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RESEARCH ARTICLE

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Season, age, and sex affect the fecal mycobiota of free-ranging Tibetan macaques (*Macaca thibetana*)

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Recent studies highlight that the gut mycobiota play essential roles in mammalian metabolic and immune systems, but to date we lack information on the forces that naturally shape the gut mycobiota of wild primates. To investigate the contributions of host and environmental factors in the taxonomic variation of the gut mycobiota, we examined the effects of age, sex, and season on the fecal mycobiota in wild-living Tibetan macaques (*Macaca thibetana*). Using next generation sequencing and a longitudinal set of fecal samples collected over 1 year, we identified a set of core fungal taxa present in the Tibetan macaque's fecal samples. The predominant genera *Aspergillus* and *Penicillium*, which promote the digestion of cellulose and hemicellulose in herbivorous mammals, were detected in this study. Similar to humans, we found age and sex effects on the macaques' fecal mycobiota. We also found that both fecal fungal composition and diversity (alpha and beta diversity) varied significantly by season. In particular, the *Penicillium* enriched mycobiota in summer samples may aid in the digestion of cellulose and hemicellulose present in mature leaves. The high alpha diversity detected in Tibetan macaques' winter fecal samples may facilitate a diet rich in fiber ingested during this season. We propose that the gut mycobiota play an important role in the macaques' ability to adapt to seasonal fluctuations in food availability and nutrient content.

KEYWORDS
fecal mycobiota, next generation sequencing, primate, seasonal variation

1 | INTRODUCTION

The mammalian gut is a complex ecological system colonized by a diverse microbial population, including bacteria, archaea, fungi, and viruses (Underhill & Iliev, 2014). Advances in high-throughput sequencing technologies have revealed an important role of the gut microbiota in host biology, including immune regulation, energy acquisition, vitamin synthesis, and disease risk (Greenblum, Turnbaugh, & Borenstein, 2012; Hooper, Littman, & Macpherson, 2012; Turnbaugh et al., 2006). The composition of the gut microbial community is influenced by a range of host- and group-specific intrinsic and extrinsic factors such as genotype, diet, health, social interactions, and group demography (Amato et al., 2013, 2015; David et al., 2014; Degnan et al., 2012; Howard et al., 2010; Ley et al., 2008; Maurice et al., 2015; Tung et al., 2015). In general, studies of the gut microbiome have focused solely on the bacterial component (Qin et al., 2010). The role of fungi, which also appear to affect host health

and nutrition, are poorly understood (Huffnagle & Noverr, 2013; Sokol et al., 2016; Underhill & Iliev, 2014).

Fungi are normal inhabitants of the mammalian gut. The total number of fungal cells present in the gut, however, is orders of magnitude smaller than that of the bacterial microbiota (Qin et al., 2010; Underhill & Iliev, 2014). In general, the gut mycobiota appears to function in altering the bacterial composition of the gut, the production of fungal metabolites, and in interacting with immune cells to counter the effects of pathogenic microbes that compromise host health. For example, gut fungi are reported to modulate both innate and adaptive immune responses (Hajishengallis et al., 2011; Iliev & Underhill, 2012; Rizzetto, De, & Cavalieri, 2014), and imbalances in healthy gut mycobiota are associated with a range of pathologies, including metabolic disorders (obesity), colorectal adenomas, and Inflammatory Bowel Diseases (IBDs) (Luan et al., 2015; Mar et al., 2016; Sokol et al., 2016; Wheeler et al., 2016).

As a new and emerging field of research, the study of the gut mycobiota offers insights into our understanding of the role of the gut microbiota (Huffnagle & Noverr, 2013) in host health and nutrition ecology. However, to date, the specific factors that shape the gut mycobiota in mammals remain unclear. Compared to other groups of mammals, non-human primates (NHPs) share broadly similar morphological, physiological and genetic characteristics with humans. NHPs are important animal model systems for understanding many aspects of human behavior, cognition, physiology, and health (McCord et al., 2014; Ren, Grieneisen, Alberts, Archie, & Wu, 2015; Phillips et al., 2014). Therefore, studies of wild NHPs have the potential to offer critical insight into ecological and evolutionary relationships among gut bacterial and fungal community structure, host diet, and host health that are missing from human and laboratory animal studies.

In this study, we used high-throughput sequencing and non-invasive genetic methods to study the fecal mycobiota of a free-ranging social group of Tibetan macaques (*Macaca thibetana*) at Mt. Huangshan, Anhui Province, China. This group has been the subject of over 30 years of behavioral research, and therefore the behavior, ecology, and demographic history of individual group members are well documented. Based on field studies, the diet of Tibetan macaques varies seasonally and is characterized by a predominance of young leaves in the spring, mature leaves in the summer, mature leaves and fruits/nuts in the fall, and mature leaves, bark, stem, and fallen nuts in the winter (Xiong & Wang, 1988; You, Yin, Zhang, Ying, & Feng, 2013). Our main objectives are (1) to characterize the composition of the fecal mycobiota of individual group members; (2) test the degree to which factors such as age, sex, and season affect the mycobiome diversity and community composition; and (3) present these results in the context of what is known about the mycobiota in other mammals to discuss their potential functions in the feeding ecology of Tibetan macaques.

2 | METHODS

2.1 | Sample collection and ethics statement

This study was carried out in the Valley of Wild Monkeys (VWM), a tourist destination located in Mt. Huangshan National Reserve, southern Anhui Province. Since 1986, we have been able to recognize all individuals in our study group. The ages of all individuals born into the group over the past 30 years are known. In addition, the age of all immigrants into our study group have been estimated based on information from known-aged individuals (Zhang, Li, Zhu, Wang, & Wang, 2010). The site represents a highly seasonal ecosystem, with an annual average temperature of 15.3 °C (highest: 34.2 °C, lowest: −13.9 °C) (Xiong & Wang, 1988; Zhao, 1999). During most winters, the temperature can remain below freezing for more than 40 consecutive days (Xiong & Wang, 1988).

We obtained a total of 95 fresh fecal samples from 31 identified individuals, including twenty adults (female: $n = 12$, male: $n = 8$; all adults of the social group) and eleven juveniles (female: $n = 6$, male: $n = 5$), representing 70.5% of the study group. Fecal samples from each age/sex class were collected during each of four seasons: spring, summer, autumn, winter (July 23, 2015 to May 24, 2016). Samples and individual information are listed in the Table S1. All fecal samples were collected, stored and shipped in RNAlater (QIA-GEN, Valencia CA). Our samples were shipped at ambient temperatures but subsequently stored at −80 °C for storage until they were sent out for analysis. This research was approved by the Institutional Animal Care and Use Committee of the Anhui Zoological Society (permit number BH20131202). We performed all experiments in accordance with the approved guidelines and regulations, and followed the American Society of Primatologists principles for the ethical treatment of primates.

2.2 | DNA extraction and sequencing

We thawed the fecal samples on ice and sliced the fecal samples into sections. To avoid soil contamination, we then extracted the DNA from feces collected from the inner part of the fecal samples. Following the protocol of Tang, Iliev, Brown, Underhill, and Funari (2015), we extracted the total DNA from frozen stool samples following lyticase treatment, bead beating, and processing using QIAmp DNA mini kit (Qiagen). We sent the total DNA extracted from 95 fecal samples to the Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for analysis. The ITS regions were identified by ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (2043R) (5′-GCTGCGTTCTTCATCGATGC-3′) primers (Bokulich & Mills, 2013). PCR reaction mixtures contained 5–100 ng of DNA template, 1× GoTaq Green master mix, 1 M $MgCl_2$, and 5 pmol of each primer. Reaction conditions consisted of an initial 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s, and a final extension of 72 °C for 5 min. After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2 × 300bp sequencing was performed using the Illlumina Miseq platform (San Diego, CA).

2.3 | Sequence analysis

We trimmed raw FASTQ sequencing data for adaptor sequence and for quality control using the sliding window approach implemented in

Trimmomatic (Bolger, Lohse, & Usadel, 2014). We merged overlapping paired-end reads using Flash software (Magoc & Salzberg, 2011), with the minimum overlap set to 10 bp and other parameters at default settings. Lastly, we clustered the quality-check of sequences into operational taxonomic units (OTUs) using the Usearch program (version 7.0 http://drive5.com/uparse/) with a cut-off of 97% sequence identity (Edgar, 2013).

We made taxonomical assignments using the Ribosomal Data base Project (RDP) classifier (Wang, Garrity, Tiedje, & Cole, 2007) implemented in Quantitative Insights Into Microbial Ecology (QIIME). We subjected the sequences representing the OTUs to BLAST searches in UNITE (https://unite.ut.ee/) (Koljalg et al., 2013) databases (Release 6.0). We deposited the sequences in the NCBI Sequence Read Archive (Accession: PRJNA447371).

2.4 | Data analysis

We calculated alpha diversity using mothur (version v.1.30.1 http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity) with an OTU similar level of 97%, and beta diversity (the distance matrices between samples) was calculated by QIIME 2. We used weighted and unweighted UniFrac distances to perform PCoA (Principal Coordinates Analysis) using the packages Made4 and Vegan (http://www.cran.r-project.org/package=vegan). PERMANOVA analysis was used to detect the influence of age, sex, and season on beta diversity (Weighted UniFrac, Unweighted UniFrac) variation, and an adonis test in the vegan package of R.

Following Maurice et al. (2015), we used linear mixed models to detect the potential multiple influences of season, age, and sex on alpha diversity, with individual ID controlled as a random intercept term. Model assumptions were checked by examining the distribution of residuals and plotting fitted values against residuals. The response variable was log-transformed or square root transformed where needed to meet the model assumptions. The same sets of predictors (age, sex, and season) were included in all starting models. Following the linear mixed models, Wilcoxon rank sum test were used to detect the differences of alpha diversity between any two groups within age, sex, and season and *p*-values were corrected for multiple comparison using the false discovery rate correction (FDR) (Benjamini & Hochberg, 1995). To detect fungal taxa (including phylum, family and genus) with significantly different abundances within age, sex, and season, we used LEfSe (Linear discriminant analysis effect size) analyses according to the online protocol (https://huttenhower.sph.harvard.edu/galaxy/).

3 | RESULTS

3.1 | General patterns of the fecal fungal profile

After quality filtering, we acquired 3,485,311 high-quality filtered reads (average length of amplicons is 258.56 bp), corresponding to 36,687.48 ± 4,831.91 reads per monkey from a total of 95 fecal samples from 8 adult males, 12 adult females, 5 juvenile males, and 6 juvenile females. The information regarding these samples is presented in Table S1. Taxonomic assignment revealed representatives from six known fungal phyla at 97% sequence identity. Similar to humans, the Tibetan macaque fecal mycobiota was dominated by Ascomycota (x = mean ± Std. Deviation, x = 76.25.43 ± 8.51%) and Basidiomycota (x = 9.46 ± 13.63 %). Other phyla represented were Zygomycota (x = 0.23 ± 0.092 %), Rozellomycota (x = 0.056 ± 0.196%), Glomeromycota (x = 0.013 ± 0.062%), and Chytridiomycota (x = 0.0091 ± 0.055%) (Figure 1a). The dominant identified family was Trichocomaceae (x = 24.70 ± 24.33%). The predominant known genera of fungi isolated from the monkeys' fecal samples were *Aspergillus* (x = 12.46 ± 14.36%) and *Penicillium* (x = 10.72 ± 18.82%).

We examined evidence for a core set of fungal taxa in the Tibetan macaque fecal mycobiota. We defined core genera and families as present in more than 90% of fecal samples and at an average relative abundance >0.01. Our results indicated the existence of three core taxa at the family level (Trichocomaceae, Nectriaceae, and Davidiellaceae) (Figure 1b) and three at the genus level (*Aspergillus*, *Penicillium*, and *Fusarium*) (Figure 1c).

3.2 | Variation of the fecal mycobiota diversity across age, sex, and season

We used linear mixed models to estimate alpha diversity using the Shannon diversity index and Chao to examine the association among mycobiome alpha diversity, age, sex, and season. There was no evidence of a significant influence of season, sex, or age using Chao (Season: $F = 1.39$, $p = 0.252$; Age: $F = 2.05$, $p = 0.090$; Sex: $F = 1.96$, $p = 0.179$). However, we detected a significant influence of age and season using the Shannon diversity index (Age: $F = 4.92$, $p = 0.0076$; Sex: $F = 1.15$, $p = 0.292$; Season: $F = 9.39$, $p = 0.00002$). This is likely the result of dietary differences across age classes and seasonal differences in food availability.

Based on the results of the linear mixed models, we used Wilcoxon rank-sum test to detect the variation of alpha diversity (Shannon diversity index) associated with age and season respectively. *p*-values corrected for multiple comparisons were calculated using the FDR (Figures 2a and 2b). Although the dyads Spring/Summer, Autumn/Spring, and Autumn/Winter (Shannon, Spr/Sum, $Z = -0.629$, $p = 0.672$; Aut/Spr, $Z = -1.628$, $p = 0.059$; Aut/Win, $Z = -1.274$, $p = 0.058$) were not significantly different, for the remaining three dyads significant differences were identified (Shannon, Aut/Sum, $Z = -2.943$, $p = 0.003$; Spr/Win, $Z = -1.886$, $p = 0.018$; Sum/Win, $Z = -2.293$, $p = 0.001$) (Figure 2b). In particular, alpha diversity of the winter samples exhibited the greatest level of diversity across seasons, followed by autumn, spring, and summer samples. In addition, our analysis based on age indicated that only the adolescent macaques exhibited significant higher alpha diversity than the middle-aged adult group (Shannon, Juvenile − Middle Adult, $Z = -2.133$, $p = 0.029$) (Figure 2a).

We performed PCoA and PERMANOVA tests on weighted and unweighted unifrac dissimilarities to investigate the predictors of fecal fungal beta diversity. Our PERMANOVA tests revealed significant separation based on age for each of the dissimilarities matrix methods (Unweighted unifrac: $F = 1.917$, $R^2 = 0.059$, $p = 0.001$; Weighted

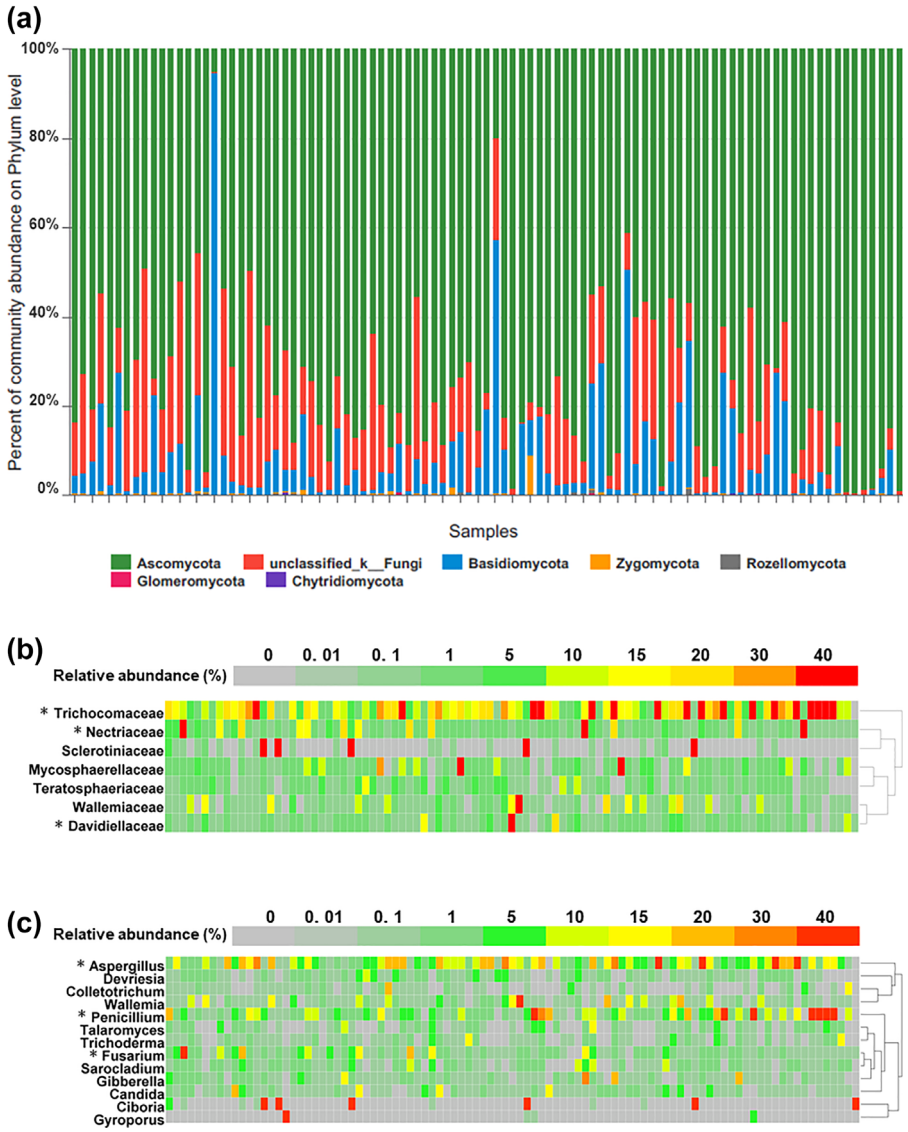

FIGURE 1 The distributions of phylum, families and genera. (a) Relative abundance of fecal fungal taxa at the phylum level. Stacked bar graphs illustrate the abundances of phyla, *x*-axis represents the samples. (b and c) The distributions of families and genera (average relative abundance >0.01). The heatmap shows family and genus patterns, respectively. (b) Genus; (c) family, *the taxa present in more than 90% and the average relative abundance >0.01 of our 95 samples (core set of fungal taxa)

unifrac: $F = 2.092$, $R^2 = 0.065$, $p = 0.026$) (Figures 3a and 3b). Significant separation based on sex was detected (Unweighted unifrac: $F = 2.832$, $R^2 = 0.030$, $p = 0.001$; Weighted unifrac ($F = 3.961$, $R^2 = 0.041$, $p = 0.007$) (Figures 3c and 3d). In addition, significant seasonal separation was detected (Unweighted unifrac: $F = 1.958$, $R^2 = 0.061$, $p = 0.001$; Weighted unifrac: $F = 2.642$, $R^2 = 0.080$, $p = 0.004$) (Figures 3e and 3f).

3.3 | Variation of fecal fungal composition across age, sex, and season

To explore variation in the fecal fungal community composition based on age, sex, and season at a broader taxonomic level, we performed LEfSe tests on the relative abundance of phyla, families, and genera across samples. The relative abundance of known phyla, families, and genera accounting for ≥1% of the sample and the level of enrichment was examined among a number of different categories (age: juvenile, young adult, middle adult, old adult; sex: male, female; season: spring, summer, autumn, and winter) (LDA > 2, *p*-value < 0.05). Among the four age groups examined, only the fungal genus, *Sarocladium*, was significantly enriched and this was in the Old Adult sample (LDA = 4.011, $p = 0.008$). We also found that four taxa were significantly enriched based on sex (LDA > 2, *p*-value < 0.05). The results indicate that two taxa (family Mycosphaerellaceae and genus *Devriesia* were significantly enriched in female samples, and two other taxa (phylum Ascomycota and family Tetraplosphaeriaceae) were significantly enriched in male samples (Figure 4a).

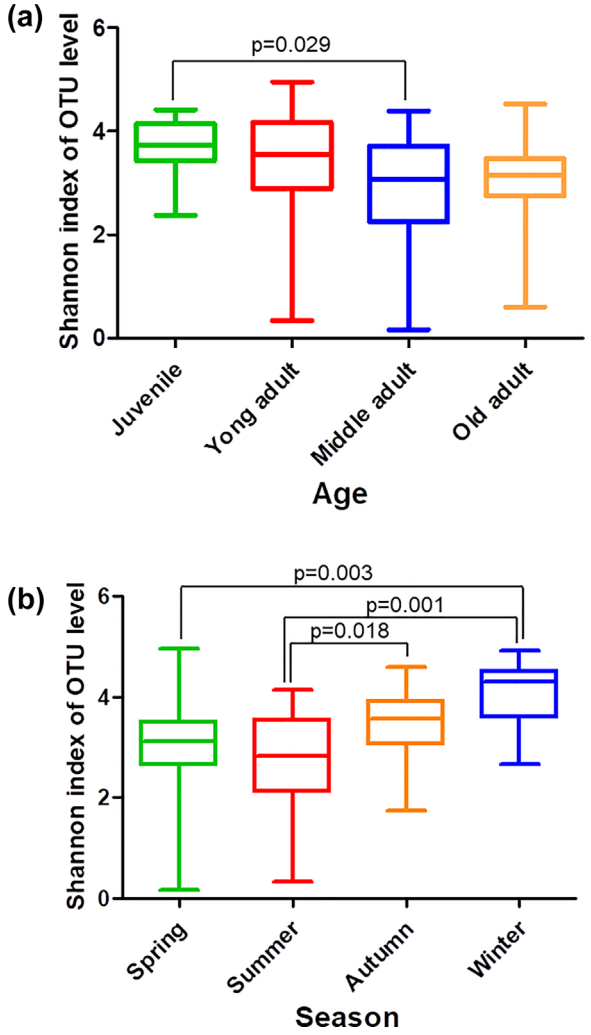

FIGURE 2 Differences in the Shannon index of fecal mycobiota across age and season in Tibetan macaques. (a) Age group; (b) season group. Wilcoxon rank-sum test, *p*-values were corrected for multiple comparisons using the FDR

We found evidence of significant seasonal variation in the Tibetan macaque mycobial community, with fifteen taxa significantly enriched in one of the four seasons (LDA > 2, *p*-value < 0.05). Two families (Wallemiaceae, Hypocreaceae) and two genera (Wallemia, *Trichoderma*) were significantly enriched in the autumn samples. Two families (Sclerotiniaceae, Nectriaceae) and five genera (*Ciboria*, *Fusarium*, *Gibberella*, *Sarocladium*, *Talaromyces*) were significantly enriched in the spring samples. One family (Trichocomaceae) and one genus (*Penicillium*) were significantly enriched in the summer samples. One genus (*Devriesia*) and one family (Teratosphaeriaceae) were significantly enriched in the winter samples. The results of the LEfSe analysis and their relative abundances are presented in Figure 4b.

4 | DISCUSSION

We found that the Tibetan macaque's fecal mycobiome was dominated by three phyla: Ascomycota, Basidiomycota, and Zygomycota. This is consistent with previous studies of the human and mouse mycobiome (Iliev & Underhill, 2012; Mar et al., 2016; Qiu et al., 2015; Sokol et al., 2016; Strati et al., 2016; Suhr, Banjara, & Hallen-Adams, 2016; Wheeler et al., 2016). At the genus level, we isolated three core genera *Aspergillus*, *Penicillium*, and *Fusarium*, suggesting that they are normal inhabitants in the Tibetan macaques' gut. In particular, the genera *Aspergillus* and *Penicillium* were abundant (12.46% and 10.72%, respectively). It is well known that anaerobic fungi of these two genera produce cellulolytic and hemicellulolytic enzymes for cellulosic biomass degradation (Boots et al., 2013; Mortensen et al., 2010; Solomon, Haitjema, & Henske, 2016; Liao, Li, Wei, Shen, & Xu, 2014; Tani, Kawaguchi, & Kobayashi, 2014). Similarly, recent evidence indicates that species of the genus *Fusarium* are robust cellulose and hemicellulose degraders (Huang, Busk, & Lange, 2015). Although small amounts of corn were provisioned to help habituate and attract monkeys at our study site, the diet of Tibetan macaques consists of a high proportion of leaves throughout the year (Spring: 82% Young leaves; Summer: 95% mature leaves; Autumn: 47% mature leaves; Winter: 43% mature leaves), supplemented by 15% bamboo shoots and twigs in Spring; 2% stems in Summer; 46% fruits/nuts in the Autumn; and 23% bark, 13% stem, 17% fallen nuts in Winter (Xiong & Wang, 1988; You et al., 2013). The leaves, bark and stems consumed by nonhuman primates commonly contain large quantities of cellulose and hemicellulose (Campbell, Glenn, Grossi, & Eisemann, 2001; Hladik, 1978). We hypothesize, that the core and abundant genera *Aspergillus*, *Penicillium*, and *Fusarium* detected in wild-living Tibetan macaque fecal samples play an important role in the digestion of cellulose and hemicellulose.

Having a diverse and responsive gut microbial community represents an important adaptive mechanism enabling individual primate species to consume a broad based diet that varies seasonally in nutrient composition (Amato et al., 2015; Sun et al., 2016). As symbionts in the mammalian gut, anaerobic fungi produce a wide range of hydrolytic enzymes such as cellulases, hemicellulases, proteases, amylases, and pectinases (Trinci et al., 1994). The prevalence of particular fungal taxa is expected to vary across seasons in direct response to changes in diet, nutritional needs, and reproductive and thermoregulartory requirements (Noma, Suzuki, & Izawa, 1998; Tsuji, Hanya, & Grueter, 2013). However at present, little is known regarding the primary and secondary functions of particular fungal mycobiota in the mammalian gut (Huffnagle and Noverr, 2013).

Our data documented evidence of marked seasonal variation in Tibetan macaques' fecal mycobiota diversity. Our study group inhabits a semideciduous forest. These Tibetan macaques experienced seasonal shifts in diet characterized by the exploitation of young leaves in the spring, mature leaves in the summer, mature leaves and fruits/nuts in the fall, and mature leaves, bark, stem, and fallen nuts in winter (Xiong & Wang, 1988, You et al., 2013). We found that the fecal mycobiota of these Tibetan macaques varied by season, likely in response to dietary shifts.

In addition to differences in fecal mycobiota diversity, we found that 15 different taxa were significantly enriched during a particular season, presumably in response to nutritional differences in the proportion of protein, lipids, and carbohydrates present in foods consumed. Given that metabolic maps and genomic databases of fungi

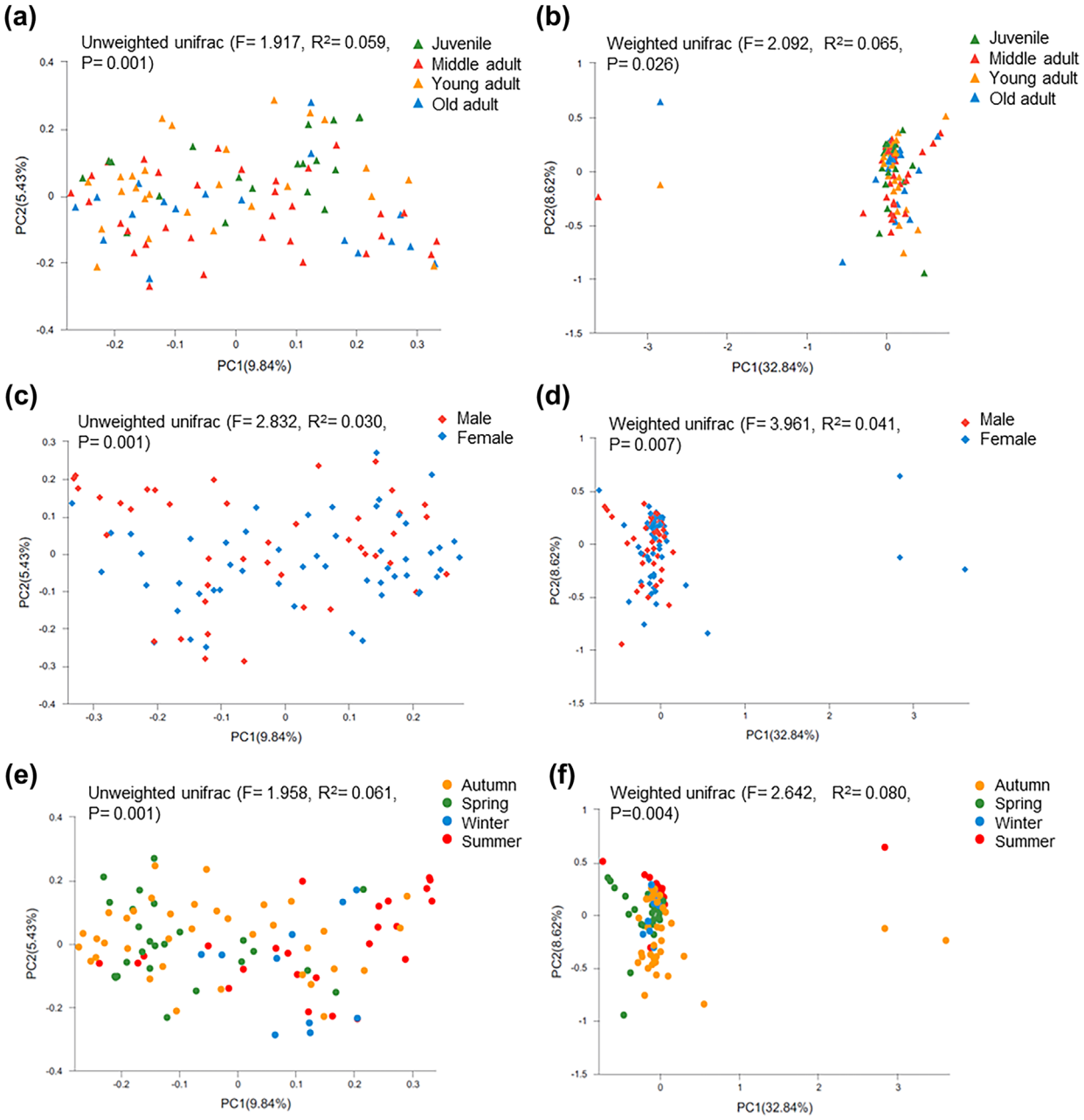

FIGURE 3 Differentiation of fecal mycobiota structure across age, sex, and season, in Tibetan macaques. PCoAs were used to show patterns across age, sex, and season. Adonis tests were performed on unweighted unifrac (a, age; c, sex, e, season) and weighted unifrac (b, age; d, sex; f, season), significant at the 0.05 level

lag significantly behind those of bacteria (Huffnagle & Noverr, 2013), and few dietary intervention studies of gut fungal communities have been performed, it is difficult to functionally explain mycobiota composition variation and forager nutritional ecology. Based on information suggesting that *Penicillium* can produce cellulolytic and hemicellulolytic enzymes for cellulosic biomass degradation (Tani et al., 2014), we hypothesize that the genus *Penicillium*, which is enriched in summer fecal samples may aid in the digestion of cellulose and hemicellulose present in mature leaves. However, during the winter, Tibetan macaques also consume a high fiber diet consisting of mature leaves, bark and stems, and *Penicillium* was not enriched in winter fecal samples. Evidence from a study of the human fecal mycobiome found that almonds and pistachio nuts can significantly decrease the abundance of *Penicillium* (Ukhanova et al., 2014). This may offer a possible explanation for this seasonal difference in the Tibetan macaque mycobiome between summer and winter.

Although the Tibetan macaque diet is characterized by its lowest plant species richness during winter (winter: 25 species, spring: 46 species, summer: 32 species, autumn: 37 species; You et al., 2013), we found the highest alpha diversity in the winter samples. It has been reported that anaerobic fungi are more prevalent in ruminants fed a stalky fibrous diet compared to those

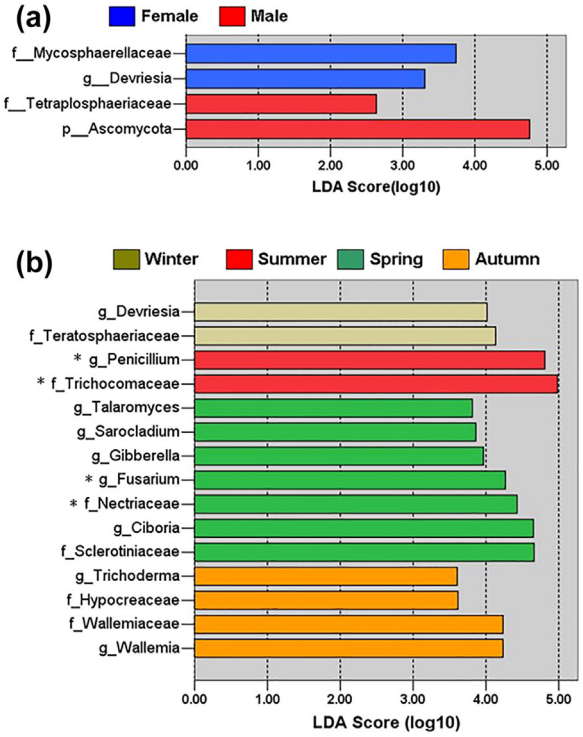

FIGURE 4 Variation in the fungal composition across sex and season of Tibetan macaque fecal mycobiota. Phyla, families, and genera present in each sex (a) and season (b) identified by LEfSe analysis (LDA > 2, $p < 0.05$), including non-parametric factorial Kruskal–Wallis sum-rank test and linear discriminant analysis (LDA). *Core set of fungal taxa, which are present in more than 90% and the average relative abundance >0.01 of our 95 samples

fed a soft leafy diet (Bauchop, 1981). In addition, cows fed a diet high in plant fiber also were characterized by high rumen fungal diversity (Denman & McSweeney, 2006). This suggests that the mycobiota diversity may increase in response to a more fibrous diet. Thus, the high alpha diversity detected in Tibetan macaques' winter fecal samples may be explained as a consequence and function of ingesting a diet rich in fiber and a broader range of plant tissues during the winter. In this regard, one study that identified an association between fungal diversity and increased plant fiber consumption indicated that individuals with high gut fungal diversity may be more efficient in the break down of plant fiber (Akin, Gordon, & Hogan, 1983).

Additionally, many studies have revealed that gut bacterial microbial diversity is most strongly affected by factors such as host, age, and sex (Amato et al., 2014, 2015; David et al., 2014; Degnan et al., 2012; Howard et al., 2010; Ley et al., 2008; Maurice et al., 2015; Tung et al., 2015). At present, only one study has examined the influence of age and sex on fecal fungal diversity in healthy humans (Strati et al., 2016). In this study, infants (0–2 years old) and children (3–10 years old) were found to have higher alpha diversity than did adults (≥18 years old). Consistent with this study, we found that juvenile Tibetan macaques exhibited significantly higher alpha diversity than middle-aged adults (but not young adults and old adults). In the mammalian gastrointestinal tract, fungi and bacteria can interact and antagonize each other (Hoarau et al., 2016; Oever & Netea, 2014), and a decrease in gut bacterial diversity can result in an increase in gut mycobiota diversity (Dollive et al., 2013). A recent study found that juvenile baboons exhibited lower bacterial alpha diversity, and a less stable bacterial microbiota compared to adults (Ren et al., 2015). In terms of Tibetan macaques, this might help to explain adult–juvenile differences, and weak bacterial competition for gut fungi in juveniles may help to explain why juvenile macaques have higher alpha diversity than adults (Koenig & Klaenhammer, 2011; Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012). We did find that the genus *Sarocladium* was enriched in old adult Tibetan macaques. Too little is known regarding the possible function of this genus in the mammalian gut, and therefore we currently cannot speculate on its relationship with age-based differences in the macaque diet.

In addition, we found significant differences in mycobiota composition and beta diversity between macaque males and females. This is consistent with results from humans studied by Strati et al. (2016). These authors speculate that sex hormones may serve to modulate mycobiota composition (Markle et al., 2013). In contrast, Bolnick et al. (2014) have suggested that sex-based differences in diet related to the nutritional costs of reproduction may help to explain mycrobiota composition. The degree to which either of these two factors drives significant differences in male and female mycobiota composition and beta diversity in Tibetan macaques requires additional study.

5 | CONCLUSIONS

To our knowledge, this study is the first to characterize the fecal fungal community of a wild primate species. Our results provide evidence that gut fungi may function to assist Tibetan macaques in exploiting and digesting a diet that is seasonally composed of high levels of cellulose and hemicellulose. Specifically, we identified seasonal shifts in community composition and the diversity of the macaque mycobiota. This leads us to speculate that a symbiotic relationship between Tibetan macaques and their gut mycobiota may represent an adaptive response to seasonal shifts in diet and nutrient requirements. In addition, host age and sex also were found to shape the macaques' fecal mycobiota, but the set of mechanisms and functions driving these differences remain unclear. Given that fungi primarily enter the gut via food colonization (Li et al., 2017), future studies are needed to examine the degree to which seasonal shifts in temperature and rainfall contribute to the seasonal changes in food availability and the fecal mycobiota of Tibetan macaques and other primates.

DATA ACCESSIBILITY

Sequences were deposited in the NCBI Sequence Read Archive (Accession: PRJNA447371).

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CONFLICTS OF INTEREST

We declare we have no competing interests.

AUTHORS' CONTRIBUTIONS

B.H. and J.H. designed the research and interpreted data. B.H., Zh.Y., W.X., Y.Z., Z.D., and D.P. prepared samples. B.H., Zh.Y., and J.H. analyzed and interpreted data. B.H., J.H., and P.A.G wrote the manuscript. B.H., M.A.H., P.A.G., and L.S. revised the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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