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Identification of enterotype for patients with Alzheimer's disease

Guang-sheng Liu[1†], Yang Song[2†], Jin-sheng Yan[1†], Yi-jie Chai[3], Yun-fei Zhao[4*] and Huan Ma[5*]

Abstract

Background Alzheimer's disease (AD) is a progressive and chronic neurodegenerative disorder of the central nervous system, characterized by behavioral and dysexecutive deficits. Its pathogenesis is closely associated with the intestinal flora. This study aimed to explore the enterotypes in AD by identifying key bacteria through machine learning and species co-occurrence network analysis.

Methods The collection of fecal samples from AD patients was followed by 16 S rRNA analysis using QIIME2. Enterotype clustering was conducted at the genus level, and deep neural network (DNN) classification models were developed for AD and healthy controls within each enterotype.

Results Analysis of three 16 S rRNA gut microbiome datasets identified three distinct enterotypes: *Escherichia_Shigella* (ET-E), *Faecalibacterium* (ET-F), and *Bacteroides* (ET-B). The ET-E is mainly characterized by the absence of *Akkermansia* in AD group. The *Akkermansia* was significantly positively correlated with *Eubacterium_coprostanoligenes_group* and negatively correlated with biosynthesis and amino acid metabolism. The ET-F highly expressed *Agathobacter*, *un_f__Lachnospiraceae*, *Lachnoclostridium*, and low expressed *Dorea* in AD group. Among them, *Agathobacter* was significantly positively correlated with *un_f__Lachnospiraceae*, and *un_f__Lachnospiraceae* was significantly positively correlated with *Lachnoclostridium*. The *Dorea* was significantly negatively correlated with *Lachnoclostridium*. The AD from ET-B group had high expression of two beneficial bacteria, *Butyricicoccus* and *Parabacteroides*. The findings suggest that the ET-E enterotype may predispose individuals to AD, with *Akkermansia* identified as a potential risk factor. Conversely, the ET-B enterotype appears to be associated with milder symptoms, with *Butyricicoccus* and *Parabacteroides* potentially serving as protective factors. Therefore, a comprehensive understanding of the species characteristics and interactions within different enterotypes is essential for modulating the gut-brain axis and mitigating AD symptoms.

Keywords Alzheimer's disease, Enterotypes, Gut microbiome, Akkermansia, Butyricicoccus, Parabacteroides, Machine learning, Co-occurrence network

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting the central nervous system [1]. The principal clinical features of AD encompass a progressive deterioration in memory, alongside cognitive and behavioral impairments [1]. Recent research has proposed a potential link between gut microbiota and Alzheimer's disease. Notably, a prior study demonstrated that individuals with AD exhibit reduced gut microbiota diversity compared to healthy individuals [2].

Recent research indicates a significant correlation between gut microbiota activity and the progression of AD [3]. Alterations in the composition of gut microbiota can initiate immune activation and systemic inflammation, potentially compromising the integrity of the blood-brain barrier and promoting neuroinflammation and β-amyloid plaque deposition, both of which are characteristic features of AD pathology [4–7]. Additionally, the gut microbiota exerts influence on cognitive functions, including learning and memory, by modulating the amygdala and regulating neurogenesis in the hippocampus [8]. The gut microbiota produces short-chain fatty acids crucial for maintaining intestinal barrier integrity and regulating metabolic and immune functions [9, 10].

Arumugam et al. introduced the concept of enterotypes, which aids in identifying structural patterns within the composition of microbial communities and the mechanisms underlying their assembly. Each enterotype possesses a distinct microbial community structure, comprising various bacterial species that coexist in a harmonious manner. These communities interact to sustain the balance of the gut ecosystem [11]. The enterotypic characteristics of AD patients are not well understood, and there is limited knowledge regarding how interactions within the gut microbiota affect the disease or how different enterotypes interact.

This study conducts a comprehensive analysis of 16 S rRNA sequencing data from AD patients, focusing on the symbiotic microbial variations among AD patients with distinct enterotypes. The SHapley Additive exPlanations (SHAP) method is employed to identify gut microbiota biomarkers that differentiate AD patients from healthy controls (HC). Furthermore, a symbiotic network of the gut microbiota is constructed, and DNNs are utilized to distinguish AD patients from HC. This study elucidates the enterotypic characteristics of AD patients, enhancing our comprehension of the microbial symbiotic networks across various enterotypes and offering insights into potential therapeutic strategies.

Methods

Data acquisition

The gut 16 S rRNA metagenomic data were sourced from the European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI) and the National Center for Biotechnology Information (NCBI). The search utilized the keywords "AD", "16S", "gut", and "Alzheimer's disease". Raw data pertaining to the AD and HC samples were downloaded from the EMBL-EBI platform. Ultimately, datasets from five studies were integrated, corresponding to the following NCBI Bioproject IDs: PRJNA489760, PRJNA554111, PRJNA734525, PRJNA792014 and PRJNA633959.

16 S rRNA data analysis

The FASTQ files containing the sequenced data were subjected to demultiplexing and quality filtering, followed by the identification of amplicon sequence variants (ASVs) using the DADA2 pipeline within the QIIME2. In DADA2, sequences were excluded if their forward and reverse median quality scores were under 20 and they had less than 2000 ASVs during noise reduction and filtering. Post-denoising, the SILVA database was employed for species-level taxonomic classification. Subsequent analyses utilized the ASV counts either in their raw form or as relative abundances. Predictive functional analysis was conducted using the PICRUSt2 plugin integrated within the QIIME2 platform [12].

We employed a Dirichlet multinomial mixture (DMM) model to analyze the enterotypes at the genus level. To reduce noise in the enterotype analysis, we used Excel's VAR function to calculate the variance of each ASV in the genus relative abundance table and then removed ASVs with a variance less than 1. The Shannon index was used to measure α-diversity from the genus-level relative abundance table, while Beta-diversity was analyzed using the Bray-Curtis approach with the R package vegan. Statistical analysis of α-diversity was conducted using the Wilcoxon test, while the permutation test from the vegan package assessed significance via the Bray-Curtis distance.

DNN model construction and SHAP analysis

DNN classification models were constructed utilizing TensorFlow's built-in APIs and trained using Keras in Python. For input data, we retained only those microbial genera exhibiting a variance greater than 1. The preprocessing module from scikit-learn was employed to normalize the variables. The data was split into 70% for training purposes and 30% for testing. Afterward, the ttest_ind function from Python's SciPy package was used to conduct statistical tests on each ASV in the training set. Only ASVs that demonstrated significant differences between AD and HC were selected for training the DNN model.

SHAP was utilized to assess the importance of each ASV. The model was then retrained using this refined dataset. The model's effectiveness was assessed on the

test set using several metrics such as AUC, accuracy, sensitivity, specificity, precision, and F1 score.

Co-occurrence network analysis and gut microbiome function

We employed the 'Corr.test' function from the 'Psych' package to compute the Spearman correlation coefficient among the top significant taxa identified by SHAP. A species co-occurrence network was constructed for correlations exceeding 0.1 with a p-value less than 0.05. The 'igraph' package in R was subsequently utilized to analyze the network properties. Additionally, we used the LEfSe tool to determine the Linear Discriminant Analysis (LDA) values distinguishing AD from Healthy Controls (HC) concerning the metabolic functions predicted by PICRUSt2. We calculated the Pearson correlation using the relative abundance tables for gut bacteria and metabolic functions derived from PICRUSt2. Additionally, a heatmap illustrating correlations was generated.

Results

Study selection

Three studies were included after applying filtering criteria, with all participants being from Chinese cohorts (Table 1). Overall, 317 samples were examined, consisting of 173 from the AD cohort and 144 from the HC cohort. Given that our study integrated three distinct datasets, we performed principal coordinates analysis (PCoA) based on species at the genus and family levels to identify potential cohort-driven biases or effects in the results. This analysis revealed statistically significant batch effects ($P<0.001$) (Supplementary Fig. S1). However, due to the limited availability of pertinent information in the public dataset, batch correction could not be conducted. Gut microbial communities exhibit robust characteristics, and minor disturbances typically exert minimal impact on the overall structure of microbial networks. Consequently, integrating networks and examining subtle changes within network submodules may constitute a reasonable approach.

Enterotype analysis

Following data processing, genera with a variance of more than 1 were selected for further enterotype analysis. In the analysis of enterotypes, it was determined that the optimal number of clusters is three (Fig. 1A). At the family level, *Lachnospiraceae* was identified as the significantly dominant taxon for enterotype 1 and enterotype 2 (Fig. 1C), while enterotype 3 was predominantly characterized by *Bacteroidaceae* (Fig. 1C). At the genus level, we identified *Escherichia-Shigella*, *Faecalibacterium*, and *Bacteroides* as the significantly dominant taxa for the three enterotypes, respectively (Fig. 1D) (Supplementary Fig.S2, $P<0.05$). The three identified enterotypes were designated based on the predominant bacterial genus: *Escherichia-Shigella* (ET-E), *Faecalibacterium* (ET-F), and *Bacteroides* (ET-B). We determined the distribution of the AD group across the ET-E, ET-F, and ET-B enterotypes, with proportions of 62.7%, 51.7%, and 27.8%, respectively (Fig. 1B). Chi-square tests revealed a statistically significant difference in the proportion of AD patients across the ET-E, ET-F, and ET-B enterotypes (Fig. 1B, $P<0.05$).

Results of intestinal flora diversity analysis

The analysis showed that there were no notable differences in Shannon diversity between the HC and AD groups (Fig. 2A). The PCoA plot demonstrated a clear separation between the AD and HC groups (PERMANOVA, $P<0.001$) (Fig. 2B). In contrast, the beta-diversity analysis of ET-F indicated a certain degree of overlap between the AD and HC groups, which was distinctly different from the clear separation observed in ET-E and ET-B (Fig. 2, $P<0.001$). Although there is some overlap between HC samples and AD samples, the gut microbiota structure exhibited significant differences. HC participants whose microbiota profiles overlapped with the AD cluster may be at increased risk of developing AD. These findings suggest that alterations in disease states may vary across different enterotypes. Further network analysis is warranted to examine the community relationships among these enterotypes.

DNN classification model performance

DNN models were constructed utilizing genus-level species data. The AUC values for the DNN models were 0.84, 0.72, and 0.75 for the total, ET-E, and ET-F participant groups, respectively (Supplementary Fig.S3). It was observed that the proportion of samples in the ET-B group exhibited significant variability, resulting in suboptimal ROC curve outcomes. Detailed metrics, including

Table 1 The table describes the three studies

Accession number	Design	Sample group	Species	Age	Gender	Country	16 S region	Seq Tech
PRJNA489760	Case–control	AD: 30 Health: 30	Homo sapiens	NA	NA	China	V3-V4	Illumina MiSeq
PRJNA554111	Case–control	AD: 43 Health: 43	Homo sapiens	55–58	Male:46 Female:40	China	V3-V4	Illumina MiSeq
PRJNA633959	Case–control	AD: 100 Health: 71	Homo sapiens	65–83	Male:78 Female:93	China	V3-V4	Illumina MiSeq

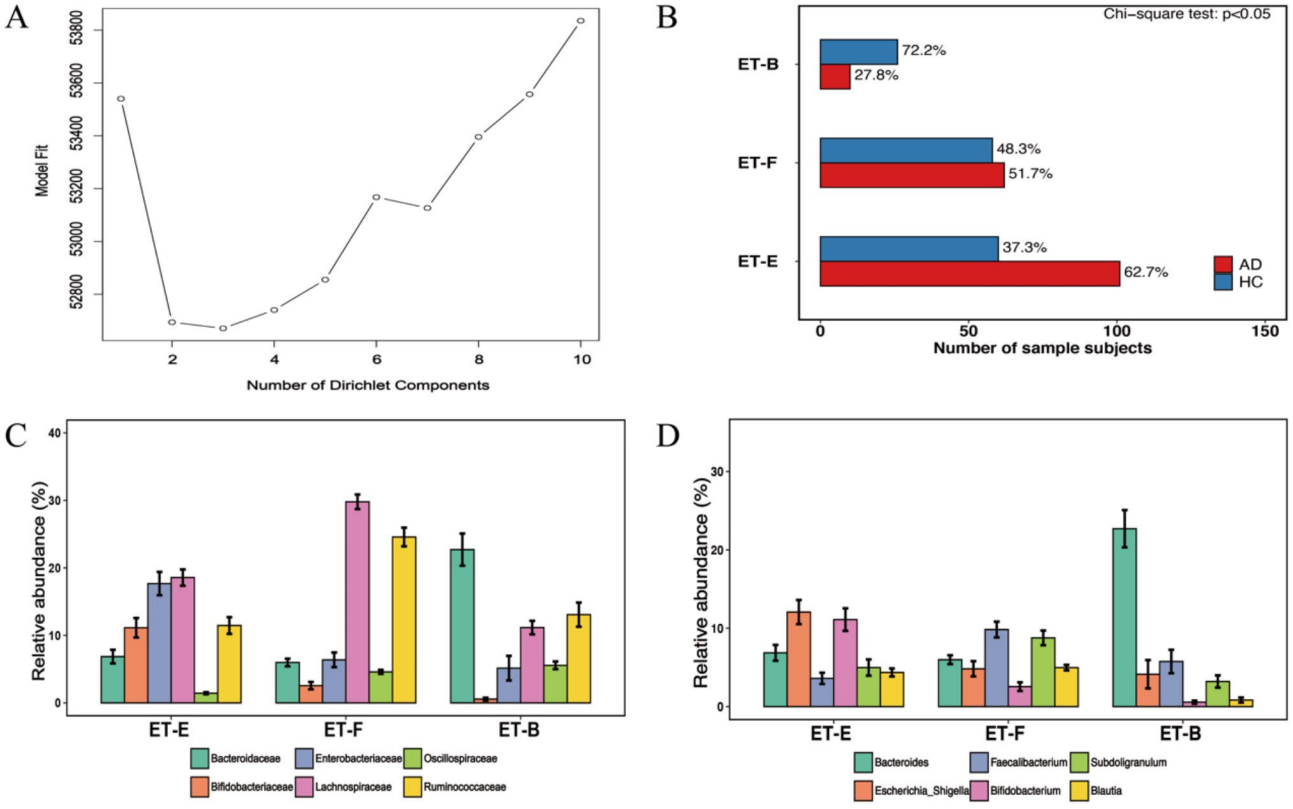

Fig. 1 Characterization and distribution of three enterotypes in AD and HC subjects based on the gut microbiome. (**A**) The model fit is shown for the given number of genua estimated from DMM using the Laplace approximation. (**B**) The number of UC and HC subjects in each enterotype. (**C**) The relative abundance of the top 6 family taxons in each enterotype. (**D**) The relative abundance of the top 6 genus taxons in each enterotype

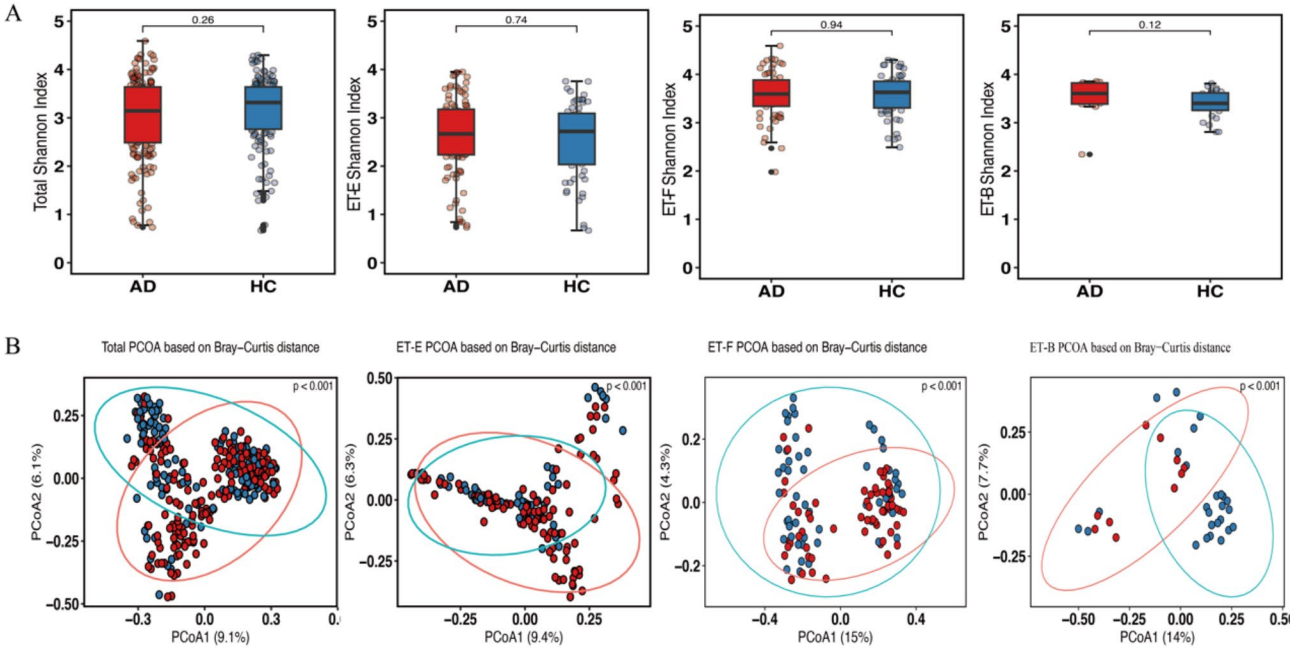

Fig. 2 α-diversity and Beta-diversity. (**A**) α-diversity Shannon index in total participants, ET-E, ET-F, and EF-B. (**B**) Beta-diversity in total participants, ET-E, ET-F, and EF-B

accuracy, sensitivity, specificity, precision, and F1 scores, are provided in Supplementary Table S1.

SHAP interpreter and co-occurrence network in the total cohort

Using the SHAP interpreter, we pinpointed the 19 most important genera of microorganisms as determined by the DNN classifier (Fig. 3A). Within the SHAP analysis of the entire cohort, *Akkermansia* and *Bifidobacterium* emerged as the two most critical species associated with AD, whereas Bacteroides and Faecalibacterium were identified as species associated with HC. Box plots were generated for these 19 taxa, and the Wilcoxon rank-sum test was performed. *Akkermansia*, *Bifidobacterium*, *Christensenellaceae_R_7_group*, *Erysipelatoclostridium*, *UBA1819* are highly expressed in AD group ($P<0.05$).

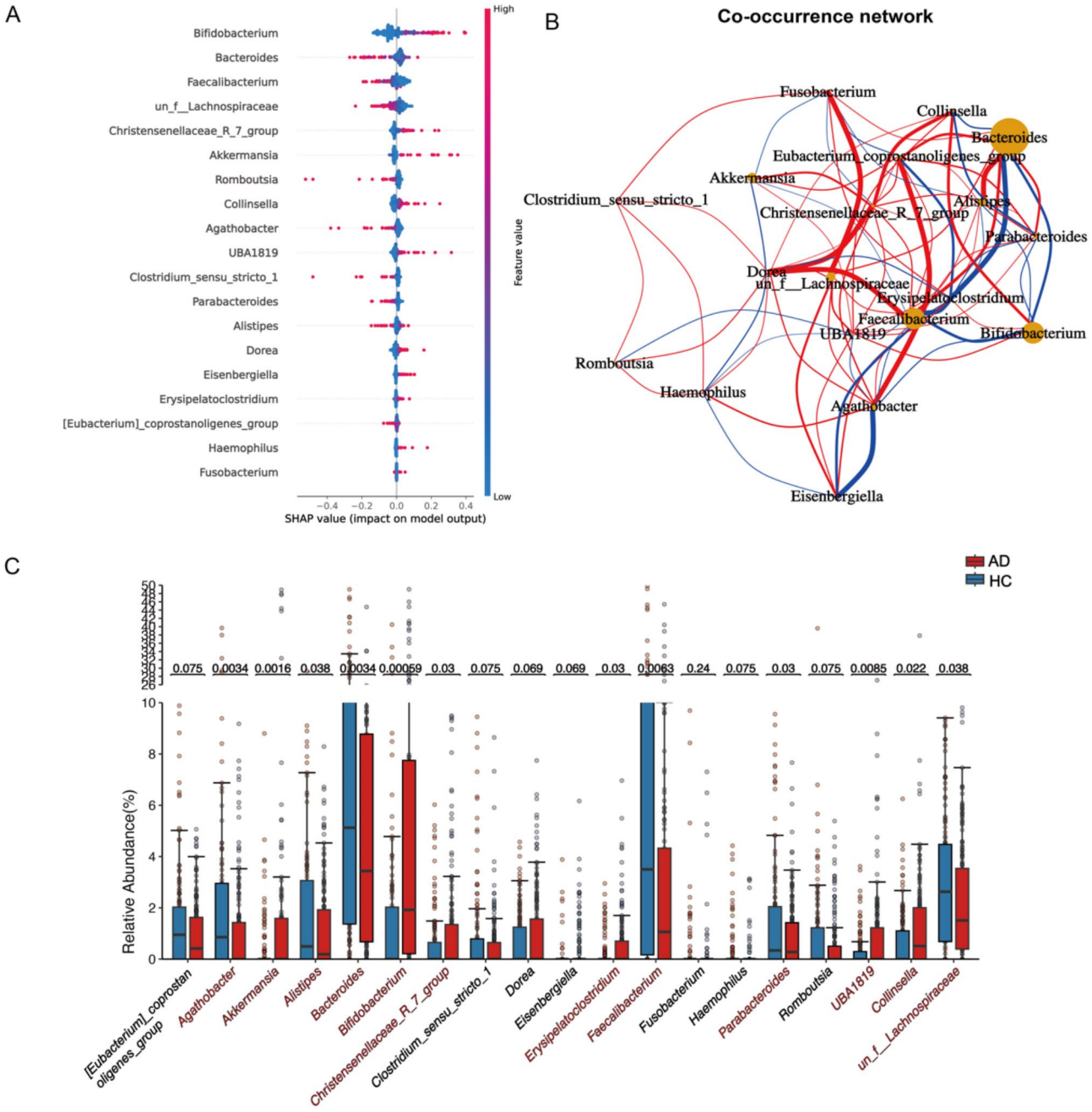

Fig. 3 The SHAP interpreter and species co-occurrence network of the total cohort. (**A**) The SHAP interpreter was used to conduct the microbial-specific importance analysis in the DNN classifier. (**B**) Box plot of the 19 taxons included in the SHAP swarm plot. (**C**) The species co-occurrence network was constructed using important gut microbes in the DNN classifier. Positive correlations are shown by red edges, negative correlations by blue edges, the thickness of the edges signifies the size of the absolute correlation coefficient, and node size indicates relative abundance

Agathobacter, *Alistipes*, *Bacteroides*, *Faecalibacterium*, *Parabacteroides* are highly expressed in HC group ($P < 0.05$) (Fig. 3C). Additionally, we visualized the bacteria selected by the DNN within a species co-occurrence network, which revealed that *Faecalibacterium* were significantly positively correlated with *Agathobacter*, *Dorea*, *Eubacterium_coprostanoligenes_group*, and *Alistipes*, but significantly negatively correlated with *Erysipelatoclostridium* and *Bifidobacterium*. The *Bacteroides* were significantly positively correlated with *Parabacteroides* and *Alistipes*. The *Christensenellaceae_R_7_group* were significantly positively correlated with *Eubacterium_coprostanoligenes_group* and *Alistipes* (Fig. 3B).

SHAP interpreter and co-occurrence network in the ET-E cohort

Using the SHAP interpreter, we pinpointed the 8 most important genera of microorganisms as determined by the DNN classifier (Fig. 4A). *Akkermansia* and *Christensenellaceae_R_7_group* are highly expressed in HC group ($P < 0.05$) (Fig. 4C). Among them, in the HC group, *Akkermansia* was significantly positively correlated with *Christensenellaceae_R_7_group*. The *Akkermansia* were significantly positively correlated with *Eubacterium_coprostanoligenes_group* and *Faecalibacterium*. The *Christensenellaceae_R_7_group* was significantly positively correlated with *Alistipes*

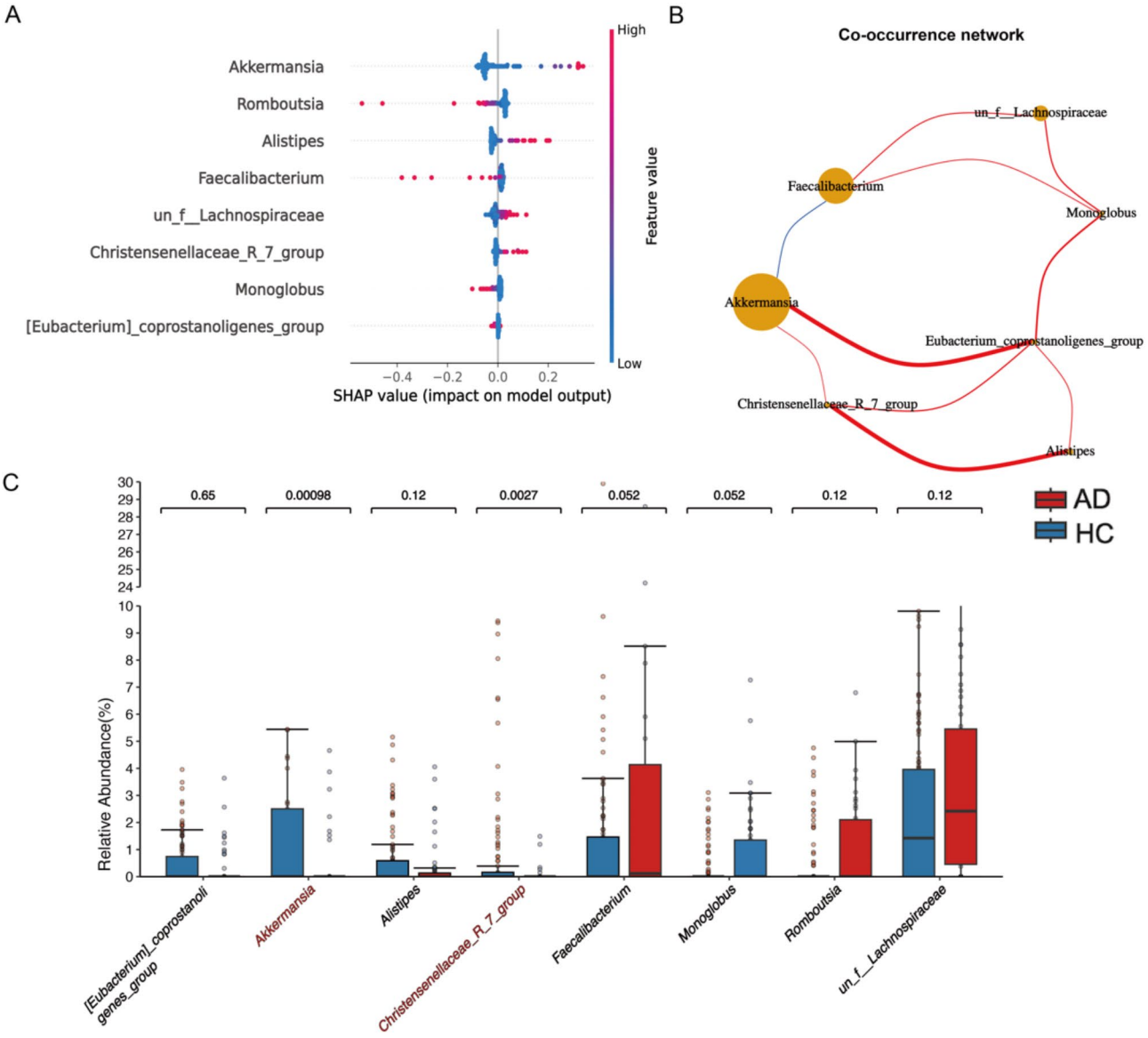

Fig. 4 The SHAP interpreter and species co-occurrence network of the ET-E cohort. (**A**) The SHAP interpreter was used to conduct the microbial-specific importance analysis in the DNN classifier. (**B**) Box plot of the 8 taxons included in the SHAP swarm plot. (**C**) The species co-occurrence network was constructed using important gut microbes in the DNN classifier. Positive correlations are shown by red edges, negative correlations by blue edges, the thickness of the edges signifies the size of the absolute correlation coefficient, and node size indicates relative abundance

(Fig. 4B). Overall, ET-E is characterized by the absence of Akkermansia.

SHAP interpreter and co-occurrence network in the ET-F cohort

Using the SHAP interpreter, we pinpointed the 4 most important genera of microorganisms as determined by the DNN classifier (Fig. 5A). *Agathobacter*, *un_f__Lachnospiraceae*, and *Lachnoclostridium* are highly expressed in AD group ($P<0.05$) (Fig. 5C). *Dorea* is highly expressed in HC group ($P<0.05$) (Fig. 5C). Among them, in the AD group, *Agathobacter* was significantly positively correlated with *un_f__Lachnospiraceae*, *un_f__Lachnospiraceae* was significantly positively correlated with *Lachnoclostridium*. The *Dorea* was significantly negatively correlated with *Lachnoclostridium* (Fig. 5B).

SHAP interpreter and co-occurrence network in the ET-B cohort

Using the SHAP interpreter, we pinpointed the 9 most important genera of microorganisms as determined by the DNN classifier (Fig. 6A). *Butyricicoccus* and *Parabacteroides* are highly expressed in AD group ($P<0.05$) (Fig. 6C). Among them, *Butyricicoccus* was significantly positively correlated with *Bacteroides*, but *negatively* correlated with *Christensenellaceae_R_7_group*. The *Christensenellaceae_R_7_group* were significantly

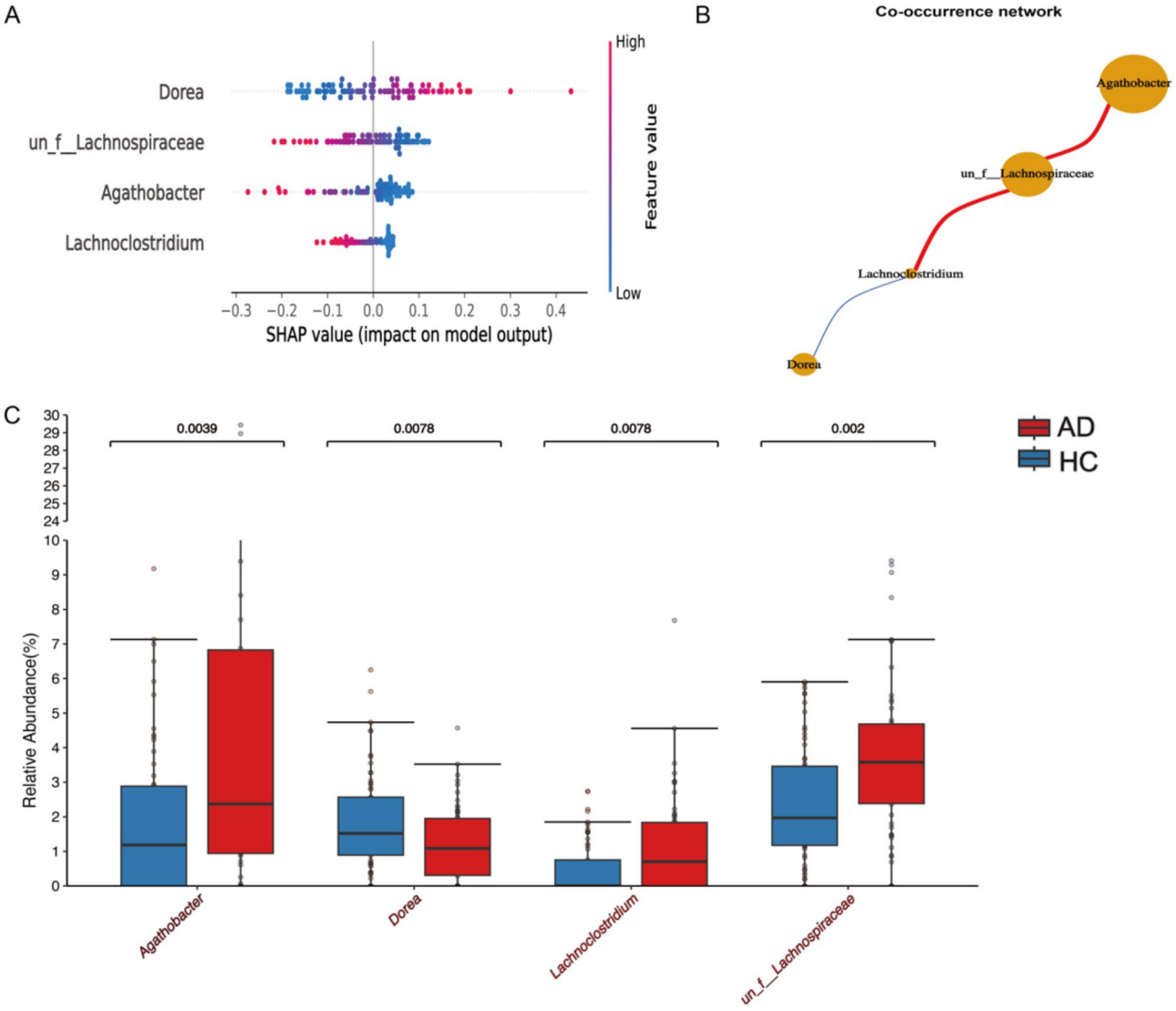

Fig. 5 The SHAP interpreter and species co-occurrence network of the ET-E cohort. (**A**) The SHAP interpreter was used to conduct the microbial-specific importance analysis in the DNN classifier. (**B**) Box plot of the 4 taxons included in the SHAP swarm plot. (**C**) The species co-occurrence network was constructed using important gut microbes in the DNN classifier. Positive correlations are shown by red edges, negative correlations by blue edges, the thickness of the edges signifies the size of the absolute correlation coefficient, and node size indicates relative abundance

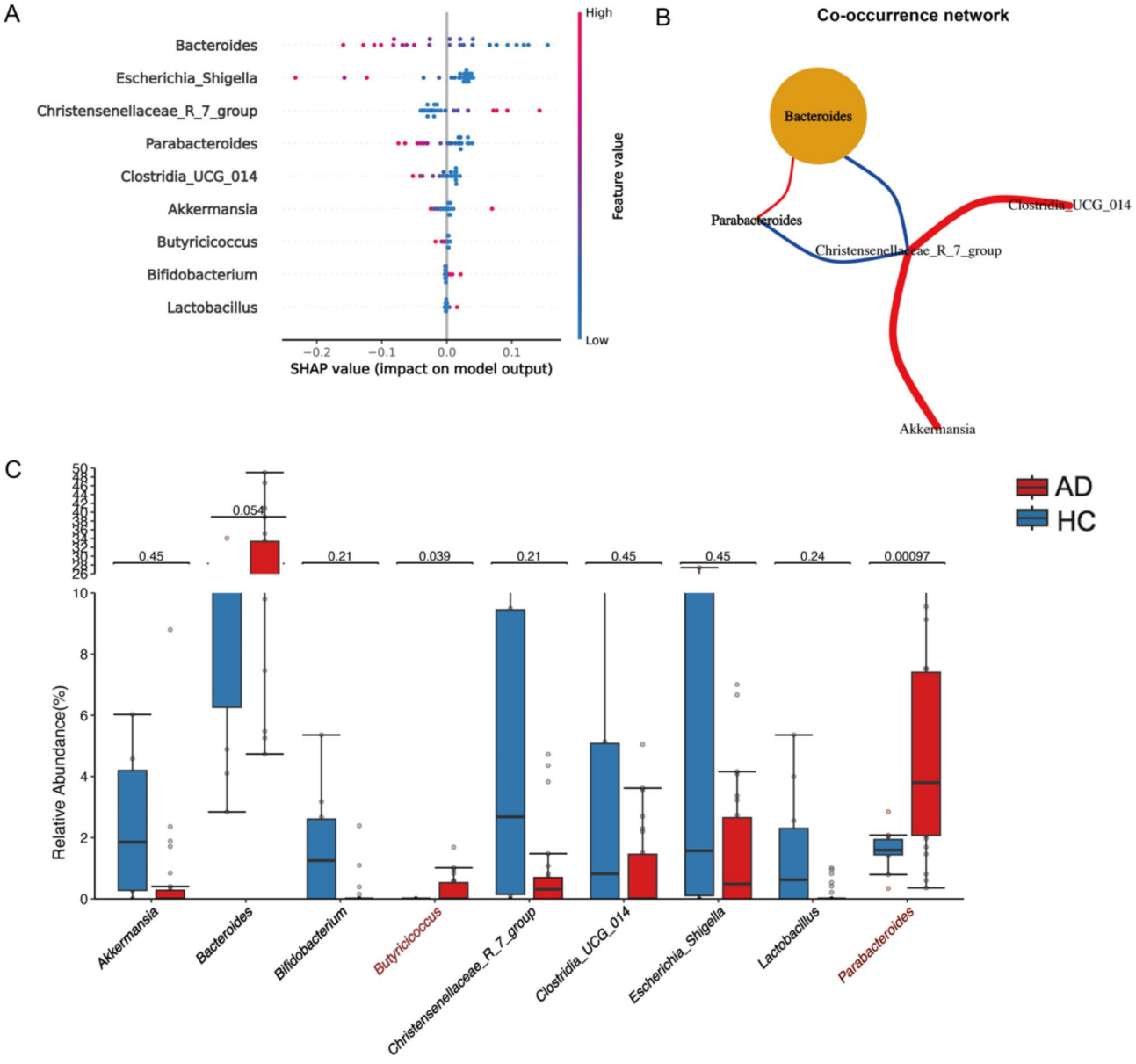

Fig. 6 The SHAP interpreter and species co-occurrence network of the ET-E cohort. (**A**) The SHAP interpreter was used to conduct the microbial-specific importance analysis in the DNN classifier. (**B**) Box plot of the 9 taxons included in the SHAP swarm plot. (**C**) The species co-occurrence network was constructed using important gut microbes in the DNN classifier. Positive correlations are shown by red edges, negative correlations by blue edges, the thickness of the edges signifies the size of the absolute correlation coefficient, and node size indicates relative abundance

positively correlated with *Akkermansia* and *Clostridia_UCG_014* (Fig. 6B).

SHAP interpreter and PICRUSt2 function prediction results in the total cohort

PICRUSt2 assessment highlighted substantial differences in the metabolic pathways of gut microbes selected for each group (Fig. 7A). While drawing a correlation heatmap, *Akkermansia* was highly negatively correlated with Nucleotide excision repair, Lysine biosynthesis, Valine leucine and isoleucine biosynthesis, Aminoacyl tRNA biosynthesis, Cell cycle Caulobacter, Alanine aspartate and glutamate metabolism, and Glycine serine and threonine metabolism. *Bacteroides* was highly positively correlated with Lipoic acid metabolism, Bisphenol degradation, Primary bile acid biosynthesis, Secondary bile acid biosynthesis, Alanine aspartate and glutamate metabolism, and Glycine serine and threonine metabolism. *Agathobacter* was highly positively correlated with Bacterial chemotaxis and Flagellar assembly. *Bifidobacterium* was highly negatively correlated with Fatty acid

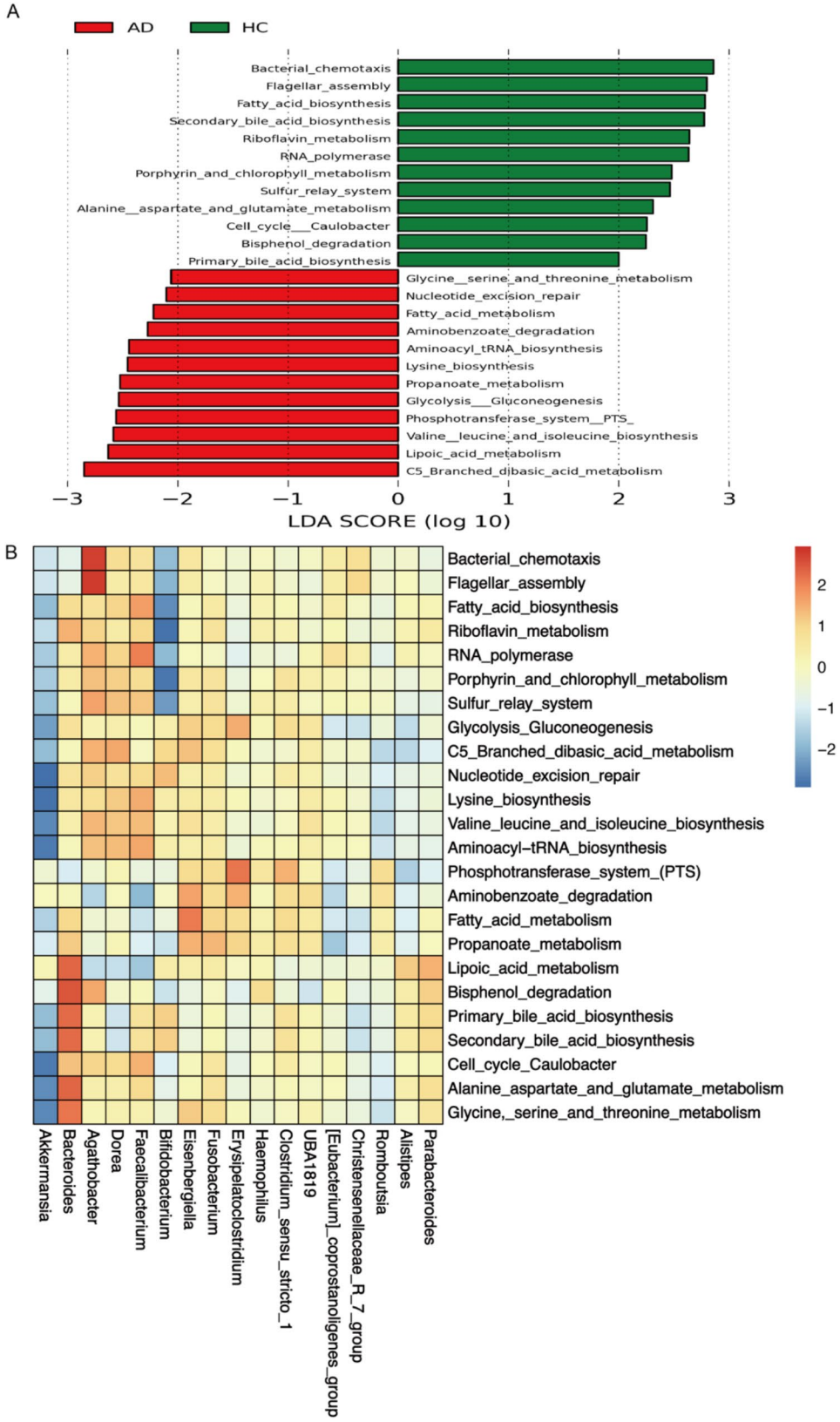

Fig. 7 PICRUSt2 analysis in the total cohort. (**A**) LDA scores of the HC and AD groups in LEfSe. (**B**) A heatmap was created to illustrate the correlation analysis between the functions with significant differences identified by LEfSe and the key bacteria of HC and AD highlighted in the SHAP analysis

biosynthesis, Riboflavin metabolism, and Porphyrin and chlorophyll metabolism (Fig. 7B).

Discussion

In our research, we identified three enterotypes using the DMM method. The findings indicated a significant prevalence of AD within the ET-E enterotype. *Escherichia-Shigella*, identified as a proinflammatory bacterium, demonstrated a strong correlation with AD and APP/PS1 mice models [13–15]. The influence of *Escherichia-Shigella* on the biosynthesis of secondary metabolites is significant, as it affects short-chain fatty acids and neurotransmitters, both of which play a crucial role in modulating the gut-brain axis [16]. The ET-E enterotype is predominantly characterized by the absence of *Akkermansia* in the AD group. There is evidence suggesting that Akkermansia may exert beneficial effects on AD [17]. Following treatment with mesenchymal stromal cells, the gut microbial balance in AD mice showed partial restoration, characterized by an increase in the neuroprotective bacterium *Akkermansia* and a decrease in the AD-associated bacterium Sphingomonas [18]. *Akkermansia* is known for its anti-inflammatory and metabolic properties, playing a crucial role in modulating the microbiota-gut-brain axis by maintaining intestinal mucosal barrier integrity and regulating metabolic processes [19–21]. In our study we found that *Akkermansia* was highly negatively correlated with Nucleotide excision repair, Lysine biosynthesis, Valine leucine and isoleucine biosynthesis, Aminoacyl tRNA biosynthesis, Cell cycle Caulobacter, Alanine aspartate and glutamate metabolism, and Glycine serine and threonine metabolism. Furthermore, *Akkermansia* has been demonstrated to enhance glucose and lipid metabolism and to produce interleukin 10 (IL-10), an anti-inflammatory cytokine [22–25]. Studies suggest that *Akkermansia* can modulate the production of metabolites such as short-chain fatty acids (SCFAs) and amino acids, which are crucial for maintaining gut barrier integrity and systemic immune regulation [26]. Specifically, *Akkermansia* has been linked to the metabolism of tryptophan, an essential amino acid, which is a precursor for serotonin and kynurenine pathways. Dysregulation of these pathways has been implicated in neuroinflammation and neurodegenerative diseases, including AD [27]. Akkermansia's ability to enhance gut barrier function and reduce systemic inflammation may mitigate neuroinflammation, a key contributor to AD pathology [2]. Akkermansia's association with amino acid metabolism and biosynthesis is relevant to AD pathology through its influence on neuroinflammation, gut-brain axis communication, and the modulation of key amino acid pathways implicated in neurodegeneration. Further research is needed to elucidate the specific mechanisms by which *Akkermansia* may contribute to AD pathogenesis. In summary, within the ET-E enterotype, the regulation of *Akkermansia* in conjunction with other bacteria, such as the *Eubacterium-coprostanoligenes* group and *Faecalibacterium*, as well as the interactions of metabolic pathways, are crucial for both the treatment and amelioration of AD.

In ET-F enterotype, *Agathobacter*, *un_f__Lachnospiraceae*, and *Lachnoclostridium* are highly expressed in AD group. *Dorea* is highly expressed in HC group. Evidence suggests that Lachnospiraceae are potent producers of butyrate [28, 29], which can influence T-cell differentiation [30, 31] and microglial function [32]. Targeting Lachnoclostridium, known as an immune mediator in inflammatory diseases or as an activator of inflammation [33], may offer a therapeutic avenue for treating atherosclerosis [34]. Agathobacter, a beneficial gut bacterium, produces SCFAs, particularly butyrate, which exhibits anti-tumor potential [35]. A decrease in Agathobacter species likely impacts both host metabolism and the commensal microbiota. Given that Dorea was associated with a reduced Mini-Mental State Examination (MMSE) score, it is suggested that these factors may constitute risk factors for the development of AD. The positive correlation between *Agathobacter* and *Lachnospiraceae* may play a significant role in AD progression. Both *Agathobacter* and *Lachnospiraceae* are known producers of SCFAs, particularly butyrate, which has been shown to exert neuroprotective effects by reducing neuroinflammation and enhancing blood-brain barrier integrity [36]. The synergistic activity of these genera may amplify SCFA production, potentially mitigating AD-related pathology. *Lachnospiraceae* has been implicated in maintaining gut barrier function, while *Agathobacter* may enhance metabolic homeostasis. Their co-occurrence could stabilize the gut environment, reducing systemic inflammation and amyloid deposition [2]. Recent studies have shown that reduced abundance of both genera is associated with cognitive decline and AD progression, suggesting their potential as biomarkers or therapeutic targets. The ET-F is influenced by *Agathobacter*, *Lacchnospiraceae*, *Dorea*, and *Lachnoclostridium*, but its role in AD remains to be investigated further.

In the ET-B enterotype, the expression levels of *Butyricicoccus* and *Parabacteroides* are significantly elevated in the AD group. A decline in cognitive function in elderly individuals has been correlated with a reduced relative abundance of *Butyricicoccus*, a bacterium known for its anti-inflammatory properties [37–39]. Both *Parabacteroides* and *Bacteroides* are producers of SCFAs, which function as anti-inflammatory agents [40, 41]. Our observation of a positive correlation between *Parabacteroides* and *Bacteroides* aligns with the existing body of literature. In conclusion, the EF-B enterotype may exhibit mild symptoms.

While our study provides insights into the association between gut microbiota and AD pathology, it is important to acknowledge potential confounders that may influence gut microbiota composition, such as diet, medication use, and other lifestyle factors. For instance, dietary patterns have been shown to significantly alter gut microbiota diversity and function [42]. Similarly, medications such as antibiotics, proton pump inhibitors, and metformin can modulate gut microbial communities [43]. Additionally, lifestyle factors like physical activity, smoking, and stress levels may also contribute to gut microbiota variability [44]. In our study, we attempted to control for some of these variables by collecting demographic and clinical data, however, detailed dietary records or medication histories were not available for all participants. This limitation may have introduced variability in gut microbiota profiles that could not be fully accounted for in our analysis. Despite these potential confounders, our findings highlight robust associations between specific gut microbiota taxa and AD-related biomarkers, suggesting that gut microbiota may play a role in AD pathology independent of these factors. Future studies should aim to collect more comprehensive data on diet, medication use, and lifestyle factors to better control for these variables and further elucidate the gut-brain axis in AD.

In conclusion, this study conducted a novel comparative analysis of gut microbial samples from individuals with AD and HC through enterotype analysis. The results demonstrated significant variations in the distribution of AD and HC across different enterotypes, identifying ET-E as an enterotype susceptible to AD and ET-B as a protective enterotype against AD. Furthermore, the study identified Akkermansia as a potential pathogen, while Butyricicoccus and Parabacteroides were recognized as potential beneficial symbionts in the context of AD. Future studies should use in co-culture experiments to clarify the interactions and mechanisms of these key species.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06343-3.

Supplementary Material 1

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Author contributions
Guang-sheng Liu, Yang Song, Yi-jie Chai, and Jin-sheng Yan developed the methodology and interpreted of data. Drafting the article: Guang-sheng Liu, Yang Song, and Jin-sheng Yan. Revising the article critically: Yun-fei Zhao and Huan Ma. All authors contributed to the article and approved the submitted version. All authors read and approved the final manuscript.

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Data availability
The datasets supporting the conclusions of this article are available in the European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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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