Inc	lex
	F	R <sup>2</sup>	FDR p	F	R <sup>2</sup>	FDR p	F	R <sup>2</sup>	FDR p
Lozupone et al. (2013)	9.131	0.154	< 0.001***	14.016	0.219	< 0.001***	6.167	0.110	< 0.001***
Dillon et al. (2014)	0.515	0.017	< 1.000	0.301	0.010	< 1.000	0.748	0.024	< 1.000
Dinh et al. (2015)	0.992	0.028	< 0.460	1.012	0.029	< 0.460	1.004	0.029	< 0.460
Vázquez-Castellanos et al. (2015)	2.691	0.124	< 0.012*	3.631	0.160	< 0.012*	2.096	0.099	< 0.012*
Dubourg et al. (2016)	9.073	0.080	< 0.001***	13.751	0.117	< 0.001***	5.916	0.054	< 0.001***
Noguera-Julian et al. (2016)	2.821	0.012	< 0.002**	4.123	0.017	< 0.002**	2.150	0.009	< 0.002**
Vesterbacka et al. (2017)	2.052	0.033	< 0.021*	2.477	0.040	< 0.021*	1.682	0.027	< 0.021*
Armstrong et al. (2018)	13.380	0.059	< 0.001***	20.417	0.087	< 0.001***	8.695	0.039	< 0.001***
Cook et al. (2019)	3.489	0.009	< 0.001***	5.237	0.014	< 0.001***	2.421	0.006	< 0.001***
Lee et al. (2018)	1.002	0.022	< 0.430	1.059	0.024	< 0.430	1.013	0.022	< 0.430
Neff et al. (2018)	1.032	0.023	< 0.390	1.079	0.024	< 0.390	1.004	0.023	< 0.390
Li et al. (2019)	0.987	0.021	< 0.430	0.962	0.020	< 0.430	1.014	0.022	< 0.430

\*FDR p < 0.05, \*\*FDR p < 0.01, and \*\*\*FDR p < 0.001.

		MSM		N	onMSM			Std Mean Difference	Std Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI	IV, Fixed, 95% Cl
14.1.1 a. All-simpson									
Armstrong et al. (2018)	0.88471142	0.10661613	125	0.91280428	0.07846719	92	12.1%	-0.29 [-0.56, -0.02]	
Dinh et al. (2015)	0.86275544	0.11449414	12	0.90748181	0.05283398	8	1.1%	-0.45 [-1.36, 0.46]	
Lee et al. (2018)	0.87937842	0.04755225	25	0.88899607	0.03392895	21	2.6%	-0.23 [-0.81, 0.36]	
Li et al. (2019)	0.85953212	0.08437036	32	0.94176135	0.0294436	16	2.1%	-1.13 [-1.78, -0.49]	
Noguera-Julian et al. (2016)	0.91907657	0.05460308	119	0.92442923	0.06066473	121	13.8%	-0.09 [-0.35, 0.16]	
Vesterbacka et al. (2017) Subtotal (95% CI)	0.90878957	0.1011604	15 328	0.93473718	0.04617443	46 304	2.6% 34.2%	-0.40 [-0.99, 0.19] -0.27 [-0.43, -0.11]	•
Heterogeneity: Chi <sup>2</sup> = 9.18, df Test for overall effect: Z = 3.32	= 5 (P = 0.10); I 2 (P = 0.0009)	² = 46%							
14.1.2 b. Restricting the anal	vsis to HIV+-fi	sher							
Armstrong et al. (2018)	40.05277654	9.00192586	90	36.03456855	7.6018104	22	4.0%	0.46 [-0.01, 0.93]	— <u> </u>
Dinh et al. (2015)	7.99977846	3.58914957	12	7.41608581	1.94958887	4	0.7%	0.17 [-0.97, 1.30]	
Lee et al. (2018)	12.61170254	2.97003735	14	12.00934799	1.34009825	12	1.5%	0.25 [-0.53, 1.02]	<del></del>
Noguera-Julian et al. (2016)	55.32236882	7.60915706	96	46.8768955	11.0343583	110	10.7%	0.88 [0.59, 1.16]	
Vesterbacka et al. (2017) Subtotal (95% CI)	59.14756546	10.28373665	12 224	54.04222725	13.04987858	34 182	2.0%	0.40 [-0.26, 1.07]	•
Heterogeneity: Chi <sup>2</sup> = 5.32, df	= 4 (P = 0.26); I	² = 25%				TOL	10.070	0.00 [0.40, 0.00]	-
Test for overall effect: Z = 6.01	(P < 0.00001)								
14.1.3 c. Restricting the anal	vsis to HIVinv	vsimpson							
Armstrong et al. (2018)	11,76235804	7.40434128	35	17.95874152	8.92358573	70	5.1%	-0.73 [-1.15, -0.31]	
Lee et al. (2018)	8.93919058	3.32016651	11	9.47314561	3.1659514	9	1.1%	-0.16 [-1.04, 0.73]	
Li et al. (2019)	10.03161821	7.21077043	20	21.02452641	10.71362307	16	1.7%	-1.20 [-1.92, -0.48]	
Noguera-Julian et al. (2016)	19.18593793	11.56171232	23	23.80192887	10.25909298	11	1.7%	-0.40 [-1.13, 0.32]	
Vesterbacka et al. (2017)	19.77667988	5.31202063	3	27.22905293	8.5742921	12	0.5%	-0.86 [-2.17, 0.46]	
Subtotal (95% CI)			92			118	10.1%	-0.70 [-0.99, -0.40]	◆
Heterogeneity: Chi <sup>2</sup> = 4.05, df	= 4 (P = 0.40); I	² = 1%							
l est for overall effect: Z = 4.61	(P < 0.00001)								
14.1.4 d. Restricting the anal	lysis to age $<$ 4	5-invsimpson							
Armstrong et al. (2018)	14.27752911	11.69310885	77	18.61011454	10.89501935	65	8.0%	-0.38 [-0.71, -0.05]	
Lee et al. (2018)	9.30230095	3.28688477	21	9.64056571	2.38258786	10	1.6%	-0.11 [-0.86, 0.65]	
Noguera-Julian et al. (2016)	17.50691113	10.98299247	81	21.42704719	11.5478913	56	7.5%	-0.35 [-0.69, -0.00]	
Vesterbacka et al. (2017)	16.11122206	10.92460261	4	21.50677665	10.65828282	20	0.8%	-0.49 [-1.57, 0.60]	
Subtotal (95% CI)	- 2 (D - 0 02); I	2 - 09/	183			151	17.8%	-0.35 [-0.57, -0.12]	•
Test for overall effect: Z = 3.05	– 3 (P – 0.92), 1 5 (P = 0.002)	0 %							
14.1.5 o. Postricting the anal	veis to ano >4	5 fichor							
Armstrong et al. (2018)	39 05174532	9 84319323	48	31 67606366	9 51536522	27	3 7%	0 75 [0 26 1 24]	
Amstrong et al. (2018)	12 097/65/1	3 17/72796	40	12 12509526	3.02787526	11	0.7%	-0.01 [-1.15, 1.14]	
Noquera- Julian et al. (2016)	56 60544739	7 47220634	38	45 10563355	11 05326683	65	4.8%	1 15 [0 72 1 59]	
Vesterbacka et al. (2017)	62,73997786	4.29928759	11	54,48984937	13.02476908	26	1.7%	0.72 [-0.01, 1.44]	
Subtotal (95% CI)			101			129	10.8%	0.88 [0.59, 1.16]	•
Heterogeneity: Chi <sup>2</sup> = 4.33, df	= 3 (P = 0.23); I	² = 31%							
Test for overall effect: Z = 6.00	) (P < 0.00001)								
14.1.6 f. Restricting the analy	ysis to BMI=24	-27.9-fisher							
Armstrong et al. (2018)	38.45202345	9.75920033	45	33.03346256	7.37524378	34	4.3%	0.61 [0.15, 1.06]	
Dinh et al. (2015)	9.00008781	3.85042924	7	8.9895864	3.04183336	4	0.6%	0.00 [-1.23, 1.23]	
Noguera-Julian et al. (2016)	56.94666709	7.20758514	32	50.21306051	8.49360644	17	2.3%	0.86 [0.25, 1.48]	· · · · · · · · · · · · · · · · · · ·
Vesterbacka et al. (2017)	58.61136583	9.2478474	7	51.0275004	12.1274772	15	1.0%	0.64 [-0.28, 1.56]	
Subtotal (95% CI)			91			70	8.2%	0.64 [0.31, 0.97]	
Heterogeneity: Chi <sup>2</sup> = 1.56, df Test for overall effect: Z = 3.84	= 3 (P = 0.67); I I (P = 0.0001)	<sup>2</sup> = 0%							
Total (95% CI)			1019			954	100.0%	0.05 [-0.05, 0.14]	•
Heterogeneity: Chi <sup>2</sup> = 152.44,	df = 27 (P < 0.0	0001); l <sup>2</sup> = 82%							
Test for overall effect: Z = 1.00	) (P = 0.32)								-Z -1 U 1 2 Enriched in NonMSM Enriched in MSM
Test for subaroup differences:	Chi <sup>2</sup> = 127.51. (	df = 5 (P < 0.00	001). F	² = 96.1%					

FIGURE 4 | Forest plots comparing MSM to non-MSM (NonMSM) individuals. Before controlling for other confounding factors, the alpha diversity of the MSM was significantly lower than that of the non-MSM individuals ( $\mathbf{a}$ , Simpson index). When restricting the analysis to HIV+ ( $\mathbf{b}$ , Fisher index), age  $\geq$ 45 years ( $\mathbf{e}$ , Fisher index), BMI = 24–27.9 ( $\mathbf{f}$ , Fisher index) individuals, MSM status was associated with an increase in alpha diversity. When restricting the analysis to HIV- individuals ( $\mathbf{c}$ , Invsimpson index) and age < 45 years ( $\mathbf{d}$ , Invsimpson index), MSM status was associated with a significant decrease in alpha diversity.

subgroup analysis, the downregulation of alpha diversity in the gut microbiome is related to HIV+ non-ART status, consistent with the results by Vesterbacka et al. (2017). However, there was evidence that ART also induces changes in the gut microbiome, unrelated to HIV infection. Some authors have implied that ART may enhance dysbiosis, which is consistent with the high frequency of gastrointestinal symptoms associated with ART (Lozupone et al., 2014; Nowak et al., 2015; Noguera-Julian et al.,

2016). In addition, severe mucosal CD4<sup>+</sup>T cell depletion is an important reason for disruption of the gut epithelial barrier and translocation of the gut microbiome in the early stage of HIV infection (Hirao et al., 2014). We also confirmed that the CD4<sup>+</sup>T cell depletion in HIV+ individuals is closely related to the gut microbiome.

Another group of people that we evaluated was MSM. Recent studies have shown that MSM status may profoundly affect the





structure of the gut microbiome, which may be stronger than HIV, and this factor may confound many studies of HIV-related gut microbiomes (Noguera-Julian et al., 2016; Kelley et al., 2017; Armstrong et al., 2018; Neff et al., 2018; Guillen et al., 2019; Hensley-McBain et al., 2019; Kehrmann et al., 2019; Li et al., 2019). In our study, a significant reduction in alpha diversity associated with HIV+ status was found in non-MSM individuals, not MSM. Further analysis revealed that there was a significant difference in the gut microbiome alpha diversity between MSM and non-MSM individuals and samples were better clustered in PCoA by MSM, rather than HIV status. These trends reflect previously published results showing that the gut microbiome of MSM has higher immune activity than men who have sex with

women (MSW), regardless of HIV infection (Neff et al., 2018; Li et al., 2019).

In addition, many cross-sectional studies have indicated that the gut microbiome shifts from *Bacteroides* to *Prevotella* predominance after HIV infection (Lozupone et al., 2013; Mutlu et al., 2014; Vázquez-Castellanos et al., 2015; Ling et al., 2016; Dillon et al., 2017; Serrano-Villar et al., 2017). However, the latest research suggests that the *Prevotella* predominance is associated with MSM rather than HIV status (Armstrong et al., 2018; Neff et al., 2018; Li et al., 2019). For the inconsistent results of the previous studies, we used LEfSe method to identify the differential genus related to HIV+ and MSM status and used DESeq2 and Random Forests method to verify. Our results



References	В	Bray-Curtis	Index	Jenser	-Shannon I	Divergence		Jaccard Ind	lex
	F	R <sup>2</sup>	FDR p	F	R <sup>2</sup>	FDR p	F	$R^2$	FDR p
Dinh et al. (2015)	1.838	0.093	< 0.020*	2.362	0.116	< 0.020*	1.531	0.078	< 0.020*
Noguera-Julian et al. (2016)	32.974	0.122	< 0.001***	55.313	0.189	< 0.001***	19.926	0.077	< 0.001***
Vesterbacka et al. (2017)	5.225	0.081	< 0.001***	8.669	0.128	< 0.001***	3.573	0.057	< 0.001***
Armstrong et al. (2018)	21.148	0.090	< 0.001***	32.809	0.132	< 0.001***	13.312	0.058	< 0.001***
Lee et al. (2018)	1.523	0.033	< 0.099	1.712	0.037	< 0.099	1.385	0.031	< 0.099
Li et al. (2019)	5.990	0.115	< 0.001***	8.281	0.153	< 0.001***	4.059	0.081	< 0.001***

#### TABLE 5 | The beta diversity results of MSM in OTU level.

\*FDR p < 0.05, \*\*FDR p < 0.01, \*\*\*FDR p < 0.001.

showed that in multiple studies ( $\geq$ 3 studies), HIV+ status was related to the abundant downregulation of *Bacteroides*, *Coprococcus*, *Faecalibacterium*, and *SMB53*, while the *Prevotella*rich and *Bacteroides*-poor were more closely related to MSM status. For the *Prevotella*-rich not seen in HIV+ individuals, a study found that *Prevotella* abundance decreased after ART initiation (Nowak et al., 2015). It is worth noting that at the species level, the pattern of microbial composition with decreased abundance of *B. caccae*, *B. ovatus*, and *B. uniformis* and increased abundance of *P. stercorea* is consistent in HIV+ individuals and MSM. *Prevotella* spp. is generally considered to have proinflammatory effects, whereas *Bacteroides* spp. has a role in promoting T-regulatory cell function. For example, studies have found evidence that enhanced  $CD4^+T$  cell HIV infection or inflammation induction is associated with experiments utilizing *P. copri* or *P. stercorea* (Dillon et al., 2016; Kaur et al., 2018). *Bacteroides* is considered to be the main genus of the core microbiome module; of which, the species with relative abundances exceeding 1% are *B. uniformis*, *B. vulgatus*, *B. caccae*, and *B. thetaiotaomicron* (Tan et al., 2019). Gauffin Cano et al. demonstrated that the *B. uniformis CECT7771* is capable of ameliorating the overweight-associated immune dysfunctions (Gauffin Cano et al., 2012). Hamady et al. (2010, 2011) found that the *B. ovatus* can prevent colitis caused by DSS in the form of improving weight loss and reducing the colon length, and downregulating the secretion of proinflammatory cytokines such



as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 (Hamady et al., 2010, 2011). These findings suggest that MSM status may be an independent factor related to dysbiosis of the gut microbiome. Meanwhile, early regulation of MSM-related gut microbiome dysbiosis is of great significance for the prevention and treatment of HIV infection and intestinal inflammation.

The results of the KEGG functional pathway analysis showed gut microbiome-gut microbiome interactions and gut microbiome-human body interactions. For example, a study using gnotobiotic mouse models showed that extracellular digestion of inulin increases the growth rate of B. ovatus. In turn, by-products from inulin catabolism can be used by F. prausnitzii and B. vulgatus (Rakoff-Nahoum et al., 2016). Our research also confirmed this result. That is, in HIV+ individuals, the abundance of carbohydrate metabolism and F. prausnitzii is significantly downregulated with the downregulation of B. ovatus. Comparative analysis of microbial genomes shows that more than 98% of all microbiomes (such as Bacteroides spp.) sequenced so far lack essential pathways or key genes for amino acid synthesis. Therefore, most microbiomes are auxotrophic and require a source of extracellular amino acids, vitamins, and/or cofactors to survive (Mee and Wang, 2012; Mee et al., 2014). Our research also shows that the reduction in the abundance of Bacteroides in HIV+ individuals and MSM was accompanied by the downregulation of amino-acid-metabolism-related pathways.

This study has some limitations. First, we did not collect all demographic and disease characteristics related to the subjects, which leads to a lack of enough datasets for our subgroup analysis. We analyzed the relationship between the gut microbiome and MSM status based on HIV-related data. There are many confounding factors in the analysis related to MSM status, such as age, disease status, and BMI, which are often not matched in HIV-related research, and our included studies are mainly from Europe and the Americas, lacking research from Asia and Africa. Moreover, the shotgun data of the gut microbiome were excluded in our analysis, which might cause flaws in our findings, especially in the bacterial functional pathway analysis. Lastly, the different studies used different variable regions and instruments for 16S rRNA gene amplicon sequencing. Although we have processed and standardized the sequences according to the characteristics of the data, different experimental techniques may still cause bias.

In conclusion, our results clarified that HIV+ status is associated with decreased alpha diversity of the gut microbiome. MSM status was an important factor affecting the study of HIV-related gut microbiomes; that is, MSM was associated with alpha diversity changes in the gut microbiome regardless of HIV infection, and the change in gut microbiome composition of MSM was more significant than that of HIV+ individuals. There was a consistent change in *B. caccae*, *B. ovatus*, *B. uniformis*, and *P. stercorea*, in HIV+ individuals and MSM. The differential expression of the gut microbiome was also accompanied by changes in functional pathways, such as carbohydrate metabolism, amino acid metabolism, and lipid metabolism, These findings might help to elucidate the effects of HIV+ and MSM status on the gut microbiome in humans.

A 🗌	Genus	Enriched in	Studies	ј В	Species	Enriched in	Studies
	Acidaminococcus		Lozupone et al. (2013)				Neff et al. (2018)
	Actualiniococcus		Li et al. (2019)		Akkermansia muciniphila		Dubourg et al. (2016)
	Adlercreutzia		Vesterbacka et al. (2017)				Armstrong et al. (2018)
			Dubourg et al. (2016)				Lozupone et al. (2013)
	Agrobacterium		Vesterbacka et al. (2017)		Bacteroides caccae		Armstrong et al. (2018)
			Dubourg et al. (2016)				Cook et al. (2019)
	Akkermansia		Vesterbacka et al. (2017)		Bacteroides fragilis		Cook et al. (2019)
			Armstrong et al. (2018)				Noguera-Julian et al. (2016)
	Anaerostipes		Dubourg et al. (2016)				Dubourg et al. (2016)
			Noguera-Julian et al. (2016)		Bacteroides ovatus		Lozupone et al. (2013)
	Pastonoidos		Lozupone et al. (2013)				Armstrong et al. (2018)
	Bacterolues		Armstrong et al. (2018)				Cook et al. (2019)
			Cook et al. (2019)				Noguera-Julian et al. (2016)
	Dig to be students		Armstrong et al. (2018)				Dillon et al. (2014)
	Bindobacterium		Dubourg et al. (2016)		Bacteroides uniformis		Lozupone et al. (2013)
	Blautia		Vesterbacka et al. (2017)				Armstrong et al. (2018)
	Bulleidia		Cook et al. (2019)				Cook et al. (2019)
	Butyricicoccus		Dubourg et al. (2016)				Lee et al. (2018)
	Butyrivibrio		Noguera-Julian et al. (2016)				Dubourg et al. (2016)
			Lozunone et al. (2013)		Bifidobacterium adolescentis		Armstrong et al. (2018)
	Catenibacterium		Noguera-Julian et al. (2016)				Cook et al. (2019)
	Citrobacter		Dubourg et al. (2016)				Lozupone et al. (2013)
- H	Clostridium		Lozupone et al. (2013)				Dubourg et al. (2016)
- H	Clostridium		Armetrong of al. (2013)		Blautia obeum		Armstrong at al. (2018)
	Ciosa idiulli		Armstrong et al. (2010)				Cook at al. (2010)
	Convog		At instrong et al. (2018) Dubouwg et al. (2016)		Pulloidio - 1620 -5		Cook et al. (2019)
	Coprococcus		Dubourg et al. (2016)		Bullelala p-1630-c5		Cook et al. (2019)
$\vdash$	Commelant		Cook et al. (2019)		Campyiobacter ureolyticus		Cook et al. (2019)
F	Corynebacterium		Cook et al. (2019)		Clostridium citroniae		Dinn et al. (2015)
	Desulfovibrio		Cook et al. (2019)				Dubourg et al. (2016)
			Lozupone et al. (2013)		Clostridium celatum		Dubourg et al. (2016)
	Dialister		Lozupone et al. (2013)		Clostridium citroniae		Armstrong et al. (2018)
	Dimister		Noguera-Julian et al. (2016)		Clostridium ruminantium		Dubourg et al. (2016)
	Dorea		Cook et al. (2019)		Clostridium saccharogumia		Lozupone et al. (2013)
	Enterococcus		Lee et al. (2018)				Dubourg et al. (2016)
	Escherichia		Dubourg et al. (2016)		Coprococcus catus		Armstrong et al. (2018)
	Euboatarium		Armstrong et al. (2018)				Cook et al. (2019)
	Eubacterium		Lozupone et al. (2013)		Desulfovibrio D168		Noguera-Julian et al. (2016)
			Armstrong et al. (2018)		Dorea formicigenerans		Lozupone et al. (2013)
	Faecalibacterium		Dubourg et al. (2016)		Eggerthella lenta		Dubourg et al. (2016)
			Cook et al. (2019)		Escherichia coli		Dubourg et al. (2016)
			Cook et al. (2019)				Lozupone et al. (2013)
	Fusobacterium		Lee et al. (2018)		Eubacterium bitorme		Armstrong et al. (2018)
	Granulicatella		Cook et al. (2019)		Eubacterium cylindroides		Cook et al. (2019)
	Holdemania		Armstrong et al. (2018)		Eubacterium dolichum		Dubourg et al. (2016)
	Lachnobacterium		Noguera-Julian et al. (2016)				Dillon et al. (2014)
			Dubourg et al. (2016)				Dubourg et al. (2016)
	Lachnospira		Cook et al. (2019)		Faecalibacterium prausnitzii		Armstrong et al. (2018)
	Megamonas		Lee et al. (2018)				Cook et al. (2019)
			Lee et al. (2018)		formicigenerans		Cook et al. (2019)
	Megasphaera		Lozupone et al. (2013)		Mitsuokella multacida		Lozupone et al. (2013)
	Mitsuokella		Lozupone et al. (2013)				Vázquez-CastellanosF et al. (2015)
			Armstrong et al. (2018)				Vesterbacka et al. (2017)
	Mogibacterium		Lozupone et al. (2013)				Neff et al. (2018)
			Dillon et al. (2014)				Li et al. (2019)
			Dinh et al. (2015)		NA		Dinh et al. (2015)
			Li et al. (2019)				Vázquez-CastellanosF et al. (2015)
	NA		Vázquez-CastellanosF et al. (2015)				Vesterbacka et al. (2017)
			Neff et al. (2018)				Lee et al. (2018)
			Dinh et al. (2015)				Li et al. (2019)
			Cook et al. (2019)				Lozupone et al. (2013)
	Oribacterium		Noguera-Julian et al. (2016)		Parabacteroidas distasenis		Armstrong et al (2013)
	Oscillocaira		Dubourg et al. (2016)		a abacter ordes distasoffis		Cook et al. (2010)
$\vdash$	Озспозріга		Dubourg et al. (2010)		Pontostrontoso 1		Cook et al. (2019)
	Darabaatensides		Lozupono et al. (2010)		prostreptococcus anaerobius		Dillon at al (2014)
	rarabacteroides		Lozupone et al. (2013)		Prevotella copri		Dinion et al. (2014)
H			Cook et al. (2019)		-		Dillon at al. (2014)
	Paraprevotella		Lozupone et al. (2013)		Deserve H. C.		Dinion et al. (2014)
$\vdash$			v azquez-CastellanosF et al. (2015)		Prevotella stercorea		Lozupone et al. (2013)
$\vdash$	Parvimonas		Cook et al. (2019)				Armstrong et al. (2018)
	Peptococcus		Lozupone et al. (2013)		Roseburia faecis		Dubourg et al. (2016)
			Noguera-Julian et al. (2016)				Armstrong et al. (2018)
	Peptostreptococcus		Cook et al. (2019)		Ruminococcus bromii		Dubourg et al. (2016)
	Phascolarctobacterium		Armstrong et al. (2018)		Ruminococcus torques		Dubourg et al. (2016)
	Prevotella		Armstrong et al. (2018)				Lee et al. (2018)
	Trevotena		Lozupone et al. (2013)		Streptococcus infantis		Dillon et al. (2014)
Ps	eudoramibacter Eubacterium		Dubourg et al. (2016)				
	Roseburia		Dubourg et al. (2016)				
	Ruminococcus		Dubourg et al. (2016)				
			Armstrong et al. (2018)				
	SMB53		Dubourg et al. (2016)				
			Cook et al. (2019)				
	Sneathia		Cook et al. (2019)				
			Cook et al. (2019)				
	Staphylococcus						
F	Staphylococcus		Dillon et al. (2014)				
	Staphylococcus Streptococcus		Dillon et al. (2014) Dubourg et al. (2016)				
	Staphylococcus Streptococcus Turicibacter		Dillon et al. (2014) Dubourg et al. (2016) Noff et al. (2018)				

FIGURE 8 | Differential genus (A) and species (B) map related to HIV status. From left to right, the first column represents the differential genus or species, and the third column represents the studies. The third column represents the research corresponding to the differential genus or species. The second column shows the differential enrichment of genus or species in HIV+ and HIV- individuals. Red indicates that the genus or species were significantly enriched in HIV+ individuals. Blue indicates that the genus or species were significantly enriched in HIV- individuals.

A	Genus	Enriched in	Studies	В	Species	Enriched in	Studies
			Noguera-Julian et al. (2016)	1 -	Akkermansia muciniphila		Vesterbacka et al. (2017)
	Adlercreutzia		Vesterbacka et al. (2017)		· · · · · · · · · · · · · · · · · · ·		Armstrong et al. (2018)
	Aggregatibactor		Li et al. (2019) Lee et al. (2018)		Alistipes indistinctus		Noguera-Julian et al. (2016) Vesterbacka et al. (2017)
	Aggregatibacter		Vesterbacka et al. (2017)		Asteroleplasma anaerobium		Noguera-Julian et al. (2017)
	Akkermansia		Armstrong et al. (2018)		rister oreplasing where obtain		Noguera-Julian et al. (2016)
	Alictinos		Noguera-Julian et al. (2016)		Pastoroidos sassas		Vesterbacka et al. (2017)
	Ausupes		Vesterbacka et al. (2017)		Dacter oldes caccae		Armstrong et al. (2018)
	Asteroleplasma		Noguera-Julian et al. (2016)				Li et al. (2019)
	Pastoroidor		Noguera-Julian et al. (2016)		Bacteroides Iragilis		Li et al. (2019) Noguere Julien et al. (2016)
	Dacteroities		Armstrong et al. (2018)		Bacteroides ovatus		Noguera-Julian et al. (2010) Vesterbacka et al. (2017)
			Noguera-Julian et al. (2016)		Dacter oldes ovatus		Armstrong et al. (2018)
	D'C labortorione		Vesterbacka et al. (2017)				Noguera-Julian et al. (2016)
	Billuobacterium		Armstrong et al. (2018)		Bacteroides uniformis		Vesterbacka et al. (2017)
			Li et al. (2019)		Ducter of des uniformis		Armstrong et al. (2018)
			Noguera-Julian et al. (2016)				Li et al. (2019)
	Bilophila		Armstrong et al. (2018)		Bifidobacterium pseudolongum		Noguera-Julian et al. (2016) Vesterbacka et al. (2017)
			Li et al. (2019)		Bifidobacterium adolescentis		Armstrong et al. (2018)
	Plantia		Noguera-Julian et al. (2016)				Li et al. (2019)
	biautia		Armstrong et al. (2018)		Blautia obeum		Noguera-Julian et al. (2016)
	Bulleidia		Noguera-Julian et al. (2016)				Armstrong et al. (2018)
			Vesterbacka et al. (2017)		Bulleidia p-1630-c5		Noguera-Julian et al. (2016)
	Butyricicoccus		Armstrong et al. (2018)				Armstrong et al. (2017)
	Butyrivibrio		Noguera-Julian et al. (2016)		Butyricicoccus pullicaecorum		Li et al. (2019)
			Noguera-Julian et al. (2016)		Clostridium alternation		Noguera-Julian et al. (2016)
			Vesterbacka et al. (2017)		Clostrialum citroniae		Armstrong et al. (2018)
	Catenibacterium		Dinh et al. (2015)		Collinsella aerofaciens		Noguera-Julian et al. (2016)
			Armstrong et al. (2018)		Coprococcus catus		Li et al. (2019)
			Armstrong et al. (2018)		Dorea formicigenerans		Armstrong et al. (2018)
	Clostridium		Li et al. (2019)		Escherichia coli		Lee et al. (2018)
	Collinsella		Noguera-Julian et al. (2016)				Noguera-Julian et al. (2016)
	Coprococcus		Armstrong et al. (2018)		Eubacterium biforme		Vesterbacka et al. (2017)
	coprototeus		Li et al. (2019)		Dubucterium bitorime		Armstrong et al. (2018)
	Desulfovibrio		Vesterbacka et al. (2017)		Fubactorium cylindroides		Li et al. (2019) Noguera-Iulian et al. (2016)
			Noguera-Julian et al. (2016)		Eubacterium cynnurolues		Armstrong et al. (2018)
	Dialister		Armstrong et al. (2018)		Faecalibacterium prausnitzii		Li et al. (2019)
	Enterococcus		Lee et al. (2018)		NA		Lee et al. (2018)
	Escherichia		Lee et al. (2018)				Noguera-Julian et al. (2016)
			Noguera-Julian et al. (2016) Vostorbacka et al. (2017)		Parabacteroides distasonis		Vesterbacka et al. (2017)
	Eubacterium		Armstrong et al. (2018)				Li et al. (2019)
			Li et al. (2019)				Noguera-Julian et al. (2016)
	Faecalibacterium		Armstrong et al. (2018)		Prevotella copri		Armstrong et al. (2018)
			Noguera-Julian et al. (2016)				Li et al. (2019)
	Holdemania		Armstrong et al. (2018) Li et al. (2019)				Vesterbacka et al. (2017) Armstrong et al. (2018)
			Noguera-Julian et al. (2016)		Prevotella stercorea		Li et al. (2019)
	Lachnospira		Li et al. (2019)				Noguera-Julian et al. (2016)
	Megasphaera		Noguera-Julian et al. (2016)		Roseburia faecis		Armstrong et al. (2018)
	Methanobrevibacter		Noguera-Julian et al. (2016)				Li et al. (2019)
	Mitenokella		Noguera-Julian et al. (2016)		Ruminococcus bromii		Armstrong et al. (2018) Noguera-Julian et al. (2016)
	Mitsuokena		Li et al. (2019)		Ruminococcus gnavus		Vesterbacka et al. (2017)
	Mogilestanium		Noguera-Julian et al. (2016)				Noguera-Julian et al. (2016)
	mogioacterium		Armstrong et al. (2018)		Ruminococcus torques		Armstrong et al. (2018)
	NA		Dinh et al. (2015)		Ctt.		Li et al. (2019)
	Odoribastor		Noguera-Julian et al. (2016) Vesterbacka et al. (2017)		Streptococcus agalactiae		Lee et al. (2018) Noquera-Iulian et al. (2016)
	Guoribacter		Armstrong et al. (2018)		Thursands vaucusis		onavia-ounan ci an (2010)
	Oribacterium		Noguera-Julian et al. (2016)				
			Noguera-Julian et al. (2016)				
	Parabacteroides		Vesterbacka et al. (2017)				
			Armstrong et al. (2018)				
	Paraprevotella		Noguera-Julian et al. (2016)				
	Peptococcus		Noguera-Julian et al. (2016)				
	ph2		Lee et al. (2018)				
			Noguera-Julian et al. (2016)				
	Phascolarctobacterium		Vesterbacka et al. (2017)				
			Armstrong et al. (2018)				
	Porphyromonas		Lee et al. (2013)				
			Noguera-Julian et al. (2016)				
			Vesterbacka et al. (2017)				
	Prevotella		Dinh et al. (2015)				
			Armstrong et al. (2018)				
			lietal (2019)	1			
	Ruminococcus		Li et al. (2019) Noguera-Julian et al. (2016)				
	Ruminococcus		Li et al. (2019) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016)				
	Ruminococcus Slackia		Li et al. (2019) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016) Vesterbacka et al. (2017)				
	Ruminococcus		Li et al. (2019) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016) Vesterbacka et al. (2017) Armstrong et al. (2018)				
	Ruminococcus Slackia SMB53		Li et al. (2019) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016) Vesterbacka et al. (2017) Armstrong et al. (2018) Armstrong et al. (2016)				
	Ruminococcus Slackia SMB53 Streptococcus		Li et al. (2019) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016) Vesterbacka et al. (2017) Armstrong et al. (2018) Armstrong et al. (2018) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016)				
	Ruminococcus Slackia SMB53 Streptococcus Succinivibrio		Li et al. (2019) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016) Vesterbacka et al. (2017) Armstrong et al. (2018) Armstrong et al. (2018) Noguera-Julian et al. (2016) Vesterbacka et al. (2017)				
	Ruminococcus Slackia SMB53 Streptococcus Succinivibrio Sutterella		Li et al. (2019) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016) Vesterbacka et al. (2017) Armstrong et al. (2018) Noguera-Julian et al. (2016) Noguera-Julian et al. (2016) Vesterbacka et al. (2017) Noguera-Julian et al. (2016)				

FIGURE 9 | Differential genus (A) and species (B) map related to MSM status. From left to right, the first column represents the differential genus or species, and the third column represents the studies. The third column represents the research corresponding to the differential genus or species. The second column shows the differential enrichment of genus or species in MSM and non-MSM. Red indicates that the genus or species were significantly enriched in MSM. Blue indicates that the genus or species were significantly enriched in non-MSM.

KEGG I	KEGG II		KEGG III
Cellular Processes	Cell Growth and Death		Cell cycle - Caulobacter
Containe 1 focesses	Transport and Catabolism		Lysosome MARK signaling nothway - yeart
Environmental Information Processing	Signal Transduction		Phosphatidylinositol signaling system
			Two-component system
	Folding Sorting and Degradation		Protein export Sulfan solar system
	Folding, Softing and Degradation		Ubiquitin system
			Base excision repair
			Chromosome DNA repair and recombination proteins
	Danlingtion and Danain		DNA replication
	Replication and Repair		DNA replication proteins
Genetic Information Processing		_	Homologous recombination Mismatch renair
			Nucleotide excision repair
	Transcription		RNA polymerase
			Aminoacvl-tRNA biosvnthesis
	Translation		Ribosome
	T T MISIACIÓN		Ribosome Biogenesis Translation factors
	Infectious Diseases		Epithelial cell signaling in Helicobacter pylori infection
Human Diseases	Mitchell Di		Vibrio cholerae pathogenic cycle
	Metabolic Diseases		Type II diabetes mellitus Alanine, aspartate and glutamate metabolism
			Amino acid related enzymes
	Amino Acid Metabolism		Arginine and proline metabolism
			Cysteine and methionine metabolism Histidine metabolism
			Valine, leucine and isoleucine biosynthesis
			beta-Lactam resistance Butirosin and neomycin biosynthesis
	Biosynthesis of Other Secondary Metabolites		Penicillin and cephalosporin biosynthesis
			Phenylpropanoid biosynthesis
		_	C5-Branched dibasic acid metabolism Galactose metabolism
			Glyoxylate and dicarboxylate metabolism
	Carbohydrate Metabolism		Inositol phosphate metabolism
			Pentose phosphate pathway
			Pyruvate metabolism
			Starch and sucrose metabolism Methane metabolism
	Energy Metabolism		Sulfur metabolism
	Enzyme Families		Peptidases Protein kinases
	Civaan Biosynthesis and Matabalism		Other glycan degradation
	Giycan biosynthesis and Metabolishi		Peptidoglycan biosynthesis
			Fatty acid metabolism
			Linoleic acid metabolism
Wietabolism	Lipid Metabolism		Secondary bile acid biosynthesis
			Sphingolipid metabolism
			Steroid hormone biosynthesis Biotin metabolism
			Folate biosynthesis
	Matabalian of Cofester and Viter		Lipoic acid metabolism
	Metabolishi of Colactors and vitamins		Porphyrin and chlorophyll metabolism
			Riboflavin metabolism
			Ubiquinone and other terpenoid-quinone biosynthesis Cvanoamino acid metabolism
			D-Alanine metabolism
	Metabolism of Other Amino Acids		D-Glutamine and D-glutamate metabolism
			Phosphonate and phosphinate metabolism
			Biosynthesis of siderophore group nonribosomal peptides
	Metabolism of Terpenoids and Polyketides		r olyketide sugar unit blosynthesis Prenyltransferases
	in the second state of the second states		Terpenoid backbone biosynthesis
			Zeatin biosynthesis Purine metabolism
	Nucleotide Metabolism		Pyrimidine metabolism
			Bisphenol degradation
	Xenobiotics Biodegradation and Metabolism		Drug metabolism - cytochrome P450
			Drug metabolism - other enzymes
			Metabolism of xenobiotics by cytochrome P450 PPAR signaling pathway
	Endocrine System		Progesterone-mediated oocyte maturation
Organismal Systems	Immune System		Antigen processing and presentation
	Nervous System		Glutamatergic synapse
	Cellular Processes and Signaling		Inorganic ion transport and metabolism
	Genetic Information Processing		Protein folding and associated processing Translation proteins
Unclassified	Matabalism		Biosynthesis and biodegradation of secondary metabolites
	De la Classica de la		Glycan biosynthesis and metabolism
	Poorly Characterized		Function unknown

FIGURE 10 | Differential KEGG functional pathways map related to HIV status. From left to right, the first column represents the KEGG I functional pathways, and the second and fourth columns represent the KEGG II and KEGG III functional pathways, respectively, under KEGG I. The third column shows the differential enrichment of the KEGG III functional pathways in HIV+ and HIV- individuals. Red indicates that the functional pathways were significantly enriched in HIV+ individuals in at least three studies. Blue indicates that the functional pathways were significantly enriched in HIV+ individuals in at least three studies. Second pathways were significantly different in at least three studies, but the direction of changes related to HIV status was inconsistent.

KEGG I	KEGG II	KEGG III
Cellular Processes	Cell Growth and Death	Cell cycle - Caulobacter
	Transport and Catabolism	Lysosome MAPK signaling pathway - vosst
Environmental Information Processing	Signal Transduction	Phosphatidylinositol signaling system
Environmental information riocessing	Signaling Molecules and Interaction	Cellular antigens
		Chaperones and folding catalysts
	Folding, Sorting and Degradation	Protein export
		Ubiquitin system Chromosome
		DNA repair and recombination proteins
		DNA replication
	Replication and Repair	DNA replication proteins Homologous recombination
Genetic Information Processing		Mismatch repair
		Non-homologous end-joining
		Aminoacyl-tRNA biosynthesis
	Translation	Ribosome Bibosomo Biogenesis
	Translation	Ribosome biogenesis in eukaryotes
		Translation factors
	Cancers	Amoebiasis
Human Disaasas	Infactions Diseases	Epithelial cell signaling in Helicobacter pylori infection
Human Diseases	Infectious Diseases	Tuberculosis
	Neurodegenerative Diseases	Alzheimer's disease
		Amino acid related enzymes
	Amino Acid Metabolism	Arginine and proline metabolism Histidine metabolism
	Annu Acta Metabolishi	Phenylalanine, tyrosine and tryptophan biosynthesis
		Valine, leucine and isoleucine biosynthesis
		Butirosin and neomycin biosynthesis
	Biosynthesis of Other Secondary Metabolites	Flavone and flavonol biosynthesis
		Penicillin and cephalosporin biosynthesis Phenylpropanoid biosynthesis
		Streptomycin biosynthesis
		Ascorbate and aldarate metabolism C5-Branched dibasic acid metabolism
		Galactose metabolism
	Cauhahuduata Matahaliam	Glyoxylate and dicarboxylate metabolism
	Cardonydrate Metadolism	Pentose and glucuronate interconversions
		Pentose phosphate pathway
		Pyruvate metabolism Starch and sucrose metabolism
		Methane metabolism
	Energy Metabolism	Photosynthesis Photosynthesis proteins
		Sulfur metabolism
	Enzyme Families	Peptidases Protein kinger
		Glycosphingolipid biosynthesis - globo series
Metabolism	Glycan Biosynthesis and Metabolism	Other glycan degradation
		Arachidonic acid metabolism
		Glycerophospholipid metabolism
	Lipid Metabolism	Linoleic acid metabolism Primary bile acid biosynthesis
		Secondary bile acid biosynthesis
		Sphingolipid metabolism Steroid hormone biosynthesis
		Biotin metabolism
		Lipoic acid metabolism
	Metabolism of Cofactors and Vitamins	One carbon pool by folate
		Porphyrin and chlorophyll metabolism Riboflavin metabolism
		Ubiquinone and other terpenoid-quinone biosynthesis
	Matabalian of Others Arrive Arriv	Cyanoamino acid metabolism
	Metabolism of Other Amino Acids	Phosphonate and phosphinate metabolism
		Biosynthesis of siderophore group nonribosomal peptides
	Metabolism of Terpenoids and Polyketides	Prenyltransferases Terpenoid backbone biosynthesis
		Zeatin biosynthesis
	Nucleotide Metabolism	Purine metabolism Pyrimidine metabolism
		Chloroalkane and chloroalkene degradation
	Xenobiotics Biodegradation and Metabolism	Drug metabolism - cytochrome P450 Metabolism of xenobiotics by sytochrome P450
		Naphthalene degradation
	Digestive System	Carbohydrate digestion and absorption
		Insulin signaling pathway
Organismal Systems	Endocrine System	PPAR signaling pathway
organishin oystems		Progesterone-mediated oocyte maturation Proximal tubule bicarbonate reclamation
	Immune System	Antigen processing and presentation
	Nervous System	Glutamatergic synapse
	Genetic Information Processing	Translation proteins
Unclassified		Biosynthesis and biodegradation of secondary metabolites
	Metabolism	Carbohydrate metabolism
		Chean biogenthesis and metabolism

FIGURE 11 | Differential KEGG functional pathways map related to MSM status. From left to right, the first column represents the KEGG I functional pathways, and the second and fourth columns represent the KEGG II and KEGG III functional pathways, respectively, under KEGG I. The third column shows the differential enrichment of the KEGG III functional pathways in MSM and non-MSM individuals. Red indicates that the functional pathways were significantly enriched in MSM in at least three studies. Blue indicates that the functional pathways were significantly enriched in non-MSM in at least three studies.

### DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the bioproject PRJNA227062, PRJEB4335, PRJNA233597, PRJEB5185, PRJEB10578, PRJNA307231, PRJNA354863, PRJEB28485, PRJNA422134, PRJNA489590, PRJEB25418, and PRJEB31328.

### **AUTHOR CONTRIBUTIONS**

JH, HL, and LY designed the study. JZ, YZ, PC, LL, HC, BL, JJ, CN, LT, and XZ participated in data acquisition. JZ contributed to data analysis. JZ, JH, and YZ participated in interpreting the results and preparing the report for publication. All authors revised the manuscripts critically and approved the final version for publication.

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### SUPPLEMENTARY MATERIAL

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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