FULL PAPER

Wildlife Science

Role of coprophagy in the cecal microbiome development of an herbivorous bird Japanese rock ptarmigan

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ABSTRACT. The transgenerational maintenance of symbiotic microbes that benefit host nutrition and health is evolutionarily advantageous. In some vertebrate lineages, coprophagy is used as a strategy for effectively transmitting microbes across generations. However, this strategy has still not been studied in birds. Accordingly, the aim of the present study was to evaluate the role of maternal cecal feces consumption by Japanese rock ptarmigan (*Lagopus muta japonica*) chicks as a strategy for acquiring essential gut microbes. Both the duration of coprophagy behavior by the chicks and the development process of the chick cecal microbiome (n=20 one- to three-week-old chicks, from three broods) were investigated. In all three broods, coprophagy behavior was only observed from 3 to 18 days of age. Furthermore, there was no significant difference in the number of bacterial operational taxonomic units (OTUs) in 1-week-old chicks (n=651) and adults (n=609), and most of the main OTUs observed in the adults were already present in the 1-week-old chicks. These results indicate that, in this precocial bird species, coprophagy may contribute to the early establishment of cecal bacteria that are essential for food digestion and, thus, chick survival. In fact, Japanese rock ptarmigan chicks consume the same food as their hens from the time of hatching. This behavior may have applications to *ex-situ* conservation.

KEY WORDS: coprophagy, gut microbiome development, herbivorous bird, microbiome transmission

Coprophagy, which is the consumption of feces, includes the consumption of feces from other species (heterospecific coprophagy), from other individuals of the same species (allocoprophagy), or from one's self (autocoprophagy) [22] and has been observed in a variety of animal groups, including vertebrates [14, 23, 44]. In herbivorous mammals, such as rodents, autocoprophagy is considered an adaptive behavior for recycling vitamins, minerals, amino acids, trace elements, and other nutrients that are excreted in the feces. However, heterospecific coprophagy and allocoprophagy are sometimes considered non-feeding behaviors that inoculate the gut with the bacteria and protozoa of other individuals [11, 13], even if these types of coprophagy also have nutritional significance [22, 36, 44]. Feeding the feces of older individuals to juvenile conspecifics is, in fact, a kind of fecal transplantation that facilitates the coevolution between host species and symbiotic gut bacteria by continuously transmitting the bacteria to subsequent generations. Since gut bacteria contribute to the digestion of fiber and degradation of toxic secondary compounds from the plants that are consumed by herbivores [19, 31, 37, 60, 63], allocoprophagy is often observed in herbivorous mammals [4, 37]. Like mammals, birds represent a diverse and evolutionarily successful lineage and occupy a wide range of food niches throughout the world [16]. However, herbivory is a rare strategy in birds (only 3% of species) [34, 35]. This may reflect the challenges of flight since large and heavy digestive tracts are needed to digest fibrous and toxic foods [8]. The main fermentation chamber of plant materials in herbivorous birds, excluding the hoatzin (*Opisthocomus hoazin*), is the hindgut, which includes a well-developed cecum [17, 28]. Therefore, herbivorous birds may use a strategy to maintain gut bacteria that is

similar to that used by herbivorous mammals. However, little is known about the mechanisms by which essential gut microbes are maintained in avian hosts over multiple generations.

The Japanese rock ptarmigan (JRP) *Lagopus muta japonica* is a domestic endangered species that has experienced population declines over the last 40 years [12]. The bird is an isolated subspecies of rock ptarmigan (*L. muta*), which is a typical herbivorous hindgut-fermenter that is characterized by a pair of well-developed ceca [41, 45]. As in leporids [43], rock ptarmigans produce two types of feces: hard, fibrotic feces ("rectal feces") and soft, pasty feces ("cecal feces"). It is easy to distinguish these two types of feces by visual examination. The mechanisms by which these feces types are formed remains unknown, but the system seems to be similar to that described in rabbits [43], in which cecal feces are formed in the ceca and are rich in cecal bacteria and their metabolites [39, 43]. The main food sources of the JRP include alpine plants, such as crowberry (*Empetrum nigrum* var. *japonica*), cowberry (*Vaccinium vitis-idaea*), yellow-flowered rhododendron (*Rhododendron aureum*), and *Aconogonon weyrichii* var. *alpinum* [29], that contain a variety of chemicals to protect them from herbivory [61]. As such, the bacteria of the bird's ceca are hypothesized to be specialized to the bird's diet. Indeed, at least some anti-herbivore chemicals are reportedly degraded by the cecal bacteria of the JRP [49, 50].

Prior to this study, JRP chicks were unintentionally observed to exhibit coprophagy toward the cecal feces of their mothers, during close surveillance in a cage protection program (see Methods section) in Mt. Norikura (N 36.10°, E 137.55°) in 2014 (Fig. 1a–d). Rock ptarmigan hens frequently brood their chicks to warm them, since chicks are incapable of thermoregulating themselves just after hatching [47]. Chicks normally follow their hen after brooding, and in the instance in which coprophagy was observed, 3-d-old chicks were observed to consume the freshly defecated cecal feces of their hens (Fig. 1a–c), leaving behind distinctive peck traces on the surface of the feces (Fig. 1d).

As mentioned above, allocoprophagy, especially toward the cecal feces of mothers, may mediate the transmission of essential gut microbiota from older generations to young chicks. Similar behavior has been reported in the koala (*Phascolarctos cinereus*), where mothers feed a special type of feces to their pups during the weaning period, in order to transmit cecal microbes [37]. Accordingly, the aim of the present study was to test the hypothesis that the coprophagy behavior of JRP chicks facilitates the transgenerational transmission of important cecal microbes. The hypothesis was tested by continuously observing the behavior of chicks after hatching, for up to 23 d, in order to determine the frequency of coprophagy toward their hen's cecal feces. Furthermore, the cecal microbiomes of the chicks were analyzed using high-throughput sequencing (HTS)-based meta 16S rRNA gene analysis, in order to investigate the development of the chick cecal microbiome. Finally, the role of allocoprophagy in the development of the chick cecal microbiome is discussed.
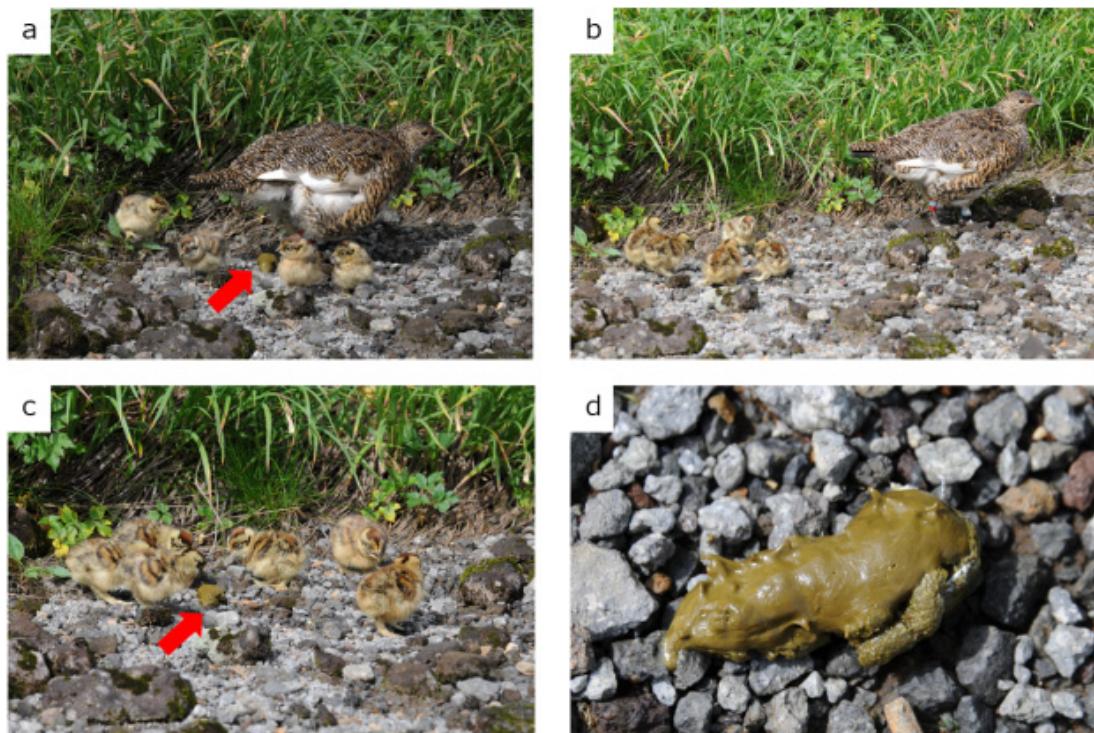
Fig. 1. Coprophagy behavior of Japanese rock ptarmigan chicks. a) Excretion of the cecal feces, just after brooding to warm the body temperature of the chicks. b) congregation of chicks around cecal feces, rather than around the hen. c) Pecking of the hen's cecal feces by chicks. Pecking behavior was observed for all chicks. d) Cecal feces left behind after pecking by the chicks. Traces of pecking are seen on the surface of the feces. Red arrows indicate the cecal feces of hens.

MATERIALS AND METHODS

Behavior observation and feces collection

Behavioral observations and the collection of cecal feces were conducted using individuals from an *in-situ* conservation program that involved temporary cage protection, under the approval of the Ministry of Environment, in order to protect young chicks from predation and severe monsoon climate conditions. In 2016, three cages were established near a mountain lodge (2900 a.s.l.) on Mt. Kita, which is located in the Southern Japanese Alps, where the extinction of the JRP is of highest concern. We introduced three wild families to the cages (one hen and brood to each cage) as soon as families were identified in the field. A total of 20 chicks and three hens were included in the study.

The protection periods for the first, second, and third cages were from 27 June to 19 July, 2016 (23 days; chicks ranged from 1 to 23 days old), from 1–19 July, 2016 (19 days; chicks ranged from 2 to 20 days old), and from 1–20 July (20 days; chicks ranged from 1 to 20 days old), respectively. The hatching dates of the chicks in the first cage were observed directly, where those of chicks in the other two cages were estimated by body size. The hens and chicks grazed freely outside the cages during the daytime (≥2 times each day, for ≤2 hr under observation), except for in cases of poor weather (heavy rain or cold, windy conditions). The hens and chicks were also fed wild plants (e.g., *Vaccinium ovalifolium*, *Stellaria nipponica*, *Oxytropis japonica*, *Aconogonon weyrichii*, *Bistorta vivipara*), which were collected in alpine areas, and frozen *Vaccinium vitis-idaea* fruits, which were collected the previous year. The natural food items were selected in accordance with previous field observations (Kobayashi, unpublished data). Mealworms (*Tenebrio molitor*) were used to replace the natural insects predated by JRPs.

As mentioned above, JRPs produce two types of feces. In the present study, allocoprophagy was recorded when peck traces were observed on the surfaces of the hens' cecal feces in the cages. Pecking by the chicks resulted in very specific markings (Fig. 1d), which were easy to detect on the surfaces of the cecal feces. Furthermore, since neither the auto- nor allocoprophagy of feces by adult rock ptarmigans had been reported in the literature, peck traces were interpreted as intentional coprophagy by chicks (Fig. 1).

Portions of fresh cecal feces were collected from chicks (4- to 22-d-old) during the first, second, and third weeks of cage protection (exact dates are reported in Table S1), and fresh cecal feces were collected from the hens at the beginning and end of the protection period. Details of the sampling procedure are provided below. Cecal feces were collected at <30 min after defecation. Both chicks and hens normally defecate both types of feces when they start feeding after sunrise, and collection was performed from as early as 5 a.m. In some cases, cecal feces were also collected under visual observation during foraging. To avoid potential contamination by environmental bacteria, a portion of the cecal feces free from soil was collected using sterile tweezers. Due to differences in the size of the hens and chicks, it was easy to distinguish the feces of the hens and chicks. Since the chicks were not marked using leg bands, cecal feces were not attributed to specific individuals, and in some cases, all the chick feces collected from a single cage were combined as a daily sample, usually because chick feces were too small to yield sufficient amounts of DNA. The collected cecal feces were preserved in sterile storage buffer [21], transported to the laboratory, and stored at 20°C for up to a month. The effectiveness of this methodology has been established by previous studies that were conducted under less-controlled conditions [27].

DNA extraction and 16S rRNA gene deep sequencing

Total DNA was extracted from stored fecal samples using a tissue DNA kit (QuickGene Mini80 system; Kurabo, Tokyo, Japan), as reported previously [55]. Briefly, the feces were recovered from the storage buffer by centrifugation and were washed using phosphate-buffered saline (PBS) to remove residual buffer solution. A portion of a fecal pellet (20–50 mg) was suspended in tissue-lysis buffer (MDT) from the kit, along with glass beads. After bead disruption, DNA was extracted following the standard protocol of the kit's manufacturer. The quantity and quality of the resulting DNA solutions were assessed using a Nano Drop ND-1000 (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and a Quant-iT dsDNA HS assay kit, with a Qubit Fluorometer (Invitrogen, Carlsbad, CA, U.S.A.). No DNA degradation was observed, and all the samples successfully passed a DNA quality check before library construction.

The construction and sequencing of 16S rRNA gene amplicon libraries were performed at BGI Japan (Kobe, Japan) using the Illumina MiSeq platform as reported previously [33]. Briefly, the V3/V4 region of the 16S rRNA gene was amplified using specific primers: 341F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The qualified libraries subject to paired-end sequencing using the MiSeq System, with sequencing strategy PE300 [33]. After sequencing, low-quality reads were removed by eliminating reads with adapter sequences, >10% unknown bases, or >50% low-quality bases (with quality values of <10). Forward and reverse reads of the paired-end library were then merged using USEARCH (version 8.0.1517) [9], and merged reads that (1) contained ambiguous nucleotides or (2) were mapped to the PhiX genome sequence, using Bowtie 2 (version 2.2.3) [32], were eliminated. Finally, high-quality reads without forward or reverse primer sequences were obtained by eliminated reads that (1) contained <250 or >450 nt or (2) were associated with low (<25) mean Phred quality scores, as calculated by the Illumina MiSeq sequencer. Sequence clustering of the high-quality reads was conducted using UCLUST (version 8.0.1517), with identity >97% and query and reference coverage >80% [9]. Chimeric OTUs were detected and removed if assigned to chimeras by both (1) UCHIME (version 6.0.307) [10], using reference mode to search against the reference gold database (http://drive5.com/uchime/gold.fa), and (2) UCHIME *de novo* mode search. The taxonomic assignment the high-quality reads was performed using RDP Classifier (version 2.12) [62]. OTUs that were represented by less than three reads were eliminated from the dataset, owing to their insignificant contribution to the total reads (on average, 86.4% of reads remained after the rare OTUs were eliminated).

The mean number of bacterial OTUs and Shannon-Wiener diversity index were calculated for both the chicks, at every week

after hatching, and adults (hens) to evaluate the alpha diversity of the cecal microbiome, and PERMANOVA was performed to evaluate the similarity of the OTU-level microbiomes from different age groups and families.

The OTUs were classified according to the detection pattern. When OTUs were detected in all growth stages, the increasing or decreasing trends of their prevalence were identified. If the fold-change observed for a particular OTU (highest proportion value was divided by the lowest proportion value) was less than ten times, the OTU was defined as "stable". However, if the prevalence changed by more than ten times between stages, the OTU was defined as "increasing" (*i.e.*, lowest at one week of age and highest in the adult) or "decreasing" (*i.e.*, the highest at 1 week of age and lowest in the adult) with respect to host growth. In addition, some OTUs were observed to sequentially increase and decrease during host growth (higher in 2- or 3-week-old chicks and lower in 1-week-old chicks and adults) or to sequentially decrease and then increase (highest in 1-week-old chicks and adults and lower in 2- or 3-week-old chicks). Statistical analyses were performed using R (version 3.5.1) [40].

Ethics statement

Fecal sampling was performed in a non-invasive manner. All the sampling methods and access to the JRPs were approved by the Ministry of the Environment (ERTDF 4-1604), and by the Agency for Cultural Affairs and the Ministry of Education, Science, Sport and Culture (27-4-365).

RESULTS

Pecking traces on the cecal feces of hens were confirmed for all broods. It is worth noting that no pecking traces were observed on the rectal feces of the hens or on the feces of the chicks. Pecking traces on the cecal feces of hens were observed while the chicks were 3–18 days old (Fig. 2), and the chicks were first observed to excrete cecal feces at 4 days after hatching. Therefore, the chicks exhibited coprophagy before excreting cecal feces.

The construction of rarefaction curves indicated that our sequencing effort was not large enough to assess the diversity of cecal bacteria, owing to the detection of single-read OTUs. On average, 30,318 reads (n=27; range: 11,914–58,668) were obtained after removing the reads corresponding to rare OTUs.

The number of bacterial OTUs detected in the cecal feces of chicks during the first week was similar to that in the cecal feces of the hens (Fig. S1), and there were no significant differences in the number of OTUs detected in the different stages (ANOVA: $F_{3, 23}=1.983$, $P=0.144$). When the number of OTUs was normalized by total read number, OTU richness tended to increase with host growth. However, significant differences were only detected the OTU richness of the 1- and 3-week-old chicks (Tukey test: $P<0.01$; Fig. 3). The same trends were observed if the rare OTUs (<3 reads) were included in the analysis (Fig. S2).

The alpha diversity of the microbiome increased with host growth (Fig. 4), as shown by the normalized OTU numbers (Fig. 3). However, significant differences in alpha diversity were only detected for the comparison of 1- and 3-week-old chicks (Tukey test: $P<0.01$). Age also affected the composition of the cecal microbiome, whereas family did not (PERMANOVA: n=27; age effect, $R^2=0.085$, $P<0.01$; family effect, $R^2=0.050$, $P=0.103$).

A total of 431 OTUs (12% of all OTUs) were common to all growth stages (Fig. 5a) and represented 77 bacterial genera such
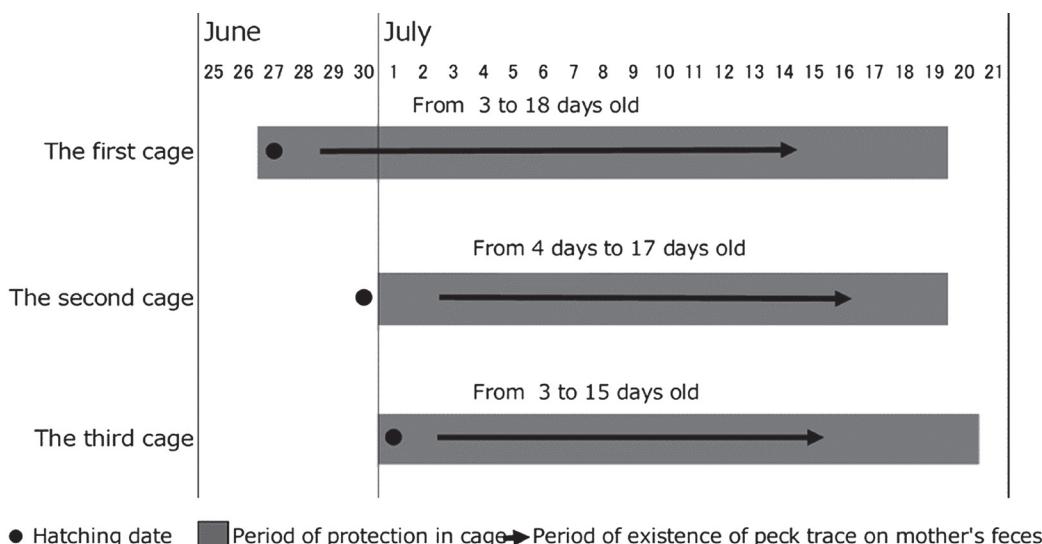
Fig. 2. Periods of cage protection and coprophagy in Japanese rock ptarmigan chicks. Three broods, with their hens, were protected in cages from 27 June to 20 July, 2016. Black dots indicate actual or estimated chick hatching dates. Gray bars indicate the period of cage protection. Black arrows indicate observations of pecking traces on the cecal feces of the hens. Coprophagy started before the first excretion of cecal feces by chicks because cecal feces were not excreted by chicks until they were 4 or 5 days old. Coprophagy ceased by 3 weeks of age in all three broods.
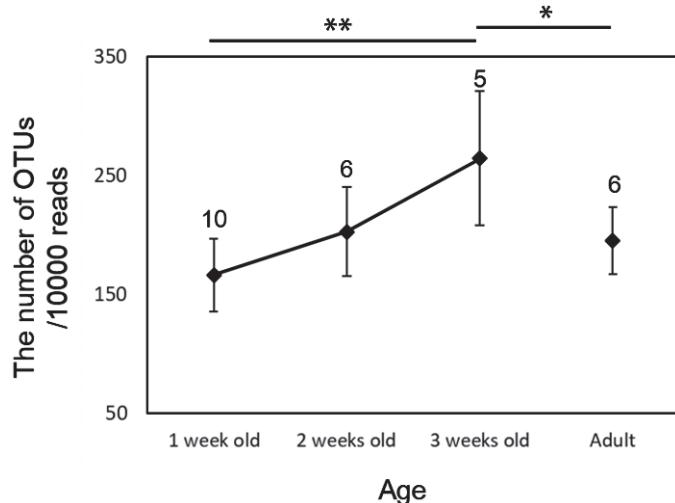
Fig. 3. Normalized abundance of bacterial operational taxonomic units (OTUs) in the cecal microbiomes of Japanese rock ptarmigans. Cecal feces were collected from 1-, 2-, and 3-week-old chicks, as well from their hens. OTU numbers were normalized by total read number, in order to avoid bias from differences in sequencing depth. Error bars indicate standard deviation values. The figures above error bars indicate the sample numbers. *$P<0.05$ between 1-week-old chicks and adult, **$P<0.01$ between 1- and 3-week-old chicks.
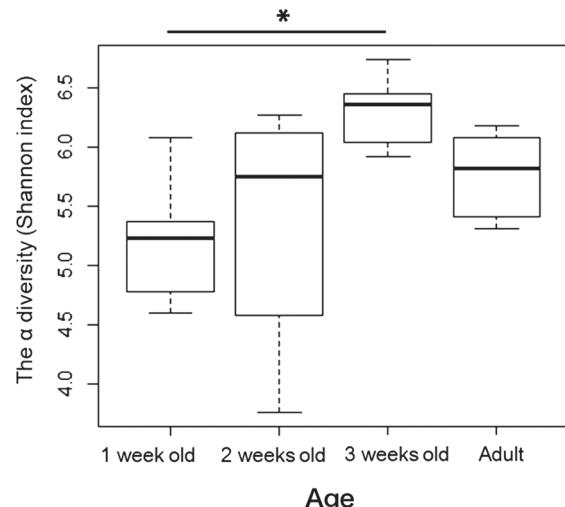
Fig. 4. Effect of age on the development of cecal microbiome alpha diversity in Japanese rock ptarmigans. Cecal feces were collected from 1-, 2-, and 3-week-old chicks, as well from their hens. *$P<0.05$ between 1- and 3-week-old chicks.

as *Olsenella*, *Actinomyces*, *Alkalibaculum*, *Slackia*, *Megasphaera*, *Shuttleworthia*, *Erysipelotrichaceae* incertae sedis, *Vallitalea*, *Robinsoniella*, and *Defluviitalea* (Table S2). The total number of reads attributed to the common OTUs accounted for 93.0, 91.5, 79.1, and 88.8% of the reads obtained for 1-, 2-, and 3-week-old chicks and for their hens (Fig. 5b).

The majority of OTUs (64.0%) common to all growth stages were stable during host growth (Table 1). For example, *Olsenella* sp. and *Alkalibaculum* sp. were consistently listed as some of the most abundant taxa (top three highest proportions), regardless of growth stage. Meanwhile, the remaining OTUs varied with the host's growth: decreasing with growth (7.4%), increasing with growth (3.7%), rising and falling (13.9%), or falling and then rising (10.9%). In the last category, OTUs that constituted a high proportion of the adult cecal microbiome (e.g., *Megasphaera* sp. and *Vallitalea* sp.) were involved.

DISCUSSION

The allocoprophagy of hen cecal feces by JRP chicks (Fig. 1) was confirmed for all three broods and was observed from a few days after the chicks hatched to about 3 weeks after hatching (Fig. 2). The duration of this period is consistent with previous observations (Fig. 1). Neither allo- nor autocoprophagy has been observed in adult JRPs toward either cecal or rectal feces, despite close observation of the last 10 years, and neither allo- nor autocoprophagy has been observed in chicks toward cecal feces after the age of one month. It is worth noting that coprophagy has not been reported in wild Tetranoids, including grouses and ptarmigan, despite close observation [60]. Indeed, no attention was paid to the coprophagy of ptarmigans in previous research because the frequency of cecal feces excretion by chicks is low and because coprophagy is only exhibited during the very early growth stages, as demonstrated by the present study. The present study is the first work to focus on the coprophagy of herbivorous birds. The cage protection protocol enabled us to exhaustively observe the feces of the hens and behavior of the chicks starting from very early growth stages. Considering that chicks first excrete cecal feces at 4 days of age when raised in captivity (Kobayashi *et al.* unpublished data), it is likely that the onset of coprophagy toward the hens' cecal feces slightly precedes the excretion of cecal feces by chicks.

Even though the depth of the present study's sequencing was not extensive, the main characteristics of the chick cecal microbiome were determined. The same number of OTUs were detected in the microbiomes of both the youngest chicks (≤1-week-old) and the hens (Fig. 3), as were the main bacterial OTUs of the hen's cecal microbiome (Fig. 5b). Most of the main adult OTUs were defined as stable (Table 1), meaning that they are acquired during early growth stages and continue to reside in the gut throughout life. Recent studies have reported that some bacteria can be detected in the intestines of bird embryos and newly hatched chicks, but those numbers are negligible [6, 7, 18, 28]. Therefore, the OTU richness of the cecal microbiome should be lower in JRP chicks before coprophagy. However, this was difficult to confirm before the first excretion of cecal feces, which occurred after the onset of coprophagy.

Unlike in JRPs, the numbers of bacterial OTUs in the feces of adult altricial species are much higher than those of chicks, even
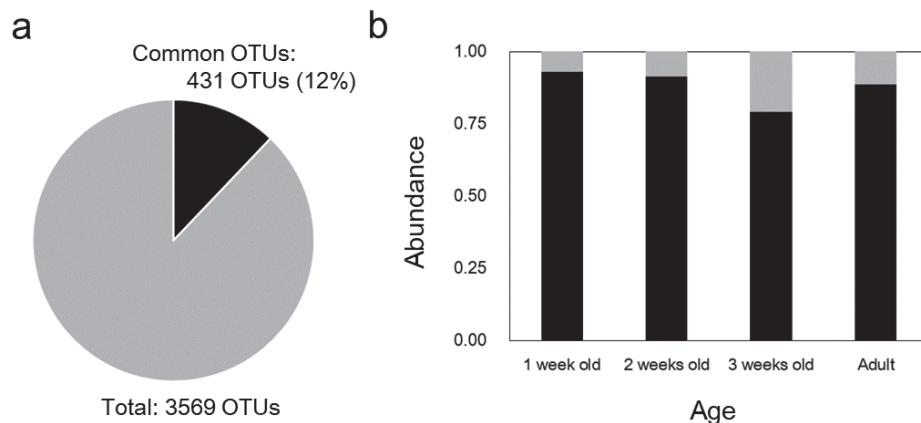
Fig. 5. Effect of age on the abundance of common bacterial operational taxonomic units (OTUs) in Japanese rock ptarmigans. a) Proportion of OTUs attributed to common OTUs. b) Effect of age on the abundance of common OTUs. Black indicates the proportion of common OTUs, and gray indicates the proportion of other OTUs. OTUs represented by less than three reads were omitted from analysis.

at several weeks after hatching [56]. As a result, the dominant OTUs of the species' gut microbiome changes drastically with host growth [56]. Indeed, this phenomenon has also been reported for the chicks of several precocial species that were artificially raised, without contact with their parents [1, 5, 58, 59]. These studies demonstrated that the gut microbiomes of these chicks were slow to establish and the chicks did not share the predominant gut bacteria of adult individuals, until at least several weeks after hatching. In contrast, JRP chicks that were raised under cage protection protocol, which allowed the chicks to perform coprophagy toward their hens' cecal feces, shared the major OTUs of the adult cecal microbiome from very early in life.

The predominant bacterial genera detected in the cecal feces of the chicks used in the present study (*e.g.*, *Olsenella*, *Actinomyces*, *Slackia,* and *Shuttleworthia*; Table 1) were also predominant in adult wild JRP in various locations [52]. These bacterial genera are all anaerobic gut bacteria, except for *Actinomyces*. This latter genus comprises several species that can be isolated from soil [38]. Even though the possibility of inoculation from other sources (*e.g.*, soil and food) does exist, the dominant bacterial OTUs are considered typical residents of the JRP gut and were likely derived from cecal feces of hens. The early onset of coprophagy in the chicks (3 to 4 days after hatching) toward the cecal feces of their hens facilitates the rapid establishment of "semi-adult"-type cecal microbiomes that include the common bacterial OTUs of adult JRPs.

The term "semi-adult"-type refers to the observation that the cecal microbiomes of the chicks were not complete during their first week. Indeed, the number of OTUs (Fig. 3) and alpha diversity (Fig. 4) of the cecal microbiome increased with host growth, and the proportion of several common OTUs changed with host growth (Table 1). Because most of the major OTUs were common to all stages, the observed increases in alpha diversity were mainly produced by quantitative changes in the microbiome (*i.e.*, changes in the number of reads for each OTU). For example, *Megasphaera* sp., which was previously identified as one of the major OTUs of the adult JRP microbiome [52], was not abundant in the chick microbiomes (Table 1). *Megasphaera* sp. is an acidophilic gut bacterium that converts lactic acid to butyric acid [20], and it is likely that this bacterium can grow with other lactic acid producers. Therefore, the proliferation of *Megasphaera* sp. is likely facilitated by increases in lactic acid bacteria. The OTUs identified as *Olsenella* sp. and *Bifidobacterium* sp., which are recognized as potent lactate producers [2, 30], were also predominant in the adult gut microbiome and may support the proliferation of *Megasphaera* sp. This might explain the later development of *Megasphaera* sp. in the gut microbiomes of the chicks.

It is worth noting that coprophagy was not exhibited immediately after hatching but, rather, started at 3 or 4 days after hatching (Fig. 2). This lag time may reflect chicks' yolk sac retention. In chickens, the residual yolk sacs of chicks are absorbed over the 3 or 4 days following hatching [42] and supply the chicks with maternal immunoglobulins [53]. If the same process occurs in the JRP, chicks are protected by maternal immunoglobulin until 3 to 4 days after hatching. In mammals, maternal immunoglobulin, mainly IgA, is supplied by the mother's milk, and intestinal levels of IgA, in particular, are lowest during weaning, since maternal immunoglobulin is no longer supplied [54]. Interestingly, the alpha diversity of mammalian gut microbiomes starts to increase by weaning, when maternal immunoglobulin is exhausted [24–26]. If the same process occurs in the JRP, the time lag for the onset of coprophagy may reflect the time required to exhaust the residual yolk sac and, thus, maternal immunoglobulin.

The acquisition of the cecal microbiome through coprophagy during early growth stages seems to be adaptive for the JRP because precocial, herbivorous chicks must consume and digest the same food as their mothers (e.g., mature leaves and stems from alpine plants) by themselves shortly after hatching. This strategy has also been reported to occur in herbivorous mammals [4, 37] and reptiles [48, 57]. Because herbivores evolved independently in each lineage [46], this behavior can be defined as a convergent strategy among vertebrates that exhibit similar digestive strategies, to facilitate the transmission of essential gut bacteria across generations. In order to elucidate the role of allocoprophagy in the survival of JRP chicks, additional studies are needed to identify the bacterial OTUs at the strain level and to determine the functions of the strains as they are transmitted *via* coprophagy.

The results of the present study are valuable to *ex-situ* conservation programs associated with herbivorous hind-gut fermenters.

Table 1. Common bacterial operational taxonomic units (OTUs) in the cecal microbiomes of Japanese rock ptarmigans at different ages

OTUs	1 week	2 weeks	3 weeks	Adult	Changing rate (highest/lowest value)
1 *Olsenella* sp. No1	0.21296	0.15836	0.08955	0.11500	2.38
Actinomyces sp. No1	0.12764	0.10284	0.06434	0.10553	1.98
Slackia sp. No1	0.04786	0.02776	0.02246	0.05692	2.53
Alkalibaculum sp. No1	0.03943	0.05674	0.04557	0.05041	1.44
Shuttleworthia sp. No1	0.07507	0.02053	0.03199	0.03780	3.66
Robinsoniella sp. No1	0.00925	0.00742	0.00552	0.01968	3.57
Syntrophococcus sp. No1	0.00601	0.00703	0.00555	0.01944	3.51
Bifidobacterium sp.	0.00713	0.00273	0.00547	0.01788	6.54
Eubacterium sp. No1	0.00673	0.00917	0.00625	0.01556	2.49
Proteinivorax sp.	0.00787	0.00609	0.00338	0.01372	4.06
Olsenella sp. No2	0.00332	0.00267	0.00364	0.00934	3.50
Slackia sp. No2	0.00808	0.00495	0.00296	0.00654	2.73
Parvibacter sp. No1	0.00511	0.00736	0.00397	0.00536	1.85
Clostridium XVIII sp. No1	0.00500	0.03369	0.01192	0.00513	6.56
Ruminococcus2 sp. No1	0.00398	0.00318	0.00240	0.00500	2.08
Lachnospiracea incertae sedis sp. No1	0.00957	0.01343	0.00311	0.00475	4.32
Clostridium XVIII sp. No2	0.00302	0.01783	0.01025	0.00473	5.90
Anaerobacterium sp. No1	0.00300	0.00173	0.00136	0.00430	3.16
Asaccharobacter sp. No1	0.03092	0.02265	0.00782	0.00377	8.20
Flavonifractor sp. No2	0.00188	0.00143	0.00456	0.00317	3.20
Anaeroglobus sp. No1	0.00116	0.00147	0.00235	0.00233	2.00
Olsenella sp. No3	0.00112	0.00097	0.00095	0.00215	2.27
Olsenella sp. No4	0.00269	0.00192	0.00108	0.00186	2.49
Clostridium XIVa sp. No1	0.00239	0.00945	0.00212	0.00170	5.55
Fusicatenibacter sp. No1	0.00299	0.00176	0.00119	0.00164	2.51
Ruminococcus2 sp. No2	0.00381	0.00636	0.00526	0.00162	3.93
Olsenella sp. No5	0.00152	0.00188	0.00150	0.00154	1.26
Olsenella sp. No6	0.00077	0.00074	0.00077	0.00148	1.92
Adlercreutzia sp. No1	0.00153	0.00058	0.00060	0.00145	2.56
Dermabacter sp. No1	0.00075	0.00033	0.00046	0.00144	4.39
Robinsoniella sp. No2	0.00039	0.00028	0.00016	0.00138	8.50
Lachnoanaerobaculum sp. No1	0.00043	0.00065	0.00046	0.00133	3.12
Olsenella sp. No7	0.00156	0.00177	0.00114	0.00132	1.55
Dermabacter sp. No2	0.00060	0.00028	0.00034	0.00126	4.48
Olsenella sp. No8	0.00163	0.00165	0.00153	0.00126	1.30
Coprococcus sp. No1	0.00035	0.00070	0.00048	0.00125	3.55
Syntrophococcus sp. No2	0.00050	0.00055	0.00045	0.00121	2.70
Olsenella sp. No9	0.00049	0.00044	0.00031	0.00119	3.87
Olsenella sp. No10	0.00054	0.00033	0.00045	0.00118	3.54
Scardovia sp.	0.00043	0.00019	0.00031	0.00118	6.17
Olsenella sp. No11	0.00046	0.00057	0.00111	0.00112	2.42
Dermabacter sp. No3	0.00071	0.00065	0.00070	0.00110	1.69
Slackia sp. No3	0.00090	0.00078	0.00045	0.00108	2.41
Alkalibaculum sp. No2	0.00159	0.00138	0.00170	0.00102	1.55
Lachnospiracea incertae sedis sp. No2	0.00214	0.00100	0.00064	0.00100	3.33
Pseudoflavonifractor sp. No1	0.00026	0.00017	0.00055	0.00100	5.79
Parvibacter sp. No2	0.00045	0.00014	0.00013	0.00097	7.28
Actinomyces sp. No2	0.00073	0.00061	0.00080	0.00097	1.58
Anaerotruncus sp. No1	0.00039	0.00193	0.00188	0.00094	4.89
Alkalibaculum sp. No3	0.00052	0.00023	0.00039	0.00093	3.97
Lachnoanaerobaculum sp. No2	0.00028	0.00023	0.00021	0.00092	4.42
Allobaculum sp.	0.00091	0.00107	0.00012	0.00086	7.56
Coprococcus sp. No2	0.00012	0.00024	0.00060	0.00086	7.43
Pseudoflavonifractor sp. No2	0.00117	0.00035	0.00032	0.00085	3.63
Olsenella sp. No12	0.00102	0.00064	0.00033	0.00083	3.10
Lachnospiracea incertae sedis sp. No3	0.00022	0.00011	0.00027	0.00081	7.43
Syntrophococcus sp. No3	0.00152	0.00075	0.00034	0.00080	4.40
Eggerthella sp. No1	0.00109	0.00094	0.00036	0.00080	2.99
Ruminococcus2 sp. No3	0.00041	0.00073	0.00051	0.00079	1.95
Hungatella sp. No1	0.00020	0.00018	0.00021	0.00077	4.25
Lachnospiracea incertae sedis sp. No4	0.00243	0.00140	0.00404	0.00077	5.23
Coriobacterium sp. No1	0.00013	0.00012	0.00028	0.00076	6.31
Clostridium XIVa sp. No2	0.00163	0.00036	0.00033	0.00076	4.97
Flavonifractor sp. No16	0.00034	0.00008	0.00014	0.00072	9.22
Dermabacter sp. No4	0.00031	0.00022	0.00009	0.00071	8.22
Flavonifractor sp. No3	0.00250	0.00214	0.00074	0.00069	3.37
Lachnospiracea incertae sedis sp. No5	0.00316	0.00430	0.00072	0.00067	5.93
Acetitomaculum sp. No1	0.00033	0.00038	0.00037	0.00066	1.77
Shuttleworthia sp. No2	0.00050	0.00029	0.00021	0.00066	3.19
Clostridium XIVa sp. No3	0.00076	0.00258	0.00202	0.00066	3.91
Intestinimonas sp. No1	0.00251	0.00232	0.00417	0.00066	6.33
Robinsoniella sp. No3	0.00028	0.00011	0.00020	0.00066	6.06
Clostridium XVIII sp. No3	0.00046	0.00318	0.00108	0.00066	6.91
Anaerobacterium sp. No2	0.00043	0.00034	0.00008	0.00065	7.98
Olsenella sp. No13	0.00080	0.00073	0.00059	0.00062	1.36
Dermabacter sp. No5	0.00115	0.00085	0.00079	0.00061	1.89
Cryptobacterium sp. No1	0.00054	0.00029	0.00023	0.00061	2.63
Clostridium IV sp. No1	0.00076	0.00232	0.00301	0.00058	5.18
Lachnospiracea incertae sedis sp. No6	0.00057	0.00019	0.00038	0.00057	3.05
Anaerobacterium sp. No3	0.00026	0.00023	0.00014	0.00057	4.08
Flavonifractor sp. No4	0.00051	0.00044	0.00126	0.00057	2.87
Lachnospiracea incertae sedis sp. No7	0.00126	0.00147	0.00017	0.00056	8.48
Alkalibaculum sp. No4	0.00035	0.00032	0.00028	0.00056	1.99
Blautia sp. No1	0.00007	0.00019	0.00006	0.00055	9.12
Christensenella sp. No1	0.00022	0.00052	0.00013	0.00054	4.21
Eggerthella sp. No2	0.00047	0.00030	0.00040	0.00054	1.82
Olsenella sp. No14	0.00080	0.00048	0.00037	0.00053	2.17
Anaerovorax sp. No1	0.00070	0.00062	0.00034	0.00053	2.09
Slackia sp. No4	0.00055	0.00030	0.00012	0.00052	4.39
Slackia sp. No5	0.00173	0.00118	0.00031	0.00052	5.52
Actinomyces sp. No3	0.00058	0.00032	0.00011	0.00051	5.32
Ruminococcus2 sp. No5	0.00092	0.00049	0.00168	0.00051	3.40
Actinomyces sp. No4	0.00014	0.00056	0.00037	0.00051	4.04
Olsenella sp. No15	0.00047	0.00038	0.00009	0.00050	5.87
Shuttleworthia sp. No3	0.00119	0.00040	0.00051	0.00049	2.96
Shuttleworthia sp. No4	0.00039	0.00010	0.00025	0.00047	4.65
Olsenella sp. No16	0.00070	0.00071	0.00035	0.00046	2.02
Ruminococcus2 sp. No6	0.00051	0.00015	0.00024	0.00046	3.31
Shuttleworthia sp. No5	0.00058	0.00015	0.00017	0.00045	3.91
Eisenbergiella sp. No1	0.00044	0.00023	0.00031	0.00042	1.90
Lachnospiracea incertae sedis sp. No8	0.00068	0.00235	0.00227	0.00041	5.69
Alkalibaculum sp. No5	0.00037	0.00031	0.00033	0.00040	1.24
Slackia sp. No6	0.00157	0.00096	0.00028	0.00039	5.62
Syntrophococcus sp. No4	0.00008	0.00018	0.00004	0.00039	9.03
Acidaminobacter sp. No1	0.00034	0.00057	0.00025	0.00039	2.31
Eisenbergiella sp. No2	0.00028	0.00009	0.00007	0.00038	5.31
Intestinimonas sp. No2	0.00024	0.00009	0.00017	0.00037	3.95
Alkalibaculum sp. No6	0.00020	0.00043	0.00031	0.00037	2.09
Actinomyces sp. No5	0.00069	0.00032	0.00013	0.00036	5.17
Acidaminobacter sp. No2	0.00017	0.00016	0.00004	0.00036	9.46
Anaerobacterium sp. No4	0.00013	0.00023	0.00019	0.00035	2.75
Anaerobacterium sp. No5	0.00024	0.00019	0.00004	0.00035	8.19
Olsenella sp. No17	0.00015	0.00007	0.00006	0.00035	5.66
Olsenella sp. No18	0.00045	0.00100	0.00197	0.00035	5.60
Slackia sp. No7	0.00027	0.00015	0.00004	0.00035	8.15
Shuttleworthia sp. No6	0.00022	0.00008	0.00005	0.00035	6.60
Coprococcus sp. No3	0.00037	0.00072	0.00019	0.00034	3.72
Fusicatenibacter sp. No2	0.00029	0.00011	0.00018	0.00034	2.99
Murimonas sp. No1	0.00046	0.00070	0.00043	0.00034	2.02
Olsenella sp. No19	0.00071	0.00042	0.00017	0.00033	4.16
Dermabacter sp. No6	0.00023	0.00005	0.00008	0.00033	6.88
Eggerthella sp. No3	0.00038	0.00013	0.00018	0.00033	2.93
Shuttleworthia sp. No7	0.00059	0.00015	0.00019	0.00033	3.94
Ruminococcus2 sp. No7	0.00047	0.00065	0.00034	0.00033	1.92
Olsenella sp. No20	0.00022	0.00017	0.00023	0.00031	1.85
Lachnospiracea incertae sedis sp. No9	0.00010	0.00005	0.00007	0.00031	5.67
Mogibacterium sp.	0.00024	0.00067	0.00021	0.00031	3.20
Dermabacter sp. No7	0.00041	0.00028	0.00008	0.00031	5.01
Paraeggerthella sp.	0.00023	0.00010	0.00007	0.00030	3.22
Shuttleworthia sp. No9	0.00203	0.00043	0.00051	0.00030	6.81
Adlercreutzia sp. No2	0.00023	0.00005	0.00035	0.00029	6.47
Shuttleworthia sp. No8	0.00049	0.00012	0.00028	0.00029	3.98
Olsenella sp. No21	0.00030	0.00040	0.00030	0.00029	1.32
Clostridium XIVa sp. No4	0.00187	0.00173	0.00094	0.00029	6.51
Adlercreutzia sp. No3	0.00008	0.00003	0.00006	0.00028	8.18
Ruminococcus2 sp. No8	0.00014	0.00008	0.00007	0.00028	4.23
Alkalibaculum sp. No7	0.00040	0.00077	0.00033	0.00028	2.77
Coriobacterium sp. No2	0.00008	0.00003	0.00010	0.00027	7.78
Shuttleworthia sp. No10	0.00089	0.00055	0.00032	0.00027	3.34
Olsenella sp. No22	0.00036	0.00023	0.00011	0.00026	3.38
Slackia sp. No8	0.00008	0.00004	0.00011	0.00026	6.34
Murimonas sp. No2	0.00018	0.00008	0.00003	0.00026	7.80
Olsenella sp. No23	0.00020	0.00020	0.00020	0.00026	1.33
Denitrobacterium sp.	0.00024	0.00022	0.00004	0.00026	4.93
Ruminococcus2 sp. No9	0.00015	0.00011	0.00004	0.00026	6.77
Shuttleworthia sp. No11	0.00038	0.00017	0.00011	0.00026	3.44

OTUs	1 week	2 weeks	3 weeks	Adult	Changing rate (highest/lowest value)
Clostridium XVIII sp. No4	0.00020	0.00073	0.00045	0.00025	3.71
Shuttleworthia sp. No12	0.00066	0.00017	0.00023	0.00025	3.93
Gordonibacter sp.	0.00021	0.00016	0.00005	0.00024	4.64
Alkalibaculum sp. No8	0.00041	0.00013	0.00036	0.00024	3.21
Coprococcus sp. No4	0.00138	0.00085	0.00136	0.00024	5.86
Anaerobacterium sp. No6	0.00014	0.00005	0.00002	0.00023	9.76
Shuttleworthia sp. No13	0.00038	0.00015	0.00013	0.00023	2.87
Coprococcus sp. No5	0.00021	0.00017	0.00011	0.00022	1.86
Ruminococcus2 sp. No10	0.00038	0.00011	0.00035	0.00022	3.53
Alkalibaculum sp. No9	0.00017	0.00003	0.00008	0.00022	8.02
Parvibacter sp. No3	0.00013	0.00009	0.00003	0.00022	6.58
Shuttleworthia sp. No14	0.00005	0.00020	0.00007	0.00021	4.47
Actinomyces sp. No6	0.00026	0.00042	0.00021	0.00020	2.05
Lachnospiracea incertae sedis sp. No10	0.00007	0.00005	0.00012	0.00020	3.61
Coprococcus sp. No6	0.00009	0.00005	0.00030	0.00020	5.44
Paraprevotella sp.	0.00016	0.00004	0.00002	0.00020	8.18
Brassicibacter sp.	0.00012	0.00008	0.00003	0.00019	6.69
Lachnospiracea incertae sedis sp. No11	0.00078	0.00030	0.00013	0.00019	5.89
Clostridium XVIII sp. No5	0.00015	0.00043	0.00005	0.00019	8.92
Dermabacter sp. No8	0.00021	0.00013	0.00007	0.00019	2.99
Flavonifractor sp. No4	0.00005	0.00008	0.00049	0.00019	9.40
Catonella sp.	0.00008	0.00003	0.00002	0.00019	7.75
Lachnospiracea incertae sedis sp. No12	0.00026	0.00014	0.00011	0.00018	2.47
Ruminococcus2 sp. No11	0.00028	0.00014	0.00020	0.00018	2.08
Shuttleworthia sp. No15	0.00038	0.00016	0.00005	0.00018	7.15
Olsenella sp. No24	0.00052	0.00018	0.00017	0.00018	3.02
Fusicatenibacter sp. No3	0.00021	0.00010	0.00021	0.00018	2.12
Robinsoniella sp. No4	0.00007	0.00005	0.00013	0.00018	3.70
Olsenella sp. No25	0.00046	0.00031	0.00019	0.00017	1.50
Clostridium IV sp. No2	0.00010	0.00008	0.00008	0.00017	2.13
Murimonas sp. No3	0.00004	0.00018	0.00004	0.00017	4.47
Olsenella sp. No26	0.00039	0.00011	0.00019	0.00017	3.56
Shuttleworthia sp. No16	0.00033	0.00011	0.00007	0.00017	4.97
Lachnospiracea incertae sedis sp. No13	0.00007	0.00003	0.00007	0.00017	4.77
Dermabacter sp. No9	0.00015	0.00004	0.00017	0.00016	4.16
Hungatella sp. No2	0.00018	0.00009	0.00009	0.00016	1.66
Ruminococcus2 sp. No12	0.00035	0.00040	0.00037	0.00015	2.73
Ruminococcus2 sp. No13	0.00022	0.00015	0.00004	0.00015	5.77
Ruminococcus2 sp. No14	0.00009	0.00005	0.00009	0.00015	3.23
Anaerovorax sp. No2	0.00007	0.00029	0.00006	0.00015	4.99
Ruminococcus2 sp. No15	0.00081	0.00009	0.00012	0.00015	8.57
Ethanoligenens sp.	0.00004	0.00013	0.00008	0.00015	4.04
Shuttleworthia sp. No17	0.00030	0.00011	0.00012	0.00014	2.75
Alkalibaculum sp. No10	0.00025	0.00008	0.00008	0.00014	3.14
Lachnospiracea incertae sedis sp. No14	0.00062	0.00037	0.00020	0.00014	4.40
Clostridium XVIII sp. No6	0.00010	0.00041	0.00040	0.00014	4.00
Robinsoniella sp. No5	0.00026	0.00025	0.00026	0.00014	1.90
Christensenella sp. No2	0.00011	0.00079	0.00074	0.00014	7.33
Eubacterium sp. No2	0.00004	0.00007	0.00002	0.00013	7.01
Lachnospiracea incertae sedis sp. No15	0.00003	0.00010	0.00015	0.00013	5.71
Flavonifractor sp. No5	0.00006	0.00029	0.00025	0.00013	5.17
Lachnospiracea incertae sedis sp. No16	0.00009	0.00005	0.00012	0.00013	2.79
Acetanaerobacterium sp.	0.00006	0.00030	0.00013	0.00013	5.29
Shuttleworthia sp. No18	0.00041	0.00059	0.00020	0.00013	4.63
Intestinimonas sp. No3	0.00002	0.00021	0.00020	0.00012	9.46
Olsenella sp. No27	0.00003	0.00005	0.00015	0.00012	4.74
Papillibacter sp. No1	0.00023	0.00035	0.00048	0.00012	4.03
Olsenella sp. No29	0.00023	0.00021	0.00012	0.00012	1.90
Olsenella sp. No28	0.00023	0.00045	0.00026	0.00012	3.76
Robinsoniella sp. No6	0.00030	0.00020	0.00006	0.00012	4.61
Shuttleworthia sp. No19	0.00021	0.00005	0.00008	0.00012	3.82
Slackia sp. No9	0.00008	0.00007	0.00003	0.00011	3.42
Slackia sp. No10	0.00011	0.00003	0.00006	0.00011	4.07
Olsenella sp. No30	0.00045	0.00020	0.00005	0.00011	8.55
Dermabacter sp. No10	0.00014	0.00009	0.00003	0.00011	4.05
Desulfitispora sp.	0.00014	0.00016	0.00007	0.00011	2.45
Ruminococcus2 sp. No16	0.00014	0.00023	0.00012	0.00011	2.14
Dermabacter sp. No11	0.00021	0.00018	0.00009	0.00011	2.23
Flavonifractor sp. No6	0.00011	0.00020	0.00039	0.00011	3.70
Clostridium XVIII sp. No7	0.00004	0.00024	0.00014	0.00011	6.10
Ruminococcus2 sp. No17	0.00006	0.00004	0.00007	0.00011	2.68
Pseudoflavonifractor sp. No3	0.00007	0.00012	0.00009	0.00010	1.54
Anaerotruncus sp. No2	0.00004	0.00007	0.00008	0.00010	2.42
Ruminococcus2 sp. No18	0.00073	0.00009	0.00010	0.00010	8.29
Shuttleworthia sp. No20	0.00044	0.00020	0.00010	0.00010	4.42

OTUs	1 week	2 weeks	3 weeks	Adult	Changing rate (highest/lowest value)
Hungatella sp. No3	0.00004	0.00009	0.00003	0.00009	3.31
Fusicatenibacter sp. No4	0.00006	0.00023	0.00053	0.00009	8.31
Olsenella sp. No31	0.00019	0.00004	0.00003	0.00009	6.51
Flavonifractor sp. No7	0.00007	0.00024	0.00032	0.00009	4.76
Ruminococcus sp.	0.00002	0.00011	0.00009	0.00009	5.20
Clostridium XVIII sp. No8	0.00008	0.00059	0.00039	0.00008	7.39
Clostridium XVIII sp. No9	0.00007	0.00018	0.00003	0.00008	5.56
Olsenella sp. No32	0.00040	0.00017	0.00030	0.00008	5.13
Olsenella sp. No33	0.00011	0.00011	0.00007	0.00008	1.60
Olsenella sp. No34	0.00020	0.00014	0.00033	0.00008	4.37
Parvibacter sp. No4	0.00027	0.00015	0.00008	0.00007	3.74
Flavonifractor sp. No8	0.00008	0.00008	0.00039	0.00007	5.31
Eisenbergiella sp. No3	0.00013	0.00010	0.00004	0.00007	3.30
Anaerosolibacter sp.	0.00012	0.00012	0.00006	0.00007	2.00
Robinsoniella sp. No7	0.00004	0.00003	0.00007	0.00007	2.25
Lachnospiracea incertae sedis sp. No17	0.00030	0.00026	0.00045	0.00006	7.17
Lachnospiracea incertae sedis sp. No18	0.00045	0.00016	0.00027	0.00006	7.40
Pseudoflavonifractor sp. No4	0.00005	0.00005	0.00022	0.00006	3.92
Shuttleworthia sp. No21	0.00021	0.00003	0.00008	0.00006	6.61
Adlercreutzia sp. No4	0.00025	0.00022	0.00003	0.00006	8.76
Clostridium XVIII sp. No10	0.00014	0.00052	0.00022	0.00005	9.82
Lachnospiracea incertae sedis sp. No19	0.00037	0.00047	0.00021	0.00005	8.83
Dermabacter sp. No12	0.00019	0.00016	0.00009	0.00005	3.90
Acetitomaculum sp. No2	0.00016	0.00003	0.00002	0.00005	8.49
Ruminococcus2 sp. No35	0.00044	0.00029	0.00006	0.00005	9.45
Coprococcus sp. No8	0.00002	0.00004	0.00003	0.00005	2.96
Coprococcus sp. No7	0.00030	0.00011	0.00010	0.00005	6.45
Ruminococcus2 sp. No19	0.00013	0.00018	0.00012	0.00004	3.96
Olsenella sp. No35	0.00007	0.00004	0.00002	0.00004	2.82
Shuttleworthia sp. No22	0.00022	0.00010	0.00005	0.00004	5.50
Clostridium XIVa sp. No5	0.00010	0.00024	0.00004	0.00004	6.05
Olsenella sp. No36	0.00006	0.00016	0.00016	0.00004	4.09
Ruminococcus2 sp. No20	0.00014	0.00037	0.00011	0.00004	9.70
Parvibacter sp. No5	0.00009	0.00007	0.00002	0.00004	4.85
Ruminococcus2 sp. No21	0.00008	0.00017	0.00004	0.00004	4.51
Olsenella sp. No37	0.00007	0.00007	0.00002	0.00004	3.77
Ruminococcus2 sp. No22	0.00020	0.00004	0.00023	0.00003	6.96
Coprococcus sp. No9	0.00009	0.00006	0.00002	0.00003	4.75
Lachnospiracea incertae sedis sp. No20	0.00022	0.00021	0.00013	0.00003	8.02
Clostridium XIVa sp. No6	0.00002	0.00004	0.00017	0.00003	6.53
Marvinbryantia sp.	0.00001	0.00010	0.00010	0.00003	8.37
Clostridium XVIII sp. No11	0.00006	0.00024	0.00010	0.00003	8.96
Parvibacter sp. No6	0.00003	0.00008	0.00006	0.00003	2.98
Olsenella sp. No38	0.00023	0.00008	0.00003	0.00003	8.02
Ruminococcus2 sp. No23	0.00004	0.00003	0.00015	0.00002	6.99
Olsenella sp. No39	0.00006	0.00003	0.00004	0.00002	2.67
Pseudoflavonifractor sp. No5	0.00011	0.00013	0.00002	0.00002	6.18
Robinsoniella sp. No8	0.00007	0.00004	0.00002	0.00002	3.66
Lachnospiracea incertae sedis sp. No21	0.00017	0.00005	0.00003	0.00002	8.62
Christensenella sp. No3	0.00008	0.00003	0.00002	0.00002	4.17
Arthrobacter sp.	0.00002	0.00006	0.00003	0.00002	2.23
Cryptobacterium sp. No2	0.00011	0.00012	0.00002	0.00002	7.40
Lachnospiracea incertae sedis sp. No22	0.00004	0.00009	0.00009	0.00001	6.54
Ruminococcus2 sp. No24	0.00005	0.00005	0.00002	0.00001	3.17
Lachnospiracea incertae sedis sp. No23	0.00010	0.00007	0.00010	0.00001	7.21
Coprococcus sp. No10	0.00006	0.00008	0.00002	0.00001	6.00
2 *Vallitalea* sp.	0.00262	0.00371	0.00958	0.02767	10.56
Hungatella sp. No4	0.00048	0.00100	0.00269	0.00860	17.98
Dialister sp.	0.00020	0.00013	0.00027	0.00395	30.95
Megamonas sp. No1	0.00005	0.00008	0.00096	0.00073	19.34
Coprococcus sp. No11	0.00001	0.00014	0.00002	0.00067	88.05
Hungatella sp. No5	0.00002	0.00007	0.00011	0.00033	15.47
Sporobacter sp. No1	0.00002	0.00009	0.00016	0.00018	10.40
Lachnospiracea incertae sedis sp. No27	0.00001	0.00005	0.00003	0.00016	22.04
Megamonas sp. No2	0.00001	0.00005	0.00013	0.00015	16.06
Murimonas sp. No4	0.00001	0.00003	0.00003	0.00014	10.81
Dermabacter sp. No14	0.00001	0.00003	0.00011	0.00009	15.11
3 *Ruminococcus2* sp. No26	0.10726	0.02147	0.03126	0.00588	18.24
Olsenella sp. No40	0.00409	0.00251	0.00066	0.00034	12.06
Olsenella sp. No41	0.00264	0.00210	0.00045	0.00021	12.49
Ruminococcus2 sp. No28	0.00277	0.00021	0.00106	0.00017	16.72
Ruminococcus2 sp. No29	0.00220	0.00050	0.00078	0.00015	14.44
Ruminococcus2 sp. No27	0.00347	0.00027	0.00096	0.00008	44.10
Ruminococcus2 sp. No31	0.00141	0.00011	0.00048	0.00007	19.07

OTUs	1 week	2 weeks	3 weeks	Adult	Changing rate (highest/lowest value)
Ruminococcus2 sp. No30	0.00204	0.00067	0.00042	0.00007	28.12
Dermabacter sp. No13	0.00076	0.00034	0.00020	0.00007	10.55
Olsenella sp. No45	0.00089	0.00053	0.00015	0.00007	13.33
Shuttleworthia sp. No23	0.00136	0.00118	0.00032	0.00007	20.37
Lachnospiracea incertae sedis sp. No24	0.00109	0.00012	0.00020	0.00005	21.37
Olsenella sp. No42	0.00098	0.00044	0.00016	0.00005	19.90
Olsenella sp. No43	0.00093	0.00054	0.00012	0.00005	19.00
Ruminococcus2 sp. No32	0.00110	0.00006	0.00006	0.00005	22.90
Olsenella sp. No44	0.00092	0.00044	0.00025	0.00005	20.06
Parvibacter sp. No7	0.00051	0.00042	0.00012	0.00005	11.17
Olsenella sp. No46	0.00065	0.00042	0.00005	0.00003	18.62
Ruminococcus2 sp. No34	0.00080	0.00110	0.00112	0.00003	33.17
Ruminococcus2 sp. No37	0.00034	0.00022	0.00004	0.00003	10.22
Lachnospiracea incertae sedis sp. No25	0.00036	0.00013	0.00009	0.00003	11.75
Shuttleworthia sp. No24	0.00069	0.00014	0.00010	0.00003	23.90
Lachnospiracea incertae sedis sp. No26	0.00032	0.00030	0.00009	0.00003	12.00
Slackia sp. No11	0.00047	0.00022	0.00002	0.00003	18.51
Ruminococcus2 sp. No38	0.00024	0.00012	0.00022	0.00002	13.52
Asaccharobacter sp. No2	0.00034	0.00026	0.00010	0.00002	18.63
Parvibacter sp. No8	0.00038	0.00007	0.00007	0.00002	21.36
Ruminococcus2 sp. No33	0.00091	0.00006	0.00034	0.00001	63.07
Lachnospira sp.	0.00060	0.00013	0.00026	0.00001	41.63
Cryptobacterium sp. No3	0.00023	0.00035	0.00017	0.00001	24.03
Ruminococcus2 sp. No36	0.00038	0.00003	0.00015	0.00001	26.13
Adlercreutzia sp. No5	0.00048	0.00004	0.00003	0.00001	36.24
4 *Megasphaera*	0.00375	0.00064	0.00777	0.05158	80.59
Erysipelotrichaceae_incertae_sedis sp. No1	0.00240	0.12181	0.03535	0.04108	50.76
Defluviitalea sp. No1	0.00278	0.00433	0.00041	0.02061	50.75
Megamonas sp. No3	0.00177	0.00453	0.03218	0.01952	18.19
Fusicatenibacter sp. No6	0.00269	0.00293	0.00060	0.01662	27.61
Alkalibaculum sp. No12	0.00326	0.00107	0.00036	0.01509	41.56
Citrobacter sp.	0.00005	0.00008	0.03688	0.01270	744.21
Flavonifractor sp. No1	0.00294	0.01398	0.02995	0.00518	10.20
Faecalitalea sp.	0.00132	0.00075	0.00024	0.00351	14.40
Anaeroglobus sp. No2	0.00022	0.00005	0.00057	0.00347	63.16
Alkalibaculum sp. No14	0.00090	0.00033	0.00017	0.00259	15.56
Anaerorhabdus sp.	0.00070	0.00058	0.00022	0.00238	10.67
Papillibacter sp. No2	0.00046	0.00020	0.00034	0.00201	10.01
Hungatella sp. No6	0.00018	0.00009	0.00005	0.00199	37.84
Anaerobacterium sp. No7	0.00127	0.00079	0.00002	0.00198	82.79
Alkalibaculum sp. No13	0.00042	0.00007	0.00005	0.00186	35.45
Defluviitalea sp. No2	0.00011	0.00010	0.00003	0.00162	56.62
Anaerospora sp.	0.00009	0.00045	0.00470	0.00135	54.11
Anaerobacterium sp. No10	0.00009	0.00017	0.00003	0.00135	40.36
Sporobacter sp. No2	0.00054	0.00005	0.00029	0.00132	23.94
Acetatifactor sp.	0.00015	0.00004	0.00046	0.00125	34.41
Robinsoniella sp. No9	0.00010	0.00015	0.00005	0.00111	23.23
Pseudoflavonifractor sp. No8	0.00031	0.00006	0.00006	0.00105	16.69
Parvibacter sp. No11	0.00033	0.00026	0.00009	0.00097	10.64
Clostridium XIVa sp. No10	0.00015	0.00148	0.00225	0.00085	14.56
Slackia sp. No13	0.00020	0.00006	0.00023	0.00079	13.30
Lachnospiracea incertae sedis sp. No32	0.00019	0.00005	0.00038	0.00077	16.84
Megamonas sp. No5	0.00009	0.00003	0.00029	0.00075	23.86
Clostridium XIVa sp. No11	0.00017	0.00043	0.00002	0.00074	38.74
Anaerobacterium sp. No8	0.00019	0.00005	0.00004	0.00071	16.62
Psychrosinus sp. No1	0.00014	0.00004	0.00007	0.00068	18.75
Acidaminobacter sp. No3	0.00005	0.00008	0.00002	0.00064	33.38
Geosporobacter sp. No2	0.00005	0.00006	0.00002	0.00063	33.07
Coprococcus sp. No16	0.00045	0.01062	0.00195	0.00063	23.78
Anaerobacterium sp. No9	0.00015	0.00023	0.00003	0.00062	21.48
Flavonifractor sp. No9	0.00025	0.00152	0.00405	0.00059	16.21
Ruminococcus2 sp. No4	0.00200	0.00223	0.00596	0.00059	10.15
Psychrosinus sp. No2	0.00004	0.00006	0.00002	0.00056	29.50
Geosporobacter sp. No1	0.00011	0.00014	0.00004	0.00055	12.85
Dermabacter sp. No16	0.00004	0.00010	0.00004	0.00055	14.38
Parvibacter sp. No9	0.00026	0.00278	0.00171	0.00050	10.65
Anaerobacterium sp. No11	0.00007	0.00006	0.00005	0.00049	10.32
Slackia sp. No14	0.00008	0.00003	0.00013	0.00049	14.04
Eisenbergiella sp. No4	0.00025	0.00028	0.00002	0.00047	24.59
Olsenella sp. No48	0.00128	0.00009	0.00022	0.00047	14.83
Adlercreutzia sp. No6	0.00045	0.00003	0.00015	0.00045	14.34
Olsenella sp. No50	0.00009	0.00003	0.00006	0.00045	13.65
Anaerotruncus sp. No3	0.00010	0.00008	0.00002	0.00034	14.31
Ruminococcus2 sp. No47	0.00223	0.00016	0.00035	0.00033	14.31
Coprococcus sp. No12	0.00080	0.00146	0.00618	0.00033	18.84
Olsenella sp. No51	0.00012	0.00028	0.00003	0.00032	11.04
Syntrophococcus sp. No5	0.00012	0.00017	0.00002	0.00028	14.40
Coprococcus sp. No20	0.00003	0.00075	0.00115	0.00027	45.06
Clostridium XIVa sp. No9	0.00026	0.01079	0.00213	0.00026	40.95
Faecalicoccus sp.	0.00029	0.00226	0.00573	0.00023	24.79
Alkalibaculum sp. No11	0.00002	0.00076	0.00038	0.00022	31.32
Anaerobacterium sp. No12	0.00026	0.00014	0.00002	0.00022	10.83
Ruminococcus2 sp. No43	0.00020	0.00541	0.01766	0.00021	88.56
Pseudoflavonifractor sp. No6	0.00058	0.00130	0.00261	0.00019	13.42
Dorea sp.	0.00226	0.00599	0.00546	0.00019	31.21
Ruminococcus2 sp. No39	0.00142	0.00216	0.00164	0.00018	12.33
Anaerovorax sp. No4	0.00002	0.00061	0.00006	0.00017	24.61
Erysipelotrichaceae_incertae_sedis sp. No3	0.00001	0.00039	0.00034	0.00015	40.79
5 *Slackia* sp. No12	0.00123	0.00079	0.00008	0.00014	15.10
Lactococcus sp. No1	0.00008	0.00131	0.00080	0.00014	15.58
Erysipelotrichaceae_incertae_sedis sp. No4	0.00001	0.00023	0.00005	0.00013	31.01
Ruminococcus2 sp. No41	0.00051	0.00225	0.01613	0.00011	152.57
Flavonifractor sp. No12	0.00002	0.00022	0.00061	0.00010	40.00
Erysipelotrichaceae_incertae_sedis sp. No2	0.00001	0.00025	0.00019	0.00009	17.28
Fusicatenibacter sp. No5	0.00013	0.00111	0.00119	0.00009	12.83
Flavonifractor sp. No15	0.00003	0.00017	0.00062	0.00009	21.49
Clostridium XVIII sp. No12	0.00003	0.00044	0.00018	0.00009	15.39
Anaerovorax sp. No3	0.00006	0.00020	0.00002	0.00009	10.30
Megamonas sp. No4	0.00001	0.00004	0.00017	0.00009	23.99
Clostridium XIVa sp. No8	0.00057	0.00331	0.00208	0.00008	40.86
Flavonifractor sp. No10	0.00004	0.00028	0.00057	0.00008	13.60
Clostridium XIVa sp. No7	0.00155	0.00529	0.00406	0.00008	67.86
Ruminococcus2 sp. No48	0.00100	0.00007	0.00035	0.00008	14.15
Coprococcus sp. No13	0.00003	0.00124	0.00014	0.00007	37.56
Coprococcus sp. No14	0.00058	0.00282	0.00108	0.00006	46.00
Olsenella sp. No52	0.00073	0.00037	0.00005	0.00006	15.18
Blautia sp. No3	0.00009	0.00058	0.00096	0.00006	16.07
Flavonifractor sp. No11	0.00002	0.00010	0.00054	0.00006	24.72
Lachnospiracea incertae sedis sp. No31	0.00091	0.00016	0.00005	0.00006	19.11
Ruminococcus2 sp. No42	0.00032	0.00043	0.00066	0.00006	11.71
Eubacterium sp. No3	0.00001	0.00013	0.00028	0.00005	23.43
Olsenella sp. No49	0.00046	0.00023	0.00002	0.00005	24.22
Ruminococcus2 sp. No46	0.00004	0.00026	0.00085	0.00005	24.08
Flavonifractor sp. No13	0.00001	0.00022	0.00075	0.00005	60.39
Olsenella sp. No47	0.00008	0.00021	0.00049	0.00005	10.69
Dermabacter sp. No15	0.00001	0.00019	0.00004	0.00005	19.64
Lachnospiracea incertae sedis sp. No30	0.00006	0.00343	0.00296	0.00005	75.56
Ruminococcus2 sp. No49	0.00044	0.00003	0.00017	0.00004	16.07
Blautia sp. No2	0.00019	0.00643	0.00396	0.00003	194.58
Pseudoflavonifractor sp. No7	0.00001	0.00008	0.00039	0.00003	41.34
Parvibacter sp. No10	0.00006	0.00038	0.00003	0.00003	11.52
Lachnospiracea incertae sedis sp. No29	0.00025	0.00188	0.01029	0.00003	357.50
Coprococcus sp. No15	0.00049	0.00229	0.00080	0.00003	79.59
Ruminococcus2 sp. No45	0.00037	0.00037	0.00316	0.00003	119.36
Lachnospiracea incertae sedis sp. No28	0.00030	0.00014	0.00070	0.00003	26.53
Coprococcus sp. No19	0.00001	0.00013	0.00016	0.00003	21.07
Butyricicoccus sp.	0.00022	0.00019	0.00055	0.00003	21.82
Shuttleworthia sp. No25	0.00008	0.00012	0.00042	0.00003	16.71
Intestinimonas sp. No4	0.00013	0.00026	0.00035	0.00002	16.36
Coprococcus sp. No17	0.00033	0.00051	0.00010	0.00002	25.55
Coprococcus sp. No18	0.00013	0.00005	0.00044	0.00002	24.32
Lactococcus sp. No2	0.00001	0.00023	0.00030	0.00002	22.82
Ruminococcus2 sp. No44	0.00007	0.00017	0.00038	0.00002	22.88
Ruminococcus2 sp. No40	0.00065	0.00456	0.04151	0.00001	2884.43
Flavonifractor sp. No14	0.00001	0.00004	0.00018	0.00001	12.83
Ruminococcus2 sp. No25	0.00002	0.00015	0.00009	0.00001	10.31
Total	0.93046	0.91477	0.79141	0.88821	

☐ <0.00010, ▨ 0.00010–0.00099, ▧ 0.00100–0.00999, ▩ 0.01000–0.09999, ■ ≥0.10000.

The numbers at the right side of the OTU identification indicate the pattern of change. 1, Stable OTUs (the ratio of the highest value to the lowest value was less than 10); 2, OTUs increased with the growth of the host; 3, OTUs decreased with the growth of the host; 4, OTUs were higher in mid-growing stage; 5, OTUs were lower in the mid-growing stage. Darker color means higher proportion in each age class.

Some studies have reported significant differences between the gut microbiomes of wild and captive individuals and that such differences are the result of diet and feed additives, such as antimicrobials [3, 51, 55, 65]. Understanding such captivity-related modifications to the gut microbiome is critical for conserving endangered species [64], considering that the loss or perturbation of a functional gut microbiome could be fatal to captive herbivores, when they need to consume wild plants that are rich in toxic and anti-herbivore chemicals [15]. The reconstruction of wild-type gut microbiomes prior to the reintroduction of captive animals may be essential to improve the results of JRP re-introduction programs. The results of the present study indicate that inoculation with fresh fecal microbes from healthy wild individuals at early life stages is one method by which to reconstruct the wild-type gut microbiome.

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