Potential impacts of pesticides on the honey bee (*Apis mellifera iberiensis*) gut microbiota

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ABSTRACT

A healthy honey bee gut microbiota has emerged as a promising avenue to protect bees against stressors. Honey bees possess a stable core microbiota, and disruptions to this microbial community (dysbiosis) within a multifactorial stressor system may serve as an indicator of adverse health conditions. This study investigated the potential impacts of two widely used pesticides, flupyradifurone (FPF) and tau-fluvalinate (TFL), and the solvent acetone on the gut microbiota of worker bees. Using full-length 16S rRNA gene sequencing (PacBio Sequel II), the microbial communities of exposed and unexposed bees were analyzed through alpha and beta diversity metrics, as well as taxonomic composition. The results revealed distinct microbial patterns across treatments. The acetone group exhibited the highest microbial richness (Chao1), but also greater interindividual variability and signs of dysbiosis, while the TFL group showed reduced diversity and increased dominance of specific taxa. FPF exposed bees maintained a more balanced microbial profile. Taxonomic analysis showed a predominance of *Lactobacillus* in pesticide-treated groups, while *Bartonella*, *Snodgrassella*, and *Commensalibacter* were reduced under acetone and TFL exposure. *Gilliamella* and *Bifidobacterium* responded differently depending on the treatment, suggesting adaptive or opportunistic dynamics. These findings demonstrate that not only pesticides, but also solvents like acetone, can significantly alter the gut microbiota of honey bees. This study highlights the need to re-evaluate the assumed inertness of formulation components in ecotoxicological risk assessments and reinforces the importance of microbiome-based indicators in pollinator health studies. The outcomes contribute to a deeper understanding of pesticide-microbiota interactions and offer insights to support more sustainable and pollinator-friendly agricultural practices.

Keywords: ecotoxicology, pollinators, agrochemicals, diversity analysis.

RESUMO

Uma microbiota intestinal saudável das abelhas melíferas tem-se revelado uma via promissora para protegê-las contra fatores de stress. As abelhas possuem uma microbiota central estável, e perturbações nesta comunidade microbiana (disbiose), num contexto multifatorial de stress, podem servir como indicador de condições adversas de saúde. Este estudo investigou os potenciais impactos de dois pesticidas amplamente utilizados, flupiradifurona (FPF) e tau-fluvalinato (TFL) na microbiota intestinal de abelhas operárias. Utilizando o sequenciamento do gene 16S rRNA de comprimento total (PacBio Sequel II), as comunidades microbianas de abelhas expostas e não expostas foram analisadas com base em métricas de diversidade alfa e beta, bem como na composição taxonômica. Os resultados revelaram padrões microbianos distintos entre os tratamentos. O grupo tratado com acetona apresentou a maior riqueza microbiana (Chao1), mas também maior variabilidade interindividual e sinais de disbiose, enquanto o grupo tratado com TFL demonstrou uma diversidade reduzida e maior dominância de táxons específicos. As abelhas expostas à FPF mantiveram um perfil microbiano mais equilibrado. A análise taxonómica revelou uma predominância de *Lactobacillus* nos grupos tratados com pesticidas, enquanto *Bartonella*, *Snodgrassella* e *Commensalibacter* apresentaram uma redução sob exposição a TFL e acetona. *Gilliamella* e *Bifidobacterium* responderam de forma variável consoante o tratamento, sugerindo dinâmicas adaptativas ou oportunistas. Estes resultados demonstram que não só os pesticidas, mas também solventes como a acetona, podem alterar significativamente a microbiota intestinal das abelhas. O estudo reforça a necessidade de reavaliar a suposta inércia dos componentes de formulação nas avaliações de risco ecotoxicológico e sublinha a importância de indicadores baseados no microbioma em estudos de saúde de polinizadores. Os dados obtidos contribuem para uma compreensão mais aprofundada das interações entre pesticidas e microbiota e oferecem orientações para práticas agrícolas mais sustentáveis e benéfico aos polinizadores.

Palavras-chave: ecotoxicologia, polinizadores, agroquímicos, análises de diversidade.

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1. INTRODUCTION

Honey bees are key organisms for the maintenance of ecosystems, playing an essential role in the pollination of agricultural crops and wild plants. It is estimated that around 75% of the world's main food crops depend, to some degree, on pollination by animals, with bees of the genus *Apis* playing a leading role in this process (REQUIER et al., 2023). Among the subspecies, *Apis mellifera iberiensis*, a subspecies endemic to the Iberian Peninsula, stands out for its ecological and economic relevance in the region, especially in agricultural systems that depend on pollination for productivity and fruit quality (UTZERI et al., 2018).

Despite their importance, honey bee populations have suffered significant declines in recent decades, a phenomenon attributed to a multitude of factors, including habitat loss, disease, climate change and, notably, the intensive use of pesticides (GOULSON et al., 2015; WARD et al., 2022). Among these compounds, insecticides and acaricides widely applied in agricultural practices have raised growing concern, since, although often considered selective, they can affect non-target organisms in a sublethal manner, impacting their physiology, behavior and microbiota (TOSI et al., 2017).

The gut microbiota of honey bee is composed of a relatively simple and specialized core of microorganisms that play key roles in digestion, immunity, and protection against pathogens (ENGEL et al., 2012; AGUADO-LÓPEZ et al., 2024). Changes in the composition and diversity of these symbiotic microorganisms have been associated with harmful effects on bee health, including increased susceptibility to diseases, reduced longevity, and metabolic dysfunctions (RAYMANN & MORAN, 2018). Thus, the study of gut microbiota emerges as a sensitive and informative tool to assess the sublethal effects of chemical agents on these pollinators.

Flupyradifurone and tau-fluvalinate are key pesticides that should be investigated for their potential effects on honey bee health. Flupyradifurone, a member of the butenolide class, acts on nicotinic acetylcholine receptors and has been promoted as a less toxic alternative to neonicotinoids (JESCHKE et al., 2013). However, recent studies indicate that it can generate relevant neurobehavioral and physiological effects, including on the microbiota (GRAY et al., 2024). Tau-fluvalinate, in turn, is a synthetic pyrethroid commonly used to control Varroa destructor, a parasitic mite widely associated with colony losses. Although frequently applied within the hives themselves, there is evidence that tau-fluvalinate can accumulate in wax and food, exposing bees to sublethal levels for prolonged periods (ERBAN et al., 2019).

In this context, the present study investigates the impacts of the insecticide flupyradifurone and the acaricide tau-fluvalinate on the intestinal microbiota of Apis mellifera iberiensis workers with the aim of evaluating the composition and diversity of the intestinal microbiota of honey bees exposed to a specific concentration of these two pesticides, comparing the data obtained with unexposed bees.

2. LITERATURE REVIEW

2.1 Honey bees

The western honey bee (*Apis mellifera*) is a cosmopolitan and social species that belongs to the order Hymenoptera and the Apidae family (EBAN-ROTHSCHILD & BLOCH, 2015). It is native to Asia, Europe, and Africa, where it inhabits desert oasis, mountains, savannas, and grasslands. Currently, honey bees are spread around the world as they are highly adaptable and have been able to successfully colonize different regions (DE LA RÚA et al., 2009).

Throughout its wide natural range, the honey bee have evolved into 31 subspecies, which have been grouped into four major evolutionary lineages, based on morphological characteristics, each native to a specific region: western and northeastern Europe and northwestern Asia (lineage M); central and southeastern Europe (lineage C); Africa (lineage A); and the Middle East and central Asia (lineage O) (RUTTNER, 1988).

Europe, with its diverse habitats, is home to 10 of these subspecies, described in Table 1. These subspecies differ in morphology and behavior and are adapted to different environmental conditions (RUTTNER, 1988). Each subspecies is essential to maintaining ecological balance and supporting agricultural productivity through pollination (MEDRZYCKI et al., 2013).

The honey bee has long been domesticated for honey and wax production and pollination. In research, it serves as a model of developmental plasticity, cognition, and social behavior (MOTTA & MORAN, 2024).

Our society gains numerous benefits from honey production, both economically and nutritionally. Honey serves as a natural sweetener with antimicrobial properties, contributes to the pharmaceutical and cosmetic industries, and supports rural economies through beekeeping activities (ALTAF & UMAIR, 2020). Additionally, bees provide essential ecological services, including pollination, which enhances biodiversity, increases agricultural yields, and supports ecosystem stability. These contributions highlight the critical role of honey bees in sustaining both human well-being and environmental health (BREEZE et al., 2011).

Table 1- European *Apis mellifera* subspecies (RUTTNER, 1988).

Lineage	**Subspecies**	**Distribution**
M	*Apis mellifera iberiensis*	Iberian Peninsula
	Apis mellifera mellifera	Northern and Western Europe
C	*Apis mellifera carnica*	Central/Eastern Europe
	Apis mellifera cecropia	Southern Greece, including the Peloponnese
	Apis mellifera ligustica	Italian Peninsula
	Apis mellifera macedonica	Bulgaria, Greece, Romania, Ukraine, Turkey
O	*Apis mellifera adami*	Europe, the Middle East, and Africa
	Apis mellifera cypria	Cyprus
A	*Apis mellifera ruttneri*	Malta
	Apis mellifera siciliana	Sicily

Among the European subspecies, one that stands out is *Apis mellifera iberiensis*, as it is valuable for beekeeping thanks to its efficiency in honey production. These bees are characterized by their high genetic diversity and adaptability to different environmental conditions, in addition to their complex social behavior (ILYASOV, 2020; PINTO et al., 2023).

Apis mellifera iberiensis has a color that varies from dark brown to jet-black. The darkness is accentuated by the low tomentum and low hairiness (Figure 1). It is adapted to hot and dry climates, has moderate to high aggressiveness, a high tendency to swarm, in addition to good honey production, mainly from wild plants (RUTTNER, 1988).

Figure 1- The Iberian honey bee (Rainwater Runoff, 2021).

Bees, particularly honey bees, play a crucial role in pollinating both crops and wild plants. However, the recent decline in their populations has raised concerns about various factors influencing their health, including the role of gut microbiota in maintaining their overall well-being. (PANZIERA et al., 2022).

2.1.1 Honey bee gut microbiota

The gut microbiota of bees is composed of a relatively small number of bacterial species (Table 2), and their abundance may vary depending on the host and ecological conditions (ROMERO et al., 2019). This flexibility allows the microbial community to adapt to different ecological niches and changes in resource availability, which is crucial for colony survival (KWONG & MORAN, 2016).

The relationship between bees and their microbiota is an example of symbiosis, where both organisms benefit. The bacteria obtain a nutrient-rich environment in the bee's gut (MARTINSON et al., 2012). Genomic and transcriptomic studies revealed that bee microbiota bacteria have adapted to metabolize sugars, taking advantage of the bees' carbohydrate-rich diet. Different strains of fermentative bacteria have distinct carbohydrate degradation capabilities, while some non-fermentative species, such as *Snodgrassella alvi*, have lost the ability to utilize sugars (TARPY et al., 2015).

On the other hand, bees benefit from the functions these bacteria perform. Microbiota plays a crucial role in protecting bees against pathogens and parasites by activating the bee's innate immune system. Indeed, the presence of certain bacteria can inhibit the growth of pathogenic microorganisms, contributing to the overall health of the colony (MOJGANI et al., 2024). The absence or disturbance of microbiota results in changes in gene expression related to bee immunity, metabolism, behavior and development. In addition to this protective role, microbiota helps to digest refractory pollen components and metabolize dietary toxins (MOTTA & MORAN, 2024).

Table 2- Composition of *Apis mellifera* gut microbiota (ROMERO et al., 2019).

Species	Class or phylum	Relative abundance (%)
Snodgrassella alvi	*Betaproteobacteria*	1–40
Gilliamella apicola	*Gammaproteobacteria*	1–39
Bartonella apis	*Alphaproteobacteria*	<4
Frischella perrara	*Gammaproteobacteria*	<13
Lactobacillus Firm-4 and 5	*L. mellis, L. mellifer, L. apis, L. helsingborgensis, L. kullabergensis, L. kimbladii, L. melliventri*	20–99
Bifidobacterium asteroides	*Actinobacteria*	<15
Parasaccharibacter apium	*Alphaproteobacteria*	<6

The microbiota also influences the development of bees, especially in the early stages of life. Young bees acquire their microbiota primarily through trophallaxis, fecal-oral transmission, and contact with hive materials, which is essential for establishing a healthy microbial community (GUO et al., 2015). As a result, the disruption of the microbiota, frequently induced by pesticides, can adversely impact bees, compromising their health and overall functionality (RIȘCU & BURA, 2013).

2.2 Pesticides

Pesticide application has become a routine and progressively expanding practice in agricultural systems, and their excessive application may pose a threat to the survival of honey bee colonies. Table 3 lists the main pesticides that can be dangerous to honey bees in all regions of the world (NAZ et al., 2022).

Strict pesticide regulations apply in the EU. They are based on Regulation (EC) No. 1107/2009, which sets out the criteria for the approval of active substances in plant protection products and ensures the protection of the environment (EUR-Lex, 2009). For example, approved pesticides include glyphosate (whose approval was recently extended until 2033) and copper (commonly used in biopesticides) (EEA, 2023). In addition, Directive 2009/128/EC aims to promote the sustainable use of these products by imposing restrictions and promoting alternative methods (EUR-Lex, 2009).

Therefore, interest in using selective pesticides that do not affect non-target organisms such as bees has been increasing. Some examples of these include the insecticide flupyradifurone (TOSI et al., 2018). Flupyradifurone (FPF) is a new insecticide that has entered the market as an alternative to neonicotinoids, which have been linked to adverse effects on bees. FPF is an insecticide that acts as a nicotinic acetylcholine receptor (nAChR) agonist, similar to neonicotinoids, but with a different chemical structure (GIORIO et al., 2021).

A study by Tosi and Nieh (2019) evaluated the lethal and sublethal effects of FPF on honey bees. The authors showed that FPF causes mortality in honey bees at specific concentrations, with more pronounced effects when combined with other pesticides. Honey bees exposed to FPF showed significant behavioral changes, including difficulties in foraging and communication, which can impact colony efficiency.

Table 3- Pesticides that are threats to honey bee colony (NAZ et al., 2022).

Types of pesticide			
Insecticide	**Acaricides**	**Fungicides**	**Herbicides**
Acephate, Carbaryl, Cypermethrin, Deltamethrin, Diazinon, Dimethoate, Imidacloprid, Lamda-cyhalothrin, Malathion, Methomyl, Permethrin, Phosmet, Pyridaben, Spinosad, Thiacloprid, Tiamethoxam, Acetamiprid, Chlorpyrifos, Endosulfan, Phosalone, Clofentezine, Formetanate.	Bifenazate, Dicofol, Spiromesifen	Azoxystrobin, Boscalid, Captan, Chlorothalonil, Cymoxanil, Fenamidone, Iprodione, Mancozeb, Myclobutanil, Pyraclostrobine, Pyrimethanil, Zineb, Zoxamide.	2, 4 D, Clomazone, Chlopyralid, Fluazifop-p-butyl, Glyphosate, Linuron, Metribuzin, Paraquat, Trifluralin.

In addition to the pesticides listed in Table 3, another example of a dangerous molecule is the acaricide tau-fluvalinate (TFL), which has been shown to be potentially harmful to bees (ALONSO-PRADOS et al., 2020). TFL is a synthetic acaricide belonging to the pyrethroid class, which is widely used in agriculture and beekeeping to control some plant pests and honey bee mites, especially the ectoparasitic mite *Varroa destructor* (BENITO-MURCIA et al., 2021). TFL acts as a neurotoxin, interfering with the sodium channels of the nerve cells of mites and insects. It has low toxicity to mammals and, when used properly, presents limited risks to bees (KAMLER et al., 2016). However, their misuse may still impact the quality of life of honey bees.

Sabová et al. (2022) investigated the effects of TFL on adult honey bees during winter, focusing on aspects of toxicity, immune response and DNA damage. The study revealed that exposure to TFL can compromise bees' ability to respond to pathogens, which is critical for colony survival. Analysis of genes related to immunity showed an altered expression, suggesting that the acaricide may interfere with the bees' immune defense.

In this context, it is essential to monitor honey bee exposure to these two pesticides, as their use may cause harm, including dysregulation of the gut microbiota (LI et al., 2015). The gut microbiota sequencing serves as a valuable tool for monitoring pesticide impact, as changes in its composition can reflect sublethal effects on bee health.

2.3 Metagenomic sequencing

Metagenomic sequencing is an advanced technique that allows the analysis of microbial diversity in environmental samples without the need for prior cultivation of microorganisms (CHRISTOFF et al., 2017). This approach is especially useful for studying complex microbial communities, such as those found in environments such as the gastrointestinal tract, soil, and water (GLENDINNING et al., 2016).

This technique involves several crucial steps that enable the analysis of microbial diversity in environmental samples. The first step is sample collection, which can be carried out in different environments. After collecting, the DNA of the microorganisms present in the sample is extracted (FRASER et al., 1998). This extraction is essential, as the quality and quantity of DNA directly influence the sequencing results. In many cases, specific DNA regions, such as the 16S rRNA gene, are amplified by polymerase chain reaction (PCR) to facilitate the identification and classification of microorganisms present in a sample (TRINGE & RUBIN, 2005).

After DNA extraction and PCR amplification, the next step is sequencing, which typically uses next-generation sequencing (NGS) technologies such as the Illumina MiSeq platform, or third-generation sequencing techniques, like PacBio and Oxford Nanopore. These technologies generate a large amount of nucleotide sequence data, enabling comprehensive microbial community analysis (FACIOLI et al., 2020).

The data obtained undergoes rigorous bioinformatic processing, which includes the removal of low-quality sequences and the identification of operational taxonomic units (OTUs). Finally, the results are analyzed to understand the composition and diversity of the microbiota, enabling comparisons with other studies and contributing to knowledge about microbial ecology and its implications for health and the environment (DE ALMEIDA & FERNANDES, 2013).

2.4 Diversity Analysis

The analysis of ecological diversity is an important endeavour for understanding the complexity of ecosystems and their underlying processes (RAFFARD et al., 2019). Among the main metrics for expressing diversity, alpha and beta indicators stand out, as they allow the assessment of local and regional biodiversity patterns. These metrics are critical not only for ecological description but also for supporting conservation and management decisions (GLOOR et al., 2017).

Alpha diversity refers to the local diversity or the community of individuals within an ecosystem. This type of analysis is commonly used to assess species richness (the total number of species in a sample) and evenness (the distribution of abundance among species) (XIA & SUN, 2023). Communities with high alpha diversity tend to be more resilient to disturbances and have a greater capacity to provide ecosystem services (WILLIS, 2019). Among the most popular metrics are the Chao1, Simpson and Shannon indices.

The Chao1 index is a nonparametric estimate of species richness, i.e., the total number of species present in a community. It corrects for underestimation of rare species by considering the frequency of taxa that occur once (singletons) or twice (doubletons) in a sample. It is defined as

$$Chao\,1 = s + \frac{F1(F1-1)}{2(F2-1)}\,, \tag{1}$$

where s is the number of observed taxa, and $F1$ and $F2$ are the number of OTU/ASV with only one sequence and two sequences (CHAO, 1984). Higher values indicate greater estimated richness, while lower values indicate that the community has few species or is undersampled. This metric is particularly useful in studies involving a large number of rare species, such as microbiome analyses (OIKONOMOU et al., 2013).

The Simpson index measures the probability that two randomly sampled units belong to the same species, reflecting dominance within a community. It is defined as

$$D = \frac{1}{\sum_{i=1}^{s} Pi^{2}}, \tag{2}$$

where s is the number of OTU/ASV and pi is the proportion of the community represented by the ith OTU/ASV. The greater the dominance of minority species, the higher the raw Simpson index (D); however, in practice, 1 - D or the inverse of D (1/D) is usually used, so that higher values indicate greater diversity (SIMPSON, 1949). Lower values indicate that the community is dominated by a few species.

The Shannon index (or Shannon entropy) measures community diversity, taking into account both species richness and the evenness of their abundance. It is defined as

$$H =- \sum_{i=1}^{s} pi * log(pi), \tag{3}$$

where s is the number of OTU/ASV and pi is the proportion of the community represented by the ith OTU/ASV (SHANNON, 1948). Higher values indicate a more diverse community with more balanced abundance, while lower values indicate lower diversity or strong dominance of certain species. This metric is sensitive to changes in rare and common taxa and is widely used in microbial ecology (LEMOS et al., 2011).

Beta diversity, on the other hand, evaluates differences in species composition between communities, samples or environments. This concept is crucial for understanding environmental heterogeneity and connectivity between different habitats (MULDER et al., 2011). According to Whittaker (1960), beta diversity can be explained as changes in species or microbial composition along environmental gradients or between locations. High beta diversity indicates a heterogeneous environment with diverse ecological niches, whereas low values suggest ecological homogeneity (LEGENDRE & LEGENDRE, 2012). In fragmented ecosystems, assessing beta diversity can help identify critical ecological corridors to maintain connectivity between populations (ANDERSON et al., 2011). The most commonly used beta diversity indices are Bray-Curtis and Jaccard (VAN DER PLAS et al., 2023).

Bray-Curtis dissimilarity is a measure of beta diversity that assesses the difference in species composition between two communities based on relative abundance (BRAY & CURTIS, 1957). It is defined as

$$BC = 1 - \frac{2Cij}{Si+Sj}, \quad (4)$$

where Cij is the sum of the smallest values for only those taxa in common between the sample i and j, and Si and Sj are the total number of taxa counted in sample i and j, respectively. Values range from 0 (same community) to 1 (completely different community). High values indicate strong differences in composition or structure, while low values indicate high similarity between communities (LO et al., 2024).

The Jaccard index is a qualitative measure used to measure the similarity between communities based on the presence/absence of species (JACCARD, 1901). It ranges from 0 (no species shared) to 1 (all species shared). It is widely used for simple categorical comparisons. High values indicate greater species overlap between communities, while low values indicate greater compositional differences (KERS & SACCENTI, 2022).

Integrated analyses of alpha and beta diversity are particularly important for conservation planning. Alpha diversity helps identify areas of high ecological value in terms of local richness, while beta diversity aids in selecting complementary sites for conservation, ensuring the representation of different ecological communities (ALLEN et al., 2016).

The combination of alpha and beta analyses is essential for generating reliable information that aids in understanding ecological patterns and developing effective environmental policies (LAKENS, 2013). Moreover, incorporating these indicators into temporal analyses enables the assessment of biodiversity changes in response to factors such as climate change, stochastic events, and human activities (CASALS-PASCUAL et al., 2020), including pesticide use.

3. OBJECTIVES

This study is focused on exploring the effects of the insecticide flupyradifurone and the acaricide tau-fluvalinate on the gut microbiota of workers of *Apis mellifera iberiensis*. Specifically, it aims to analyse the gut microbiota of workers exposed to a single concentration of these chemicals. To ensure that any observed effects are attributed to the active compounds rather than the solvent, acetone was used as a vehicle control in a separate treatment group.

The analysis focused on evaluating the relative abundance of microbial taxa and calculating diversity indexes as key parameters to assess the impact of these pesticides and the solvent.

By understanding how these commonly used pesticides—and the acetone vehicle itself—influence the gut microbiota, this study provides valuable insights into the broader implications for honey bee health and informs strategies to mitigate risks posed by agricultural practices.

4. METHODOLOGY

The data analysed in this study was obtained from previously sequenced honey bee guts. The experiment was developed at the Centro de Investigación Apícola y Agroambiental, in Spain (CIAPA), attached to the Regional Institute for Agri-Food and Forestry Research and Development of Castilla-La Mancha (IRIAF) in Guadalajara, Spain.

4.1 Principle of the test

The chronic toxicity test was performed on adult worker honey bees following OECD Test Guideline No. 245 (OECD, 2017). Newly emerged bees (≤2 days old) were continuously exposed for 10 days to a 50% (w/v) sucrose solution containing the test compound, provided ad libitum. Daily monitoring included recording mortality and feed consumption. Treatment groups were compared against two control groups: a negative control (NC) consisting of bees fed only sucrose syrup, and an acetone control (AC) where bees received syrup containing acetone (the solvent used for compound dissolution). To validate the test system's sensitivity and reliability, dimethoate (DIM) was included as a reference toxicant, as required by OECD guidelines. This experimental design allowed for comprehensive assessment of chronic toxicity effects while controlling for potential solvent interference and ensuring methodological robustness.

4.1.1. Substances tested

We evaluated the chronic toxicity of tau-fluvalinate (TFL, PESTANAL®, analytical standard, Merck, Germany) and flupyradifurone (FPF, PESTANAL®, analytical standard, Merck, Germany) on two-day-old honey bee workers. TFL was tested at 232 ppm, a concentration determined through preliminary proof-of-concept studies conducted at CIAPA. The FPF concentration was selected based on the highest field-relevant concentration reported by Tosi et al. (2021). Dimethoate (DMT, PESTANAL®, analytical standard) served as the reference toxicant at 0.7 ppm. Since pesticide formulations typically require organic solvents, we included an acetone control (AC) to account for potential solvent effects. This experimental design enables differentiation between compound-specific toxicity and potential solvent-mediated effects on either host physiology or associated microbial communities.

4.1.2. Preparation of the stock and feeding solutions

Twenty-four hours prior to initiating the toxicity test, stock solutions were prepared using HPLC-grade acetone (99.9% purity, CAS No. 67-64-1) at concentrations of 50,000 ppm, 10,000 ppm, and 1,000 ppm for TFL, FPF, and DIM, respectively. To achieve the desired concentrations in the final feeding solutions, calculated volumes of these stock solutions were added to 50 mL flasks containing 25 mL of 50% (w/v) sucrose solution and homogenized using a magnetic stirrer. Following initial mixing, additional syrup was gradually incorporated to ensure complete homogenization of the viscous matrix.

The acetone concentration in the final solutions ranged from 0.08 to 1.2 ppm, with control solutions prepared to contain a maximum of 2% (v/v) acetone. To maintain solution stability, stock feeding solutions were replaced every 72 hours and stored at -20°C between renewals. This protocol ensured consistent test compound concentrations throughout the exposure period while accounting for potential solvent effects.

4.1.3. Honey bees

The study utilized honey bees (Apis mellifera iberiensis) from three healthy colonies maintained at the apiary of the CIAPA. Prior to experimentation, all colonies underwent rigorous health assessments and showed no signs of disease. To ensure colony health status, strict sanitary protocols were implemented, including a minimum six-month withdrawal period from all acaricide and antibiotic treatments before sample collection.

Brood frames containing capped pupal cells were carefully selected from queenright colonies and transferred to a temperature-controlled incubator (Memmert® IPP500) maintained at 34 ± 0.5°C with 50-60% relative humidity. Emerging adult bees were collected within 24 hours of eclosion for use in subsequent experiments. This standardized rearing protocol ensured consistent age and physiological status of experimental subjects while minimizing potential confounding effects of field conditions.

4.1.4. Test design and data collection

Twenty-four hours prior to test initiation, two-day-old worker honey bees were gently transferred to experimental cages (20 bees per cage) without anesthesia, in accordance with OECD guidelines (2017). Each cage was equipped with a 2 mL sterile pipette tip feeder containing 50% (w/v) sucrose solution provided ad libitum. The experimental design included three replicate cages per treatment, along with negative controls (50% sucrose only) and solvent controls (50% sucrose + 2% v/v acetone). To account for evaporative losses, six additional empty cages (three per control type) were included in the incubation chamber. All cages were maintained in a precision incubator (Memmert HCP240; temperature stability ±0.1°C, humidity stability ±0.5% RH) at 33°C and 65% relative humidity, with daily environmental fluctuations limited to ≤2 hours.

Daily syrup consumption was quantified using an analytical balance (Sartorius CP225D). Feeders were weighed immediately before placement and again after 24 hours (±2 h). Consumption values were corrected for evaporative losses (determined from empty cages) and normalized to the number of surviving bees in each cage. Mortality was recorded daily during feeder replacement, when deceased bees were removed. Surviving bees were collected at study termination and preserved at -80°C for subsequent molecular analyses.

4.2 Molecular Analysis

4.2.1. DNA Extraction

At the CIAPA laboratory, all samples were processed in a laminar flow hood (Telstar AV-30/70). The bees were first washed with ethanol and subsequently with sterile Milli-Q water to remove potential surface bacteria. For molecular analyses, we processed 15 intestinal tracts per treatment group. Meanwhile, each intestine was carefully extracted by pulling the last segment of the abdomen using sterile forceps. Each sample was then deposited into a well of a 96-well plate containing five 2-mm diameter glass beads (Sigma-Aldrich, MO, USA) along with 180 µL of PCR-grade water.

Once the samples were placed in the plate with the glass beads, they were homogenized in a TissueLyser (Qiagen®, Hilden, Germany) for 90 seconds at 30 Hz. Subsequently, a 150 µL aliquot was taken from each sample and transferred to a 96-well

Deepwell plate (BioSprint 96 DNA Blood Kit, 940057, Qiagen®, Hilden, Germany) containing ribonuclease A (10 mg/mL) and incubated at 37°C for 15 minutes to remove RNA. After incubation, 20 µL of proteinase K was added, followed by incubation at 56°C for 1 hour at 300 rpm in a thermomixer (Thermomixer Comfort).

A MASTER MIX was then prepared in a sterile Falcon tube, containing the following per well: 200 µL of Buffer AL, 200 µL of isopropanol, and 30 µL of MagAttract Suspension G magnetic particles (Qiagen, Hilden, Germany). A volume of 430 µL of the MASTER MIX was dispensed into each well. DNA extraction and purification were performed using the automated BioSprint system with the commercial BioSprint® 96 DNA Blood Kit and the BS96 DNA Tissue program (Qiagen, Hilden, Germany).

In all cases, negative extraction controls (1 per 20 samples) were included and processed in parallel as described. After extraction, DNA concentration was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Finally, the DNA extracts were stored at -20°C until subsequent shipment to Novogene Co. (Beijing, China) for metataxonomic analysis.

4.2.2. Library Preparation, Sequencing, and Taxonomic Assignment

Novogene Co. performed PCR amplification using the primers 27F and 1492R, targeting the full-length 16S rRNA gene. Additionally, the company carried out genetic library construction, PCR product quality control, and sequencing using the PacBio Sequel II/Ile system (Pacific Biosciences, CA, USA), following the effective library concentration and required data volume.

Sequences with lengths below 1,340 bp or above 1,640 bp were discarded. The remaining sequences, falling within the expected amplicon size range, were retained in FASTQ format. Primer sequences were then trimmed to refine the dataset, ensuring uniform nucleotide lengths and generating validated final sequences for downstream analysis. Subsequently, taxonomic assignment of the obtained Amplicon Sequence Variants (ASVs) was performed, and those with fewer than 5 reads were filtered out.

4.3 Quality filtering

Sequences with average quality scores less than 25, shorter than 50% of the expected length, that contain ambiguous base calls (where it cannot be determined with certainty which nucleotide is present) and do not match the primers or barcodes used to label the samples, were removed. The remaining sequences were aligned against a 16S rRNA database to assign taxonomies. Sequences that do not fit into the alignment window were discarded (ENGEL et al., 2013).

4.4 Contaminant identification

To identify potential contaminants in the samples, the decontam package (Davis et al., 2018) was used within the R programming environment (version 4.4.3). The analysis was performed using the prevalence method, which compares the detection frequency of each taxon in true biological samples versus negative controls. No taxa were identified as contaminants under the prevalence criterion, suggesting the absence of detectable contamination among the negative controls included in the dataset. The result of the decontam analysis is available in the Appendices.

4.5 Data analysis

The platform QIIME 2, especially popular in microbiome studies, allowing the analysis of microbial communities from amplicon sequencing data, such as 16S rRNA (ESTAKI et al., 2020), was used for the procedures described below. Alpha diversity and beta diversity analysis were done.

The alpha diversity analysis was performed using the (i) Chao1 index, an abundance-based nonparametric estimator of taxa Richness, (ii) Simpson Index, an estimator of taxa diversity that combines richness and evenness and (iii) Shannon Entropy, also an estimator of taxa diversity, combining richness and evenness. The results were displayed in a table with the diversity indices for each sample and in graphs showing the comparison of the alpha diversity indices between groups.

Statistical comparisons between treatment groups were performed using the non-parametric Kruskal-Wallis test, suitable for datasets that do not assume normal distribution. This test was applied to the alpha diversity indices to evaluate significant differences across groups. In the Kruskal–Wallis test the H statistic represents the test statistic calculated by ranking all values across groups and evaluating if the distributions show a significant distinction. A higher H value suggests a larger difference between the compared groups. The p-value reflects the probability that the observed differences (or more extreme) would occur if the null hypothesis assumes no difference among the groups. Generally, a p-value less than 0.05 is regarded as statistically meaningful. The q-value denotes the FDR-adjusted p-value applicable for multiple comparisons (KRUSKAL & WALLIS, 1952).

The beta diversity analysis was implemented using the e (i) Bray-Curtis metric, which measures the compositional dissimilarity between the microbial communities of two samples based on counts on each sample and the similarity for two communities and (ii) Jaccard, which also measures the similarity for two communities. The results were displayed through a distance matrix, where each cell represents the dissimilarity between two samples based on the chosen metric, and graphics showing the intra-group and inter-group distances were built. All these metrics were implemented in the samples exposed to the two pesticides and in the controls for comparison purposes.

5. RESULTS AND DISCUSSION

5.1 Validation of the assays

Ten days post-exposure, the average mortality across replicates for the untreated control and solvent control groups was ≤15 %, and the average mortality in the reference substance treated group was ≥ 50 %, guaranteeing the validity of the chronic toxicity tests.

The accumulated consumption after 10 days was (in mg/honey bee): NC = 19.45, AC = 18.95, TFL = 23.09, FPF = 21.82, and DIM = 29.45. These metrics confirmed that the bees consumed the daily offered food.

5.2 Taxonomic composition of the gut microbiota

Before evaluating microbial diversity metrics it is necessary to investigate the taxonomic composition of the gut microbiota of honey bees in different treatment groups. The relative abundance of the main bacterial genera across treatments is summarized in Table 4. To assess whether the treatments influenced the abundance of each genus, the Kruskal-Wallis test was performed, as the data did not meet the assumptions of normality (Shapiro-Wilk, $p < 0.05$). For genera with significant overall differences ($p < 0.05$), pairwise comparisons were conducted using the Mann-Whitney U test5.1

Table 4- Relative abundance (mean ± standard deviation) of dominant bacterial genera in the gut microbiota of *Apis mellifera iberiensis* across experimental treatments (FPF = flupyradifurone, TFL = tau-fluvalinate, C = untreated control, CA = solvent control treated with acetone). Different lowercase letters (a, b) within the same row indicate statistically significant differences between treatment groups ($p < 0.05$; Mann-Whitney U test).

Taxon (%)	**FPF**	**TFL**	**C**	**CA**
Lactobacillus	57.2 ± 24 ^{a}	65.8 ± 25 ^{a}	26.0 ± 18 ^{b}	45.3 ± 35 ^{a}
Bartonella	19.6 ± 24 ^{a}	6.2 ± 15 ^{a}	31.9 ± 34 ^{a}	9.7 ± 20 ^{a}
Bifidobacterium	10.5 ± 9 ^{a}	9.4 ± 6 ^{a}	2.5 ± 3 ^{b}	6.7 ± 8 ^{a}
Commensalibacter	4.7 ± 7 ^{a}	9.7 ± 27 ^{a}	7.2 ± 16 ^{a}	0.8 ± 2 ^{a}
Gilliamella	4.8 ± 10 ^{a}	1.8 ± 4 ^{a}	4.7 ± 6 ^{a}	14.2 ± 31 ^{a}
Snodgrassella	3.1 ± 8 ^{b}	7.1 ± 8 ^{a}	27.7 ± 32 ^{a}	23.3 ± 31 ^{a}

Among all genera, *Lactobacillus* dominated the microbial communities, representing 57.2% and 65.8% of the sequences in the flupyradifurone (FPF) and tau-fluvalinate (TFL) groups, respectively. These proportions were lower in the acetone (45.3%) and control (26.0%) groups. The proliferation of *Lactobacillus* under pesticide exposure may reflect a protective or opportunistic response, as members of this genus are known for their stress resistance and their beneficial roles in gut barrier integrity and carbohydrate fermentation (ZHANG et al., 2022).

Bartonella was abundant in the control group (31.9%) but decreased in the TFL (6.2%) and acetone (9.7%) groups. This decrease suggests that *Bartonella* may be sensitive to pesticides and solvents. Previous studies have reported that *Bartonella* is a core but highly variable member of the bee gut microbiota and may be involved in nitrogen cycling and immune regulation (LIU et al., 2022).

Curiously, the relative abundance of *Bifidobacterium* increased in the pesticide-treated groups (FPF, 10.5%; TFL, 9.4%) compared to the control group (2.5%). This trend may reflect a compensatory mechanism or adaptive expansion in response to dysbiosis. Certain strains of *Bifidobacterium* have been associated with detoxification pathways and immune stimulation in bees (CHEN et al., 2021).

Gilliamella, a key bacterial species in carbohydrate metabolism and pollen digestion, was particularly abundant in the acetone group (14.2%), but not in the FPF group (4.8%), TFL group (1.8%), and control group (4.7%). This may indicate that solvent-induced disruption of intestinal homeostasis triggered selective reproduction of this genera (YANG et al., 2025).

Commensal bacteria (*Commensalibacter*), known to maintain the integrity of the intestinal epithelium (BOTERO et al., 2023), were particularly abundant in the TFL group (9.7%) and control group (7.2%), but were almost completely absent in the acetone group (0.8%). Such reduction may point to the disruptive effects of acetone on microbiota stability, reinforcing concerns about the inertness of commonly used solvents (TOMÉ et al., 2023).

Finally, *Snodgrassella*, a core member involved in biofilm formation and immune priming (QUINN et al., 2024), was abundant in the control group (27.7%), remained high in the acetone group (23.3%), but decreased significantly in the FPF group (3.1%). Although *Snodgrassella* has been reported to decrease under xenobiotic stress, its partial retention in the acetone group suggests that its response varies depending on the specific chemical stressor (ROUZÉ et al., 2019).

These taxonomic changes highlight that both pesticides and solvents can significantly affect the composition of the bee gut community. Each compound exerts specific selection pressures, resulting in different microbial profiles. The observed patterns also emphasize the importance of integrating taxonomic insights with diversity metrics to capture the potential ecological impact of chemical exposure.

5.3 Alpha diversity analysis

The alpha diversity indices revealed distinct patterns among the experimental groups. Variations in the richness and evenness of the microbial communities were observed, as indicated by the Chao1, Shannon and Simpson indices. The results are illustrated in the boxplots (Figures 2–4) and summarized in Table 8, allowing a direct comparison of the microbial diversity between the groups. Tables 5-7 shows the H statistic, p-values, and FDR-adjusted q-values used to evaluate statistical significance.

The Chao1 richness index revealed notable differences in microbial richness across the experimental groups (Figure 2). The acetone-treated group exhibited the highest richness (10.3 ± 3.37), clearly standing out from the others. In contrast, the groups treated with FPF and TFL showed lower and similar richness values (6.42 ± 1.24 and 6.46 ± 1.27, respectively), comparable to the control group (6.83 ± 1.19). These results suggest that acetone, used as a solvent for pesticides, may have contributed to an increase in microbial richness within the gut community of *Apis mellifera iberiensis*.

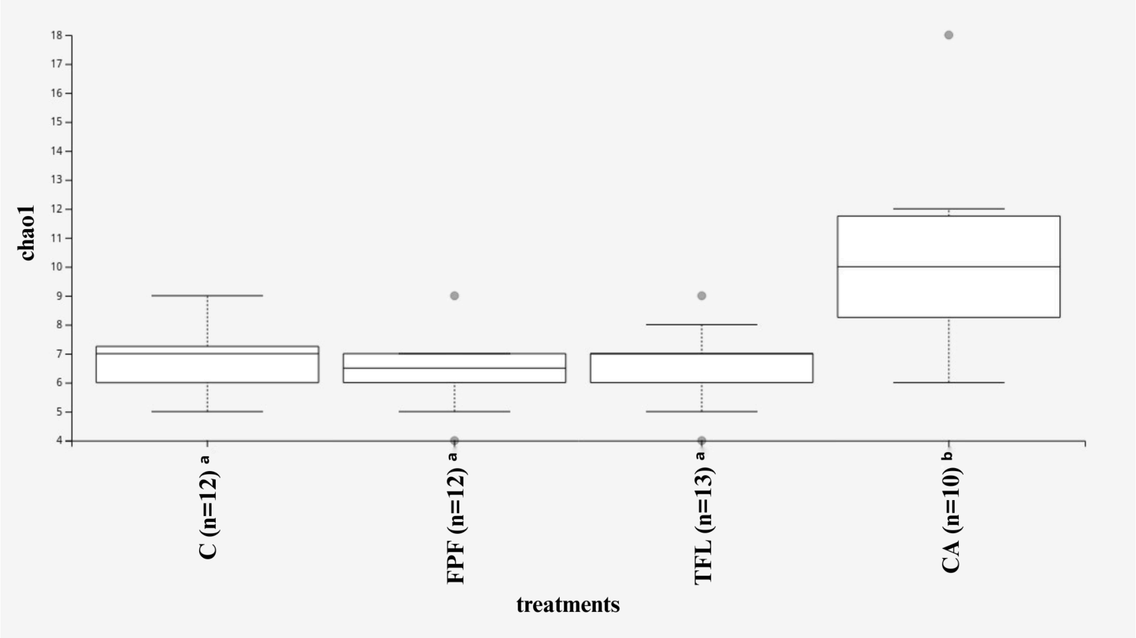

Figure 2- Boxplot of Chao1 index of the gut microbiota of *Apis mellifera iberiensis* across treatment groups (C = untreated control, FPF = flupyradifurone, TFL = tau-fluvalinate, CA = solvent control treated with acetone). The central line represents the median; boxes indicate the interquartile range (IQR), and whiskers extend to 1.5× IQR. Outliers are shown as individual dots. Different lowercase letters (a, b) within the same row indicate statistically significant differences between treatment groups ($p < 0.05$; Kruskal–Wallis test).

This increase could be related to a stress-induced effect or a selective alteration of less abundant taxa, resulting in a greater number of observed or predicted taxa. Similar trends of elevated microbial richness under chemical stressors have been reported in honey bees (EFSA SCIENTIFIC COMMITTEE, et al., 2021), suggesting a compensatory expansion of rare taxa or microbial imbalance.

On the other hand, the similar richness observed in the control, FPF, and TFL treated groups may indicate that these pesticide exposures, under the tested conditions, did not significantly affect the richness component of alpha diversity. Previous studies also found limited impact of certain pesticide treatments on richness, despite significant shifts in composition or evenness (CHMIEL et al., 2020).

In Table 5, the acetone-treated group differed significantly from both the control group and the FPF treated group, with higher richness observed in the acetone group, reinforcing the results seen in the boxplot. This finding may be associated with dysbiosis induced by acetone exposure, a phenomenon rarely investigated in bees but previously reported in microbial communities subjected to solvent treatments, where alterations in microbial richness and membrane integrity have been observed (DYRDA et al., 2019).

The H statistics were also highest in comparisons involving the acetone group, supporting the idea of significant variation. Other comparisons did not show significant differences (q > 0.5), indicating a relatively stable richness under those conditions.

Table 5- Kruskal–Wallis pairwise comparisons of Chao1 richness index across treatment groups (FPF = flupyradifurone, TFL = tau-fluvalinate, C = untreated control, CA = solvent control treated with acetone). Asterisks indicate statistically significant differences between groups (p < 0.05; q < 0.05).

	H	**p-value**	**q-value**
C vs FPF	0.824	0.364	0.546
C vs TFL	0.389	0.533	0.639
C vs CA	8.258	0.004*	0.008*
FPF vs TFL	0.082	0.775	0.775
FPF vs CA	9.929	0.002*	0.005*
TFL vs CA	9.707	0.002*	0.005*

Shannon entropy index which takes into account both richness and evenness of microbial communities, showed moderate differences between the experimental groups (Figure 3). The FPF treated group had the highest average diversity (1.33 ± 0.56), while the control group followed closely behind (1.32 ± 0.47). These values point to a reasonably intact and diverse microbial community structure in both scenarios. In comparison, the TFL treated group had the lowest average Shannon index with 1.11 ± 0.53, suggesting lower diversity and increased dominance of fewer taxa.

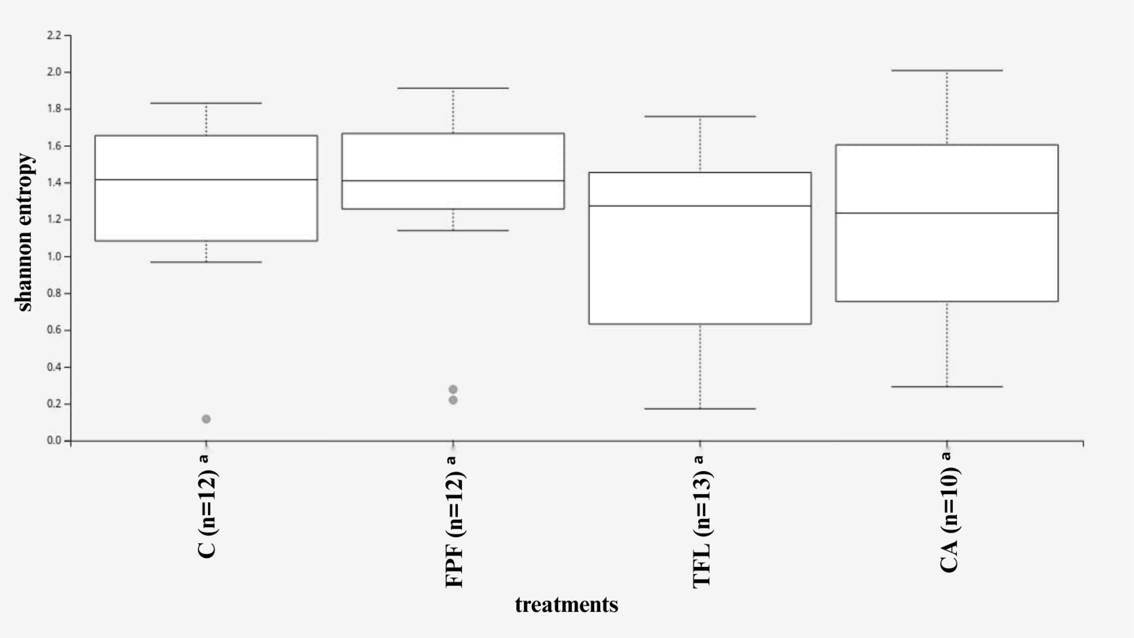

Figure 3- Boxplot of Shannon entropy of the gut microbiota of *Apis mellifera iberiensis* across treatment groups (C = untreated control, FPF = flupyradifurone, TFL = tau-fluvalinate, CA = solvent control treated with acetone). The central line represents the median; boxes indicate the interquartile range (IQR), and whiskers extend to 1.5× IQR. Outliers are shown as individual dots. Groups sharing the same letter are not significantly different ($p > 0.05$, Kruskal–Wallis test).

The acetone treated group is also noteworthy as they demonstrated increased observation of dominant taxa, with lower average diversity of 1.17 ± 0.59. This suggests that the solvent employed for pesticide dilution may have further altered the balance within the microbial community. While these shifts do not appear to be absolute, the TFL and acetone groups do suggest decreased diversity indicative of a potential stress response or selective suppression of certain taxa.

The Shannon index pairwise comparisons (Table 6) showed no statistically significant differences between any treatment groups ($q > 0.80$). The H values were low across all comparisons and the corresponding p-values were far from the significance threshold. These results suggest that, although richness might vary due to treatment (as shown by Chao1), the overall balance in taxa abundance distributions remained relatively stable. This stability indicates that the microbial communities retained a comparable diversity structure, even under chemical exposure.

Table 6- Kruskal–Wallis pairwise comparisons of Shannon diversity index across treatment groups (FPF = flupyradifurone, TFL = tau-fluvalinate, C = untreated control, CA = solvent control treated with acetone).

	H	**p-value**	**q-value**
C vs FPF	0.120	0.729	0.804
C vs TFL	0.959	0.327	0.804
C vs CA	0.156	0.692	0.804
FPF vs TFL	1.183	0.277	0.804
FPF vs CA	0.278	0.598	0.804
TFL vs CA	0.061	0.804	0.804

The calculation using Simpson's index showed similar results across all treatment groups with some variation (Figure 4). However, the FPF treatment group had the highest mean Simpson value of 0.50 ± 0.22, while the control group had a value of 0.49 ± 0.18. Both values indicate a moderately balanced dominance distribution in the microbial community. These results show that both treatment groups sustained a relatively balanced microbial profile without extreme dominance from a single group.

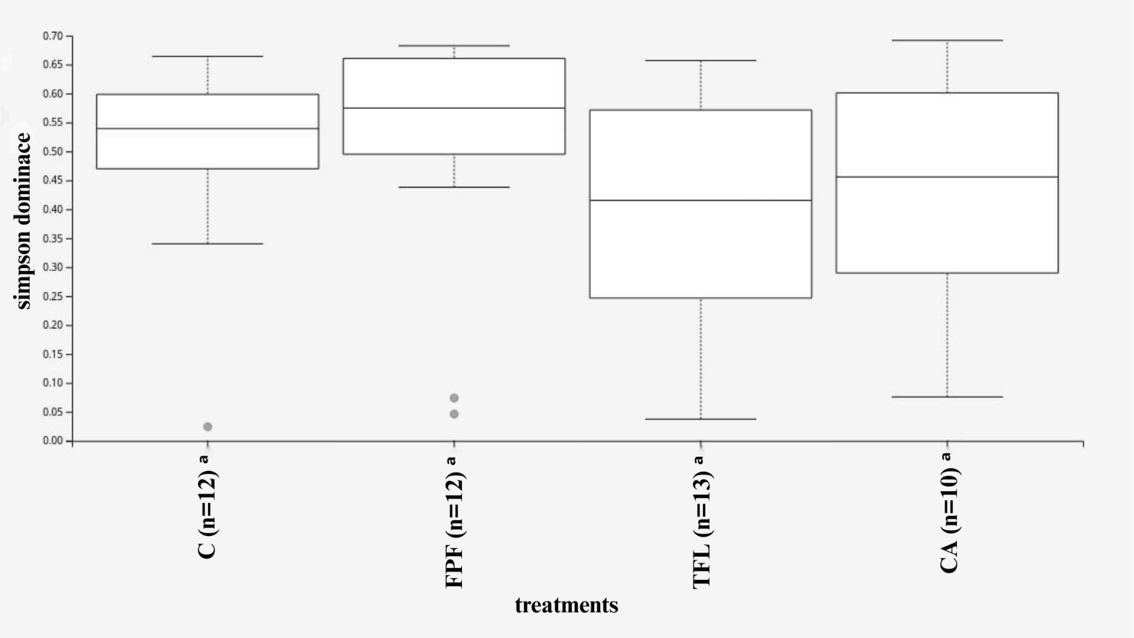

Figure 4- Boxplot of Simpson dominance index of the gut microbiota of *Apis mellifera iberiensis* across treatment groups (C = untreated control, FPF = flupyradifurone, TFL = tau-fluvalinate, CA = solvent control treated with acetone). The central line represents the median; boxes indicate the interquartile range (IQR), and whiskers extend to 1.5× IQR. Outliers are shown as individual dots. Groups sharing the same letter are not significantly different ($p > 0.05$, Kruskal–Wallis test).

On the other hand, the group treated with TFL had the lowest value of the Simpson index (0.40 ± 0.20), indicating an increase in dominance by specific taxa with a relative decrease in evenness. The acetone treated group also showed a decrease in the dominance index (0.42 ± 0.22). These reductions can imply that both TFL and acetone treatments disturbed the natural balance of the microbial community, possibly allowing a few taxa to proliferate disproportionately.

The pairwise comparisons for Simpson's index results (Table 7) further support the notion of stability in microbial dominance patterns across treatments. No comparisons reached statistical significance ($q > 0.42$), and H values remained modest (maximum $H = 3.03$ for FPF vs. TFL), suggesting only minor variations in the degree of taxa dominance. This index, which is sensitive to highly dominant taxa, did not indicate an increased prevalence of single dominant genera in any treatment group.

Table 7- Kruskal–Wallis pairwise comparisons of Simpson dominance index across treatment groups (FPF = treated with flupyradifurone, TFL = treated with tau-fluvalinate, C = untreated control, CA = solvent control treated with acetone).

	H	**p-value**	**q-value**
C vs FPF	0.563	0.453	0.612
C vs TFL	1.704	0.192	0.575
C vs CA	0.435	0.510	0.612
FPF vs TFL	3.030	0.082	0.490
FPF vs CA	0.626	0.429	0.612
TFL vs CA	0.138	0.710	0.710

Table 8 presents the summary statistics for the alpha diversity indices (Chao1, Shannon entropy, and Simpson dominance) calculated for each experimental group. These numerical results complement the visual trends observed in the boxplots.

Table 8- Mean ± Standard Deviation of Alpha Diversity Metrics, allowing a clearer comparison of microbial richness, diversity, and dominance across treatments (FPF = treated with flupyradifurone, TFL = treated with tau-fluvalinate, C = untreated control, CA = solvent control treated with acetone).

Treatment groups	**Chao1**	**Shannon**	**Simpson**
C	6.83 ± 1	1.32 ± 0.5	0.49 ± 0.2
CA	10.3 ± 3	1.17 ± 0.6	0.42 ± 0.2
FPF	6.42 ± 1	1.33 ± 0.6	0.50 ± 0.2
TFL	6.46 ± 1	1.11 ± 0.5	0.40 ± 0.2

Alpha diversity analyses revealed significant variations in the richness and evenness of gut microbial communities among the experimental groups. The Chao1 index indicated that the acetone treated group had the highest microbial richness, surpassing the untreated, FPF treated, and TFL treated groups. This increase in richness may be attributed to a stress effect induced by acetone, leading to the proliferation of less abundant taxa or the introduction of opportunistic microorganisms. Previous studies suggest that organic solvents, such as acetone, can disrupt the gut microbiota, promoting imbalances in the microbial community (DESCLOS LE PELEY et al., 2024).

The Shannon index showed that the FPF-treated group had the highest diversity, followed by the untreated group. In contrast, the TFL and acetone treated groups exhibited lower diversity. These results suggest that FPF may have a less disruptive impact on the gut microbial community structure. Recent studies indicate that FPF, although a systemic insecticide, may have limited sublethal effects on the gut microbiota of honey bees when compared to other pesticides (AL NAGGAR et al., 2022). However, it is important to note that FPF may still affect the abundance of certain taxa, such as the observed increase in *Bifidobacterium* in FPF treated bees.

The Simpson index revealed that the group treated with FPF had the highest value, indicating a more balanced community with no predominance of a few taxa. On the other hand, the group treated with TFL had the lowest value, suggesting a greater dominance of certain species and, therefore, less uniformity. The group treated with acetone also had a reduced value, reinforcing the hypothesis that acetone can cause imbalances in the microbial community. Exposure to TFL, a widely used acaricide, has been associated with changes in the composition of the intestinal microbiota of bees, including the reduction of beneficial taxa such as *Snodgrassella alvi* and *Gilliamella apicola*, essential for the digestion and immunity of bees (KAKUMANU et al., 2016), as observed in the present study.

5.4 Beta diversity analysis

The beta diversity analysis provided insights into the differences in microbial community composition between the experimental groups. Using the Bray-Curtis dissimilarity and Jaccard distance metrics, principal coordinates analysis (PCoA) plots (Figures 5 and 6) and intra and inter-group distances (Tables 9 and 10) were generated to visualize the degree of similarity or divergence among samples. These visualizations enabled the assessment of whether the pesticide treatments and the acetone control caused measurable shifts in the structure of the gut microbiota of *Apis mellifera iberiensis*.

The Bray–Curtis PCoA plot (Figure 5) reveals two main clusters of samples; however, these clusters are composed of individuals from different treatment groups, with no clear separation between them. This pattern suggests that the treatment alone does not fully explain the variation in microbial community structure. The observed distribution may reflect high interindividual variability in gut microbiota composition, potentially influenced by factors such as initial microbiota differences, individual responses to chemical exposure, or unmeasured variables like colony genetics or environmental conditions. The acetone treated samples (blue) form a distinct and dispersed cluster, with greater variation along the two main axes, suggesting greater interindividual variability and differences. On the other hand, samples from the TFL (green) and FPF (orange) groups tend to cluster closer together, indicating relatively more similar microbial compositions within each treatment. The untreated group (red) presents a moderate dispersion, partially overlapping with the FPF and TFL samples, but clearly differentiating from the acetone-treated samples.

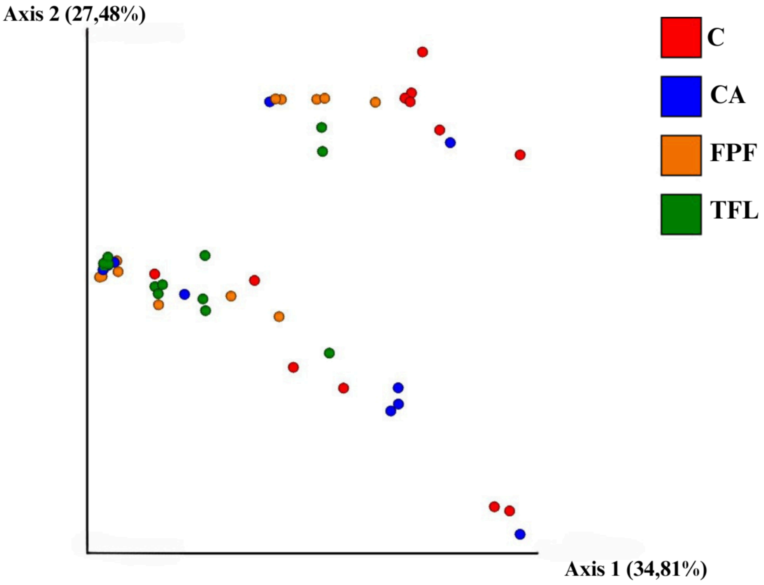

Figure 5- Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity. Each point represents a sample of *Apis mellifera iberiensis*, and colors indicate the treatment groups (C = untreated control, FPF = flupyradifurone, TFL = tau-fluvalinate, CA = solvent control treated with acetone). The first two axes explain 34.81% and 27.48% of the total variation in microbial community structure, respectively.

The table of Bray-Curtis distances (Table 9) reinforces the visual trends observed in the PCoA. The acetone group exhibited the highest intra-group dissimilarity (mean = 0.85), indicating great variability among individuals exposed only to the solvent. In contrast, the TFL group showed the lowest intra-group distance (mean = 0.33), suggesting a more consistent microbial profile within this group. When analyzing inter-group distances, the most pronounced dissimilarities were between the acetone group and all others, particularly acetone vs TFL (mean = 0.98) and acetone vs control (mean = 0.95).

Table 9- Mean and Standard Deviation of Intra and inter-group Bray-Curtis distances (FPF = treated with flupyradifurone, TFL = treated with tau-fluvalinate, C = untreated control, CA = solvent control treated with acetone).

	Mean	**Std Dev**
FPF	0.44	0.2
TFL	0.33	0.2
C	0.52	0.2
CA	0.85	0.3
FPF vs TFL	0.42	0.1
FPF vs C	0.55	0.1
CA vs FPF	0.95	0.1
C vs TFL	0.54	0.1
CA vs TFL	0.98	0.03
CA vs C	0.95	0.06

Based on the beta diversity analysis using the Jaccard index, it was observed that the clusters in the PCoA plot (Figure 6) did not present very clear separations between treatments, although there was a slight tendency for the samples treated with TFL to cluster. This observation suggests that, although the microbial compositions share many taxa between the groups, there are subtle variations in the profiles present in each treatment. The considerable dispersion of the samples in the three-dimensional space indicates considerable intra-group variability, especially between the control and acetone treated groups.

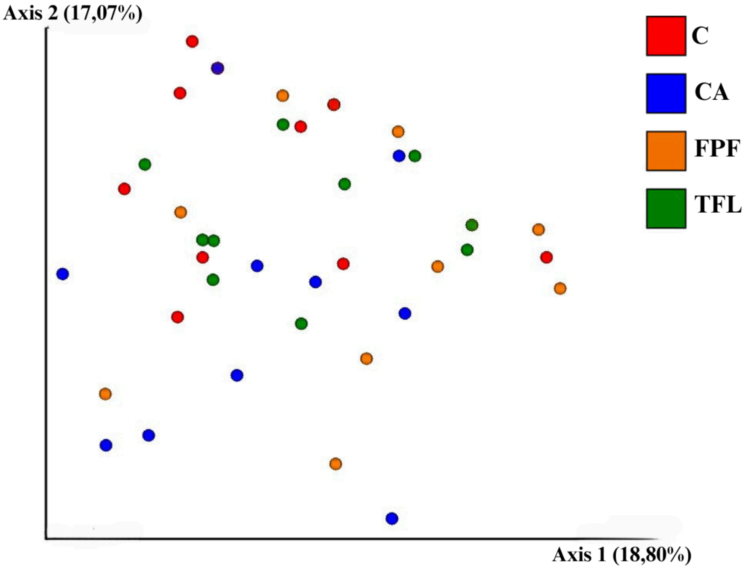

Figure 6- Principal Coordinates Analysis (PCoA) based on Jaccard dissimilarity. Each point represents a sample of *Apis mellifera iberiensis*, and colors correspond to the treatment groups (C = untreated control, FPF = flupyradifurone, TFL = tau-fluvalinate, CA = solvent control treated with acetone).The first two axes explain 18,80% and 17,07% of the total variation in microbial community structure, respectively.

The results of the Jaccard distance matrix (Table 10) reinforce this interpretation. The greatest intra-group dissimilarity was observed in the samples treated with acetone (mean = 0.551), indicating a large variation in the microbial composition within this group. In contrast, the TFL group presented the smallest intra-group distance (mean = 0.359), suggesting greater uniformity among the samples. The largest inter-group distances were recorded between the FPF and Acetone groups (0.541), as well as between TFL and Acetone (0.511), revealing that the acetone treatment induced marked changes in the microbial composition when compared to the pesticides themselves.

Table 10- Mean and Standard Deviation of Intra and inter-group Jaccard distances (FPF = treated with flupyradifurone, TFL = treated with tau-fluvalinate, C = untreated control, CA = solvent control treated with acetone).

	Mean	**Std Dev**
FPF	0.47	0.2
TFL	0.36	0.1
C	0.37	0.2
CA	0.55	0.1
FPF vs TFL	0.43	0.1
FPF vs C	0.45	0.2
CA vs FPF	0.54	0.1
C vs TFL	0.38	0.1
CA vs TFL	0.51	0.1
CA vs C	0.50	0.1

The Bray–Curtis and Jaccard indices not only showed treatment-level clustering but also strong variability in groups, especially for the bees exposed to acetone. The high variation of this group in the Bray-Curtis PCoA is in line with the chemical stress-induced dysbiosis hypothesis of a destabilized microbial system (MA et al., 2020; DESCLOS LE PELEY et al., 2024).

For intra-group variation, the greatest distance was recorded in the acetone group, suggesting that this group had the greatest variability in community composition. This acetone variability could be linked to the solvent's indiscriminate perturbation of gut ecological niches, leading to distinct microbial adaptive strategies. In comparison, the lowest intra-group distance was found in the TFL group, implying that tau-fluvalinate may have a more uniform restraining influence, thus driving the system towards a streamlined but stable microbiota. These results are in line with findings by Kakumanu et al. (2016) who showed that exposure to TFL reduced beneficial taxa, altering the community structure and functional redundancy.

The observations made were complemented by the Jaccard index, which focuses on presence/absence instead of abundance. The acetone group again showed the highest intra-group dissimilarity, while the TFL group displayed the lowest. The FPF and control groups clustered more closely, reinforcing the idea that FPF, under the tested conditions, preserved a greater portion of the natural microbial landscape. Additionally, the inter-group comparisons showed that acetone treatment induced greater divergence than either pesticide alone. The highest dissimilarities were noted with the acetone and FPF or TFL groups.

These findings collectively suggest that acetone is not microbiologically inert and should be carefully considered in experimental design, especially when interpreting sublethal effects of pesticides. Additionally, TFL's suppressive pattern contrasts with FPF's apparently neutral or slightly supportive effect on gut microbial integrity. This reinforces the need for multi-metric diversity analysis, as each index contributes distinct layers of ecological insight.

For detailed consultation of the data derived from the microbial diversity analyses, the distance matrices and alpha diversity tables are included in the Appendices.

6. CONCLUSIONS

This study investigated the effects of the insecticide flupyradifurone (FPF), the acaricide tau-fluvalinate (TFL), and the solvent acetone on the gut microbiota of *Apis mellifera iberiensis* worker bees. Through full-length 16S rRNA sequencing and comprehensive ecological analyses, the results revealed that both the active substances and the solvent can alter the gut microbial composition which may have a significant impact on bee health.

Alpha diversity analysis showed variations in richness and evenness among the treatment groups. The acetone group presented the highest microbial richness, suggesting a stress-induced disruption in microbial balance. Conversely, the TFL group exhibited the lowest diversity and evenness, indicating increased dominance of specific taxa. Beta diversity metrics (Bray-Curtis and Jaccard) confirmed distinct microbial community profiles between the groups, particularly highlighting the divergence of acetone exposed honey bees from the others.

The analysis of the taxonomic composition indicated significant changes across different treatments. In honey bees treated with pesticides, *Lactobacillus* was identified as the prevailing genus, hinting at a potential adaptive reaction to chemical stressors. Both *Bartonella* and *Snodgrassella*, key components of the core microbiota, were found to be significantly diminished in the TFL and acetone groups, raising alarms about their susceptibility. There was a considerable rise in *Gilliamella* in bees receiving acetone treatment, whereas *Bifidobacterium* seemed to proliferate under pesticide influence, potentially serving as a compensatory response. These changes illustrate that each chemical, whether it is the active substance or the solvent, exerts unique selective influences on the microbial population.

Collectively, these results highlight that solvents frequently utilized in pesticide mixtures, including acetone, are not inactive from a microbiological standpoint and can disrupt the natural gut microbiota similarly or even more than the pesticides. Furthermore, although FPF displayed relatively milder effects, TFL was linked to a negative impact on the diversity and stability of microbial communities.

Understanding how these agrochemicals affect the gut microbiota is essential, as these microbial communities play crucial roles in digestion, immunity, and protection against pathogens in bees. Disruptions in this system may compromise colony health and reduce pollination efficiency, posing risks not only to apiculture but also to broader agricultural sustainability.

Future studies should build on these results by investigating the functional consequences of the noted changes, beginning by expanding the analysis to include functional predictions or metatranscriptomic profiling, which would provide insights not only into which taxa are affected, but also how their metabolic capabilities and functional roles are altered by exposure to pesticides and solvents. This would help clarify whether observed taxonomic shifts translate into functional dysbiosis or compensatory microbial responses.

Additionally, long-term studies assessing the temporal stability and recovery of the gut microbiota after chemical exposure would provide critical information about the resilience of these microbial communities. Such studies could clarify whether the dysbiosis is transient or persistent, and whether recovery is influenced by the type or concentration of the chemical.

Investigating the impact of various solvents utilized in pesticide mixtures would also be beneficial, as the current study indicates that acetone alone can markedly change gut microbiota. Comparative research might help in identifying safer ingredients that cause less disturbance to microbial communities.

Lastly, merging microbiota information with host physiological and behavioral metrics, such as immune gene expression, metabolic state, and foraging habits, could clarify the mechanistic connections between microbiota disruption and the decline in bee health. Such comprehensive strategies are crucial for formulating evidence-based regulations for pesticide safety and fostering sustainable agricultural methods that protect pollinator well-being.

This research provides meaningful contributions to the understanding of how commonly used agrochemicals and their solvents influence the gut microbiota of *Apis mellifera iberiensis*. By combining ecological diversity metrics with taxonomic insights, this work highlights the often-overlooked impact of sublethal exposures on microbial homeostasis, a key element of bee health. The results emphasize the importance of evaluating both the active substances and formulation elements in ecotoxicological evaluations, providing a scientific foundation for developing more sustainable agricultural practices. In the end, this research contributes to the broader effort of protecting pollinator populations and ensuring the long-term stability of the ecosystems and food systems that depend on them.

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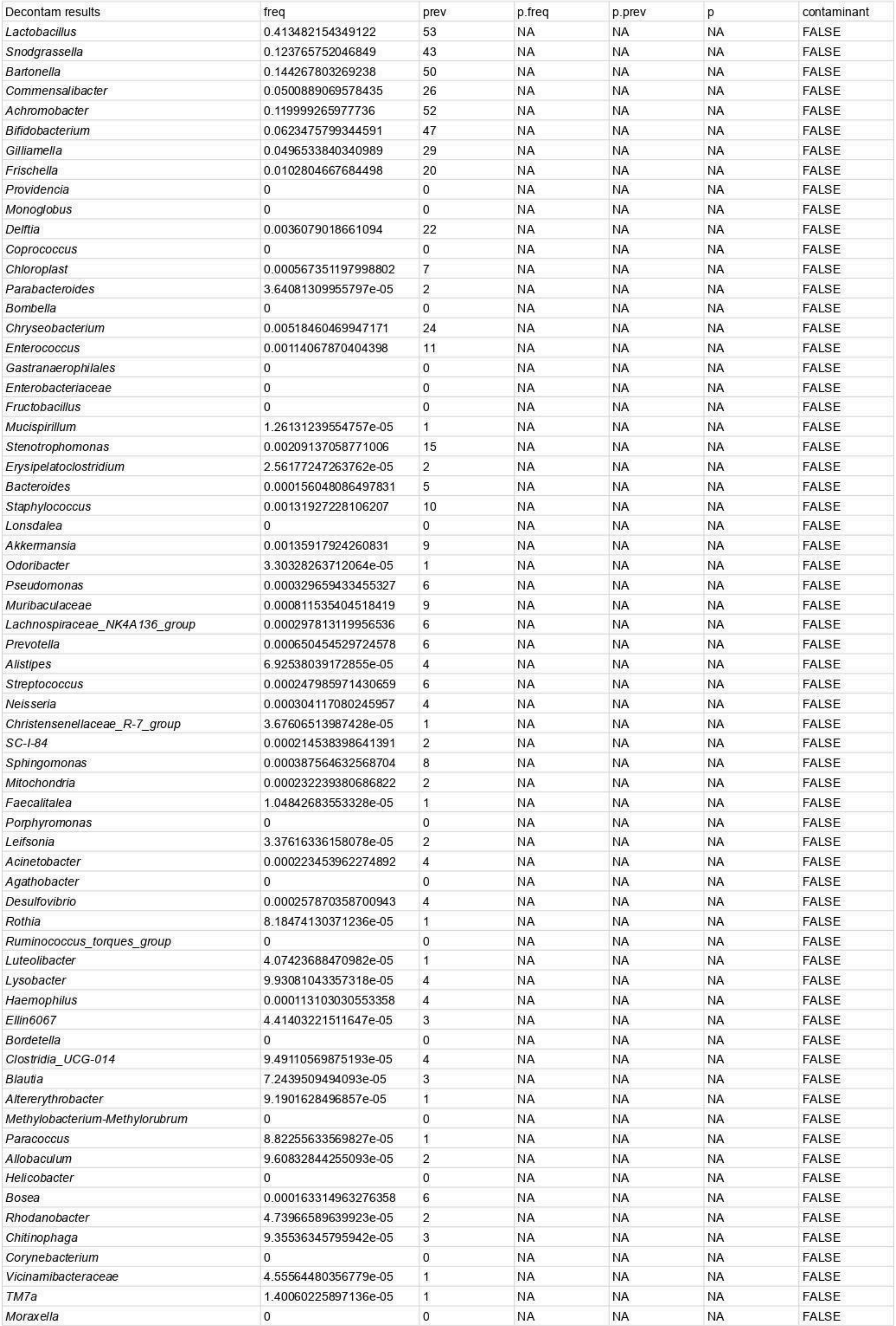
APPENDICES



Decontam results	freq	prev	p.freq	p.prev	p	contaminant
Lactobacillus	0.413482154349122	53	NA	NA	NA	FALSE
Snodgrassella	0.123765752046849	43	NA	NA	NA	FALSE
Bartonella	0.144267803269238	50	NA	NA	NA	FALSE
Commensalibacter	0.0500889069578435	26	NA	NA	NA	FALSE
Achromobacter	0.119999265977736	52	NA	NA	NA	FALSE
Bifidobacterium	0.0623475799344591	47	NA	NA	NA	FALSE
Gilliamella	0.0496533840340989	29	NA	NA	NA	FALSE
Frischella	0.0102804667684498	20	NA	NA	NA	FALSE
Providencia	0	0	NA	NA	NA	FALSE
Monoglobus	0	0	NA	NA	NA	FALSE
Delftia	0.0036079018661094	22	NA	NA	NA	FALSE
Coprococcus	0	0	NA	NA	NA	FALSE
Chloroplast	0.000567351197998802	7	NA	NA	NA	FALSE
Parabacteroides	3.64081309955797e-05	2	NA	NA	NA	FALSE
Bombella	0	0	NA	NA	NA	FALSE
Chryseobacterium	0.00518460469947171	24	NA	NA	NA	FALSE
Enterococcus	0.00114067870404398	11	NA	NA	NA	FALSE
Gastranaerophilales	0	0	NA	NA	NA	FALSE
Enterobacteriaceae	0	0	NA	NA	NA	FALSE
Fructobacillus	0	0	NA	NA	NA	FALSE
Mucispirillum	1.26131239554757e-05	1	NA	NA	NA	FALSE
Stenotrophomonas	0.00209137058771006	15	NA	NA	NA	FALSE
Erysipelatoclostridium	2.56177247263762e-05	2	NA	NA	NA	FALSE
Bacteroides	0.000156048086497831	5	NA	NA	NA	FALSE
Staphylococcus	0.00131927228106207	10	NA	NA	NA	FALSE
Lonsdalea	0	0	NA	NA	NA	FALSE
Akkermansia	0.00135917924260831	9	NA	NA	NA	FALSE
Odoribacter	3.30328263712064e-05	1	NA	NA	NA	FALSE
Pseudomonas	0.000329659433455327	6	NA	NA	NA	FALSE
Muribaculaceae	0.000811535404518419	9	NA	NA	NA	FALSE
Lachnospiraceae_NK4A136_group	0.000297813119956536	6	NA	NA	NA	FALSE
Prevotella	0.000650454529724578	6	NA	NA	NA	FALSE
Alistipes	6.92538039172855e-05	4	NA	NA	NA	FALSE
Streptococcus	0.000247985971430659	6	NA	NA	NA	FALSE
Neisseria	0.000304117080245957	4	NA	NA	NA	FALSE
Christensenellaceae_R-7_group	3.67606513987428e-05	1	NA	NA	NA	FALSE
SC-I-84	0.000214538398641391	2	NA	NA	NA	FALSE
Sphingomonas	0.000387564632568704	8	NA	NA	NA	FALSE
Mitochondria	0.000232239380686822	2	NA	NA	NA	FALSE
Faecalitalea	1.04842683553328e-05	1	NA	NA	NA	FALSE
Porphyromonas	0	0	NA	NA	NA	FALSE
Leifsonia	3.37616336158078e-05	2	NA	NA	NA	FALSE
Acinetobacter	0.000223453962274892	4	NA	NA	NA	FALSE
Agathobacter	0	0	NA	NA	NA	FALSE
Desulfovibrio	0.000257870358700943	4	NA	NA	NA	FALSE
Rothia	8.18474130371236e-05	1	NA	NA	NA	FALSE
Ruminococcus_torques_group	0	0	NA	NA	NA	FALSE
Luteolibacter	4.07423688470982e-05	1	NA	NA	NA	FALSE
Lysobacter	9.93081043357318e-05	4	NA	NA	NA	FALSE
Haemophilus	0.000113103030553358	4	NA	NA	NA	FALSE
Ellin6067	4.41403221511647e-05	3	NA	NA	NA	FALSE
Bordetella	0	0	NA	NA	NA	FALSE
Clostridia_UCG-014	9.49110569875193e-05	4	NA	NA	NA	FALSE
Blautia	7.2439509494093e-05	3	NA	NA	NA	FALSE
Altererythrobacter	9.1901628496857e-05	1	NA	NA	NA	FALSE
Methylobacterium-Methylorubrum	0	0	NA	NA	NA	FALSE
Paracoccus	8.82255633569827e-05	1	NA	NA	NA	FALSE
Allobaculum	9.60832844255093e-05	2	NA	NA	NA	FALSE
Helicobacter	0	0	NA	NA	NA	FALSE
Bosea	0.000163314963276358	6	NA	NA	NA	FALSE
Rhodanobacter	4.73966589639923e-05	2	NA	NA	NA	FALSE
Chitinophaga	9.35536345795942e-05	3	NA	NA	NA	FALSE
Corynebacterium	0	0	NA	NA	NA	FALSE
Vicinamibacteraceae	4.55564480356779e-05	1	NA	NA	NA	FALSE
TM7a	1.40060225897136e-05	1	NA	NA	NA	FALSE
Moraxella	0	0	NA	NA	NA	FALSE

SWB02	4.20437465182522e-05	1	NA	NA	NA	FALSE
Veillonella	0.000129536356953297	3	NA	NA	NA	FALSE
Faecalibaculum	6.92699568078347e-05	3	NA	NA	NA	FALSE
Gemmatimonas	4.06292157081803e-05	3	NA	NA	NA	FALSE
Tepidisphaerales	4.55564480356779e-05	1	NA	NA	NA	FALSE
Leptotrichia	8.27112488724844e-05	2	NA	NA	NA	FALSE
Clostridium_sensu_stricto_10	0	0	NA	NA	NA	FALSE
Saccharimonadales	2.54791351703488e-05	2	NA	NA	NA	FALSE
WD2101_soil_group	1.43862467481088e-05	1	NA	NA	NA	FALSE
Gemella	8.26906618813559e-05	4	NA	NA	NA	FALSE
Terrimonas	3.02800908402725e-05	1	NA	NA	NA	FALSE
env.OPS_17	0	0	NA	NA	NA	FALSE
Flavisolibacter	5.69224178989958e-05	2	NA	NA	NA	FALSE
Eubacterium_coprostanoligenes_group	2.20218842474709e-05	1	NA	NA	NA	FALSE
Noviherbaspirillum	3.30328263712064e-05	1	NA	NA	NA	FALSE
AKAU4049	0	0	NA	NA	NA	FALSE
Prevotellaceae_UCG-001	1.92691487165371e-05	1	NA	NA	NA	FALSE
Capnocytophaga	3.64536984421405e-05	1	NA	NA	NA	FALSE
Flavobacterium	4.0761032452975e-05	1	NA	NA	NA	FALSE
Faecalibacterium	2.57324559791199e-05	1	NA	NA	NA	FALSE
Parasutterella	0	0	NA	NA	NA	FALSE
Fusicatenibacter	0	0	NA	NA	NA	FALSE
LWQ8	0	0	NA	NA	NA	FALSE
Eubacterium_siraeum_group	0	0	NA	NA	NA	FALSE
Pirellula	1.37636776546693e-05	1	NA	NA	NA	FALSE
Filifactor	0	0	NA	NA	NA	FALSE
Paenarthrobacter	4.40437684949418e-05	1	NA	NA	NA	FALSE
MND1	6.07735350009472e-05	3	NA	NA	NA	FALSE
Clostridium_sensu_stricto_1	1.19885389567573e-05	1	NA	NA	NA	FALSE
Prosthecobacter	0	0	NA	NA	NA	FALSE
Sphingobium	3.57855619021403e-05	1	NA	NA	NA	FALSE
Flavitalea	1.05109366295631e-05	1	NA	NA	NA	FALSE
Rikenellaceae_RC9_gut_group	2.32685530101536e-05	2	NA	NA	NA	FALSE
Nitrospira	5.56929790529049e-05	2	NA	NA	NA	FALSE
Lapillicoccus	0	0	NA	NA	NA	FALSE
Lacunisphaera	3.35679090789206e-05	1	NA	NA	NA	FALSE
Arthrobacter	5.14649119582399e-05	1	NA	NA	NA	FALSE
Bacillus	0	0	NA	NA	NA	FALSE
Chujaibacter	3.85382974330741e-05	1	NA	NA	NA	FALSE
NB1-j	0	0	NA	NA	NA	FALSE
Anaerotruncus	0	0	NA	NA	NA	FALSE
Arenimonas	5.44849293715201e-05	2	NA	NA	NA	FALSE
Novosphingobium	0	0	NA	NA	NA	FALSE
Terrisporobacter	2.58973908378731e-05	1	NA	NA	NA	FALSE
Iamia	2.15793701221632e-05	1	NA	NA	NA	FALSE
TM7	5.14649119582399e-05	1	NA	NA	NA	FALSE
Chryseolinea	0	0	NA	NA	NA	FALSE
Microlunatus	0	0	NA	NA	NA	FALSE
Unknown_Family	4.77888468183656e-05	1	NA	NA	NA	FALSE
TRA3-20	3.57855619021403e-05	1	NA	NA	NA	FALSE
Parafilimonas	0	0	NA	NA	NA	FALSE
Romboutsia	2.39937011765118e-05	2	NA	NA	NA	FALSE
Peptostreptococcus	2.78763576322251e-05	1	NA	NA	NA	FALSE
Chthoniobacter	7.2546213300883e-05	2	NA	NA	NA	FALSE
Escherichia-Shigella	0.000126303090802242	5	NA	NA	NA	FALSE
Opitutus	4.77888468183656e-05	1	NA	NA	NA	FALSE
vadinHA49	0	0	NA	NA	NA	FALSE
Pedobacter	2.20563908392457e-05	1	NA	NA	NA	FALSE
Frateuria	0	0	NA	NA	NA	FALSE
Subgroup_10	3.57855619021403e-05	1	NA	NA	NA	FALSE
Alloprevotella	8.82893791279575e-05	3	NA	NA	NA	FALSE
RF39	7.33648032282108e-05	3	NA	NA	NA	FALSE
NS9_marine_group	2.87724934962176e-05	1	NA	NA	NA	FALSE
Pseudoflavitalea	0	0	NA	NA	NA	FALSE
Luteimonas	3.30328263712064e-05	1	NA	NA	NA	FALSE
Comamonas	1.40060225897136e-05	1	NA	NA	NA	FALSE

Blfdi19	4.41127816784913e-05	1	NA	NA	NA	FALSE
Fimbriiglobus	4.41127816784913e-05	1	NA	NA	NA	FALSE
Inquilinus	0	0	NA	NA	NA	FALSE
Burkholderia-Caballeronia-Paraburkholderia	2.47746197784048e-05	1	NA	NA	NA	FALSE
Massilia	0	0	NA	NA	NA	FALSE
Dubosiella	3.40564759802642e-05	2	NA	NA	NA	FALSE
Aggregatibacter	0	0	NA	NA	NA	FALSE
Pantoea	0	0	NA	NA	NA	FALSE
Candidatus_Arthromitus	0	0	NA	NA	NA	FALSE
Amb-16S-1323	0	0	NA	NA	NA	FALSE
Oscillibacter	5.94076120760725e-05	2	NA	NA	NA	FALSE
Ruminococcus	4.975976085833e-05	2	NA	NA	NA	FALSE
Taibaiella	4.04367165386171e-05	1	NA	NA	NA	FALSE
Polyangium	2.14433520247885e-05	1	NA	NA	NA	FALSE
Family_XIII_UCG-001	0	0	NA	NA	NA	FALSE
Turicibacter	2.62834143498542e-05	2	NA	NA	NA	FALSE
Candidatus_Kaiserbacteria	3.02800908402725e-05	1	NA	NA	NA	FALSE
ADurb.Bin063-1	0	0	NA	NA	NA	FALSE
Gaiella	0	0	NA	NA	NA	FALSE
Selenomonas	3.02800908402725e-05	1	NA	NA	NA	FALSE
Oribacterium	0	0	NA	NA	NA	FALSE
OM190	6.05180068929154e-05	3	NA	NA	NA	FALSE
Olsenella	0	0	NA	NA	NA	FALSE
Ramlibacter	0	0	NA	NA	NA	FALSE
Ellin6055	2.75273553093387e-05	1	NA	NA	NA	FALSE
Rhodopseudomonas	0	0	NA	NA	NA	FALSE
Candidatus_Solibacter	0	0	NA	NA	NA	FALSE
Phenylobacterium	1.28660112148731e-05	1	NA	NA	NA	FALSE
Fimbriimonadales	0	0	NA	NA	NA	FALSE
WS2	0	0	NA	NA	NA	FALSE
Lachnospiraceae_UCG-004	0	0	NA	NA	NA	FALSE
Listeria	1.84981363127665e-05	1	NA	NA	NA	FALSE
Arachidicoccus	2.10218732591261e-05	1	NA	NA	NA	FALSE
Parvimonas	0	0	NA	NA	NA	FALSE
MB-A2-108	2.39770779135147e-05	1	NA	NA	NA	FALSE
Lachnospiraceae_UCG-008	1.92691487165371e-05	1	NA	NA	NA	FALSE
Granulicatella	2.12528558525052e-05	1	NA	NA	NA	FALSE
Treponema	2.39770779135147e-05	1	NA	NA	NA	FALSE
Eubacterium_fissicatena_group	4.94562130838635e-05	3	NA	NA	NA	FALSE
Castellaniella	0	0	NA	NA	NA	FALSE
Meiothermus	2.47746197784048e-05	1	NA	NA	NA	FALSE
Pedosphaeraceae	1.70022846820041e-05	1	NA	NA	NA	FALSE
Hirschia	1.89196859332135e-05	1	NA	NA	NA	FALSE
Atopobium	0	0	NA	NA	NA	FALSE
Variovorax	2.47746197784048e-05	1	NA	NA	NA	FALSE
Brevundimonas	0	0	NA	NA	NA	FALSE
Megasphaera	2.94085211189942e-05	1	NA	NA	NA	FALSE
Rubellimicrobium	2.94085211189942e-05	1	NA	NA	NA	FALSE
Fusobacterium	2.94085211189942e-05	1	NA	NA	NA	FALSE
Roseimicrobium	0	0	NA	NA	NA	FALSE
Intrasporangium	2.94085211189942e-05	1	NA	NA	NA	FALSE
Rokubacteriales	3.3671094805434e-05	2	NA	NA	NA	FALSE
Caulobacter	0	0	NA	NA	NA	FALSE
Mesorhizobium	0	0	NA	NA	NA	FALSE
PLTA13	5.28628610256633e-05	3	NA	NA	NA	FALSE
Elev-16S-573	0	0	NA	NA	NA	FALSE
Gemmata	1.71546816198308e-05	1	NA	NA	NA	FALSE
Nordella	0	0	NA	NA	NA	FALSE
Ellin517	1.68174986073009e-05	1	NA	NA	NA	FALSE
Marvinbryantia	1.71546816198308e-05	1	NA	NA	NA	FALSE
Roseisolibacter	3.08741499075437e-05	2	NA	NA	NA	FALSE
Tahibacter	1.65164131856032e-05	1	NA	NA	NA	FALSE
MM2	2.20218842474709e-05	1	NA	NA	NA	FALSE
Herminiimonas	1.71546816198308e-05	1	NA	NA	NA	FALSE
TK10	2.20218842474709e-05	1	NA	NA	NA	FALSE
Pseudolabrys	0	0	NA	NA	NA	FALSE

Tuzzerella	2.74670247928914e-05	2	NA	NA	NA	FALSE
A0839	0	0	NA	NA	NA	FALSE
Clostridioides	1.5010346417352e-05	1	NA	NA	NA	FALSE
UCG-010	0	0	NA	NA	NA	FALSE
Steroidobacter	0	0	NA	NA	NA	FALSE
Latescibacteraceae	1.92691487165371e-05	1	NA	NA	NA	FALSE
Catonella	1.92691487165371e-05	1	NA	NA	NA	FALSE
Ochrobactrum	1.5010346417352e-05	1	NA	NA	NA	FALSE
IMCC26256	0	0	NA	NA	NA	FALSE
Puia	0	0	NA	NA	NA	FALSE
Longimicrobiaceae	0	0	NA	NA	NA	FALSE
Clostridium_sensu_stricto_12	2.99908247289313e-05	2	NA	NA	NA	FALSE
Lachnospiraceae_NC2004_group	0	0	NA	NA	NA	FALSE
0319-6G20	1.37636776546693e-05	1	NA	NA	NA	FALSE
Candidatus_Gottesmanbacteria	0	0	NA	NA	NA	FALSE
RB41	0	0	NA	NA	NA	FALSE
Haliangium	1.47153112813883e-05	1	NA	NA	NA	FALSE
67-14	0	0	NA	NA	NA	FALSE
Lineage_IIa	0	0	NA	NA	NA	FALSE
Streptomyces	0	0	NA	NA	NA	FALSE
JG36-TzT-191	0	0	NA	NA	NA	FALSE
KF-JG30-B3	0	0	NA	NA	NA	FALSE
Blrii41	1.47153112813883e-05	1	NA	NA	NA	FALSE
Anaerostipes	0	0	NA	NA	NA	FALSE
Acidibacter	0	0	NA	NA	NA	FALSE
MBNT15	0	0	NA	NA	NA	FALSE
Pajaroellobacter	0	0	NA	NA	NA	FALSE
Cutibacterium	3.32384672767402e-05	3	NA	NA	NA	FALSE
Labrys	0	0	NA	NA	NA	FALSE
Candidatus_Alysiosphaera	1.67839545394603e-05	1	NA	NA	NA	FALSE
Muribaculum	0	0	NA	NA	NA	FALSE
Eubacterium_oxidoreducens_group	1.92691487165371e-05	1	NA	NA	NA	FALSE
Singulisphaera	1.26131239554757e-05	1	NA	NA	NA	FALSE
Polaromonas	2.20563908392457e-05	1	NA	NA	NA	FALSE
Dokdonella	1.19885389567573e-05	1	NA	NA	NA	FALSE
Pla4_lineage	1.27517135115031e-05	1	NA	NA	NA	FALSE
Blastococcus	0	0	NA	NA	NA	FALSE
Eubacterium_yurii_group	0	0	NA	NA	NA	FALSE
Sumerlaea	1.5946631938446e-05	1	NA	NA	NA	FALSE
JG30-KF-AS9	0	0	NA	NA	NA	FALSE
Clostridia_vadinBB60_group	1.40309850920783e-05	1	NA	NA	NA	FALSE
Mucilaginibacter	0	0	NA	NA	NA	FALSE
Bdellovibrio	1.43862467481088e-05	1	NA	NA	NA	FALSE
Ferruginibacter	0	0	NA	NA	NA	FALSE
Lachnoclostridium	3.5820068493915e-05	2	NA	NA	NA	FALSE
Pseudarthrobacter	0	0	NA	NA	NA	FALSE
Fimbriimonas	0	0	NA	NA	NA	FALSE
Agromyces	0	0	NA	NA	NA	FALSE
Holophaga	0	0	NA	NA	NA	FALSE
mle1-7	0	0	NA	NA	NA	FALSE
Sorangium	0	0	NA	NA	NA	FALSE
Roseibacillus	1.43862467481088e-05	1	NA	NA	NA	FALSE
Bryobacter	1.65164131856032e-05	1	NA	NA	NA	FALSE
Devosia	1.28660112148731e-05	1	NA	NA	NA	FALSE
Harryflintia	0	0	NA	NA	NA	FALSE
UA11	0	0	NA	NA	NA	FALSE
Prevotellaceae_NK3B31_group	0	0	NA	NA	NA	FALSE
Subgroup_7	1.83803256993714e-05	1	NA	NA	NA	FALSE
Fimbriimonadaceae	2.17326181361297e-05	2	NA	NA	NA	FALSE
AKYH767	0	0	NA	NA	NA	FALSE
Tannerella	0	0	NA	NA	NA	FALSE
Ilumatobacteraceae	0	0	NA	NA	NA	FALSE
Candidatus_Saccharimonas	0	0	NA	NA	NA	FALSE
Dorea	1.1692487576732e-05	1	NA	NA	NA	FALSE
Candidatus_Berkiella	0	0	NA	NA	NA	FALSE
UCG-005	0	0	NA	NA	NA	FALSE

Decontam results

Candidatus_Levybacteria	1.06264279262526e-05	1	NA	NA	NA	FALSE
Rhodopila	1.37636776546693e-05	1	NA	NA	NA	FALSE
Parasegetibacter	0	0	NA	NA	NA	FALSE
JGI_0001001-H03	0	0	NA	NA	NA	FALSE
Ensifer	0	0	NA	NA	NA	FALSE
Nitrosomonas	0	0	NA	NA	NA	FALSE
Lachnospiraceae_UCG-006	2.57522166114267e-05	2	NA	NA	NA	FALSE
CCD24	1.05109366295631e-05	1	NA	NA	NA	FALSE
WCHB1-41	0	0	NA	NA	NA	FALSE
Incertae_Sedis	0	0	NA	NA	NA	FALSE
Duganella	1.06264279262526e-05	1	NA	NA	NA	FALSE
A4b	1.05109366295631e-05	1	NA	NA	NA	FALSE
Candidatus_Woykebacteria	0	0	NA	NA	NA	FALSE
Peptococcus	0	0	NA	NA	NA	FALSE
Colidextribacter	1.83803256993714e-05	1	NA	NA	NA	FALSE
Stomatobaculum	0	0	NA	NA	NA	FALSE
Mogibacterium	1.37636776546693e-05	1	NA	NA	NA	FALSE
Subgroup_22	2.42746142842324e-05	2	NA	NA	NA	FALSE
Ilumatobacter	0	0	NA	NA	NA	FALSE
Roseburia	0	0	NA	NA	NA	FALSE
UBA1819	1.42521020407495e-05	2	NA	NA	NA	FALSE
Family_XIII_AD3011_group	1.45647685705394e-05	2	NA	NA	NA	FALSE
Synechococcus_CC9902	0	0	NA	NA	NA	FALSE
Others	0.00147595031916956	9	NA	NA	NA	FALSE

Chao1

	chao1
IPF11	6.0
IPF13	6.0
IPF14	9.0
IPF21	7.0
IPF22	7.0
IPF23	6.0
IPF24	6.0
IPF25	4.0
IPF31	7.0
IPF32	7.0
IPF34	7.0
IPF35	5.0
IPTA11	6.0
IPTA12	5.0
IPTA13	7.0
IPTA14	8.0
IPTA15	6.0
IPTA21	7.0
IPTA22	7.0
IPTA23	7.0
IPTA24	6.0
IPTA31	4.0
IPTA32	7.0
IPTA33	6.0
IPTA35	9.0
IPCN12	7.0
IPCN13	6.0
IPCN14	7.0
IPCN15	7.0
IPCN21	9.0
IPCN23	7.0
IPCN24	8.0
IPCN25	5.0
IPCN31	7.0
IPCN32	8.0
IPCN34	6.0
IPCN35	5.0
IPCA11	10.0
IPCA12	11.0
IPCA13	9.0
IPCA14	7.0
IPCA24	18.0
IPCA25	6.0
IPCA31	10.0
IPCA32	12.0

Chao1

IPCA34	8.0
IPCA35	12.0

Shannon

	shannon_entropy
IPF11	1.823764
IPF13	1.612787
IPF14	0.219226
IPF21	1.328797
IPF22	1.600627
IPF23	1.904157
IPF24	1.295150
IPF25	1.138906
IPF31	0.277371
IPF32	1.911426
IPF34	1.398717
IPF35	1.419830
IPTA11	1.426850
IPTA12	0.631675
IPTA13	1.695660
IPTA14	1.292508
IPTA15	0.540503
IPTA21	1.201356
IPTA22	1.272604
IPTA23	1.717309
IPTA24	1.758071
IPTA31	0.708813
IPTA32	0.172209
IPTA33	0.541550
IPTA35	1.454096
IPCN12	1.656182
IPCN13	1.335506
IPCN14	1.829883
IPCN15	1.752954
IPCN21	1.494544
IPCN23	1.313619
IPCN24	1.088319
IPCN25	1.066947
IPCN31	0.967552
IPCN32	1.653671
IPCN34	1.593669
IPCN35	0.116918
IPCA11	0.291704
IPCA12	0.444984
IPCA13	1.407348
IPCA14	0.739290
IPCA24	1.669873
IPCA25	1.352412
IPCA31	1.115070
IPCA32	0.799342

Shannon

IPCA34	1.858873
IPCA35	2.007276

Simpson

	functools.partial(<function _simpsons_dominance at 0x7241496bc700>)
IPF11	0.659792
IPF13	0.662687
IPF14	0.046199
IPF21	0.524696
IPF22	0.612776
IPF23	0.682466
IPF24	0.514124
IPF25	0.438062
IPF31	0.073916
IPF32	0.672531
IPF34	0.561802
IPF35	0.587743
IPTA11	0.573596
IPTA12	0.246564
IPTA13	0.571489
IPTA14	0.415324
IPTA15	0.179027
IPTA21	0.408520
IPTA22	0.469062
IPTA23	0.639689
IPTA24	0.657020
IPTA31	0.296173
IPTA32	0.037369
IPTA33	0.191866
IPTA35	0.501390
IPCN12	0.654828
IPCN13	0.497083
IPCN14	0.622616
IPCN15	0.664190
IPCN21	0.531396
IPCN23	0.547218
IPCN24	0.389035
IPCN25	0.500831
IPCN31	0.340388
IPCN32	0.590459
IPCN34	0.577138
IPCN35	0.024183
IPCA11	0.075829
IPCA12	0.110459
IPCA13	0.570554
IPCA14	0.293032
IPCA24	0.611248
IPCA25	0.478799
IPCA31	0.433008
IPCA32	0.288887

IPCA34	0.669836
IPCA35	0.691743

Bray-Curtis

	IPF11	IPF13	IPF14	IPF21	IPF22	IPF23	IPF24	IPF25	IPF31	IPF32	IPF34	IPF35	IPTA11	IPTA12	IPTA13
IPF11	0.000000	0.611220	0.750132	0.633069	0.149311	0.196810	0.621837	0.606529	0.748225	0.560166	0.250047	0.199861	0.541147	0.624026	0.502135
IPF13	0.611220	0.000000	0.618538	0.492898	0.530861	0.563998	0.257653	0.463543	0.636706	0.451453	0.584752	0.527275	0.384509	0.475698	0.464714
IPF14	0.750132	0.618538	0.000000	0.349458	0.650666	0.670006	0.357072	0.315122	0.056380	0.553703	0.533448	0.552039	0.422666	0.143622	0.458943
IPF21	0.633069	0.492898	0.349458	0.000000	0.561924	0.535028	0.247580	0.069214	0.379094	0.393996	0.500623	0.465422	0.303105	0.221424	0.344308
IPF22	0.149311	0.530861	0.650666	0.561924	0.000000	0.094310	0.544270	0.529042	0.670548	0.500756	0.158111	0.151014	0.540264	0.557713	0.470658
IPF23	0.196810	0.563998	0.670006	0.535028	0.094310	0.000000	0.576109	0.501268	0.655038	0.499937	0.175758	0.197353	0.572833	0.579583	0.405005
IPF24	0.621837	0.257653	0.357072	0.247580	0.544270	0.576109	0.000000	0.195922	0.386065	0.414591	0.516016	0.451904	0.294349	0.233472	0.356783
IPF25	0.606529	0.463543	0.315122	0.069214	0.529042	0.501268	0.195922	0.000000	0.347209	0.348469	0.464840	0.429657	0.259977	0.179769	0.293472
IPF31	0.748225	0.636706	0.056380	0.379094	0.670548	0.655038	0.386065	0.347209	0.000000	0.576609	0.540248	0.566044	0.450124	0.182840	0.453645
IPF32	0.560166	0.451453	0.553703	0.393996	0.500756	0.499937	0.414591	0.348469	0.576609	0.000000	0.481481	0.482491	0.263796	0.470112	0.248710
IPF34	0.250047	0.584752	0.533448	0.500623	0.158111	0.175758	0.516016	0.464840	0.540248	0.481481	0.000000	0.108957	0.510590	0.518174	0.394208
IPF35	0.199861	0.527275	0.552039	0.465422	0.151014	0.197353	0.451904	0.429657	0.566044	0.482491	0.108957	0.000000	0.448042	0.456009	0.389372
IPTA11	0.541147	0.384509	0.422666	0.303105	0.540264	0.572833	0.294349	0.259977	0.450124	0.263796	0.510590	0.448042	0.000000	0.279576	0.247474
IPTA12	0.624026	0.475698	0.143622	0.221424	0.557713	0.579583	0.233472	0.179769	0.182840	0.470112	0.518174	0.456009	0.279576	0.000000	0.360085
IPTA13	0.502135	0.464714	0.458943	0.344308	0.470658	0.405005	0.356783	0.293472	0.453645	0.248710	0.394208	0.389372	0.247474	0.360085	0.000000
IPTA14	0.609932	0.575934	0.223310	0.280025	0.585960	0.533602	0.322063	0.238819	0.229607	0.348266	0.486503	0.504580	0.271342	0.166986	0.235908
IPTA15	0.651507	0.530199	0.095493	0.264912	0.550033	0.578226	0.269456	0.223140	0.138219	0.464864	0.516680	0.466875	0.328466	0.054338	0.354182
IPTA21	0.669285	0.544823	0.273046	0.218427	0.605150	0.600390	0.273907	0.169687	0.307139	0.315928	0.483054	0.534254	0.334953	0.268095	0.327953
IPTA22	0.597025	0.513736	0.320268	0.272978	0.548592	0.594354	0.246459	0.228867	0.352470	0.223877	0.506446	0.479139	0.154807	0.253191	0.267367
IPTA23	0.154067	0.518399	0.605731	0.539983	0.085905	0.158012	0.526024	0.507023	0.626819	0.408019	0.144514	0.155722	0.455455	0.534833	0.379420
IPTA24	0.216990	0.510029	0.599451	0.545128	0.156434	0.216892	0.523503	0.511244	0.621594	0.373494	0.215724	0.224627	0.420486	0.534111	0.352261
IPTA31	0.602427	0.424972	0.225922	0.180473	0.531449	0.553292	0.192398	0.132865	0.263193	0.441258	0.490587	0.425498	0.221443	0.077343	0.322971
IPTA32	0.984133	0.984738	0.982609	0.751108	0.976750	0.932773	0.986196	0.860423	0.985424	0.895929	0.955809	0.985901	0.984115	0.984992	0.951755
IPTA33	0.648080	0.523757	0.076972	0.266537	0.571613	0.591871	0.270774	0.226276	0.118677	0.487904	0.532360	0.471054	0.333874	0.062713	0.382174
IPTA35	0.618764	0.370207	0.347247	0.181587	0.513983	0.501140	0.118463	0.123150	0.381038	0.308443	0.463036	0.430024	0.283209	0.238194	0.293025
IPCN12	0.660299	0.397461	0.613199	0.609293	0.614490	0.656925	0.398064	0.584766	0.631972	0.268607	0.583271	0.611539	0.411699	0.601538	0.447127
IPCN13	0.246440	0.703018	0.745643	0.736561	0.281270	0.346393	0.710305	0.720164	0.758950	0.621422	0.322429	0.293381	0.661286	0.730410	0.622717
IPCN14	0.220337	0.698970	0.783493	0.729860	0.288720	0.333937	0.707233	0.714031	0.795437	0.514164	0.330211	0.305061	0.606835	0.749203	0.591245
IPCN15	0.573274	0.536491	0.624075	0.307670	0.526082	0.512168	0.548719	0.390890	0.644012	0.290216	0.557260	0.560928	0.423650	0.550299	0.342398
IPCN21	0.244720	0.697649	0.741890	0.727882	0.285754	0.335348	0.704557	0.712111	0.743963	0.597674	0.318574	0.290000	0.635352	0.722451	0.581446
IPCN23	0.650815	0.564522	0.501867	0.497329	0.603996	0.647785	0.454504	0.462865	0.529055	0.235454	0.508706	0.528453	0.291377	0.486811	0.324167
IPCN24	0.303824	0.765797	0.806633	0.785602	0.335139	0.370286	0.771801	0.772587	0.800774	0.755022	0.358258	0.324958	0.777090	0.780620	0.730313
IPCN25	0.735502	0.599071	0.465301	0.225205	0.626374	0.594426	0.460131	0.301323	0.487412	0.434077	0.476546	0.527842	0.455013	0.463294	0.390760
IPCN31	0.840901	0.797037	0.989475	0.923723	0.893397	0.920038	0.803036	0.921801	0.992451	0.611655	0.980746	0.925649	0.735805	0.921956	0.810629
IPCN32	0.342063	0.806045	0.879297	0.873476	0.416328	0.475917	0.811801	0.867329	0.887775	0.579426	0.446035	0.419184	0.694443	0.880336	0.752310
IPCN34	0.156172	0.650987	0.736906	0.712428	0.197289	0.273214	0.660433	0.692251	0.752052	0.575510	0.263222	0.219604	0.644933	0.705095	0.599227
IPCN35	0.923115	0.987857	0.991443	0.993451	0.978435	0.995755	0.988954	0.996028	0.992866	0.754303	0.994311	0.995364	0.821082	0.992824	0.899354
IPCA11	0.994992	0.722897	0.981857	0.992429	0.983167	0.995391	0.761551	0.996058	0.988805	0.940817	0.995582	0.995211	0.994010	0.994780	0.993654
IPCA12	0.702665	0.590186	0.105492	0.305595	0.612528	0.611135	0.312496	0.265544	0.125600	0.513500	0.475339	0.511177	0.378551	0.107018	0.378297
IPCA13	0.257903	0.572148	0.517009	0.451218	0.185246	0.225129	0.454468	0.415683	0.537412	0.488645	0.111742	0.057848	0.451873	0.459652	0.392975
IPCA14	0.915782	0.827259	0.989977	0.993547	0.972006	0.996727	0.831762	0.996538	0.992147	0.678988	0.995107	0.996216	0.802579	0.992968	0.888695
IPCA24	0.652431	0.715259	0.723637	0.730964	0.685341	0.640535	0.722869	0.715673	0.710881	0.452277	0.694255	0.725043	0.537088	0.725807	0.496499
IPCA25	0.545646	0.443963	0.313014	0.227452	0.527340	0.571991	0.179923	0.180338	0.345362	0.268231	0.509640	0.447042	0.164350	0.195505	0.246275
IPCA31	0.612548	0.364741	0.291737	0.200641	0.544679	0.564297	0.214796	0.158818	0.329202	0.458227	0.503376	0.439655	0.181418	0.159732	0.342875
IPCA32	0.645181	0.475084	0.114751	0.263142	0.583603	0.603547	0.277511	0.229265	0.155472	0.502552	0.545504	0.485173	0.290736	0.057592	0.400935
IPCA34	0.163226	0.749444	0.800984	0.760431	0.248039	0.286364	0.756937	0.743383	0.790568	0.510368	0.293391	0.327753	0.553130	0.757225	0.578637
IPCA35	0.639183	0.488856	0.742491	0.653167	0.647558	0.635740	0.501491	0.632130	0.760515	0.329708	0.693305	0.710086	0.523662	0.704741	0.539969

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IPTA14	IPTA15	IPTA21	IPTA22	IPTA23	IPTA24	IPTA31	IPTA32	IPTA33	IPTA35	IPCN12	IPCN13	IPCN14	IPCN15	IPCN21	IPCN23
0.609932	0.651507	0.669285	0.597025	0.154067	0.216990	0.602427	0.984133	0.648080	0.618764	0.660299	0.246440	0.220337	0.573274	0.244720	0.650815
0.575934	0.530199	0.544823	0.513736	0.518399	0.510029	0.424972	0.984738	0.523757	0.370207	0.397461	0.703018	0.698970	0.536491	0.697649	0.564522
0.223310	0.095493	0.273046	0.320268	0.605731	0.599451	0.225922	0.982609	0.076972	0.347247	0.613199	0.745643	0.783493	0.624075	0.741890	0.501867
0.280025	0.264912	0.218427	0.272978	0.539983	0.545128	0.180473	0.751108	0.266537	0.181587	0.609293	0.736561	0.729860	0.307670	0.727882	0.497329
0.585960	0.550033	0.605150	0.548592	0.085905	0.156434	0.531449	0.976750	0.571613	0.513983	0.614490	0.281270	0.288720	0.526082	0.285754	0.603996
0.533602	0.578226	0.600390	0.594354	0.158012	0.216892	0.553292	0.932773	0.591871	0.501140	0.656925	0.346393	0.333937	0.512168	0.335348	0.647785
0.322063	0.269456	0.273907	0.246459	0.526024	0.523503	0.192398	0.986196	0.270774	0.118463	0.398064	0.710305	0.707233	0.548719	0.704557	0.454504
0.238819	0.223140	0.169687	0.228867	0.507023	0.511244	0.132865	0.860423	0.226276	0.123150	0.584766	0.720164	0.714031	0.390890	0.712111	0.462865
0.229607	0.138219	0.307139	0.352470	0.626819	0.621594	0.263193	0.985424	0.118677	0.381038	0.631972	0.758950	0.795437	0.644012	0.743963	0.529055
0.348266	0.464864	0.315928	0.223877	0.408019	0.373494	0.441258	0.895929	0.487904	0.308443	0.268607	0.621422	0.514164	0.290216	0.597674	0.235454
0.486503	0.516680	0.483054	0.506446	0.144514	0.215724	0.490587	0.955809	0.532360	0.463036	0.583271	0.322429	0.330211	0.557260	0.318574	0.508706
0.504580	0.466875	0.534254	0.479139	0.155722	0.224627	0.425498	0.985901	0.471054	0.430024	0.611539	0.293381	0.305061	0.560928	0.290000	0.528453
0.271342	0.328466	0.334953	0.154807	0.455455	0.420486	0.221443	0.984115	0.333874	0.283209	0.411699	0.661286	0.606835	0.423650	0.635352	0.291377
0.166986	0.054338	0.268095	0.253191	0.534833	0.534111	0.077343	0.984992	0.062713	0.238194	0.601538	0.730410	0.749203	0.550299	0.722451	0.486811
0.235908	0.354182	0.327953	0.267367	0.379420	0.352261	0.322971	0.951755	0.382174	0.293025	0.447127	0.622717	0.591245	0.342398	0.581446	0.324167
0.000000	0.160786	0.190030	0.169325	0.486268	0.451714	0.191494	0.947097	0.190796	0.271390	0.502287	0.674315	0.622784	0.424373	0.637091	0.389330
0.160786	0.000000	0.262253	0.248006	0.527525	0.526528	0.133055	0.982728	0.036276	0.256255	0.595963	0.723613	0.741942	0.544405	0.715331	0.481249
0.190030	0.262253	0.000000	0.194799	0.508303	0.502377	0.227516	0.876211	0.290650	0.183383	0.480200	0.655202	0.646276	0.422214	0.656981	0.388385
0.169325	0.248006	0.194799	0.000000	0.448366	0.406421	0.213183	0.985107	0.276243	0.232129	0.363682	0.638380	0.575935	0.424983	0.615049	0.240888
0.486268	0.527525	0.508303	0.448366	0.000000	0.074414	0.508492	0.978355	0.548861	0.497141	0.513770	0.266553	0.273957	0.444513	0.271146	0.501421
0.451714	0.526528	0.502377	0.406421	0.074414	0.000000	0.506666	0.983127	0.548680	0.499431	0.472256	0.319088	0.304161	0.403876	0.301486	0.458057
0.191494	0.133055	0.227516	0.213183	0.508492	0.506666	0.000000	0.985899	0.139103	0.195322	0.582971	0.716969	0.737841	0.526254	0.708974	0.461269
0.947097	0.982728	0.876211	0.985107	0.978355	0.983127	0.985899	0.000000	0.983335	0.911460	0.983713	0.985747	0.957943	0.624536	0.985208	0.985800
0.190796	0.036276	0.290650	0.276243	0.548861	0.548680	0.139103	0.983335	0.000000	0.261485	0.612182	0.737416	0.755365	0.565247	0.729209	0.503208
0.271390	0.256255	0.183383	0.232129	0.497141	0.499431	0.195322	0.911460	0.261485	0.000000	0.469242	0.696494	0.667926	0.448527	0.689745	0.429949
0.502287	0.595963	0.480200	0.363682	0.513770	0.472256	0.582971	0.983713	0.612182	0.469242	0.000000	0.649935	0.593461	0.491563	0.633003	0.157553
0.674315	0.723613	0.655202	0.638380	0.266553	0.319088	0.716969	0.985747	0.737416	0.696494	0.649935	0.000000	0.150351	0.644812	0.026647	0.641356
0.622784	0.741942	0.646276	0.575935	0.273957	0.304161	0.737841	0.957943	0.755365	0.667926	0.593461	0.150351	0.000000	0.593548	0.148741	0.597940
0.424373	0.544405	0.422214	0.424983	0.444513	0.403876	0.526254	0.624536	0.565247	0.448527	0.491563	0.644812	0.593548	0.000000	0.617988	0.477932
0.637091	0.715331	0.656981	0.615049	0.271146	0.301486	0.708974	0.985208	0.729209	0.689745	0.633003	0.026647	0.148741	0.617988	0.000000	0.623167
0.389330	0.481249	0.388385	0.240888	0.501421	0.458057	0.461269	0.985800	0.503208	0.429949	0.157553	0.641356	0.597940	0.477932	0.623167	0.000000
0.767702	0.778300	0.789643	0.766044	0.375894	0.434359	0.769500	0.985290	0.786304	0.759526	0.786207	0.114321	0.215362	0.765544	0.112521	0.779888
0.438970	0.463597	0.328708	0.451972	0.580716	0.579134	0.434944	0.579481	0.478634	0.361108	0.595145	0.735719	0.748916	0.221194	0.730453	0.478578
0.828290	0.915431	0.872569	0.699487	0.845975	0.816063	0.920340	0.992143	0.922311	0.803838	0.458673	0.895564	0.778667	0.803841	0.874509	0.514305
0.761137	0.872791	0.745432	0.648321	0.395311	0.431232	0.875753	0.972516	0.882917	0.791890	0.597718	0.246767	0.128339	0.744885	0.248603	0.630473
0.660180	0.697554	0.623384	0.610049	0.202262	0.259179	0.688804	0.982990	0.713691	0.643136	0.625479	0.143832	0.118043	0.625545	0.162392	0.628283
0.888640	0.986994	0.928795	0.801297	0.922187	0.890925	0.995260	0.991987	0.991435	0.987482	0.613696	0.929311	0.859292	0.884140	0.912546	0.585105
0.994838	0.993085	0.935959	0.964128	0.988142	0.986306	0.995083	0.992374	0.992086	0.874576	0.784059	0.976019	0.950589	0.994782	0.976659	0.962575
0.139540	0.101007	0.214744	0.267347	0.566235	0.558918	0.170810	0.980076	0.133123	0.298286	0.580589	0.720038	0.758939	0.590608	0.703664	0.461951
0.459535	0.457814	0.479178	0.446451	0.171524	0.237759	0.429657	0.969127	0.474340	0.414095	0.616877	0.290394	0.285186	0.558607	0.291177	0.506225
0.878499	0.986406	0.869529	0.756134	0.914053	0.877333	0.996165	0.991706	0.991541	0.874658	0.418025	0.908861	0.809154	0.871574	0.892254	0.515516
0.575273	0.718211	0.651371	0.517377	0.637934	0.593357	0.712744	0.981944	0.732359	0.708542	0.324106	0.676065	0.641767	0.590255	0.648357	0.283076
0.163085	0.217668	0.167681	0.077750	0.447202	0.410374	0.153229	0.985191	0.226719	0.172749	0.416867	0.640547	0.578487	0.422514	0.617489	0.315949
0.266881	0.212237	0.274362	0.236554	0.521830	0.521200	0.090866	0.981971	0.215077	0.217704	0.590580	0.723634	0.741750	0.539924	0.713291	0.474453
0.211530	0.061620	0.310500	0.296253	0.561013	0.561270	0.112068	0.982360	0.044253	0.281516	0.621241	0.743413	0.760980	0.577057	0.734501	0.515347
0.615367	0.749419	0.719002	0.539167	0.228163	0.259645	0.743340	0.980658	0.764319	0.739518	0.581237	0.312983	0.198159	0.607202	0.291395	0.570121
0.567950	0.697360	0.561608	0.477543	0.608403	0.568804	0.690664	0.925976	0.712585	0.518643	0.158689	0.663215	0.560943	0.510716	0.647716	0.281026

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IPCN24	IPCN25	IPCN31	IPCN32	IPCN34	IPCN35	IPCA11	IPCA12	IPCA13	IPCA14	IPCA24	IPCA25	IPCA31	IPCA32	IPCA34	IPCA35
0.303824	0.735502	0.840901	0.342063	0.156172	0.923115	0.994992	0.702665	0.257903	0.915782	0.652431	0.545646	0.612548	0.645181	0.163226	0.639183
0.765797	0.599071	0.797037	0.806045	0.650987	0.987857	0.722897	0.590186	0.572148	0.827259	0.715259	0.443963	0.364741	0.475084	0.749444	0.488856
0.806633	0.465301	0.989475	0.879297	0.736906	0.991443	0.981857	0.105492	0.517009	0.989977	0.723637	0.313014	0.291737	0.114751	0.800984	0.742491
0.785602	0.225205	0.923723	0.873476	0.712428	0.993451	0.992429	0.305595	0.451218	0.993547	0.730964	0.227452	0.200641	0.263142	0.760431	0.653167
0.335139	0.626374	0.893397	0.416328	0.197289	0.978435	0.983167	0.612528	0.185246	0.972006	0.685341	0.527340	0.544679	0.583603	0.248039	0.647558
0.370286	0.594426	0.920038	0.475917	0.273214	0.995755	0.995391	0.611135	0.225129	0.996727	0.640535	0.571991	0.564297	0.603547	0.286364	0.635740
0.771801	0.460131	0.803036	0.811801	0.660433	0.988954	0.761551	0.312496	0.454468	0.831762	0.722869	0.179923	0.214796	0.277511	0.756937	0.501491
0.772587	0.301323	0.921801	0.867329	0.692251	0.996028	0.996058	0.265544	0.415683	0.996538	0.715673	0.180338	0.158818	0.229265	0.743383	0.632130
0.800774	0.487412	0.992451	0.887775	0.752052	0.992866	0.988805	0.125600	0.537412	0.992147	0.710881	0.345362	0.329202	0.155472	0.790568	0.760515
0.755022	0.434077	0.611655	0.579426	0.575510	0.754303	0.940817	0.513500	0.488645	0.678988	0.452277	0.268231	0.458227	0.502552	0.510368	0.329708
0.358258	0.476546	0.980746	0.446035	0.263222	0.994311	0.995582	0.475339	0.111742	0.995107	0.694255	0.509640	0.503376	0.545504	0.293391	0.693305
0.324958	0.527842	0.925649	0.419184	0.219604	0.995364	0.995211	0.511177	0.057848	0.996216	0.725043	0.447042	0.439655	0.485173	0.327753	0.710086
0.777090	0.455013	0.735805	0.694443	0.644933	0.821082	0.994010	0.378551	0.451873	0.802579	0.537088	0.164350	0.181418	0.290736	0.553130	0.523662
0.780620	0.463294	0.921956	0.880336	0.705095	0.992824	0.994780	0.107018	0.459652	0.992968	0.725807	0.195505	0.159732	0.057592	0.757225	0.704741
0.730313	0.390760	0.810629	0.752310	0.599227	0.899354	0.993654	0.378297	0.392975	0.888695	0.496499	0.246275	0.342875	0.400935	0.578637	0.539969
0.767702	0.438970	0.828290	0.761137	0.660180	0.888640	0.994838	0.139540	0.459535	0.878499	0.575273	0.163085	0.266881	0.211530	0.615367	0.567950
0.778300	0.463597	0.915431	0.872791	0.697554	0.986994	0.993085	0.101007	0.457814	0.986406	0.718211	0.217668	0.212237	0.061620	0.749419	0.697360
0.789643	0.328708	0.872569	0.745432	0.623384	0.928795	0.935959	0.214744	0.479178	0.869529	0.651371	0.167681	0.274362	0.310500	0.719002	0.561608
0.766044	0.451972	0.699487	0.648321	0.610049	0.801297	0.964128	0.267347	0.446451	0.756134	0.517377	0.077750	0.236554	0.296253	0.539167	0.477543
0.375894	0.580716	0.845975	0.395311	0.202262	0.922187	0.988142	0.566235	0.171524	0.914053	0.637934	0.447202	0.521830	0.561013	0.228163	0.608403
0.434359	0.579134	0.816063	0.431232	0.259179	0.890925	0.986306	0.558918	0.237759	0.877333	0.593357	0.410374	0.521200	0.561270	0.259645	0.568804
0.769500	0.434944	0.920340	0.875753	0.688804	0.995260	0.995083	0.170810	0.429657	0.996165	0.712744	0.153229	0.090866	0.112068	0.743340	0.690664
0.985290	0.579481	0.992143	0.972516	0.982990	0.991987	0.992374	0.980076	0.969127	0.991706	0.981944	0.985191	0.981971	0.982360	0.980658	0.925976
0.786304	0.478634	0.922311	0.882917	0.713691	0.991435	0.992086	0.133123	0.474340	0.991541	0.732359	0.226719	0.215077	0.044253	0.764319	0.712585
0.759526	0.361108	0.803838	0.791890	0.643136	0.987482	0.874576	0.298286	0.414095	0.874658	0.708542	0.172749	0.217704	0.281516	0.739518	0.518643
0.786207	0.595145	0.458673	0.597718	0.625479	0.613696	0.784059	0.580589	0.616877	0.418025	0.324106	0.416867	0.590580	0.621241	0.581237	0.158689
0.114321	0.735719	0.895564	0.246767	0.143832	0.929311	0.976019	0.720038	0.290394	0.908861	0.676065	0.640547	0.723634	0.743413	0.312983	0.663215
0.215362	0.748916	0.778667	0.128339	0.118043	0.859292	0.950589	0.758939	0.285186	0.809154	0.641767	0.578487	0.741750	0.760980	0.198159	0.560943
0.765544	0.221194	0.803841	0.744885	0.625545	0.884140	0.994782	0.590608	0.558607	0.871574	0.590255	0.422514	0.539924	0.577057	0.607202	0.510716
0.112521	0.730453	0.874509	0.248603	0.162392	0.912546	0.976659	0.703664	0.291177	0.892254	0.648357	0.617489	0.713291	0.734501	0.291395	0.647716
0.779888	0.478578	0.514305	0.630473	0.628283	0.585105	0.962575	0.461951	0.506225	0.515516	0.283076	0.315949	0.474453	0.515347	0.570121	0.281026
0.000000	0.796899	0.959199	0.305184	0.225354	0.989210	0.986874	0.768808	0.325717	0.985669	0.781869	0.767558	0.773399	0.789394	0.333720	0.777435
0.796899	0.000000	0.993060	0.865784	0.725284	0.992507	0.993451	0.423148	0.474625	0.992623	0.711947	0.454780	0.447535	0.493853	0.785705	0.675897
0.959199	0.993060	0.000000	0.708473	0.862814	0.245128	0.868117	0.982333	0.937786	0.068290	0.449296	0.741599	0.918413	0.922617	0.743018	0.362147
0.305184	0.865784	0.708473	0.000000	0.227793	0.787711	0.922353	0.860977	0.404253	0.701501	0.662600	0.700134	0.875942	0.884424	0.226430	0.601795
0.225354	0.725284	0.862814	0.227793	0.000000	0.936612	0.949384	0.707571	0.217840	0.891986	0.676421	0.599503	0.696894	0.721029	0.229949	0.620322
0.989210	0.992507	0.245128	0.787711	0.936612	0.000000	0.987442	0.985177	0.994545	0.245267	0.489080	0.867194	0.992352	0.990980	0.802733	0.546128
0.986874	0.993451	0.868117	0.922353	0.949384	0.987442	0.000000	0.984818	0.993545	0.823974	0.988195	0.943912	0.961869	0.987616	0.995553	0.784446
0.768808	0.423148	0.982333	0.860977	0.707571	0.985177	0.984818	0.000000	0.477656	0.983745	0.671778	0.259681	0.245026	0.153337	0.749682	0.720816
0.325717	0.474625	0.937786	0.404253	0.217840	0.994545	0.993545	0.477656	0.000000	0.994751	0.733128	0.449711	0.442580	0.487642	0.341875	0.696654
0.985669	0.992623	0.068290	0.701501	0.891986	0.245267	0.823974	0.983745	0.994751	0.000000	0.434972	0.809008	0.990938	0.989544	0.781385	0.351331
0.781869	0.711947	0.449296	0.662600	0.676421	0.489080	0.988195	0.671778	0.733128	0.434972	0.000000	0.590025	0.715509	0.732269	0.567035	0.266547
0.767558	0.454780	0.741599	0.700134	0.599503	0.867194	0.943912	0.259681	0.449711	0.809008	0.590025	0.000000	0.178040	0.242131	0.611790	0.528294
0.773399	0.447535	0.918413	0.875942	0.696894	0.992352	0.961869	0.245026	0.442580	0.990938	0.715509	0.178040	0.000000	0.170517	0.748423	0.694378
0.789394	0.493853	0.922617	0.884424	0.721029	0.990980	0.987616	0.153337	0.487642	0.989544	0.732269	0.242131	0.170517	0.000000	0.769976	0.718522
0.333720	0.785705	0.743018	0.226430	0.229949	0.802733	0.995553	0.749682	0.341875	0.781385	0.567035	0.611790	0.748423	0.769976	0.000000	0.563790
0.777435	0.675897	0.362147	0.601795	0.620322	0.546128	0.784446	0.720816	0.696654	0.351331	0.266547	0.528294	0.694378	0.718522	0.563790	0.000000

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	IPF11	IPF13	IPF14	IPF21	IPF22	IPF23	IPF24	IPF25	IPF31	IPF32	IPF34	IPF35	IPTA11	IPTA12	IPTA13
IPF11	0.000000	0.500000	0.636364	0.700000	0.375000	0.500000	0.500000	0.750000	0.700000	0.375000	0.375000	0.428571	0.500000	0.428571	0.375000
IPF13	0.500000	0.000000	0.636364	0.375000	0.142857	0.285714	0.285714	0.333333	0.700000	0.142857	0.375000	0.428571	0.500000	0.428571	0.375000
IPF14	0.636364	0.636364	0.000000	0.400000	0.545455	0.636364	0.500000	0.700000	0.666667	0.545455	0.545455	0.600000	0.333333	0.444444	0.545455
IPF21	0.700000	0.375000	0.400000	0.000000	0.444444	0.375000	0.555556	0.428571	0.727273	0.444444	0.444444	0.500000	0.555556	0.500000	0.444444
IPF22	0.375000	0.142857	0.545455	0.444444	0.000000	0.375000	0.375000	0.428571	0.727273	0.000000	0.250000	0.500000	0.375000	0.285714	0.250000
IPF23	0.500000	0.285714	0.636364	0.375000	0.375000	0.000000	0.500000	0.333333	0.555556	0.375000	0.142857	0.166667	0.500000	0.428571	0.142857
IPF24	0.500000	0.285714	0.500000	0.555556	0.375000	0.500000	0.000000	0.571429	0.555556	0.375000	0.555556	0.428571	0.285714	0.428571	0.555556
IPF25	0.750000	0.333333	0.700000	0.428571	0.428571	0.333333	0.571429	0.000000	0.777778	0.428571	0.428571	0.500000	0.571429	0.500000	0.428571
IPF31	0.700000	0.700000	0.666667	0.727273	0.727273	0.555556	0.555556	0.777778	0.000000	0.727273	0.600000	0.500000	0.555556	0.666667	0.600000
IPF32	0.375000	0.142857	0.545455	0.444444	0.000000	0.375000	0.375000	0.428571	0.727273	0.000000	0.250000	0.500000	0.375000	0.285714	0.250000
IPF34	0.375000	0.375000	0.545455	0.444444	0.250000	0.142857	0.555556	0.428571	0.600000	0.250000	0.000000	0.285714	0.375000	0.285714	0.000000
IPF35	0.428571	0.428571	0.600000	0.500000	0.500000	0.166667	0.428571	0.500000	0.500000	0.500000	0.285714	0.000000	0.428571	0.333333	0.285714
IPTA11	0.500000	0.500000	0.333333	0.555556	0.375000	0.500000	0.285714	0.571429	0.555556	0.375000	0.375000	0.428571	0.000000	0.166667	0.375000
IPTA12	0.428571	0.428571	0.444444	0.500000	0.285714	0.428571	0.428571	0.500000	0.666667	0.285714	0.285714	0.333333	0.166667	0.000000	0.285714
IPTA13	0.375000	0.375000	0.545455	0.444444	0.250000	0.142857	0.555556	0.428571	0.600000	0.250000	0.000000	0.285714	0.375000	0.285714	0.000000
IPTA14	0.444444	0.444444	0.583333	0.500000	0.333333	0.250000	0.600000	0.500000	0.636364	0.333333	0.125000	0.375000	0.444444	0.375000	0.125000
IPTA15	0.500000	0.500000	0.333333	0.375000	0.375000	0.500000	0.500000	0.571429	0.700000	0.375000	0.375000	0.428571	0.285714	0.166667	0.375000
IPTA21	0.375000	0.375000	0.666667	0.600000	0.250000	0.375000	0.555556	0.625000	0.600000	0.250000	0.250000	0.500000	0.555556	0.500000	0.250000
IPTA22	0.375000	0.375000	0.400000	0.600000	0.250000	0.555556	0.142857	0.625000	0.600000	0.250000	0.444444	0.500000	0.142857	0.285714	0.444444
IPTA23	0.375000	0.142857	0.545455	0.444444	0.000000	0.375000	0.375000	0.428571	0.727273	0.000000	0.250000	0.500000	0.375000	0.285714	0.250000
IPTA24	0.285714	0.285714	0.500000	0.555556	0.142857	0.500000	0.285714	0.571429	0.700000	0.142857	0.375000	0.428571	0.285714	0.166667	0.375000
IPTA31	0.571429	0.333333	0.555556	0.428571	0.428571	0.333333	0.333333	0.400000	0.625000	0.428571	0.428571	0.200000	0.333333	0.200000	0.428571
IPTA32	0.700000	0.555556	0.545455	0.600000	0.444444	0.555556	0.555556	0.625000	0.600000	0.444444	0.444444	0.666667	0.375000	0.500000	0.444444
IPTA33	0.500000	0.500000	0.333333	0.555556	0.375000	0.500000	0.285714	0.571429	0.555556	0.375000	0.375000	0.428571	0.000000	0.166667	0.375000
IPTA35	0.500000	0.333333	0.363636	0.400000	0.222222	0.500000	0.333333	0.555556	0.666667	0.222222	0.400000	0.600000	0.333333	0.444444	0.400000
IPCN12	0.375000	0.142857	0.545455	0.444444	0.000000	0.375000	0.375000	0.428571	0.727273	0.000000	0.250000	0.500000	0.375000	0.285714	0.250000
IPCN13	0.285714	0.285714	0.500000	0.555556	0.142857	0.500000	0.285714	0.571429	0.700000	0.142857	0.375000	0.428571	0.285714	0.166667	0.375000
IPCN14	0.375000	0.142857	0.545455	0.444444	0.000000	0.375000	0.375000	0.428571	0.727273	0.000000	0.250000	0.500000	0.375000	0.285714	0.250000
IPCN15	0.555556	0.375000	0.400000	0.250000	0.250000	0.375000	0.555556	0.428571	0.727273	0.250000	0.250000	0.500000	0.375000	0.285714	0.250000
IPCN21	0.333333	0.500000	0.363636	0.545455	0.400000	0.500000	0.333333	0.700000	0.545455	0.400000	0.400000	0.444444	0.333333	0.444444	0.400000
IPCN23	0.555556	0.555556	0.400000	0.444444	0.444444	0.700000	0.555556	0.625000	0.833333	0.444444	0.600000	0.666667	0.555556	0.500000	0.600000
IPCN24	0.250000	0.600000	0.583333	0.636364	0.500000	0.600000	0.600000	0.800000	0.750000	0.500000	0.500000	0.555556	0.600000	0.555556	0.500000
IPCN25	0.625000	0.625000	0.727273	0.666667	0.500000	0.428571	0.777778	0.500000	0.666667	0.500000	0.285714	0.571429	0.625000	0.571429	0.285714
IPCN31	0.375000	0.375000	0.400000	0.444444	0.250000	0.555556	0.375000	0.625000	0.727273	0.250000	0.444444	0.500000	0.375000	0.285714	0.444444
IPCN32	0.444444	0.250000	0.454545	0.333333	0.125000	0.444444	0.444444	0.500000	0.750000	0.125000	0.333333	0.555556	0.444444	0.375000	0.333333
IPCN34	0.285714	0.285714	0.500000	0.555556	0.142857	0.500000	0.285714	0.571429	0.700000	0.142857	0.375000	0.428571	0.285714	0.166667	0.375000
IPCN35	0.428571	0.428571	0.600000	0.666667	0.285714	0.625000	0.428571	0.714286	0.666667	0.285714	0.500000	0.571429	0.428571	0.333333	0.500000
IPCA11	0.666667	0.400000	0.416667	0.454545	0.454545	0.545455	0.400000	0.600000	0.692308	0.454545	0.583333	0.636364	0.545455	0.636364	0.583333
IPCA12	0.454545	0.454545	0.461538	0.363636	0.363636	0.454545	0.583333	0.636364	0.714286	0.363636	0.363636	0.545455	0.583333	0.545455	0.363636
IPCA13	0.500000	0.333333	0.615385	0.400000	0.400000	0.333333	0.500000	0.555556	0.666667	0.400000	0.400000	0.444444	0.636364	0.600000	0.400000
IPCA14	0.555556	0.555556	0.400000	0.600000	0.444444	0.700000	0.375000	0.777778	0.600000	0.444444	0.600000	0.666667	0.375000	0.500000	0.600000
IPCA24	0.666667	0.736842	0.650000	0.750000	0.684211	0.736842	0.666667	0.842105	0.750000	0.684211	0.684211	0.722222	0.666667	0.722222	0.684211
IPCA25	0.285714	0.285714	0.500000	0.555556	0.142857	0.500000	0.285714	0.571429	0.700000	0.142857	0.375000	0.428571	0.285714	0.166667	0.375000
IPCA31	0.666667	0.545455	0.538462	0.454545	0.583333	0.400000	0.545455	0.600000	0.583333	0.583333	0.454545	0.500000	0.545455	0.636364	0.454545
IPCA32	0.714286	0.714286	0.384615	0.538462	0.642857	0.714286	0.615385	0.769231	0.733333	0.642857	0.642857	0.692308	0.500000	0.583333	0.642857
IPCA34	0.250000	0.444444	0.692308	0.636364	0.333333	0.444444	0.600000	0.666667	0.750000	0.333333	0.333333	0.555556	0.600000	0.555556	0.333333
IPCA35	0.615385	0.500000	0.500000	0.538462	0.416667	0.615385	0.615385	0.666667	0.812500	0.416667	0.538462	0.692308	0.615385	0.583333	0.538462

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IPTA14	IPTA15	IPTA21	IPTA22	IPTA23	IPTA24	IPTA31	IPTA32	IPTA33	IPTA35	IPCN12	IPCN13	IPCN14	IPCN15	IPCN21	IPCN23
0.444444	0.500000	0.375000	0.375000	0.375000	0.285714	0.571429	0.700000	0.500000	0.500000	0.375000	0.285714	0.375000	0.555556	0.333333	0.555556
0.444444	0.500000	0.375000	0.375000	0.142857	0.285714	0.333333	0.555556	0.500000	0.333333	0.142857	0.285714	0.142857	0.375000	0.500000	0.555556
0.583333	0.333333	0.666667	0.400000	0.545455	0.500000	0.555556	0.545455	0.333333	0.363636	0.545455	0.500000	0.545455	0.400000	0.363636	0.400000
0.500000	0.375000	0.600000	0.600000	0.444444	0.555556	0.428571	0.600000	0.555556	0.400000	0.444444	0.555556	0.444444	0.250000	0.545455	0.444444
0.333333	0.375000	0.250000	0.250000	0.000000	0.142857	0.428571	0.444444	0.375000	0.222222	0.000000	0.142857	0.000000	0.250000	0.400000	0.444444
0.250000	0.500000	0.375000	0.555556	0.375000	0.500000	0.333333	0.555556	0.500000	0.500000	0.375000	0.500000	0.375000	0.375000	0.500000	0.700000
0.600000	0.500000	0.555556	0.142857	0.375000	0.285714	0.333333	0.555556	0.285714	0.333333	0.375000	0.285714	0.375000	0.555556	0.333333	0.555556
0.500000	0.571429	0.625000	0.625000	0.428571	0.571429	0.400000	0.625000	0.571429	0.555556	0.428571	0.571429	0.428571	0.428571	0.700000	0.625000
0.636364	0.700000	0.600000	0.600000	0.727273	0.700000	0.625000	0.600000	0.555556	0.666667	0.727273	0.700000	0.727273	0.727273	0.545455	0.833333
0.333333	0.375000	0.250000	0.250000	0.000000	0.142857	0.428571	0.444444	0.375000	0.222222	0.000000	0.142857	0.000000	0.250000	0.400000	0.444444
0.125000	0.375000	0.250000	0.444444	0.250000	0.375000	0.428571	0.444444	0.375000	0.400000	0.250000	0.375000	0.250000	0.250000	0.400000	0.600000
0.375000	0.428571	0.500000	0.500000	0.500000	0.428571	0.200000	0.666667	0.428571	0.600000	0.500000	0.428571	0.500000	0.500000	0.444444	0.666667
0.444444	0.285714	0.555556	0.142857	0.375000	0.285714	0.333333	0.375000	0.000000	0.333333	0.375000	0.285714	0.375000	0.375000	0.333333	0.555556
0.375000	0.166667	0.500000	0.285714	0.285714	0.166667	0.200000	0.500000	0.166667	0.444444	0.285714	0.166667	0.285714	0.285714	0.444444	0.500000
0.125000	0.375000	0.250000	0.444444	0.250000	0.375000	0.428571	0.444444	0.375000	0.400000	0.250000	0.375000	0.250000	0.250000	0.400000	0.600000
0.000000	0.444444	0.333333	0.500000	0.333333	0.444444	0.500000	0.500000	0.444444	0.454545	0.333333	0.444444	0.333333	0.333333	0.454545	0.636364
0.444444	0.000000	0.555556	0.375000	0.375000	0.285714	0.333333	0.555556	0.285714	0.333333	0.375000	0.285714	0.375000	0.142857	0.333333	0.375000
0.333333	0.555556	0.000000	0.444444	0.250000	0.375000	0.625000	0.444444	0.555556	0.400000	0.250000	0.375000	0.250000	0.444444	0.400000	0.600000
0.500000	0.375000	0.444444	0.000000	0.250000	0.142857	0.428571	0.444444	0.142857	0.222222	0.250000	0.142857	0.250000	0.444444	0.222222	0.444444
0.333333	0.375000	0.250000	0.250000	0.000000	0.142857	0.428571	0.444444	0.375000	0.222222	0.000000	0.142857	0.000000	0.250000	0.400000	0.444444
0.444444	0.285714	0.375000	0.142857	0.142857	0.000000	0.333333	0.555556	0.285714	0.333333	0.142857	0.000000	0.142857	0.375000	0.333333	0.375000
0.500000	0.333333	0.625000	0.428571	0.428571	0.333333	0.000000	0.625000	0.333333	0.555556	0.428571	0.333333	0.428571	0.428571	0.555556	0.625000
0.500000	0.555556	0.444444	0.444444	0.444444	0.555556	0.625000	0.000000	0.375000	0.400000	0.444444	0.555556	0.444444	0.444444	0.545455	0.727273
0.444444	0.285714	0.555556	0.142857	0.375000	0.285714	0.333333	0.375000	0.000000	0.333333	0.375000	0.285714	0.375000	0.375000	0.333333	0.555556
0.454545	0.333333	0.400000	0.222222	0.222222	0.333333	0.555556	0.400000	0.333333	0.000000	0.222222	0.333333	0.222222	0.222222	0.200000	0.400000
0.333333	0.375000	0.250000	0.250000	0.000000	0.142857	0.428571	0.444444	0.375000	0.222222	0.000000	0.142857	0.000000	0.250000	0.400000	0.444444
0.444444	0.285714	0.375000	0.142857	0.142857	0.000000	0.333333	0.555556	0.285714	0.333333	0.142857	0.000000	0.142857	0.375000	0.333333	0.375000
0.333333	0.375000	0.250000	0.250000	0.000000	0.142857	0.428571	0.444444	0.375000	0.222222	0.000000	0.142857	0.000000	0.250000	0.400000	0.444444
0.333333	0.142857	0.444444	0.444444	0.250000	0.375000	0.428571	0.444444	0.375000	0.222222	0.250000	0.375000	0.250000	0.000000	0.400000	0.444444
0.454545	0.333333	0.400000	0.222222	0.400000	0.333333	0.555556	0.545455	0.333333	0.200000	0.400000	0.333333	0.400000	0.400000	0.000000	0.400000
0.636364	0.375000	0.600000	0.444444	0.444444	0.375000	0.625000	0.727273	0.555556	0.400000	0.444444	0.375000	0.444444	0.444444	0.400000	0.000000
0.545455	0.444444	0.500000	0.500000	0.500000	0.444444	0.666667	0.636364	0.600000	0.454545	0.500000	0.444444	0.500000	0.500000	0.300000	0.500000
0.375000	0.625000	0.285714	0.666667	0.500000	0.625000	0.714286	0.500000	0.625000	0.600000	0.500000	0.625000	0.500000	0.500000	0.600000	0.666667
0.500000	0.375000	0.444444	0.250000	0.250000	0.142857	0.428571	0.600000	0.375000	0.400000	0.250000	0.142857	0.250000	0.444444	0.400000	0.250000
0.400000	0.444444	0.333333	0.333333	0.125000	0.250000	0.500000	0.500000	0.444444	0.300000	0.125000	0.250000	0.125000	0.333333	0.454545	0.333333
0.444444	0.285714	0.375000	0.142857	0.142857	0.000000	0.333333	0.555556	0.285714	0.333333	0.142857	0.000000	0.142857	0.375000	0.333333	0.375000
0.555556	0.428571	0.285714	0.285714	0.285714	0.166667	0.500000	0.500000	0.428571	0.444444	0.285714	0.166667	0.285714	0.500000	0.444444	0.500000
0.615385	0.545455	0.583333	0.454545	0.454545	0.545455	0.600000	0.583333	0.545455	0.272727	0.454545	0.545455	0.454545	0.454545	0.416667	0.583333
0.416667	0.454545	0.363636	0.500000	0.363636	0.454545	0.636364	0.615385	0.583333	0.333333	0.363636	0.454545	0.363636	0.363636	0.333333	0.363636
0.454545	0.500000	0.400000	0.545455	0.400000	0.500000	0.555556	0.666667	0.636364	0.363636	0.400000	0.500000	0.400000	0.400000	0.363636	0.545455
0.636364	0.375000	0.444444	0.250000	0.444444	0.375000	0.625000	0.444444	0.375000	0.222222	0.444444	0.375000	0.444444	0.444444	0.222222	0.444444
0.700000	0.666667	0.684211	0.611111	0.684211	0.666667	0.777778	0.684211	0.666667	0.578947	0.684211	0.666667	0.684211	0.684211	0.500000	0.684211
0.444444	0.285714	0.375000	0.142857	0.142857	0.000000	0.333333	0.555556	0.285714	0.333333	0.142857	0.000000	0.142857	0.375000	0.333333	0.375000
0.500000	0.545455	0.583333	0.583333	0.583333	0.666667	0.600000	0.583333	0.545455	0.416667	0.583333	0.666667	0.583333	0.454545	0.416667	0.692308
0.571429	0.500000	0.733333	0.538462	0.642857	0.615385	0.666667	0.538462	0.500000	0.500000	0.642857	0.615385	0.642857	0.538462	0.500000	0.538462
0.400000	0.600000	0.333333	0.500000	0.333333	0.444444	0.666667	0.636364	0.600000	0.454545	0.333333	0.444444	0.333333	0.500000	0.454545	0.636364
0.571429	0.500000	0.538462	0.538462	0.416667	0.500000	0.666667	0.642857	0.615385	0.384615	0.416667	0.500000	0.416667	0.416667	0.500000	0.538462

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IPCN24	IPCN25	IPCN31	IPCN32	IPCN34	IPCN35	IPCA11	IPCA12	IPCA13	IPCA14	IPCA24	IPCA25	IPCA31	IPCA32	IPCA34	IPCA35
0.250000	0.625000	0.375000	0.444444	0.285714	0.428571	0.666667	0.454545	0.500000	0.555556	0.666667	0.285714	0.666667	0.714286	0.250000	0.615385
0.600000	0.625000	0.375000	0.250000	0.285714	0.428571	0.400000	0.454545	0.333333	0.555556	0.736842	0.285714	0.545455	0.714286	0.444444	0.500000
0.583333	0.727273	0.400000	0.454545	0.500000	0.600000	0.416667	0.461538	0.615385	0.400000	0.650000	0.500000	0.538462	0.384615	0.692308	0.500000
0.636364	0.666667	0.444444	0.333333	0.555556	0.666667	0.454545	0.363636	0.400000	0.600000	0.750000	0.555556	0.454545	0.538462	0.636364	0.538462
0.500000	0.500000	0.250000	0.125000	0.142857	0.285714	0.454545	0.363636	0.400000	0.444444	0.684211	0.142857	0.583333	0.642857	0.333333	0.416667
0.600000	0.428571	0.555556	0.444444	0.500000	0.625000	0.545455	0.454545	0.333333	0.700000	0.736842	0.500000	0.400000	0.714286	0.444444	0.615385
0.600000	0.777778	0.375000	0.444444	0.285714	0.428571	0.400000	0.583333	0.500000	0.375000	0.666667	0.285714	0.545455	0.615385	0.600000	0.615385
0.800000	0.500000	0.625000	0.500000	0.571429	0.714286	0.600000	0.636364	0.555556	0.777778	0.842105	0.571429	0.600000	0.769231	0.666667	0.666667
0.750000	0.666667	0.727273	0.750000	0.700000	0.666667	0.692308	0.714286	0.666667	0.600000	0.750000	0.700000	0.583333	0.733333	0.750000	0.812500
0.500000	0.500000	0.250000	0.125000	0.142857	0.285714	0.454545	0.363636	0.400000	0.444444	0.684211	0.142857	0.583333	0.642857	0.333333	0.416667
0.500000	0.285714	0.444444	0.333333	0.375000	0.500000	0.583333	0.363636	0.400000	0.600000	0.684211	0.375000	0.454545	0.642857	0.333333	0.538462
0.555556	0.571429	0.500000	0.555556	0.428571	0.571429	0.636364	0.545455	0.444444	0.666667	0.722222	0.428571	0.500000	0.692308	0.555556	0.692308
0.600000	0.625000	0.375000	0.444444	0.285714	0.428571	0.545455	0.583333	0.636364	0.375000	0.666667	0.285714	0.545455	0.500000	0.600000	0.615385
0.555556	0.571429	0.285714	0.375000	0.166667	0.333333	0.636364	0.545455	0.600000	0.500000	0.722222	0.166667	0.636364	0.583333	0.555556	0.583333
0.500000	0.285714	0.444444	0.333333	0.375000	0.500000	0.583333	0.363636	0.400000	0.600000	0.684211	0.375000	0.454545	0.642857	0.333333	0.538462
0.545455	0.375000	0.500000	0.400000	0.444444	0.555556	0.615385	0.416667	0.454545	0.636364	0.700000	0.444444	0.500000	0.571429	0.400000	0.571429
0.444444	0.625000	0.375000	0.444444	0.285714	0.428571	0.545455	0.454545	0.500000	0.375000	0.666667	0.285714	0.545455	0.500000	0.600000	0.500000
0.500000	0.285714	0.444444	0.333333	0.375000	0.285714	0.583333	0.363636	0.400000	0.444444	0.684211	0.375000	0.583333	0.733333	0.333333	0.538462
0.500000	0.666667	0.250000	0.333333	0.142857	0.285714	0.454545	0.500000	0.545455	0.250000	0.611111	0.142857	0.583333	0.538462	0.500000	0.538462
0.500000	0.500000	0.250000	0.125000	0.142857	0.285714	0.454545	0.363636	0.400000	0.444444	0.684211	0.142857	0.583333	0.642857	0.333333	0.416667
0.444444	0.625000	0.142857	0.250000	0.000000	0.166667	0.545455	0.454545	0.500000	0.375000	0.666667	0.000000	0.666667	0.615385	0.444444	0.500000
0.666667	0.714286	0.428571	0.500000	0.333333	0.500000	0.600000	0.636364	0.555556	0.625000	0.777778	0.333333	0.600000	0.666667	0.666667	0.666667
0.636364	0.500000	0.600000	0.500000	0.555556	0.500000	0.583333	0.615385	0.666667	0.444444	0.684211	0.555556	0.583333	0.538462	0.636364	0.642857
0.600000	0.625000	0.375000	0.444444	0.285714	0.428571	0.545455	0.583333	0.636364	0.375000	0.666667	0.285714	0.545455	0.500000	0.600000	0.615385
0.454545	0.600000	0.400000	0.300000	0.333333	0.444444	0.272727	0.333333	0.363636	0.222222	0.578947	0.333333	0.416667	0.500000	0.454545	0.384615
0.500000	0.500000	0.250000	0.125000	0.142857	0.285714	0.454545	0.363636	0.400000	0.444444	0.684211	0.142857	0.583333	0.642857	0.333333	0.416667
0.444444	0.625000	0.142857	0.250000	0.000000	0.166667	0.545455	0.454545	0.500000	0.375000	0.666667	0.000000	0.666667	0.615385	0.444444	0.500000
0.500000	0.500000	0.250000	0.125000	0.142857	0.285714	0.454545	0.363636	0.400000	0.444444	0.684211	0.142857	0.583333	0.642857	0.333333	0.416667
0.500000	0.500000	0.444444	0.333333	0.375000	0.500000	0.454545	0.363636	0.400000	0.444444	0.684211	0.375000	0.454545	0.538462	0.500000	0.416667
0.300000	0.600000	0.400000	0.454545	0.333333	0.444444	0.416667	0.333333	0.363636	0.222222	0.500000	0.333333	0.416667	0.500000	0.454545	0.500000
0.500000	0.666667	0.250000	0.333333	0.375000	0.500000	0.583333	0.363636	0.545455	0.444444	0.684211	0.375000	0.692308	0.538462	0.636364	0.538462
0.000000	0.700000	0.500000	0.545455	0.444444	0.555556	0.615385	0.416667	0.454545	0.500000	0.555556	0.444444	0.615385	0.571429	0.400000	0.571429
0.700000	0.000000	0.666667	0.555556	0.625000	0.571429	0.750000	0.545455	0.600000	0.666667	0.789474	0.625000	0.636364	0.785714	0.555556	0.692308
0.500000	0.666667	0.000000	0.125000	0.142857	0.285714	0.583333	0.363636	0.545455	0.444444	0.684211	0.142857	0.692308	0.538462	0.500000	0.538462
0.545455	0.555556	0.125000	0.000000	0.250000	0.375000	0.500000	0.272727	0.454545	0.500000	0.700000	0.250000	0.615385	0.571429	0.400000	0.461538
0.444444	0.625000	0.142857	0.250000	0.000000	0.166667	0.545455	0.454545	0.500000	0.375000	0.666667	0.000000	0.666667	0.615385	0.444444	0.500000
0.555556	0.571429	0.285714	0.375000	0.166667	0.000000	0.636364	0.545455	0.600000	0.285714	0.722222	0.166667	0.750000	0.692308	0.555556	0.583333
0.615385	0.750000	0.583333	0.500000	0.545455	0.636364	0.000000	0.500000	0.416667	0.454545	0.666667	0.545455	0.461538	0.625000	0.615385	0.428571
0.416667	0.545455	0.363636	0.272727	0.454545	0.545455	0.500000	0.000000	0.333333	0.500000	0.619048	0.454545	0.500000	0.562500	0.416667	0.466667
0.454545	0.600000	0.545455	0.454545	0.500000	0.600000	0.416667	0.333333	0.000000	0.545455	0.650000	0.500000	0.416667	0.687500	0.454545	0.500000
0.500000	0.666667	0.444444	0.500000	0.375000	0.285714	0.454545	0.500000	0.545455	0.000000	0.611111	0.375000	0.583333	0.538462	0.636364	0.538462
0.555556	0.789474	0.684211	0.700000	0.666667	0.722222	0.666667	0.619048	0.650000	0.611111	0.000000	0.666667	0.666667	0.571429	0.700000	0.695652
0.444444	0.625000	0.142857	0.250000	0.000000	0.166667	0.545455	0.454545	0.500000	0.375000	0.666667	0.000000	0.666667	0.615385	0.444444	0.500000
0.615385	0.636364	0.692308	0.615385	0.666667	0.750000	0.461538	0.500000	0.416667	0.583333	0.666667	0.666667	0.000000	0.625000	0.615385	0.533333
0.571429	0.785714	0.538462	0.571429	0.615385	0.692308	0.625000	0.562500	0.687500	0.538462	0.571429	0.615385	0.625000	0.000000	0.666667	0.666667
0.400000	0.555556	0.500000	0.400000	0.444444	0.555556	0.615385	0.416667	0.454545	0.636364	0.700000	0.444444	0.615385	0.666667	0.000000	0.571429
0.571429	0.692308	0.538462	0.461538	0.500000	0.583333	0.428571	0.466667	0.500000	0.538462	0.695652	0.500000	0.533333	0.666667	0.571429	0.000000