



SOCIO-SEXUAL BEHAVIOUR AND THE VAGINAL MICROBIOME IN FEMALE BONOBOS

EFFECTS OF AGE, HORMONAL CONTRACEPTION AND
BEHAVIOUR-MICROBIOME INTERACTIONS



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Abstract (Dutch)

Bonobo's (*Pan paniscus*) staan bekend om hun frequente socioseksuele interacties, die vermoedelijk een belangrijke rol spelen in sociale binding en spanningsregulatie. Ondanks uitgebreid onderzoek naar het gedrag van bonobo's blijft weinig bekend over hoe leeftijd en hormonale anticonceptie socioseksueel gedrag en het vaginale microbioom beïnvloeden, en hoe deze gedragingen samenhangen met de vaginale microbiële gemeenschap.

Deze studie onderzocht de effecten van leeftijd en hormonale anticonceptie op socioseksueel gedrag en het vaginale microbioom bij vrouwelijke bonobo's in dierentuinen, evenals de relatie tussen socioseksueel gedrag en vaginale microbiota. Hiervoor werden individuele, gedrags- en microbiële gegevens verzameld van 71 vrouwelijke bonobo's verspreid over acht Europese zoölogische instellingen. Socioseksueel gedrag werd gekwantificeerd aan de hand van gedragsobservaties en vaginale microbiomstalen werden geanalyseerd met behulp van shotgun metagenomische sequencing.

Er werd geen niet-lineair effect van leeftijd gevonden op socioseksueel gedrag. Hormonale anticonceptie bleek daarentegen wel consistent geassocieerd met socioseksueel gedrag. Vrouwtjes die een pil met enkel progestageen (POP) kregen, vertoonden minder socioseksuele interacties en een trend naar minder seksuele partners in vergelijking met vrouwtjes die een gecombineerde orale anticonceptiepil (COC) gebruikten of geen hormonale anticonceptie kregen.

Leeftijd was geassocieerd met variatie in de samenstelling van het vaginale microbioom, hoewel de effecten op α -diversiteit niet eenduidig waren. Het gebruik van een POP werd geassocieerd met een meer gelijkmatige verdeling van bacteriële taxa binnen het vaginale microbioom, terwijl dit patroon niet werd waargenomen bij COC-gebruikers. Daarnaast vertoonden vrouwtjes met meer seksuele partners en hogere frequenties van socioseksuele interacties minder gelijkmatige microbiële gemeenschappen, gedomineerd door minder bacteriële taxa. Socioseksuele interactiefrequenties verklaarden geen microbiële gelijkens tussen vrouwelijke dyads.

Samen tonen deze bevindingen aan dat leeftijd en hormonale status bijdragen aan variatie van socioseksueel gedrag en het vaginale microbioom van bonobo's. Tenslotte suggereert deze studie dat socioseksueel gedrag en het vaginale microbioom nauw met elkaar verbonden zijn bij vrouwelijke bonobo's.

Abstract (English)

Bonobos (*Pan paniscus*) are known for their frequent socio-sexual behaviour, which contributes to social bonding and tension regulation. Despite extensive research on bonobo behaviour, little remains known about how age and hormonal contraception influence socio-sexual interactions, the vaginal microbiome and how these behaviours relate to their vaginal microbial community.

This study investigated the effects of age and hormonal contraception on socio-sexual behaviour and the vaginal microbiome in zoo-housed female bonobos, while exploring the relationship between socio-sexual behaviour and vaginal microbiota. It combined individual, behavioural and microbial data from 71 female bonobos housed across eight European zoological institutions. Socio-sexual behaviour was quantified through behavioural observations and vaginal microbiome samples were analysed using shotgun metagenomic sequencing.

No clear non-linear effect of age was detected on socio-sexual behaviour. In contrast, hormonal contraception was consistently associated with socio-sexual behaviour. Females receiving a progestin-only-pill (POP) engaged less in socio-sexual interactions. Furthermore, these females showed a trend towards fewer sexual partners compared to females receiving a combined-oral-contraceptive pill (COC) or no hormonal contraception.

Age was associated with variation in vaginal microbiome composition, although effects on α -diversity were inconclusive. The use of a POP was associated with a more even distribution of bacterial taxa within the vaginal microbiome, whereas this was not observed for females using a COC.

In addition, females with more sexual partners and higher frequencies of socio-sexual interactions showed less even microbial communities, dominated by fewer bacterial taxa. However, similarity between vaginal microbial communities of dyads was not explained by socio-sexual interaction frequencies between females of that dyad.

Together, these findings demonstrate that age and hormonal state contribute to variation of socio-sexual behaviour and the vaginal microbiome in bonobos, while their effects differ in strength. Moreover, it showed that socio-sexual behaviour and the vaginal microbiome are interconnected in female bonobos.

Lay summary

Bonobos are one of our closest living relatives and are well known for their frequent sexual-behaviour. Unlike in many other animals, bonobos use sexual interactions not only for reproduction, but also to form social bonds, reduce tension and maintain peaceful group relationships. However, little is known about how factors, such as age and hormonal contraceptives, influence this behaviour, and the bacteria living in the vaginal tract. In addition, it remains unclear whether socio-sexual behaviour and these bacterial communities are linked.

In this study, 71 female bonobos from eight European zoos were observed to investigate how age and hormonal contraception affect socio-sexual behaviour and microbes living inside the vagina. Socio-sexual behaviour was measured through behavioural observations. Vaginal swab samples were collected and analysed to identify the bacteria present in the vaginal environment. This study also explored whether females with more socio-sexual interactions showed differences in their vaginal microbial communities and whether females interacting frequently together, shared more similar vaginal bacteria.

The results showed that age did not affect socio-sexual behaviour, in contrast to hormonal contraceptive use. Females receiving a progestin-only pill (POP) showed lower frequencies of socio-sexual behaviour and tended to have fewer sexual partners. Age was, however, linked to differences in the vaginal bacterial communities of bonobos. Additionally, more sexually active females and females with more partners showed bacterial communities in which fewer bacterial groups became more abundant. Surprisingly, females that interacted more frequently with each other did not show more similar vaginal bacterial communities.

This study was the first to combine behavioural observations and analyses of vaginal microbes in female bonobos. It contributed to the understanding of how behaviour, hormones and microbes interact in a social species.

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Table of contents

Abstract (Dutch)	i
Abstract (English)	ii
Lay summary	iii
Acknowledgments	iv
Table of contents	v
1. Introduction	1
1.1 Socio-sexual behaviour	1
1.1.1 The effect of age on socio-sexual behaviour	2
1.1.2 The effect of hormonal contraception on socio-sexual behaviour.....	3
1.2 The vaginal microbiome	5
1.2.1 The effect of age on the vaginal microbiome	6
1.2.2 The effect of hormonal contraception on the vaginal microbiome	7
1.3 Influence of socio-sexual behaviour on the vaginal microbiome.....	8
2. Scientific research objectives	9
3. Materials and methods	12
3.1 Study subjects and housing	12
3.2 Behavioural data collection	12
3.3 Microbiome data collection	13
3.3.1 Sample collection	13
3.3.2 Metagenomic sequencing	14
3.3.3 Calculation of microbiome metrics.....	14
3.4 Statistical approach	15
3.4.1 Socio-sexual behaviour	15
3.4.2 Vaginal microbiome	17
3.5 Ethics statement.....	18
4. Results	19
4.1 Socio-sexual behaviour	19
4.1.1 Factors influencing socio-sexual degree	19
4.1.2 Factors influencing frequency of socio-sexual behaviour.....	21
4.1.3 Case study: the effect of switching contraception type on the frequency of socio-sexual behaviour	22
4.2 The vaginal microbiome	23

4.2.1	Description of the vaginal microbiome	23
4.2.2	Factors influencing the inverse Simpson index	24
4.2.3	Factors influencing Bray-Curtis similarity	27
5.	Discussion	31
5.1	Factors influencing socio-sexual behaviour	31
5.2	Overview of the vaginal microbiome composition.....	33
5.3	Factors influencing the vaginal microbiome	34
5.4	Influence of socio-sexual behaviour on the vaginal microbiome.....	37
5.5	Future research perspectives	40
6.	Conclusion	42
7.	References	43
8.	Appendix.....	60

1. Introduction

1.1 Socio-sexual behaviour

Socio-sexual behaviour, or non-conceptive sexual behaviour, is widespread throughout the animal kingdom with observations in over 1500 species across major animal clades such as Mammalia, Cephalopoda, Reptilia and Echinodermata (Bailey & Zuk, 2009; Monk et al., 2019; Orille, 2020). These behaviours are thought to be useful for relationship development or to reduce tension, while some believe they are practices for later reproductive events (Bailey & Zuk, 2009).

Within primates, sexual behaviour frequently occurs outside reproductive contexts and has been associated with a range of social functions, including the formation and maintenance of social bonds in humans (Douglas et al., 2016; Fleischman, 2021; Vaglio et al., 2021; Clarke et al., 2022). Furthermore, it has been found that participating in socio-sexual behaviours may positively impact one's physical and emotional well-being (Diamond & Huebner, 2012; Dubé et al., 2020; Clarke et al., 2022). Overall, socio-sexual behaviour plays an important social role in many primate species that exhibit complex social systems (Kappeler & Van Schaik, 2002; Neumann et al., 2010; Clarke et al., 2022; Brooker et al., 2025). For example, stump-tailed macaques (*Macaca arctoides*) show higher rates of socio-sexual behaviour during post-conflict reunions (Call et al., 2002). In species like marmosets (Callitrichidae), social odours, such as the scent of a novel ovulating female, play a key role in both social and sexual behaviour (Snowdon et al., 2006).

Therefore, primates provide a valuable comparative framework for investigating the evolutionary origins and function of socio-sexual behaviours (Brooker et al., 2025). Moreover, studying these patterns in non-human primates can clarify the significance of such behaviours across species, including our own (Brooker et al., 2025).

As one of our closest living relatives, bonobos (*Pan paniscus*) represent a particularly informative model for investigating proximate factors underlying socio-sexual behaviour. They are well known for their rich and heightened sexual behaviour that plays a prominent feature in the social lives of both females and males (Hohmann & Fruth, 2000; Gruber & Clay, 2016). Bonobo societies are considered to be female-centred, even though female bonobos typically lack strong genetic relatedness to other females within their groups (Sommer et al., 2011). Nevertheless, they form strong coalitions with each other and with other males in which socio-sexual behaviour plays an important role (Furuichi, 1989; Hohmann et al., 1999). Female bonobos are continuously sexually receptive over the duration of their cycles, with frequencies of socio-sexual behaviour reaching a peak around maximum tumescence of the sexual swelling (Hohmann & Fruth, 2000; Paoli et al.,

2006). Their sexual repertoire, however, is not constrained to reproductive sex alone (Clay & De Waal, 2015; Clarke et al., 2022). For example, it also includes mounting without intromission, oral sex and genital massage between both female-male and same-sex dyads (a social unit consisting of two individuals and the interactions between them) (Clay & De Waal, 2015). These dyads may also differ in strength and in frequencies of socio-sexual behaviours (Clay & De Waal, 2015). Moreover, female bonobos interact most notably in genito-genital rubbing during which two females embrace ventro-ventrally and swing their hips laterally while keeping their vulva in contact (Hohmann & Fruth, 2000; Figure 1).

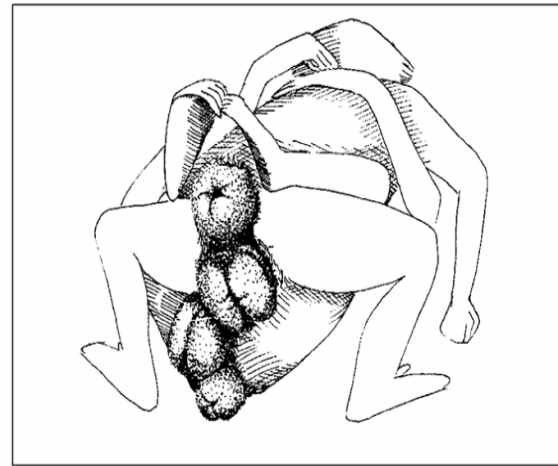


Figure 1. Two female bonobos interacting in genito-genital rubbing (Hohmann & Fruth, 2000).

1.1.1 The effect of age on socio-sexual behaviour

These behaviours have been shown to occur across wild and captive settings, even in juveniles (De Waal, 1990; Hashimoto, 1997; Woods & Hare, 2011; Clay & De Waal, 2015). Socio-sexual behaviour emerges early in development, even before puberty, as bonobo infants younger than one year old have been observed rubbing their genitals together (Hashimoto, 1997; Woods & Hare, 2011). Strikingly, there appears to be no need for adults to initiate or to act as models for observational learning (Woods & Hare, 2011). Moreover, the use of socio-sexual behaviour occurs in a wide range of contexts, including play, feeding anticipation and conflict management (De Waal, 1990; Enomoto, 1990; Hohmann & Fruth, 2000; Clay & De Waal, 2015). The function of socio-sexual behaviour is not yet fully understood, but it is hypothesised to play an important social function in reconciliation and to relieve social tension (Clay & De Waal, 2015). Additionally, it is thought to be an important aspect in the bonding of unrelated females and the facilitation of peaceful co-existence between group members (Wrangham, 1993). Thus, unlike reproductive sexual behaviour, socio-sexual behaviour is not primarily aimed at increasing fitness through reproductive success, but serves important social functions within bonobo populations. Interestingly, socio-sexual behaviour seems to have similar functions across great apes and humans, which may suggest that affiliative genital contact could already have been present in our last common ancestor to help manage social relationships or during periods of social tension (Hohmann & Fruth, 2000; Brooker et al., 2025).

1.1.2 The effect of hormonal contraception on socio-sexual behaviour

Although genito-genital rubbing in female bonobos is often seen as a socio-affiliative behaviour, little is known about its proximate regulation (Clay & De Waal, 2015). In many mammals, reproductive hormones fluctuating through the reproductive cycle (i.e. estrogens and progesterone) play a primary role in activating pathways underlying sexual motivation (Roney & Simmons, 2013; Kondracki et al., 2025). Therefore, if genito-genital rubbing or other forms of socio-sexual behaviour were foremost hormonally driven, suppression of ovulation through hormonal contraceptives might reduce their frequency. Given that socio-sexual behaviour frequently occurs even outside of the fertile window in bonobos, these behaviours might only be partially explained by cyclical hormonal changes in the ovarian hormones (Hohmann et al., 1999).

Hormonal contraceptives are often used in zoo-housed primates as a way to manage the size and genetic variation of populations (Karaskiewicz et al., 2023). As captive-born primates live longer and breed more successfully due to improved nutrition and medical care, controlling reproduction is important to allow institutions to care for all offspring both financially and physically (Plowman et al., 2005; Karaskiewicz et al., 2023). These hormonal treatments can then also be opportunistically used to investigate proximate endocrinological drivers of socio-sexual behaviour. Moreover, from an applied perspective, understanding how hormonal contraceptives may affect behaviours involved in social bonding, such as socio-sexual behaviour, is particularly important in a species such as bonobos.

In European zoo-housed bonobos, the most common form of contraception used is the combined oral contraceptive pill (COC) (data collected during this study). They consist of synthetic forms of both estrogen and progestin in varying doses that inhibit pituitary gonadotropins, subsequently leading to the suppression of follicular growth and ovulation (Goldzieher, 1994). Both estrogen and progestin alone are capable of inhibiting follicle-stimulating hormone and luteinizing hormone to prevent ovulation (Goldzieher, 1994). Combining the two of them together, however, increases the antigonadotropic and ovulation-inhibitory effects (Goldzieher, 1994). For bonobos, particularly, the COC consists of either desogestrel or levonorgestrel as the progestin component with ethinyl estradiol serving as the estrogenic component. Here, ethinyl estradiol contributes to the antigonadotropic effect of progestin and prevents irregular shedding of the endometrium (Lobo & Stanczyk, 1994). Next to the COCs, progestin-only pills (POPs) are also administered to female bonobos due to their ability to block midcycle rise in luteinizing hormone secretion and their consideration to be safer, especially for lactating individuals (Goldzieher, 1994; Nederlof et al., 2024). In these cases, the progestin component consists most often of desogestrel. Because of their genetic closeness, the contraceptives that bonobos receive are the same contraceptives

available for humans (Malcolm, 2008). Thus far, no adverse effects of using these have yet been documented, indicating that human safety studies can be applied to apes as well (Malcolm, 2008).

In several primate species, the use of contraception has been demonstrated to alter both attraction to males and social behaviour in females (reviewed by Karaskiewicz et al., 2023). In a study with ring-tailed lemurs (*Lemur catta*), males preferred the scent of females not taking hormonal contraceptives over the scent of females receiving a POP (Crawford et al., 2011). Male hamadryas baboons (*Papio hamadryas*) were also less likely to mate with females using a POP than with females not using any contraceptives (Guy et al., 2008). Interestingly, this species relies heavily on visual cues as females display elaborate visual signs of ovulation (Guy et al., 2008). These findings suggest that contraceptives can modify key sexual signals through variable sensory cues (Karaskiewicz et al., 2023).

In addition to male responses, studies in macaques indicate that contraceptives may directly affect female behaviour (Karaskiewicz et al., 2023). In Barbary macaques (*Macaca sylvanus*), the use of POPs was correlated with increased antagonistic behaviour and increased received grooming (Maijer & Semple, 2016). The possibility of females on contraception becoming more aggressive was also found in stump-tailed macaques, especially toward male conspecifics (Linn & Steklis, 1990). However, oral contraceptives did not affect female aggression in crab-eating macaques (*Macaca fascicularis*) (Shively et al., 1990). Instead, treated females spent less time in body contact than untreated females.

Together, these studies show that hormonal contraceptives can influence both signalling and social interactions, possibly through underlying hormonal pathways. However, most of the existing research focuses on male responses or male-female interactions that are typically linked to specific phases of the menstrual cycle, especially ovulation. Therefore, the effects on female-female socio-sexual interactions, which occur throughout the entire menstrual cycle, remain largely unexplored (Hohmann & Fruth, 2000).

Moreover, findings in great apes do not seem to be as consistent as those of other primate studies. For instance, in gorillas (*Gorilla gorilla gorilla*), the use of COCs or POPs did not alter the rate of initiation of sexual interactions for both males and females, but instead it did have an effect on their timing (LeFauve & Margulis, 2023). This suggests that the timing of sexual interactions may be altered when these interactions are primarily hormone-driven. In chimpanzees (*Pan troglodytes*), the use of a COC reduced rates of sexual interactions, but its effect was related to an individual's motivation to engage in sexual behaviour while on a natural cycle (Nadler et al.,

1993). Individuals with strong dyads and high rates of sexual activity continued to show high rates of sexual behaviour. Only individuals in weak dyads, with little interaction, ceased to copulate. The same study showed no effect of the COC on the acceptance of male copulatory attempts compared to before receiving contraception. Lastly, in bonobos, only one study has yet looked at the effects of hormonal contraception on sexual behaviour (Agnew et al., 2016). In this study, a survey was given to zookeepers, but behavioural observations were not consistently recorded. Most sexual activity of female bonobos on COCs was scored as unchanged after being put on hormonal contraceptives. In conclusion, the effects of both COCs and especially POPs on socio-sexual behaviour remain to be investigated in bonobos.

1.2 The vaginal microbiome

The same endocrine changes potentially involved in socio-sexual behaviour may also shape the vaginal environment in which microbial communities establish. Because of their promiscuous behaviour, bonobos open up an especially interesting avenue for the investigation of the vaginal microbiome. Their diverse sexual networks make way for a heightened possibility of transmitting microorganisms, leading to potentially greater individual microbial diversity (Yildirim et al., 2014).

The vaginal microbiome is a dynamic microecosystem that harbours billions of microorganisms (Chen et al., 2021). It is predominantly composed of bacteria, but it also includes smaller portions of archaea and eukaryotic microorganisms, which provide antimicrobial and anti-inflammatory functions (Mitrevva, 2012; Stumpf et al., 2013; Chen et al., 2021). Across primate phylogeny, distinct differences can be found in both microbiome composition and diversity, related to factors such as sexual behaviour, mating group size and gestation time (Yildirim et al., 2014). Commonly, the vaginal microbiome forms a crucial and mutualistic relationship with its host, profoundly affecting physiology, reproduction, survival and eventually evolution (Stumpf et al., 2013; Chen et al., 2021).

During sexual interactions, females are particularly susceptible to microbes, including pathogens, through their vaginal environment (Stumpf et al., 2013). Therefore, protective microorganisms residing in the microbiome play an important role in preventing the colonisation of maladaptive microbes like those responsible for sexually transmitted infections (Wren, 2000; Gupta & Maiden, 2001). Given its involvement in reproduction, such microbial dynamics could have a key role in the evolution of sexual behaviour in primates (Stumpf et al., 2013). Thus, depending on the mating system, the primate vaginal microbiome might shift in composition to enhance its resistance to pathogenic microorganisms (Stumpf et al., 2013). Commonly, in primates, this includes generating an acidic, protective environment (Stumpf et al., 2013).

In humans, the low vaginal pH (approximately 3.5-4.4) is facilitated by *Lactobacillus*, a bacterial genus that metabolises glycogen into lactic acid (Boskey et al., 2001; Stumpf et al., 2013). A decrease of *Lactobacillus* in humans increases other bacterial species, which leads to a disrupted vaginal pH, which then might increase the chance of yeast infections and sexually transmitted infections (Mirmonsef et al., 2012; Stumpf et al., 2013). Interestingly, non-human primates do not show the same abundance of *Lactobacillus* (Yildirim et al., 2014). Instead, they might possess other lactic-acid-producing bacteria to lower vaginal pH (Miller et al., 2016). For example, the vaginal microbiome of chimpanzees shows a *Lactobacillus* proportion of less than 3.50% and a much higher taxonomic diversity than humans (Stumpf et al., 2013; Yildirim et al., 2014). Similarly, bonobos show a higher average vaginal pH of approximately 6.1 compared to humans, potentially reflecting these differences in vaginal microbial composition (Samartino et al., 2024).

For bonobos, the vaginal microbiome has only been investigated in a single study to date, and despite providing valuable insights, it was limited due to the inclusion of only two female individuals (Samartino et al., 2024). Similar to other non-human primates, *Lactobacillus* was present at very low abundances (< 1.00%) (Yildirim et al., 2014). However, other lactic-acid-producing genera, such as *Aerococcus* (0.33%) and *Streptococcus* (0.07%), were found, which likely contribute to their acidic vaginal environment. In this study, the most abundant genus to be found was *Gardnerella*, which is linked to disrupted vaginal microbial communities and bacterial vaginosis in humans (Ling et al., 2010; Samartino et al., 2024).

Notably, the bonobo vaginal community shows an abundance of bacterial genera that would typically be linked to suboptimal health in humans, particularly related to bacterial vaginosis (Mirmonsef et al., 2014; Samartino et al., 2024). This might mean that the vaginal microbiome in bonobos may be shaped by different host-related or environmental factors compared to humans. Thus, this leaves a need to investigate potential drivers such as effects of age, contraception and (socio-)sexual behaviour on the vaginal microbiome in bonobos.

1.2.1 The effect of age on the vaginal microbiome

In humans, age-associated changes in the vaginal microbiome are relatively well characterised (Stumpf et al., 2013; Lebeer et al., 2023; Maneesha et al., 2025; Quin et al., 2025). Important shifts include the colonisation of microbes right after birth, transitions from juvenile to adult stages and changes associated with menopause (Stumpf et al., 2013). The changes during puberty are mostly a response to rising estrogen levels and may serve as preparation for risks associated with sexual contact (Marrazzo et al., 2010; Thoma et al., 2011). In contrast, the menopause is associated with

declining estrogen levels and corresponding shifts in microbial composition (Marrazzo et al., 2010). Interestingly, every life stage is accompanied by its own differentiation in microbial signatures and pathogenic risks (Maneesha et al., 2025).

Similar patterns are found in non-human primates such as olive baboons (*Papio anubis*) and rhesus macaques (*Macaca mulatta*), with both species showing a decreased diversity of the vaginal microbiome upon reaching adulthood (Uchihashi et al., 2015; Janiak et al., 2021). Interestingly, in macaques, no difference was found in the diversity of the vaginal microbiome between younger adults compared to their older conspecifics. This suggests that there may not be a substantial shift for non-human primates like the menopause in humans. However, studies investigating age effects on the vaginal microbiome remain largely scarce in non-human primates, particularly in great apes.

1.2.2 The effect of hormonal contraception on the vaginal microbiome

Aside from long-term changes derived from age, the menstrual cycle introduces short-term fluctuations in the vaginal microbiome. This cycle is paired with periods of high estrogen and progesterone levels, which contribute to keeping the vaginal microbiome stable (Gajer et al., 2012). Throughout the menstrual cycle, vaginal microbial communities can differ significantly in composition depending on the menstrual phase (Miller et al., 2017). As seen in rhesus macaques and humans, the phase of their cycle was a strong predictor of the overall bacterial taxa richness in the vaginal microbiome (Hallmaier-Wacker et al., 2019; Lebeer et al., 2023). This diversity may either peak outside of the fertile period or right before ovulation (Miller et al., 2017). These findings indicate that cyclic hormonal fluctuations contribute to variation in the diversity and composition of the vaginal microbiome.

Hormonal contraceptives may alter these dynamics by suppressing natural cyclic hormone levels and ovulation (Rivera et al., 1999). However, their effects on the vaginal microbiome remain understudied in primates and unexplored in *Pan*. Limited evidence from baboons suggests that the use of a levonorgestrel-releasing intrauterine system does not significantly affect microbial composition, however the sample size in this study was rather limited (n = 3) (Hashway et al., 2014). Studies in humans have found contradicting outcomes on the effects of hormonal contraception on the vaginal microbiome (Song et al., 2020; Lebeer et al., 2023; Tuddenham et al., 2023). Changes are subtle, but found for COCs leading to either less or more dominance of taxa such as *Lactobacillus* (Song et al., 2020; Lebeer et al., 2023; Tuddenham et al., 2023). No significant effects were found for POPs or progestin implants (Bastianelli et al., 2021; Lebeer et

al., 2023). In addition, next to hormonal contraceptives, the administration of other medicines like antibiotics may further influence microbial diversity (Tuddenham et al., 2023).

1.3 Influence of socio-sexual behaviour on the vaginal microbiome

Alongside these effects, socio-sexual behaviour and, in particular, levels of promiscuity, have been associated with variations in prevalence and diversity of microbes (Freeland, 1976). Microorganisms can be directly transmitted between sexual partners and, in turn, may affect microbial composition and vaginal health (Marrazzo et al., 2009). Supporting this, sexual promiscuity has been linked with greater instability and increased risk of bacterial vaginosis in humans (Schwebke et al., 1999). More recently, studies have continued to highlight the effects of sexual behaviour on the human vaginal microbiome, demonstrating its important role in forming the composition of genital communities (Plummer et al., 2019; Toh et al., 2023; Magoutas et al., 2025). Research has further shown that sexual partners and individuals with a shared sexual history tend to have greater similarity of their vaginal microbiome (Schwebke et al., 1999; Miller et al., 2017). Moreover, group-living and repeated social interactions have been found to explain more similar microbial profiles among conspecifics (Hallmaier-Wacker et al., 2019). Together, these findings show that behavioural pathways affect the diversity and composition of the vaginal microbiome both directly and indirectly. However, these transmission dynamics may depend on the frequency of social interactions as well as individual behavioural variation.

Nevertheless, it is through such transmissions that the vaginal microbiome of primates may be shaped, leading to species-specific differences across the primate clade (Yildirim et al., 2014). In turn, the vaginal microbial community may be involved in the evolution of mating behaviour as research on host-microbe interactions suggests potential microbial influences on primate evolution (Hooper et al., 2012; Stumpf et al., 2013; Henry et al., 2021).

2. Scientific research objectives

Because of their promiscuous socio-sexual behaviour, bonobos offer a valuable and unique comparative framework for studying aspects of socio-sexual behaviour and the vaginal microbiome. Their strong female social bonds and close evolutionary relationship to humans further highlight their value as a model species for investigating factors underlying socio-sexual behaviour in highly social primates.

Although socio-sexual behaviour has been widely investigated in both captivity and the wild in bonobos, previous research has failed to explore the effects of age and hormonal contraception. Furthermore, research on the vaginal microbiome of female bonobos is close to non-existent, leaving a wide gap for this study to focus on.

Therefore, this study aims to investigate the effects of age and hormonal contraception on socio-sexual behaviour, as well as on the diversity and composition of the vaginal microbiome in zoo-housed female bonobos. Additionally, it aims to characterise the overall structure of the vaginal microbiome and to explore the relationship between socio-sexual behaviour and vaginal microbial diversity to assess whether both are interconnected.

The first objective of this study is to quantify the socio-sexual behaviour of female bonobos through *ad libitum* behavioural observations. Next, to assess which factors impact socio-sexual behaviour, individual data will be collected, which includes age, contraception stability (yes or no) and contraception type (COC or POP) (see Table S1). To investigate the effects of hormonal contraception, a cross-sectional approach will be combined with a longitudinal pilot study including a subset of females that switched contraception type during the observational period. This enables the investigation of changes in socio-sexual behaviour within individuals before and after the switch, while accounting for individual differences in behavioural responses to contraception. This leads to the formulation of the following research question:

1. Does age and/or the use of hormonal contraceptives affect the **socio-sexual degree** and/or **frequency of socio-sexual behaviour** in zoo-housed female bonobos?
 - Age: Based on previous studies, there is no significant effect of age expected on the number of sexual partners. However, this study does expect to find that the frequencies of socio-sexual behaviour in females will follow a U-shaped curve across age. This way, socio-sexual activity is expected to increase and peak during early adulthood when most females reproduce, followed by a decrease as females get older.

- Hormonal contraception: No significant difference is expected in the socio-sexual degree between female bonobos receiving hormonal contraception and those who do not. However, it is expected that the frequency of socio-sexual behaviour will be significantly negatively impacted for users of hormonal contraception compared to non-users. In addition, no significant differences are expected between COC and POP users on the frequency of these behaviours.

The second objective of this study is to collect samples of the vaginal microbiome of the observed zoo-housed female bonobos and to discover whether factors such as age and hormonal contraception affect their bacterial diversity and composition. The data collected for the previous research questions will then be used to test which factors impact the vaginal microbiome.

2. Does age and/or the use of hormonal contraceptives affect the **diversity and/or composition of the vaginal microbiome**, both **within individuals** and **between individuals** of zoo-housed female bonobos?

- Age: As bonobos already get exposed to genital microbiota early in life when they participate in socio-sexual behaviour, subtle shifts in their vaginal microbiome are expected when transitioning from juveniles to adults in the form of increased dominance of certain species rather than major changes in overall diversity or composition.
- Hormonal contraception: Based on the literature, this study does not expect to find significant differences in the α -diversity and composition of the vaginal microbiome between females receiving hormonal contraception and those who do not. Lastly, it is hypothesised that there will be no difference in the diversity of microbial genera in the vaginal microbiome of individuals using a COC or POP.

The final objective of this study is to explore the relationship between socio-sexual behaviour and the vaginal microbiome. To this end, the socio-sexual behaviour of each included individual will be linked to the diversity of their vaginal microbiome to assess whether behavioural variation is associated with within-individual α -diversity. Additionally, microbiome similarity between females will be examined to determine whether the frequency of socio-sexual interactions within dyads is associated with increased similarity compared to other dyads.

3. Does **individual variation in socio-sexual behaviour** affect the **diversity of the vaginal microbial community** in female bonobos?

- Individual variation: Higher levels of socio-sexual behavioural interactions within bonobo groups are expected to contribute to the transmission of microbial communities between individuals of the same group. Therefore, it is hypothesised that individuals with more sexual partners will show a more even distribution of microbial taxa for their vaginal microbiome compared to individuals with fewer sexual partners. Furthermore, a more diverse vaginal microbiome is expected for individuals with higher rates of socio-sexual behaviour compared to individuals with lower rates.
4. Does the **frequency of socio-sexual interactions between dyads** predict **similarity of the vaginal microbiome** between individuals?
- Similarity between dyads: An increased similarity of the vaginal microbiome of females forming dyads is expected when this dyad has stronger affiliative relationships (i.e. more frequent sexual contact) compared to dyads with a weaker relationship.

Together, these objectives contribute to the investigation of a potential interplay between all components. In conclusion, this study expects to find that age and hormonal contraceptives affect both the socio-sexual behaviour and the vaginal microbiome of zoo-housed bonobos. Moreover, socio-sexual behaviour is hypothesised to influence the vaginal microbiome both directly and indirectly through age- and contraception-associated changes. This way, a multi-directional relationship can be explored.

3. Materials and methods

3.1 Study subjects and housing

This study focused on 71 female bonobos (*Pan paniscus*), ranging from 1 to 60 years old, housed across eight different European zoos.

Data were collected between February 2025 and September 2025, in the following institutions: Apenheul Primate Park (The Netherlands), Berlin Zoo (Germany), Frankfurt Zoo (Germany), Dierenpark Ouwehands (The Netherlands), Planckendael Zoo (Belgium), Twycross Zoo (England), Vallée des Singes (France) and Wuppertal Zoo (Germany). All observed individuals were housed according to the guidelines of the EAZA Ex situ Program (EEP).

In total, 50 females were of reproductive age (i.e. between 8 and 45 years old). Eight years old is the youngest age at which a female received contraception in this study, and has given birth in the captive population (Lafaut et al., 2026), while the oldest female known to give birth within captivity was 44 years old (Lafaut et al., 2026).

During this study, 40 of the above-mentioned 50 females received the same type of contraception (none, COC or POP) over the whole observational period. Of those, 13 females did not receive any type of contraception of which four were pregnant, four were lactating, and five were cycling normally (with one having received a bilateral tubal ligation, which does not affect hormone levels). Ten individuals received a POP, while 17 received a COC.

The type of hormonal contraception changed for 10 females during the observational period, of which five individuals changed from a POP to a COC and four individuals changed from no hormonal contraception to a COC. Only one female changed from no hormonal contraception to a POP.

Almost all groups included individuals of both sexes and juveniles and/or infants (individuals younger than eight years old), with the exception of one group in Wuppertal Zoo, which consisted of only two adult females. The number of potential sexual partners for a female bonobo varied from 1 to 21 individuals.

3.2 Behavioural data collection

Behavioural data collection was obtained through eight different observers who underwent rigorous training for at least 2 weeks. These observers were all tested for inter-observer reliability by scoring the same 10-minute bonobo behavioural video, which resulted in a mean $r = 0.85$, indicating high reliability of observations (Martin & Bateson, 2007). Each group was observed for

about 5 days per week between roughly 9:00 AM and 5:00 PM, for a period of 5 to 12 weeks, depending on group size.

Socio-sexual behaviour of females was scored *ad libitum* throughout the observation period and included all copulatory mounts (heterosexual mount accompanied by pelvic thrusts and intromission between two sexually mature partners) and non-copulatory mounts (subject mounts receiver without intromission of the penis in the vagina, or without thrusting of the pelvis; or any sexual contact between 2 individuals of the same sex; or sexual contact involving immature individuals). For each interaction, the individuals participating were noted down using Observer XT software on a laptop (Noldus, the Netherlands). In total, 776 hours of observations were performed, wherein socio-sexual interactions were scored.

Behavioural data were used to calculate socio-sexual metrics. First, the socio-sexual degree of an individual was calculated, defined as the total number of sexual partners observed during the study period. Secondly, the frequency of socio-sexual behaviour was quantified as the total number of socio-sexual interactions standardised by total observation time. This last metric was calculated on both the individual and dyadic level.

3.3 Microbiome data collection

3.3.1 Sample collection

Bonobos were trained prior to the observational period to allow the keepers to collect a swab of the vaginal cavity. During actual sampling, zookeepers wore gloves and used sterile swabs (eNAT swab, Copan, Italy) to collect samples from the vaginal cavities of each female, once at the beginning and once at the end of the observational period. A sterile cotton swab was inserted into the opening of the vaginal canal and rotated several times to accumulate enough biomass, following the procedure used in human women (Lebeer et al., 2023). For juveniles or anxious females where swabbing within the vaginal tract was not possible, sampling was performed by rolling and rubbing the swab externally over the sexual swelling. Afterwards, the swab was placed in the eNAT vial with DNA-



Figure 2: Collection of a vaginal swab sample of a female bonobo

preserving eNAT buffer and temporarily placed in a freezer on site at around -20°C. Within five days, the samples were transported while remaining frozen to the laboratory of Zoo Antwerp, where they were stored at -20°C until further analyses.

3.3.2 Metagenomic sequencing

Microbial genomic DNA was extracted from the samples using the DNeasy PowerSoil Pro Kit (Qiagen). Before starting extractions, the eNAT tube with the swab was vortexed for 15 seconds, in order to loosen bacteria from the swab into the eNAT buffer. Next, 400 µL of the buffer solution with suspended bacteria was transferred into the PowerBead Pro Tube, and further steps were done following the manufacturer's protocol.

Shotgun metagenomic sequencing was outsourced to Eurofins Genomics. At the time of doing the analyses for this thesis, not all samples were sequenced yet: a subset of 63 samples, mainly from the end of each observation period, were already sequenced and were thus used to do the subsequent analyses shown here. Shotgun metagenomic analysis was performed using the MGIEasy Fast FS DNA Library Prep Set V2.0 kit, following the manufacturer's guidelines, and a DNBSEQ-T7 Sequencing system to achieve ~45Gb of data per sample. Host-trimmed data were provided in fq.gz format by Eurofins Genomics. All microbial reads were assigned to a species using the Genome Taxonomy Database (GTDB) version R214, and only bacterial reads were used for further analyses. Next, the read counts, or number of DNA sequences (reads), found in the control samples were checked. Actual samples had to contain at least 10 times more reads than the negative control with the highest read count, which meant all samples needed to have at least 1 155 890 reads. This quality control led to the exclusion of 3 samples, which were all swabs from juvenile female bonobos (see Table S2).

3.3.3 Calculation of microbiome metrics

For each sample, the species-level inverse Simpson index was calculated as a measure of the α -diversity or overall diversity of a sample. This community-level bacterial diversity index accounts for both the number of taxa or species present and how evenly distributed they are. Lower values represent a lower diversity, often dominated by one or a few taxa. Higher values show a higher diversity with taxa being more evenly distributed. For this, the *add_alpha* function from the *tidytacos* package was used (Wittouck et al., 2025).

Second, the species-level Bray-Curtis similarity was calculated between all individuals and between all possible female dyads per institution to compare microbiome similarity between individuals, using the *distance* function from the *phyloseq* package (McMurdie & Holmes, 2013). The Bray-Curtis similarity measures how similar two samples are based on their composition. It

is used to measure β -diversity and ranges between 0 and 1, with higher values meaning higher similarities.

3.4 Statistical approach

All statistical analyses were performed in R v 4.5.2 (R Core Team, 2025).

3.4.1 Socio-sexual behaviour

Behavioural data were collected for a total number of 71 individuals, of whom 50 individuals were of reproductive age. To examine the effects of age and contraception on socio-sexual behaviour, analyses of the cross-sectional part were restricted to 40 individuals of reproductive age who were stable in their type of contraception throughout the observational period. Individuals who were not of reproductive age did not receive hormonal contraception and were therefore excluded to avoid confounding between age and contraceptive use.

For the longitudinal case study, nine females of reproductive age were included whose contraception type was changed during the observational period, to assess the effects of changing hormonal contraception on socio-sexual behaviour. Four of these females switched from no hormonal contraceptive to a COC, while five females switched from a POP to a COC. One female was excluded in this case study, because she was the only one who switched from no hormonal contraception to a POP, limiting statistical power.

Cross-sectional design: effect of age and contraception

To test the effects of age and contraceptive use on socio-sexual behaviour, generalised additive models (GAMs) were built, using the *mgcv* package, assuming Poisson distributions (Wood, 2000). Model diagnostics were checked using the *gam.check()* function from the *mgcv* package and the *appraise* function from the *gratia* package (Wood, 2000; Simpson, 2024). Lastly, all built models were tested for overdispersion and zero-inflation using the *testDispersion* and *testZeroInflation* functions from the *DHARMa* package before continuing (Hartig, 2016).

Model 1: the effects of age and contraception on the socio-sexual degree

The number of sexual partners or socio-sexual degree was added as the response variable, and contraception type as an explanatory factor with three levels (none, COC and POP). Group size was also added in this model to control for the possible number of sexual partners in each group. Age was included in the model as a smooth term with a dimension of $k = 5$. This decision was made because a flexible non-linear relationship between age and socio-sexual degree was expected. The total hours observed were added as a log-transformed offset to the model to

account for differences in observation times across females. Lastly, zoo identity was added as a random effect to control for the non-independence of females living in the same zoo.

To compare the effects of different types of contraception to each other, a pairwise comparison was performed of the model using the *emmeans* function from the *emmeans* package (Lenth & Piaskowski, 2017).

Model 2: the effects of age and contraception on the frequency of socio-sexual behaviour

This model was fitted with the number of socio-sexual interactions as the response variable and contraception type as a fixed effect. In addition, age, zoological institution and total hours observed were included as seen above in model 1. Because this model showed overdispersion, the family was changed from Poisson to negative binomial.

The effects of different types of contraception were then compared using the *emmeans* function.

Longitudinal case study: the effect of changing hormonal contraception

This analysis included nine females, of whom four switched from no hormonal contraception to a COC, and five switched from a POP to a COC. There was no possibility to include the switch from no contraception to a POP, as data from only one individual limited the statistical power. This dataset was used for a case study to investigate whether results from the cross-sectional study were applicable to a longitudinal study.

Model 3: The effect of changing hormonal contraception

This model was fitted with a generalised linear mixed model (GLMM) using the *lme4* package, assuming a Poisson distribution (Bates et al., 2003). Diagnostics were checked using the *TestDispersion* and *TestZeroInflation* functions from the *DHARMa* package. The model included the number of socio-sexual interactions as the response variable, 'before and after' stage, type of contraception switch and their interaction as fixed effects. To account for repeated measures per individual and variation in housing, ID and zoological institution were included as random effects. Lastly, total observed hours were added as a log-transformed offset to the model as well.

To compare whether the frequency of socio-sexual behaviour before and after a switch is different according to the type of switch, a pairwise comparison was performed of the model using the *emmeans* function from the *emmeans* package.

3.4.2 Vaginal microbiome

Alpha diversity of the vaginal microbiome: the inverse Simpson index

To investigate factors affecting the inverse Simpson index, three separate GAM models were fitted assuming a Gaussian distribution. The effects of degree and frequency of socio-sexual behaviour were separately tested for the full dataset, for the purpose of easier interpretation and possible correlation. The effects of the type of contraception were tested on the subset of adult individuals, excluding juveniles who are not eligible for contraception.

Diagnostics were checked using the same tests as mentioned for models 1 and 2. The analysis showed abnormal distribution and problematic diagnostics, for which the response variable was adjusted by using the natural logarithm of the inverse Simpson index, which solved this problem.

Model 4: the effect of the socio-sexual degree on the inverse Simpson index

This model used the inverse Simpson index as the response variable with the socio-sexual degree and type of hormonal contraception as fixed effects. Age was included as a smooth term with a dimension of $k = 5$, and zoological institutions as a random effect to control for non-independence. Lastly, the use of antibiotics was controlled for, as this is expected to have an impact on the vaginal microbiome, given prior microbiome research (Tuddenham et al., 2023).

Model 5: the effect of the frequency of socio-sexual behaviour on the inverse Simpson index

Similarly, model 5 included the same variables as model 4, only now the frequency of socio-sexual behaviour was included instead of the socio-sexual degree.

Model 6: the effect of hormonal contraception on the inverse Simpson index

This model used a subset of female bonobos of reproductive age to test the effects of the type of contraception. It was built with the inverse Simpson index as the response variable and contraception type, antibiotics and age as fixed effects. The zoological institution was included as a random variable as well to control for differences in housing.

Beta diversity of the vaginal microbiome: Bray-Curtis similarity between individuals

To examine whether age and/or hormonal contraception impact the vaginal microbial community, permutational multivariate analyses of variance (PERMANOVA) were performed using the *adonis2* function in the *vegan* package (Oksanen et al., 2001). Marginal effects were evaluated using 10.000 permutations.

Model 7: The effect of age on the composition of the vaginal microbiome

This model included the Bray-Curtis distance matrix as a response variable and age class (adult or juvenile), antibiotic use and zoological institution as categorical factors.

Model 8: The effect of contraception on the composition of the vaginal microbiome

In this analysis a subset of the full dataset was used to contain only individuals of reproductive age (ages ranging from 8 years old to 45 years old).

For this model, the Bray-Curtis distance matrix was used as a response variable and type of contraception, antibiotic use and zoological institution as categorical factors. In addition, the sampling site (vulva or vagina) was included as a categorical factor to test whether sampling location could explain variation between samples. Previous research showed differences in microbial composition dependent on sampling location (Stumpf et al., 2013).

Beta diversity of the vaginal microbiome: Bray-Curtis similarity between dyads

Model 9: the effect of socio-sexual behaviour between dyads and their Bray-Curtis similarity

Lastly, to compare Bray-Curtis similarity in female dyads, a GLMM was constructed assuming beta distribution. The beta distribution is a probability distribution which is suitable for modelling the proportion of continuous variables, such as the Bray-Curtis similarity, that are restricted to values between 0 and 1 (Ferrari & Cribari-Neto, 2004). This model included Bray-Curtis similarity as the response variable and the combination of both individuals' ages, antibiotic use and frequency of socio-sexual behaviour as fixed effects. Zoological institution and ID were included as random effects to account for repeated measures.

3.5 Ethics statement

This study conformed to the ASAB guidelines for animal behaviour research and obtained ethical approval from the scientific advisory board of the Royal Zoological Society of Antwerp to collect behavioural data and vaginal samples.

4. Results

4.1 Socio-sexual behaviour

4.1.1 Factors influencing socio-sexual degree

Model 1 showed that group size had a significant positive effect on the socio-sexual degree ($\beta = 0.06641 \pm 0.022$; $z = 3.037$; $p = 0.002$). This indicates that individuals in larger groups interact with more sexual partners and supports our inclusion of this factor into the model as a control factor. There was no significant effect of age on the degree (edf = 1.000; $\chi^2 = 0.152$; $p = 0.697$; see Figure 3).

There was no overall significant effect of hormonal contraceptive type on the socio-sexual degree (df = 2.000; $\chi^2 = 3.683$; $p = 0.159$). Pairwise comparison using estimated marginal means confirmed there was no significant difference between females with and without hormonal contraception or between individuals receiving different types of hormonal contraception (all $p > 0.200$, see Figure 4).

Lastly, the random effect of zoological institution explained a significant proportion of the variation found in socio-sexual degree (edf = 4.247; $\chi^2 = 15.915$; $p < 0.001$). The overall deviance of the socio-sexual degree explained by model 1 was 63.20%.

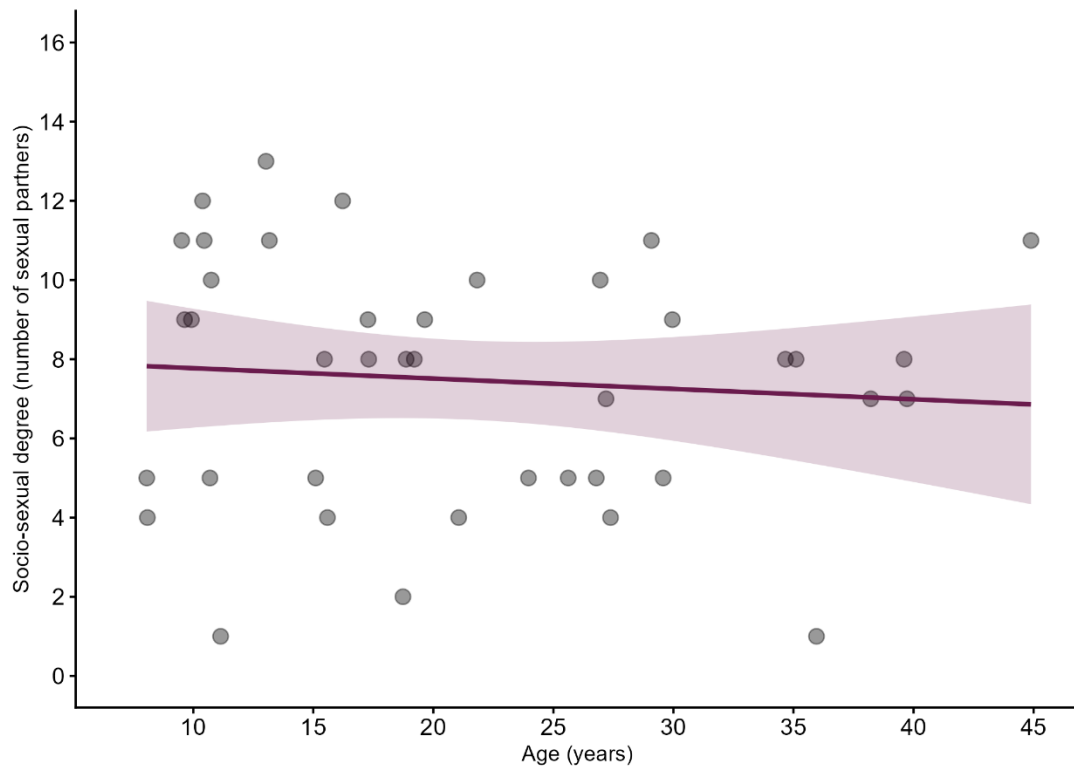


Figure 3: The effect of age on the socio-sexual degree of female bonobos. No significant effect of age was found on the number of sexual partners. The lighter purple area represents the confidence interval of 95.00%.

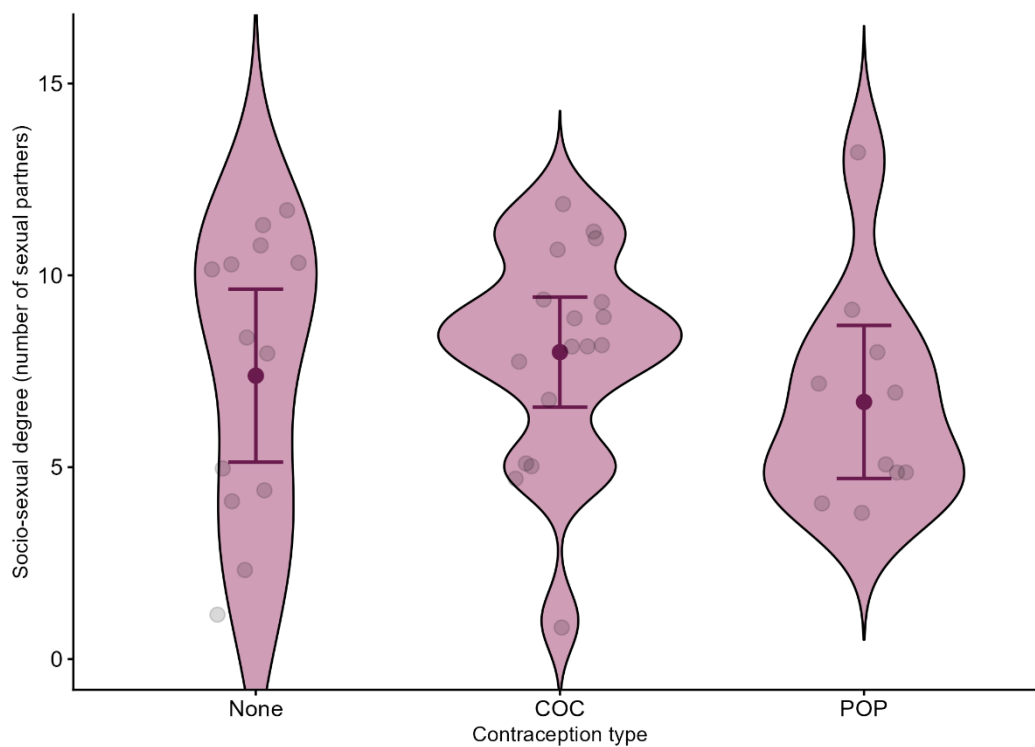


Figure 4: The effect of hormonal contraception types on the socio-sexual degree of female bonobos. No significant effect of hormonal contraception type was found on the number of sexual partners. The violin plots represent the distribution and density of individual observations within contraception type. The overlaid points represent individual samples. The dark purple points and error bars represent the predicted mean values and 95.00% confidence interval.

4.1.2 Factors influencing frequency of socio-sexual behaviour

In model 2, age did not have a significant non-linear effect on the frequency of socio-sexual behaviour in female bonobos ($\text{edf} = 1.997$; $\chi^2 = 1.919$; $p = 0.347$). In Figure 5, however, an increase is seen when an individual approaches the age of 20 years old, after which the frequency of socio-sexual behaviour slowly seems to decline.

The model revealed a significant overall effect of hormonal contraception type on the frequency of socio-sexual behaviour ($\text{df} = 2.000$; $\chi^2 = 8.104$; $p = 0.017$). A pairwise comparison using estimated marginal means revealed no significant difference between females without hormonal contraception and females using COC (estimate = 0.001 ± 0.216 ; $\text{df} = 28.5$; $p = 0.997$) whereas both untreated females and females treated with COC differed significantly from POP users (estimate = 0.880 ± 0.330 ; $\text{df} = 28.5$; $p = 0.019$ and estimate = 0.880 ± 0.325 ; $\text{df} = 28.5$; $p = 0.019$ respectively; see Figure 6). Together, these results indicate that females receiving a POP engaged significantly less frequently in socio-sexual interactions.

Again in this model, the zoological institution explains a significant proportion of the variation ($\text{edf} = 6.468$; $\chi^2 = 35.913$; $p < 0.001$). The model explains 65.80% of the deviance.

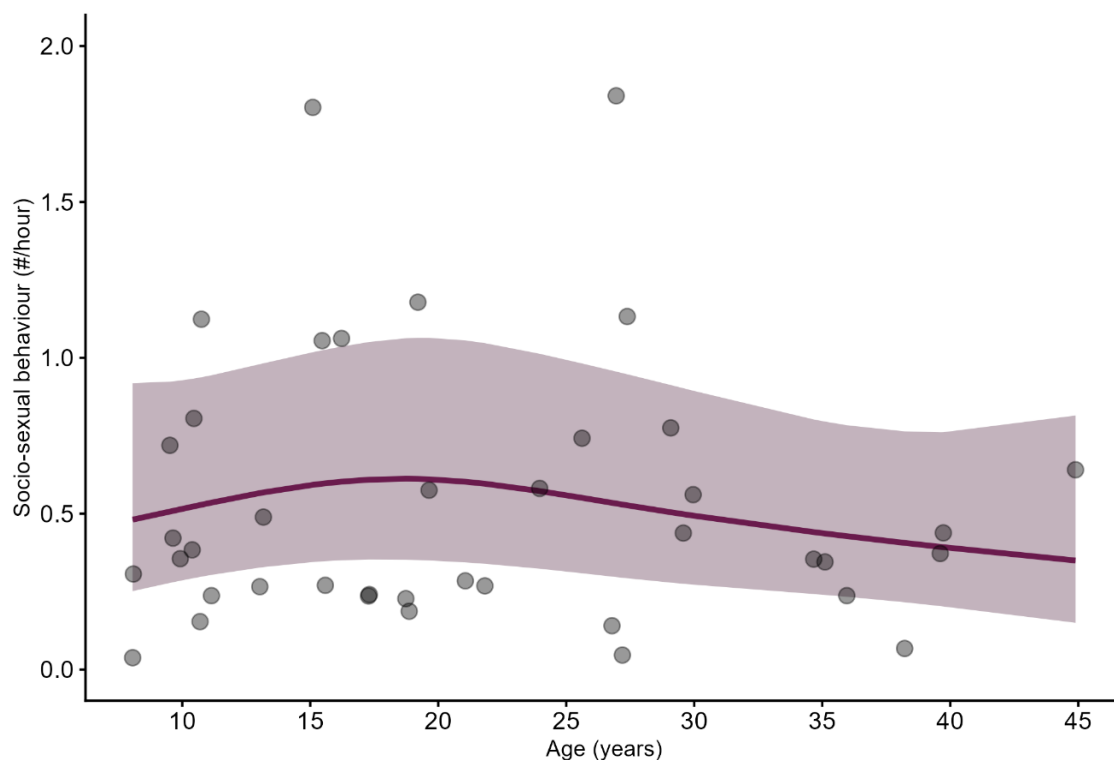


Figure 5: The effect of age on the frequency of socio-sexual behaviour per hour in female bonobos. There was no significant effect of age on the frequency of socio-sexual behaviour, although socio-sexual frequency seems to peak around 20 years old after which it declines. The lighter purple area represents the 95.00% confidence interval.

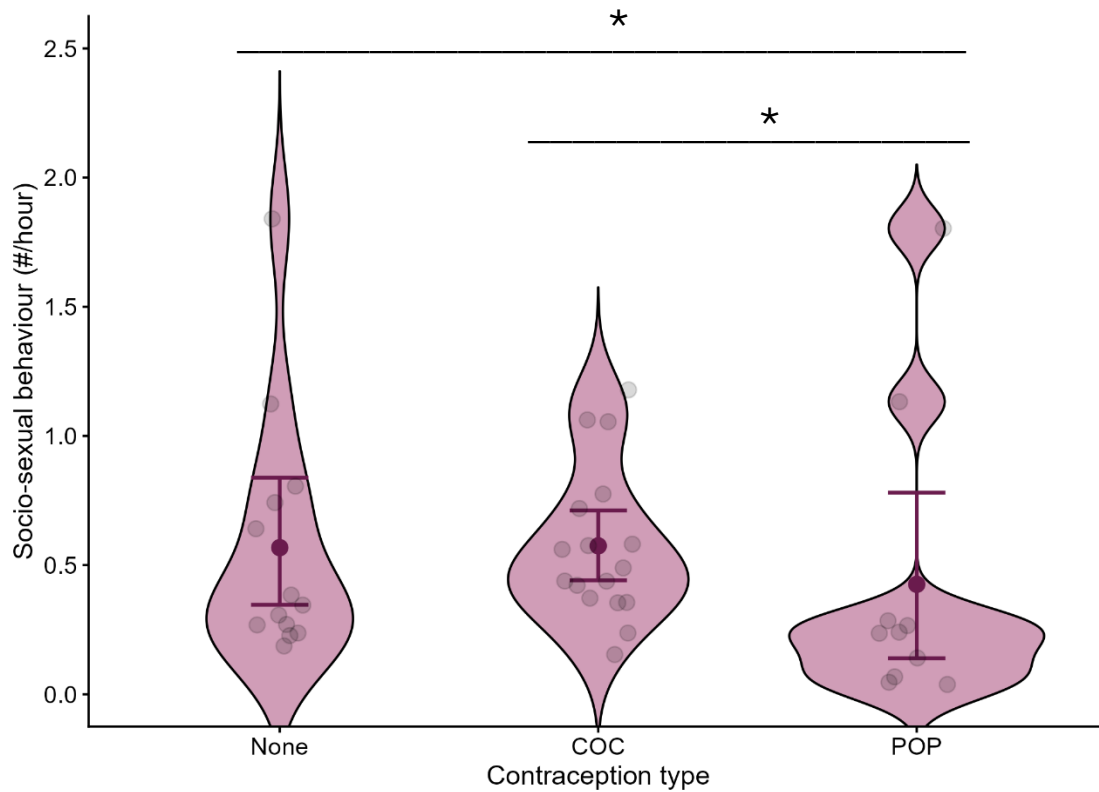


Figure 6: The effect of different types of contraception on the frequency of socio-sexual behaviour in female bonobos. Compared to females using a COC or no hormonal contraception, individuals using a POP showed a significantly lower frequency of socio-sexual behaviour (* $p < 0.050$). The violin plots represent the distribution and density of individual observations within contraception type. The overlaid points represent individual samples. The dark purple points and error bars represent the predicted mean values and 95.00% confidence interval.

4.1.3 Case study: the effect of switching contraception type on the frequency of socio-sexual behaviour

Model 3 showed a significant interaction between stage (before and after) and type of switch on the frequency of socio-sexual behaviour ($df = 1.000$; $\chi^2 = 26.181$; $p < 0.001$), meaning that changes in socio-sexual behaviour between stages depended on the type of contraception switch. Pairwise comparisons found that individuals previously receiving a POP and now a COC showed an increase in frequency of socio-sexual behaviour (estimate = 2.120 ± 0.206 ; $df = \text{inf}$; $p < 0.001$, see Figure 7). In contrast, individuals did not show an altered rate of socio-sexual behaviour when they first received no hormonal contraception compared to a COC (estimate = 0.995 ± 0.113 ; $df = \text{inf}$; $p = 1.000$).

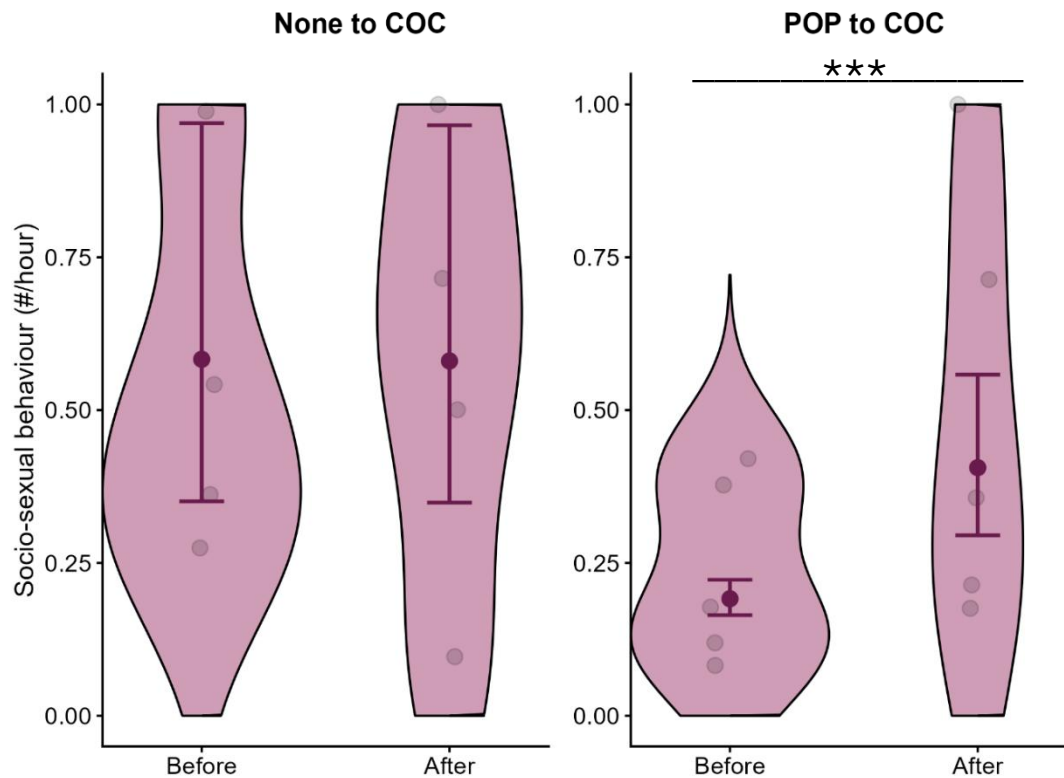


Figure 7: The effect of switching contraception type on the frequency of socio-sexual behaviour. A significant increase was found in the frequency of socio-sexual behaviour for individuals before and after switching from a POP to a COC (** $p < 0.001$). The violin plots represent the distribution and density of individual observations before and after switching contraception type. The overlaid points represent individual samples. The dark purple points and error bars represent the predicted mean values and 95.00% confidence interval.

4.2 The vaginal microbiome

4.2.1 Description of the vaginal microbiome

The most abundant bacterial species in the vaginal microbiome of both adults and juveniles were *Bifidobacterium swidsinskii* and *Bifidobacterium vaginale*, with an overall abundance of 24.45%, and 3.90% over all samples respectively. Second, *Sneathia vaginalis* accounted for a mean relative abundance of 4.21% over all samples. Even though this species was found in adults and juveniles, Figure 8 does not show its presence in juveniles because of relatively lower abundance. *Prevotella amnii* was also found for both age classes with a mean relative abundance of 2.75%, followed by *Corynebacterium sp.* (2.41%). The last common two species with a mean relative abundance higher than 2.00% were *Fannyhessea vaginae*; (2.17%) and *Burkholderia contaminans* (2.11%). Visual inspection shows high inter-individual variation in dominant species, despite the presence of several shared species across females. Overall, adults seem to show a more even distribution of species, while the juvenile microbial community seemed characterised by a larger number of low-abundance species.

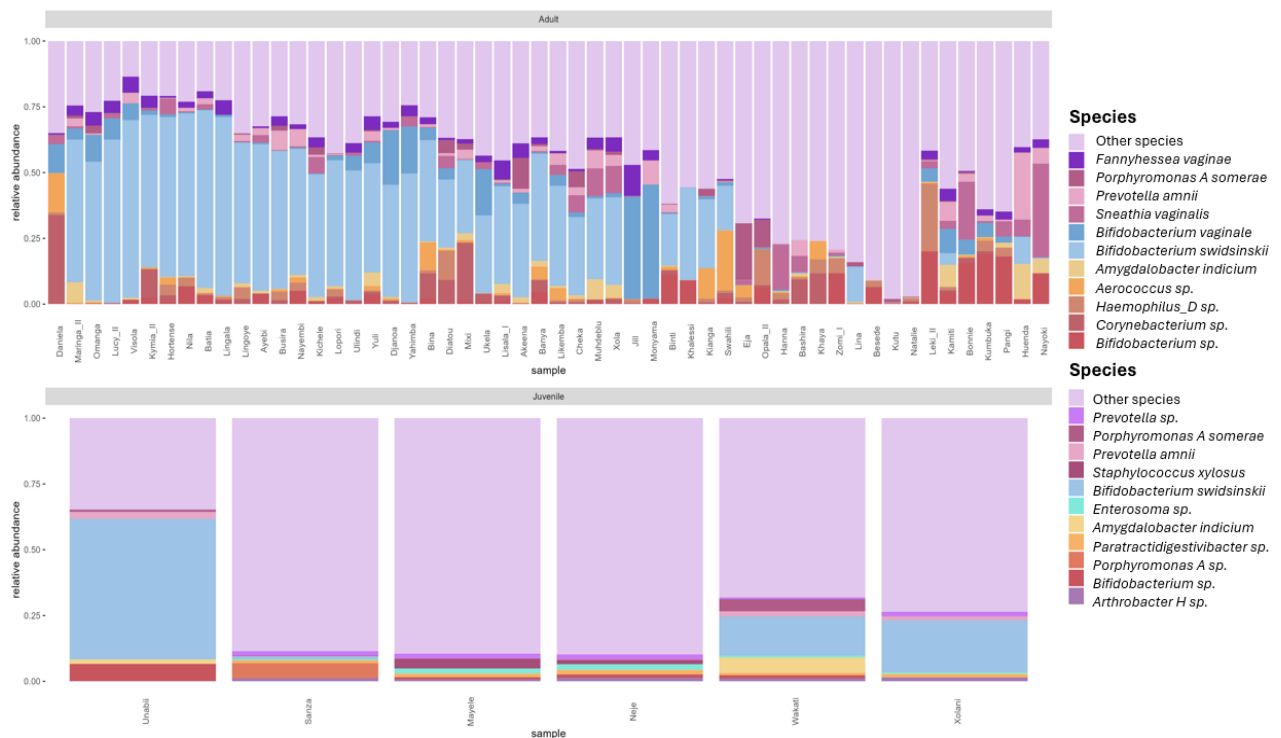


Figure 8: The relative abundance of bacterial species present in the vaginal sample of zoo-housed female bonobos. The 'other species' contain species at very low abundances. An enlarged version of this figure is provided in the appendix.

4.2.2 Factors influencing the inverse Simpson index

Effects of age

Age effects on the inverse Simpson index were investigated in models 4 and 5. In model 4, age was not significantly associated with differences in the inverse Simpson index (edf = 2.851; $F = 2.205$; $p = 0.136$), whereas a significant effect of age was detected in model 5 (edf = 3.380; $F = 3.368$; $p = 0.037$).

Effects of hormonal contraception

To examine the effects of hormonal contraceptive types on the inverse Simpson index, model 6 was constructed. Hormonal contraceptive type significantly affected the inverse Simpson index ($df = 2.000$; $\chi^2 = 4.894$; $p = 0.012$). Pairwise comparisons showed that females receiving a POP showed a significantly higher inverse Simpson index (ratio = 0.513 ± 0.117 ; $df = 44.8$; $p = 0.015$) compared to females receiving no hormonal contraception. This was also the case compared to females receiving a COC (ratio = 1.946 ± 0.483 ; $df = 44.8$; $p = 0.027$). There was no such effect between non-users and COC-users (ratio = 0.997 ± 0.208 ; $df = 44.8$; $p = 1.000$). Estimated means indicated that individuals using a POP have a more even community in the vaginal microbiome, with less dominating taxa compared to COC users (see Figure 9).

Effects of socio-sexual behaviour

The socio-sexual degree investigated in model 4 showed a significant negative impact on the inverse Simpson index ($\beta = -0.056 \pm 0.0154$; $t = -3.647$; $p < 0.001$; see Figure 10). This indicates that individuals with more sexual partners had a lower inverse Simpson index than individuals with fewer sexual partners. In model 5, the frequency of socio-sexual behaviour was significantly negatively associated with the inverse Simpson index ($\beta = -0.246 \pm 0.109$; $t = -2.489$; $p = 0.029$; see Figure 11). Thus, the vaginal microbiome of individuals interacting in socio-sexual behaviour more frequently consists of a less even and less diverse community.

Effects of other factors

In addition, the zoological institution significantly explained variation for the inverse Simpson index in models 5 and 6 (edf = 3.881; $F = 1.494$; $p = 0.0207$ and edf = 4.195; $F = 1.697$; $p = 0.017$), whereas in model 4 only a trend was found (edf = 3.185; $F = 0.878$; $p = 0.087$).

Lastly, the use of antibiotics showed a negative trend in model 4 ($\beta = -0.240 \pm 0.152$; $t = -1.573$; $p = 0.122$) and a significant negative effect in models 5 and 6 ($\beta = -0.310 \pm 0.148$; $t = -2.008$; $p = 0.050$ and $\beta = -0.297 \pm 0.125$; $t = -2.376$; $p = 0.022$, respectively). This confirmed the need to control for this variable while investigating the vaginal microbiome.

Model 4 explained 48.9% of the deviance, model 5 explained 48.50%, and this was 41.70% for model 6.

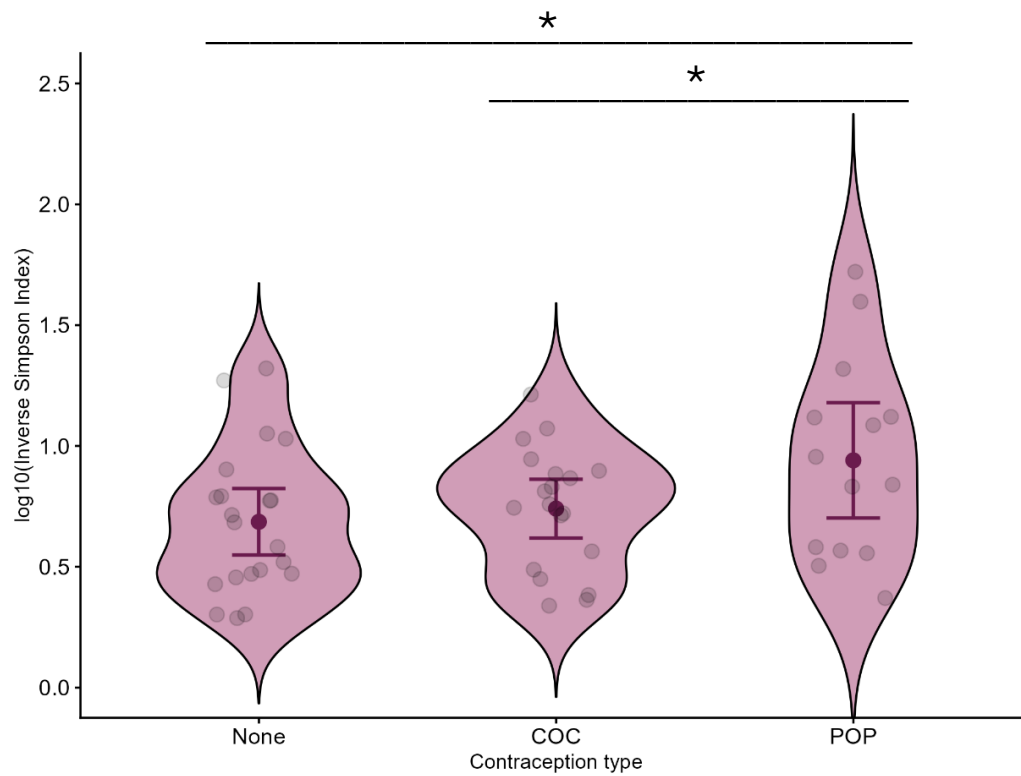


Figure 9: The effect of different types of contraception on the log10-transformed inverse Simpson index in female bonobos. Individuals using a POP showed a significantly higher inverse Simpson index in model 5 (* $p < 0.050$). The violin plots represent the distribution and density of individual observations within each contraceptive group. The overlaid points represent individual samples. The dark purple points and error bars represent the predicted mean values and 95.00% confidence interval.

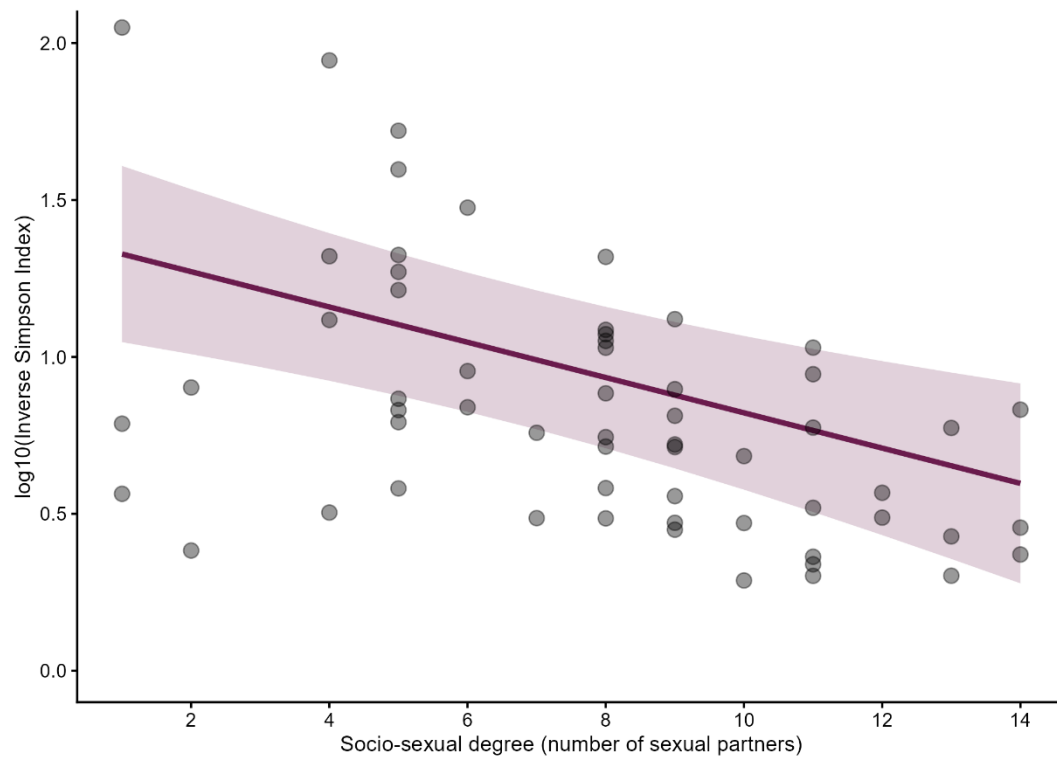


Figure 10: The effect of socio-sexual degree on the log10-transformed inverse Simpson index. Individuals with more sexual partners show a significantly lower Inverse Simpson Index. The lighter purple area represents the 95.00% confidence interval.

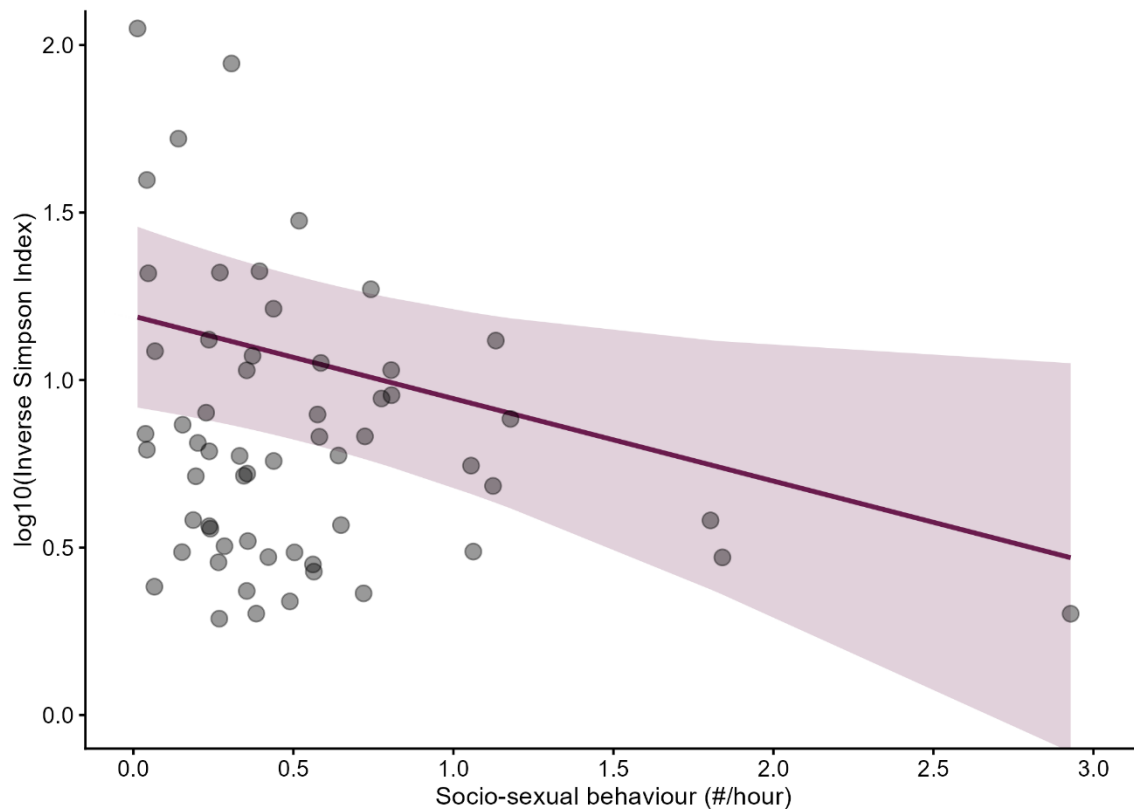


Figure 11: The effect of socio-sexual behaviour on the $\log_{10}$ -transformed inverse Simpson index in female bonobos. Individuals with higher frequencies of socio-sexual behaviour showed a significantly lower inverse Simpson Index. The lighter purple area represents the 95.00% confidence interval.

4.2.3 Factors influencing Bray-Curtis similarity

Between individuals PERMANOVA

In model 7, the effect of age significantly explained 3.55% of the variation in samples ($R^2 = 0.0355$; $F = 2.542$; $p = 0.004$). This suggests a difference between adult and juvenile vaginal microbiome communities. The largest predictor for variation, however, came from being housed in different zoological institutions ($R^2 = 0.247$; $F = 2.524$; $p < 0.001$). Almost 25% of the variation between samples could be attributed to their institutional environment (see Figure 12). Interestingly, differences could not be linked to antibiotic use ($R^2 = 0.008$; $F = 0.575$; $p = 0.945$). It is important to note that only five out of the 60 samples were taken from females on antibiotics, which could have limited statistical power.

Model 8 was fitted with the subset of females of reproductive age to test whether the type of hormonal contraception had a significant influence on the vaginal microbial composition. Analysis showed no such effect ($R^2 = 0.029$; $F = 0.905$; $p = 0.597$). Similarly, in this model, the use of antibiotics was controlled for leading to the same result as mentioned above ($R^2 = 0.011$; $F = 0.658$; $p = 0.854$). In addition, this model tested whether sampling location (vulva or vagina) had an impact on the found microbial community, as previous research concluded this was the case

(Stumpf et al., 2013). This model did not confirm these findings ($R^2 = 0.014$; $F = 0.842$; $p = 0.629$). Lastly, the zoological institution again explained 25% of the variation ($R^2 = 0.252$; $F = 2.255$; $p < 0.001$).

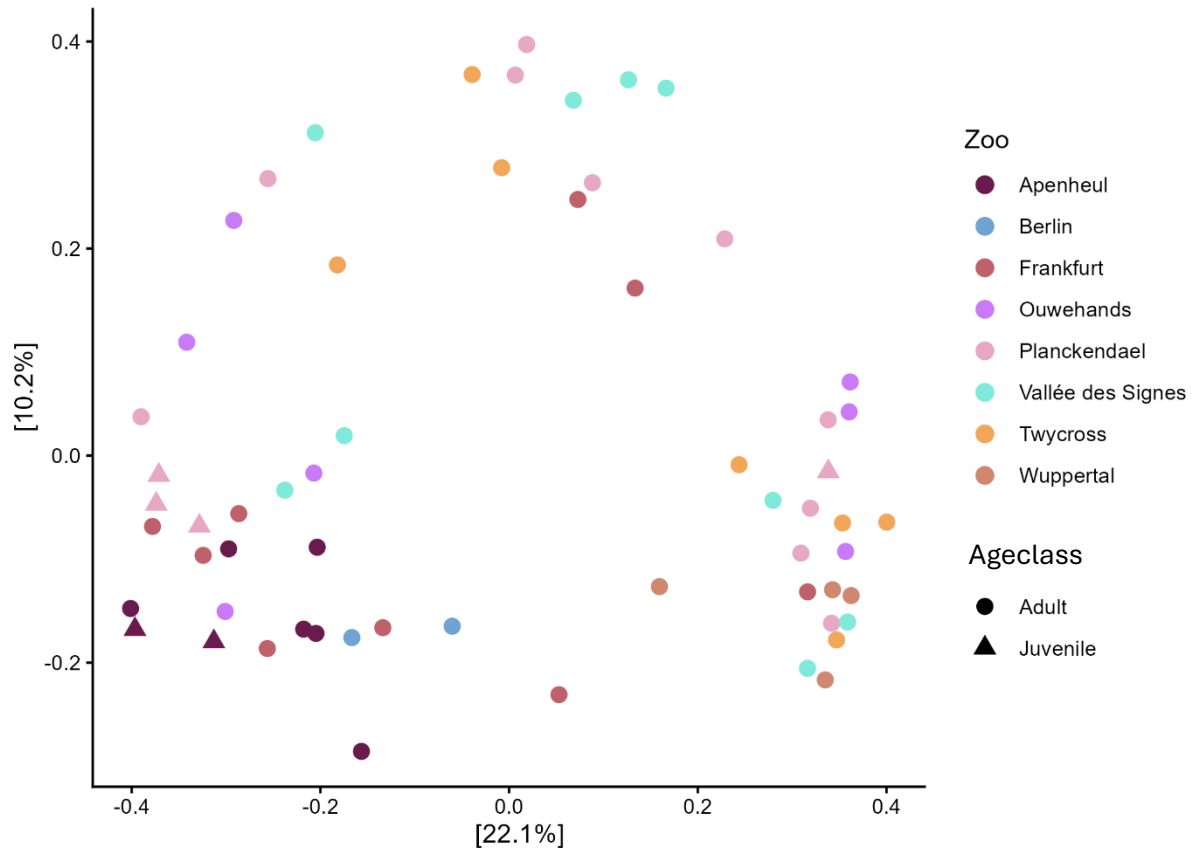


Figure 13: Principal coordinates analysis based on Bray-Curtis distances for zoo-housed female bonobos. Every colour represents a zoological institution. In addition, each datapoint is shaped according to age class (adult or juvenile).

GLMM: beta distributed

Analysis of model 9 showed a significantly lower similarity of samples between individuals when at least one bonobo of the pair was a juvenile ($\beta = -0.662 \pm 0.279$; $z = -2.372$; $p = 0.018$; see Figure 14). The composition of the vaginal microbiome thus differs between bonobos of reproductive age and those who are younger.

The same effect was found when at least one of both received a POP compared to when both received either no contraception or a COC ($\beta = -0.363 \pm 0.163$; $z = -2.227$; $p = 0.026$; see Figure 15). There was no difference in similarity between two bonobos when one of the individuals was receiving antibiotics compared to their similarity when both were not using antibiotics ($\beta = 0.074 \pm 0.204$; $z = 0.363$; $p = 0.717$).

Lastly, the frequency of socio-sexual behaviour of a dyad did not affect the similarity of their vaginal microbiome ($\beta = 0.539 \pm 0.773$; $z = 0.698$; $p = 0.485$).

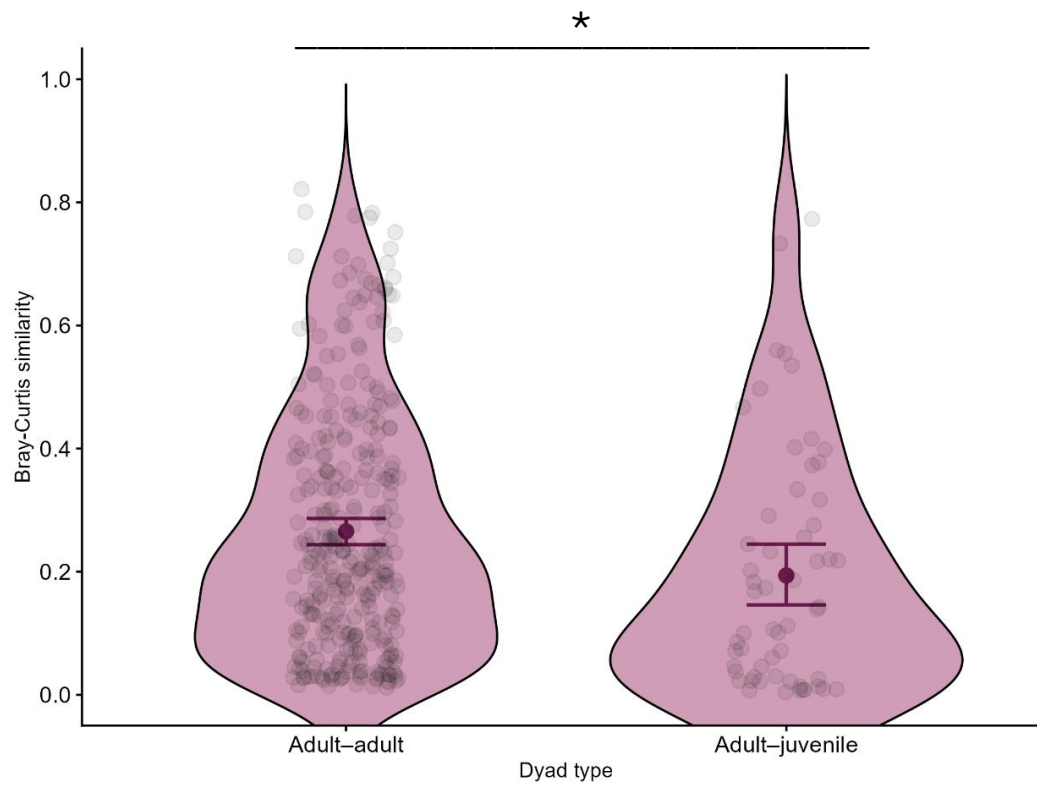


Figure 14: The Bray-Curtis similarity in adult-adult versus juvenile-containing dyads. Dyads between adults had a significantly higher similarity in the composition of their vaginal microbiome than dyads that include at least one juvenile (* $p < 0.050$). The violin plots represent the distribution and density of individual observations within each dyad type. The overlaid points represent individual samples. The dark purple points and error bars represent the predicted mean values and 95.00% confidence interval.

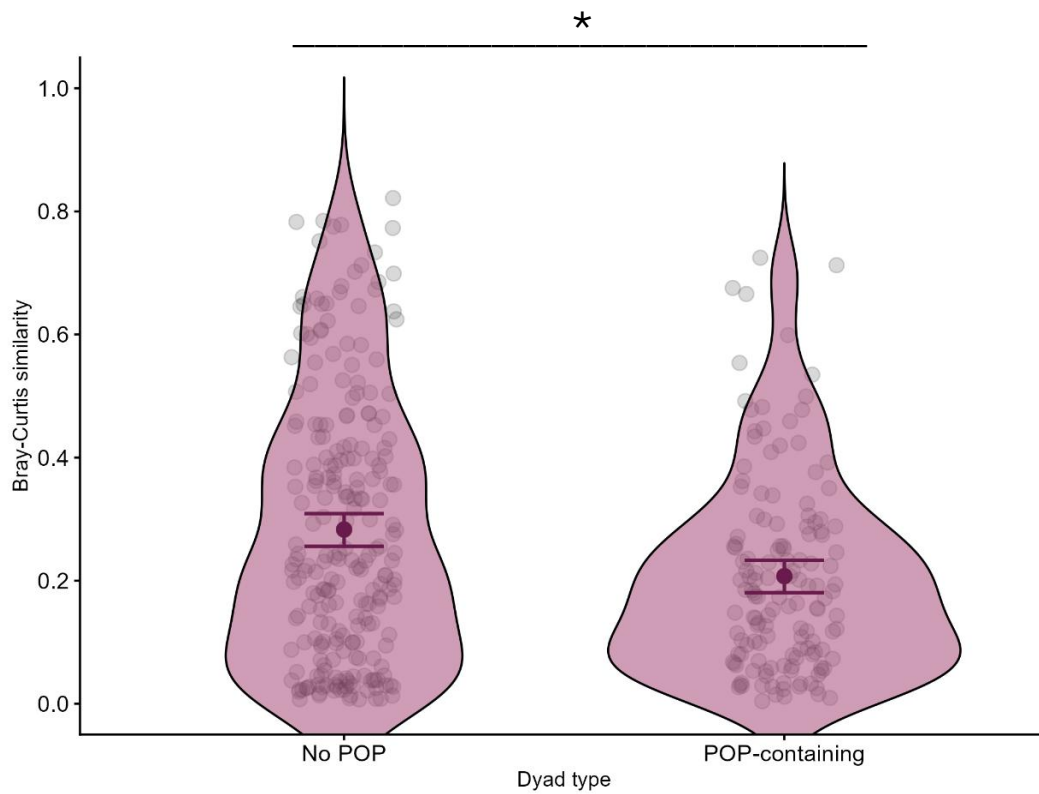


Figure 15: The effect of at least one individual in the dyad receiving a POP on the similarity of the composition of their vaginal microbiome. In dyads where at least one bonobo receives a POP, the similarity of their vaginal microbiome reduces significantly compared to dyads where both are not using a POP ($* p < 0.050$). The violin plots represent the distribution and density of individual observations within each contraceptive group. The overlaid points represent individual samples. The dark purple points and error bars represent the predicted mean values and 95.00% confidence interval.

5. Discussion

The objectives of this study were to examine the effects of age and hormonal contraception on socio-sexual behaviour and the vaginal microbiome of zoo-housed female bonobos. Moreover, it aimed to discover whether socio-sexual behaviour and the vaginal microbiome were interlinked in this species. To this end, behavioural, individual and microbial data were collected for 71 females housed across eight European zoological institutions.

For the analysis of behaviour, the number of sexual partners was calculated for each individual, and a weighted matrix was constructed of all sexual interactions. Next, microbial samples were collected from these females to describe the vaginal microbiome. Effects of age, hormonal contraception and other variables on socio-sexual behaviour and the vaginal microbiome were then statistically tested. Lastly, the effect of behavioural data were tested against the vaginal samples to discover potential relationships with the vaginal microbiome.

5.1 Factors influencing socio-sexual behaviour

The first aim of this study was to investigate the effects of age and contraception on socio-sexual behaviour. This included an analysis of both the socio-sexual degree and the frequency of interactions for zoo-housed female bonobos.

The effect of age

A first finding is that for female bonobos, aged between 8 and 45 years old, there is **no significant difference in the number of partners** they interact with in socio-sexual behaviour, supporting the hypothesis that age would not affect the socio-sexual degree. Surprisingly, there was also **no significant non-linear effect of age on the socio-sexual frequency**. In contrast, Staes et al. (2017) did find significant effect of age on socio-sexual behaviour. However, a trend seems to suggest a peak of socio-sexual behaviour right before 20 years old, partially supporting the hypothesis of a peak in socio-sexual frequency during early adulthood (Figure 5). This is remarkable, as previous studies have found the mean first birth rate to be around 10 years old in captivity, potentially confirming the social functions of socio-sexual behaviour rather than exclusive use for reproduction (Clay & De Waal, 2015; Clarke et al., 2022; Lafaut et al., 2026).

The increasing frequencies of socio-sexual behaviour when growing older might therefore be important for their social integration and as a way to form close ties with other females in their group. Furthermore, the decrease in socio-sexual behaviour after 20 years old may reflect maintenance of their now stronger position in the social network. Female bonobos are known to form coalitions with each other through affiliative behaviours, including grooming and socio-

sexual behaviour, which enables them to dominate the physically stronger males (Vervaecke et al., 2000). Supporting these studies' findings and hypothesis, grooming interactions in bonobos also peak around middle age, coinciding with the age at which their dominance rank is highest (Torfs et al., 2023). This indeed indicates that affiliative behaviours play a key role in establishing and maintaining female social status.

The effect of hormonal contraception

In line with the hypothesis, the use of **hormonal contraception did not significantly impact the socio-sexual degree**. Interestingly, individuals using a POP did show a trend towards fewer sexual partners compared to individuals using a COC.

The cross-sectional dataset confirmed the hypothesis that **hormonal contraception has a significant effect on the frequency of socio-sexual behaviour**. However, the frequency of socio-sexual behaviour was only altered by the usage of a POP. Individuals receiving a POP participated in 0.88 less socio-sexual interactions per hour than individuals using a COC or no hormonal contraception at all. Interestingly, the use of a COC had no such effect. This causal link was consistent with results of the longitudinal pilot case study: switching from a POP to a COC was followed by an increase in socio-sexual behaviour, whereas the switch from no hormonal contraceptives to a COC did not have any effect.

As female sexual motivation in bonobos is strongly linked to the balance between estrogens and progesterone, an imbalance may lead to higher progesterone and lower estrogens, which can disrupt sexual behaviour (Roney & Simmons, 2013; Kondracki et al., 2025). Furthermore, progestins not only bind to progesterone receptors, but also to other steroid hormone receptors such as those of androgens, estrogens and glucocorticoids. Using this pathway, they might suppress testosterone activity depending on the androgenic properties of the progestin (Bullock et al., 1978; Sitruk-Ware, 2006). This could explain lower frequencies of socio-sexual behaviour due to progestin-dominated contraception, especially between females, as a previous study found a positive relationship between urinary testosterone metabolite levels and genito-genital rubbing (Sannen et al., 2005).

Alternatively, hormonal contraception may alter a female bonobo's attractiveness and thus socio-sexual behaviour, as it may influence perineal skin swellings. Like a lot of other non-human primates, female bonobos show sexual swellings, previously thought to signal males when they are ovulating (Douglas et al., 2016). However, the understanding that the tumescence of this swelling always peaks during ovulation has been decoupled (Douglas et al., 2016). Research has shown that these swellings in bonobos are only loosely correlated with ovulation, when estrogens

are highest (Douglas et al., 2016). Unfortunately, most evidence linking hormone levels to swelling dynamics remains limited and inconsistent. Nevertheless, in chimpanzees, progestin-based contraception altered swelling patterns, but this effect disappeared after several months of using the POP (Bettinger et al., 1997). This might imply that this is not the reason for reduced frequencies of socio-sexual behaviour in the long-term. Moreover, olive baboons engage in sexual behaviour when they are non-fertile and sexual swelling is flattest (Vaglio et al., 2021). This further suggests that sexual attraction or the presence of maximal tumescent sexual swellings is not a reason for the findings of this study.

5.2 Overview of the vaginal microbiome composition

The second aim was to characterize the overall structure of the vaginal microbiome and to investigate how age and hormonal contraception may influence the vaginal microbiome of zoo-housed female bonobos. To this end, the inverse Simpson index as a measure for the α -diversity was calculated, while microbial composition and the Bray-Curtis similarity were analysed to assess the β -diversity.

The vaginal microbiome of bonobos seemed to differ remarkably from the vaginal microbiome typically observed in humans. In humans, vaginal bacterial communities of females are generally dominated by *Lactobacillus* species, which lower vaginal pH and supposedly protect against pathogenic colonisation (Boskey et al., 2001; Lebeer et al., 2023; Stumpf et al., 2013). In contrast, abundances of *Lactobacillus* in non-human primates are rather limited (Stumpf et al., 2013; Yildirim et al., 2014).

Comparative studies across primates have shown that the vaginal microbiome of non-human primates, including chimpanzees, vervets and baboons, is generally more diverse and contains anaerobic bacterial taxa commonly associated with bacterial vaginosis in humans, such as *Gardnerella*, *Prevotella* and *Sneathia* (Stumpf et al., 2013; Yildirim et al., 2014). Similarly, the female bonobos in this study showed prevalence of *Bifidobacterium*, *Sneathia* and *Prevotella*. These findings are supported by the only previous study covering the vaginal microbiome of bonobos to date (Samartino et al., 2024).

The most abundant genus was *Bifidobacterium*, mostly represented by *Bifidobacterium swidsinskii* and *Bifidobacterium vaginale*. Typically, *Bifidobacterium* is primarily known as a gastrointestinal taxon, but strains have been isolated from the mammalian vagina, including humans (Albert et al., 2015; Schell et al., 2002). Certain *Bifidobacterium* species have been associated with immune regulation, reduction of inflammation and may contribute to maintaining mucosal homeostasis (Gavzy et al., 2023).

In addition, several bacterial taxa commonly associated with bacterial vaginosis in humans were found in both adult and juvenile bonobos, including *Sneathia*, *Prevotella* and *Fannyhessea*. In humans, *S. vaginalis* has been linked to inflammatory conditions and infections, whereas *Prevotella* and *Fannyhessea* are associated with bacterial vaginosis (Albert et al., 2015; Bridy et al., 2025). Importantly, the presence of these taxa does not necessarily indicate pathology or dysbiosis in bonobos. Even healthy asymptomatic women exhibit substantial variation in microbial density and composition (Albert et al., 2015). Furthermore, these taxa might not retain the same functions or ecological roles as seen in humans.

Interestingly, studies have found that the primate vaginal microbiome composition strongly correlates with socio-ecological and sexual characteristics such as promiscuity and social structure (Yildirim et al., 2014). As bonobos are known for their frequent genito-genital rubbing between females, this may cause distinct selective pressures on the vaginal ecosystem compared to humans or other less socio-sexually active primates.

5.3 Factors influencing the vaginal microbiome

The effect of age

The analysis of **the α -diversity** or evenness of the vaginal microbiome **was inconsistent in finding differences between young individuals and individuals of reproductive age**. Although both models (Model 4 and 5) explained approximately the same deviance, age-related variance may have been captured differently. Thus, these results should be interpreted more cautiously. Therefore, the hypothesis that age would influence the evenness of the vaginal microbiome was only partially supported.

However, the brief examination of the overall vaginal microbial community suggests that there indeed seems to be a difference between the evenness of taxa found in adults compared to juveniles. Adult microbial communities of female bonobos seem to consist of fewer, more abundant species than juveniles, for whom a larger portion of their community consists of species in smaller abundances. Similarly, in adult baboons, microbial diversity was found to be significantly lower than in peripubertal baboons due to decreased relative abundance of minor taxa (Uchihashi et al., 2015). However, this pattern was not found for rhesus macaques, suggesting that age-related vaginal microbial shifts may differ between primate species (Hallmaier-Wacker et al., 2019).

Variation in vaginal microbial communities measured as **the β -diversity was significantly predicted by age**, in contrast to what was expected. This is in line with findings of a large-scale citizen science project in adult women, which found that a small but significant portion of

variation between samples was explained by age (Lebeer et al., 2023). Importantly, this study did not include juvenile or prepubertal individuals, making direct comparisons more difficult. In contrast, findings in rhesus macaques show that age was not a predictor of variation of vaginal microbial composition (Hallmaier-Wacker et al., 2019; Janiak et al., 2021). In addition, this study found a greater dissimilarity between samples when a pair of samples included an adult and a juvenile, further confirming this trend found with age.

There are several potential causes as to why juveniles and adults may differ in vaginal microbial composition. Developmental shifts from juvenility through puberty to adulthood are accompanied by endocrine changes that alter the vaginal environment. In adult women, this means an increased production of estrogens, thickening of the vaginal epithelium, shifts in vaginal pH and glycogen accumulation (France et al., 2022). All of these could contribute to determining which bacterial taxa are able to colonise and dominate the vaginal microbiome (Maneesha et al., 2025). Although bonobos do not appear to contain the same vaginal microbial communities as humans, transitioning to reproductive age may still affect the vaginal environment, favouring specific taxa.

In addition, age-related maturation of the immune system may further shape the vaginal microbiome (Maneesha et al., 2025; Santoro et al., 2020). The immune system continuously interacts with microbial communities and may select or suppress certain taxa through inflammatory and mucosal responses (Santoro et al., 2020). In turn, microbial taxa may affect local immune activity, suggesting a bidirectional interaction between the immune system and the vaginal microbiome (Santoro et al., 2020). Consequently, maturation of the immune system throughout development could promote the establishment of a more stable microbial community in adult females (Santoro et al., 2020).

Lastly, behavioural changes may also be a part of age-related changes. Increased affiliative interactions and stronger social integration in adults may shape exposure to microbes. As mentioned above, comparative primate studies suggest that the vaginal microbiome is associated with socio(-sexual) factors (Yildirim et al., 2014). Thus, juvenile-adult differences may not only reflect physiological changes but may also be influenced by social and sexual interactions.

It is important to note, however, that the sample size of juveniles in this study was rather limited ($n = 6$). Moreover, these results may have been further shaped by which zoological institutions they were part of. Four out of six juveniles included in this analysis were housed in Planckendael Zoo, and the other two individuals were both housed in Apenheul. It is well-documented that living

environments alter the primate microbiome, in both captive and wild environments (Clayton et al., 2016; Grieneisen et al., 2021). As zoological institutions predicted almost 25.00% of the variation between samples, further investigation, including more variation in zoos and a larger sample size, could help disentangle these findings and to draw a more robust conclusion. Keeping this in mind, these findings still remain plausible as age-related changes in hormones, vaginal structure, immune function, and alterations in socio-sexual behaviour could all contribute to distinct juvenile and adult vaginal microbial profiles.

The effect of hormonal contraception

Contrary to what was expected, **certain hormonal contraceptives had a positive effect on the α -diversity**. Females receiving a POP showed a significantly more even distribution of taxa within the vaginal microbial community compared to other types of contraception (none or COC), indicating that their community is not as dominated by a few individual taxa. Interestingly, this pattern was not observed for females receiving a COC compared to untreated females.

In support of the hypothesis, **hormonal contraception had no effect on the composition of the vaginal microbiome**. This is in line with research in other primates such as baboons and humans (Hashway et al., 2014; Donders et al., 2017; Lebeer et al., 2023). Instead, the largest proportion of variation was explained by differences between zoological institutions. However, there was a small but significant difference between dyads in which no female of the pair received a POP compared to dyads in which at least one female received a POP. Together with the findings for the β -diversity, this suggests subtle changes towards resemblance rather than large compositional shifts. Hormonal contraception may therefore alter the balance between already present taxa rather than contributing to completely different microbial communities.

Circulating estrogen levels throughout the menstrual cycle have been known to indirectly increase glycogen accumulation in the vaginal epithelium, making more glycogen available for the bacteria residing there (Song et al., 2020). This leads to an increased dominance of lactic-acid-producing taxa such as *Lactobacillus* in humans, which use glycogen-derived products as an energy resource (Mirmonsef et al., 2016; Song et al., 2020). A possibility that has not yet been studied in primates altogether, is that POPs may disrupt these glycogen availability processes in the vaginal mucosa and, in turn, reduce the dominance advantage of lactic acid-producing bacteria, giving other taxa the opportunity to compete, leading to a more even microbial distribution. Supporting this hypothesis, progestins have been associated with hypo-estrogenic effects and reduced glycogen levels in previous research (Mirmonsef et al., 2016; Wessels et al., 2018). Moreover, sex hormones are tightly linked to local immune regulation of the female genital

tract, and progestin-based contraceptives have previously been associated with increased susceptibility to sexually transmitted infections (Wessels et al., 2018). This way, POPs may alter the vaginal environment through immune and inflammatory pathways, leading to alterations of the structure and stability of the vaginal microbiome. In contrast, this pattern has not been observed in COCs (Wessels et al., 2018).

For bonobos, the consequences of altered glycogen availability or alterations of local immune modulation may differ from those observed in humans, as their vaginal profiles are not dominated by *Lactobacillus*. Evidence for POP-associated changes in vaginal microbial diversity remains scarce, as this topic is still highly understudied and shows conflicting results in humans. For example, Lebeer et al. (2023) found no effect of POPs, but did find a significant influence of COCs on the α -diversity of the vaginal microbiome, which was not supported by the findings of this study. In contrast, Noël-Romas et al. (2020) found that the use of a progestin-based contraceptive was associated with a lower α -diversity of the vaginal microbiome compared to women using COCs and women not using any hormonal contraceptives. Although the direction of the diversity shift differed from the results in this study, both studies suggest that progestin-based contraceptives may affect the α -diversity without altering overall microbial composition.

Importantly, all females receiving a POP in this study, except one, used the same variant of POP. Nevertheless, exogenous hormones in contraceptives may have different effects on vaginal microbiota depending on the release method and presence or absence of a hormone-free period (Song et al., 2020). Moreover, differences in contraceptive formulation may further influence results, particularly as POPs can consist of different formulation types with potentially distinct effects (Song et al., 2020). Furthermore, if socio-sexual behaviour and the vaginal microbiome are closely interlinked, the use of a POP might indirectly affect the vaginal microbiome through less frequent socio-sexual interactions.

5.4 Influence of socio-sexual behaviour on the vaginal microbiome

The last aim of this study was to investigate a potential relation between the socio-sexual behaviour of zoo-housed female bonobos and their vaginal microbiome. Therefore, behavioural and microbial data were examined together to explore whether both are interlinked.

As expected, the **socio-sexual degree affected the α -diversity of the vaginal microbiome**. However, contrary to the hypothesis, Individuals with more sexual partners had a lower inverse Simpson index compared to individuals with fewer sexual partners. This suggests that the vaginal microbiome of more socio-sexually active females was less evenly distributed, meaning that some taxa were more abundant and dominant compared to other present bacterial taxa. Indeed,

unexpectedly, **females with higher socio-sexual frequencies showed a significant negative impact on the α -diversity** as well. There was no examination of which taxa in particular were more dominant in females with more socio-sexual behaviour. Thus, it can only be speculated whether those are species involved in protection against potential sexually transmitted infections or species associated with an imbalance of the vaginal microbiome. While socio-sexual behaviour affected the diversity, the **frequency of socio-sexual interactions in a dyad did not affect the similarity of the vaginal microbiome composition** of females in that dyad, which was also not expected. Interestingly, genera such as *Prevotella* and *Sneathia*, commonly associated with sexual activity in humans, particularly female-female sexual interactions and higher numbers of sexual partners, were indeed abundant within the vaginal microbiome of bonobos (Ling et al., 2010; Bradshaw et al., 2014).

Similarly, socio-sexual behaviour has been shown to significantly alter the α -diversity of the vaginal microbiome in humans. In contrast to the findings of this study for bonobos, women with more sexual partners or recent sexual interactions showed a higher α -diversity, reflecting a more even distribution of bacterial taxa (Lebeer et al., 2023). Importantly, sexual behaviour studies in humans, such as the one mentioned above, assess sexual activity through questionnaires, whereas socio-sexual behaviour in bonobos was directly observed over prolonged periods. Thus, this contradicting pattern may be partially due to differences in behavioural quantification and underlying ecological and/or physiological drivers that might shape vaginal microbiomes. Particularly, these factors could include species-specific differences, hormonal patterns or immune responses.

From another perspective, the findings of this study might be interpreted through metacommunity theory, which describes how dispersal links microbial communities across space and time. A common prediction, in this context, is that the α -diversity is highest at intermediate levels of dispersal through social transmission (Cadotte, 2006; Venail et al., 2008). High rates of dispersal may overwhelm local processes, leading to community homogenization (Mouquet & Loreau, 2002). This interpretation may explain why studies seemingly report conflicting positive, negative or no relationships between the social connectedness of an individual and the α -diversity (Debray et al., 2024). Furthermore, it may indicate a non-linear relationship between the diversity and rates of social interactions, which accommodate dispersal of microbiota (Debray et al., 2024).

According to this theory, individuals that interact more frequently are expected to develop more homogeneous microbial community compositions due to increased dispersal between hosts (Debray et al., 2024). Indeed, chimpanzees showed a more homogenous gut microbiome in

periods in which they spent more time with each other (Moeller et al., 2016). In this study, the Bray-Curtis similarity between female dyads did not significantly increase with more frequent socio-sexual interactions. A possible explanation is that high dispersal rates associated with higher frequencies of socio-sexual interactions may have promoted the transmission of dominant taxa, which could outcompete less abundant taxa. This way leading to a lowered evenness of the vaginal microbial community.

Alternatively, it might also be that socio-sexual behaviour does not necessarily promote transmission of bacterial species. Interestingly, it was indeed not bacterial species, but bacterial species strains that became more similar in the human gut and oral microbiome (Valles-Colomer et al., 2023). The absence of a more similar microbiome composition in frequently interacting female dyads may thus not exclude social transmission altogether, but this might occur at a different level than this study was able to investigate. Supporting this, social partners in baboons not only shared more bacterial species than non-partners, but also more genetically similar strains (Debray et al., 2024). Aside from direct host-to-host transmission, vaginal microbiota may also be exchanged between sexual partners when they are more likely to use the same substrate than non-partners.

Together, these findings suggest that socio-sexual behaviour may indeed impact the vaginal microbiome directly. However, socio-sexual behaviour may contribute to the vaginal environment indirectly as well. Repeated socio-sexual interactions are known to change vaginal pH, cause inflammation and potentially small tears in the vaginal wall, which could further influence the vaginal microbiome by impacting the colonisation of certain taxa that may be more adapted to these conditions (Lin et al., 2021).

A last possible indirect pathway may involve stress-related hormonal responses. Longterm exposure to hormones released during stress responses has been associated with an increased susceptibility to several infections, including those of the genital tracts (Amabebe & Anumba, 2018). For example, elevated cortisol concentrations may inhibit vaginal glycogen deposition or promote inflammation, both of which are known to increase the risk of vaginal infections (Amabebe & Anumba, 2018). For bonobos, socio-sexual behaviour plays a key role in the regulation of social tension associated with stress and may therefore reduce prolonged exposure to these hormones (Hohmann & Fruth, 2000; Brooker et al., 2025). Hence, socio-sexual behaviour might contribute to the regulation of vaginal health, which could reflect the concept of social immunity seen in highly social animals (Debray et al., 2024).

5.5 Future research perspectives

Although this study contributes to the understanding of age-related and hormonal contraceptive effects on behavioural and microbial levels, several questions remain unresolved and warrant future investigation.

First, future research should investigate whether hormonal contraception influences a broader range of behaviours beyond socio-sexual interactions. Particularly, it would be valuable to assess effects on antagonistic behaviours, as well as behaviours related to social tolerance and dominance hierarchies. For example, in some species, there seems to be a trend of aggression towards male conspecifics during the use of a progestin-based hormonal contraceptive in females (Linn & Steklis, 1990; Shively et al., 1990; Maijer & Semple, 2016). This may be particularly relevant in bonobos, as females strongly shape social dynamics. Moreover, it is especially relevant now, as a recent large-scale study has challenged the previously thought peaceful nature of bonobos compared to chimpanzees (Bryon et al., 2026).

Second, the effects of hormonal contraception on the vaginal microbiome should also be further examined to understand why POPs, particularly, are associated with a more even vaginal microbial community. Longitudinal sampling of the same individuals combined with behavioural data could disentangle within-individual change from inter-individual variation. In addition, larger sample sizes are needed to improve statistical power and to allow for more robust conclusions regarding age-related effects on the vaginal microbiome.

Third, the relation between socio-sexual behaviour and the vaginal microbiome has been shown to be an interesting avenue to pursue. Further research should focus on examining which taxa become more dominant in more sexually active females, and whether such associations may reflect potential protective mechanisms against sexually transmitted infections or to debunk this hypothesis altogether. Bonobos provide a relevant comparative model in this context, given the ongoing debate regarding whether female-female sexual activity is associated with microbial states resembling bacterial vaginosis in humans (Bradshaw et al., 2014). Future studies could also examine microbial strain sharing between individuals. Integrating strain-resolved sequencing with longitudinal sampling makes it possible to track microbial strains through social networks, which could be particularly informative to do during changes in zoo group structure. This way, social transmission may be disentangled from other drivers of microbiome similarity. Although current strain-profiling methods remain limited in detecting low-abundance newly acquired strains, newer approaches seem to show great potential for identifying specific microbial strains and tracking their transmission between individuals (Ghazi et al., 2022; Olm et al., 2021).

Finally, investigating the interconnection of socio-sexual behaviour and other microbiomes might be very meaningful. In particular, the gut microbiome may offer a dynamic and potentially reversible source of variation in sexual behaviour across the lifespan. Recent studies show that microbial communities in the gut may directly shape systemic sex steroid availability through modulation, which could be a driver for the evolution of such sexual divergence as seen in bonobos and chimpanzees (Saha et al., 2024; Veerus et al., 2026).

Altogether, future work should integrate behavioural, hormonal and microbial data within standardized longitudinal designs to address these multi-layered interactions.

6. Conclusion

This study was the first to examine effects of age and hormonal contraception on socio-sexual behaviour and the vaginal microbiome in zoo-housed female bonobos, and to explore how socio-sexual behaviour may impact the vaginal microbiome.

Overall, there was not a clear non-linear effect of age on socio-sexual behaviour. Contrary, hormonal contraception was consistently associated with variation in socio-sexual behaviour. In particular, the use of a progestin-only-pill was linked to reduced frequencies of socio-sexual interactions and a trend towards fewer sexual partners.

The effect of age was inconclusive to predict within-sample diversity of the vaginal microbiome, but was associated with differences in community composition between individuals. Hormonal contraception also influenced microbial structure, with the use of POPs leading to a more even distribution of bacterial taxa. In contrast, the use of combined-oral-contraceptives did not show this pattern.

Importantly, socio-sexual behaviour was significantly associated with variation of vaginal microbial communities. Females with higher levels of socio-sexual activity and a higher number of sexual partners showed a less even microbial distribution, with a vaginal profile more dominated by few taxa. However, dyadic similarity of the vaginal microbiome could not be explained by the frequency of socio-sexual interactions. This might suggest that socio-sexual behaviour may influence the vaginal microbiome through indirect mechanisms, rather than direct bacterial transmission alone. Alternatively, and not mutually exclusive, bacterial transmission through socio-sexual interactions may occur at another taxonomic level than captured in this study. Strain-level analyses and characterisation of dominant taxa may further clarify these relationships.

Together, these findings highlight that age and hormonal state contribute to variation of socio-sexual behaviour and the vaginal microbiome in bonobos, while their effects differ in strength. Moreover, this study demonstrated that socio-sexual behaviour and the vaginal microbiome are interconnected in female bonobos. Finally, it contributed to a broader understanding of the interplay between behaviour, physiology and microbiology in a social species.

7. References

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8. Appendix

Table 1: All individuals for whom behavioural data were gathered and their adjoining individual data.

Zoo	ID	Age	Degree	Sex frequency	Stable contraception?	Type contraception	Groupsize - 1
APH	Bonnie	49	8	0.586	YES	None	12
APH	Jill	40	8	0.372	YES	COC	12
APH	Kumbuka	26	5	0.742	YES	None	12
APH	Besede	19	8	1.178	YES	COC	12
APH	Pangi	16	4	0.270	YES	None	12
APH	Leki_II	11	5	0.154	YES	COC	12
APH	Neje	4	3	0.081	YES	None	12
APH	Mayele_Frans	1	1	0.013	YES	None	12
BLN	Opala_II	27	4	1.133	YES	POP	5
BLN	Monyama	15	5	1.803	YES	POP	5
BLN	Samani	5	5	1.098	YES	None	5
FRA	Natalie	60	4	0.042	YES	None	18
FRA	Kamiti	38	7	0.068	YES	POP	18
FRA	Zomi_I	27	7	0.046	YES	POP	18
FRA	Mixi	23	6	0.152	NO	None > COC	18
FRA	Bashira	19	4	0.042	NO	None > POP	18
FRA	Hanna	17	8	0.241	YES	POP	18
FRA	Nayoki	13	13	0.266	YES	POP	18
FRA	Ayebi	10	12	0.384	YES	None	18
FRA	Xola	8	5	0.038	YES	POP	18
FRA	Cheza	2	8	0.084	YES	None	18
FRA	Ema	1	7	0.089	YES	None	18
OUW	Lisala_I	45	11	0.641	YES	None	14
OUW	Eja	35	8	0.354	YES	COC	14
OUW	Kutu	27	5	0.141	YES	POP	14
OUW	Lingoye	17	9	0.236	YES	POP	14
OUW	Omanga	16	12	1.062	YES	COC	14
OUW	Lopori	13	11	0.489	YES	COC	14
OUW	Visola	10	9	0.422	YES	COC	14
OUW	Nginga	7	5	0.219	YES	None	14
OUW	Yanonge	2	7	1.214	YES	None	14
PLD	Hortense	47	2	0.066	YES	COC	21
PLD	Banya	35	8	0.345	YES	None	21
PLD	Djanaoa	30	9	0.561	YES	COC	21
PLD	Binti	30	5	0.438	YES	COC	21
PLD	Busira	21	4	0.285	YES	POP	21
PLD	Kianga	20	9	0.575	YES	COC	21
PLD	Nayembi	19	8	0.187	YES	None	21
PLD	Swahili	10	11	0.806	YES	None	21

PLD	Bina	10	9	0.356	YES	COC	21
PLD	Nila	10	11	0.719	YES	COC	21
PLD	Sanza	8	4	0.306	YES	None	21
PLD	Balina	5	4	0.438	YES	None	21
PLD	Unabii	5	8	0.503	YES	None	21
PLD	Wakati	4	6	0.518	YES	None	21
PLD	Xolani	3	5	0.394	YES	None	21
TWY	Diatou	48	6	0.806	YES	POP	12
TWY	Lina	40	7	0.438	YES	COC	12
TWY	Cheka	29	11	0.775	YES	COC	12
TWY	Maringa_II	27	10	1.840	YES	None	12
TWY	Likemba	15	8	1.055	YES	COC	12
TWY	Yuli	11	10	1.124	YES	None	12
TWY	Batia	11	11	2.929	NO	None > COC	12
TWY	Lola	6	8	0.886	YES	None	12
VDS	Daniela	57	13	0.332	YES	None	19
VDS	Ukela	39	9	0.201	NO	POP > COC	19
VDS	Ulindi	32	12	0.649	NO	POP > COC	19
VDS	Khaya	24	9	0.183	NO	POP > COC	19
VDS	Lingala	22	10	0.268	YES	None	19
VDS	Lucy_II	21	14	0.354	NO	POP > COC	19
VDS	Yahimba	16	11	0.358	NO	None > COC	19
VDS	Khalessi	12	14	0.705	NO	POP > COC	19
VDS	Kymia_II	8	13	0.564	NO	None > COC	19
VDS	Bembé	3	8	0.261	YES	None	19
VDS	Kemba	3	11	0.422	YES	None	19
VDS	Yibambé	2	9	0.276	YES	None	19
VDS	Kipépéo	2	16	0.436	YES	None	19
WUP1	Muhdeblu	24	5	0.581	YES	COC	5
WUP1	Huenda	19	2	0.227	YES	None	5
WUP2	Kichele	36	1	0.237	YES	COC	1
WUP2	Akeena	11	1	0.237	YES	None	1

Table 2: All individuals for whom microbial data were available after quality check and their adjoining individual data.

Zoo	ID	Age	Niche	Antibiotics	Type contraception	ISI
APH	Mayele	1	Vulva	NO	None	112.070
APH	Neje	4	Vulva	NO	None	142.494
APH	Leki_II	10	Vagina	NO	COC	7.357
APH	Pangi	16	Vagina	NO	None	20.934
APH	Besede	19	Vagina	NO	COC	7.657
APH	Kumbuka	26	Vagina	NO	None	18.666
APH	Jill	40	Vagina	NO	COC	11.810
APH	Bonnie	49	Vagina	NO	None	11.250

BLN	Monyama	15	Vagina	NO	POP	3.811
BLN	Opala_II	27	Vagina	NO	POP	13.117
FRA	Xola	8	Vagina	NO	POP	6.912
FRA	Ayebi	10	Vagina	YES	None	2.009
FRA	Nayoki	13	Vagina	YES	None	2.859
FRA	Hanna	17	Vagina	NO	POP	3.601
FRA	Bashira	19	Vagina	NO	POP	39.561
FRA	Mixi	23	Vagina	NO	None	3.065
FRA	Zomi_I	27	Vagina	NO	POP	20.830
FRA	Kamiti	38	Vagina	NO	POP	12.197
FRA	Natalie	60	Vagina	NO	None	6.196
OUW	Visola	10	Vagina	YES	None	2.962
OUW	Lopori	13	Vagina	NO	COC	2.184
OUW	Omanga	16	Vagina	YES	COC	3.076
OUW	Lingoye	17	Vagina	NO	POP	13.196
OUW	Kutu	27	Vagina	NO	POP	52.545
OUW	Eja	35	Vagina	NO	COC	10.697
OUW	Lisala_I	45	Vulva	NO	None	5.953
PLD	Xolani	3	Vulva	NO	None	21.129
PLD	Wakati	4	Vulva	NO	None	29.898
PLD	Unabii	5	Vagina	NO	None	3.059
PLD	Sanza	8	Vulva	NO	None	88.067
PLD	Nila	10	Vagina	NO	COC	2.308
PLD	Bina	10	Vagina	NO	COC	5.259
PLD	Swahili	11	Vagina	NO	None	10.707
PLD	Nayembi	19	Vagina	NO	None	3.820
PLD	Kianga	20	Vagina	NO	COC	7.893
PLD	Busira	22	Vagina	YES	POP	3.193
PLD	Binti	30	Vulva	NO	COC	16.325
PLD	Djanaoa	30	Vagina	NO	COC	2.816
PLD	Banya	35	Vulva	NO	None	5.179
PLD	Hortense	47	Vagina	NO	COC	2.416
TWY	Batia	11	Vulva	NO	None	2.007
TWY	Yuli	11	Vulva	NO	None	4.830
TWY	Likemba	16	Vulva	NO	COC	5.553
TWY	Maringa_II	27	Vulva	NO	None	2.955
TWY	Cheka	29	Vulva	NO	COC	8.810
TWY	Lina	40	Vagina	NO	COC	5.733
TWY	Diatou	47	Vulva	YES	POP	9.013
VDS	Kymia_II	8	Vagina	NO	None	2.680
VDS	Khalessi	12	Vagina	NO	POP	6.790
VDS	Yahimba	16	Vagina	NO	None	3.306
VDS	Lucy_II	21	Vagina	NO	POP	2.347
VDS	Lingala	22	Vagina	NO	None	1.940
VDS	Khaya	24	Vagina	NO	COC	5.163
VDS	Ulindi	32	Vagina	NO	POP	3.691

VDS	Ukela	40	Vagina	NO	COC	6.492
VDS	Daniela	57	Vagina	NO	None	5.938
WUP1	Huenda	19	Vagina	NO	None	7.990
WUP1	Muhdeblu	24	Vagina	NO	COC	6.771
WUP2	Akeena	11	Vagina	NO	None	6.127
WUP2	Kichele	36	Vagina	NO	COC	3.662

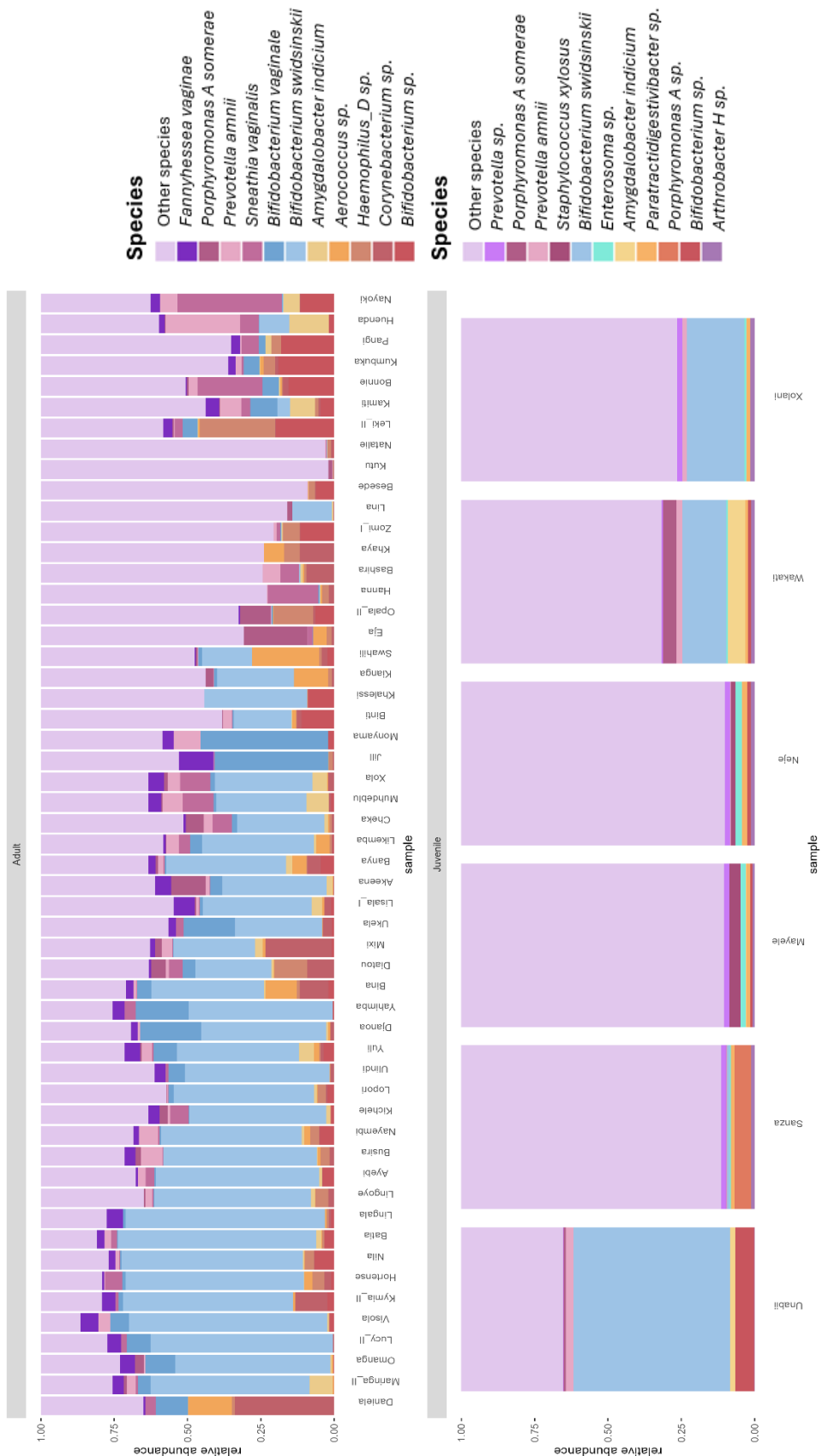


Figure 8: The relative abundance of bacterial species present in the vaginal sample of zoo-housed female bonobos. The ‘other species’ contain species at very low abundances.