

Influence of Biotic and Abiotic Factors on the Gut Microbiome of the Western Honey Bee

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Abstract

The gut microbiome of the honey bee plays key roles in digestion, growth and immunity. While there is some evidence that pathogens, such as *Nosema*, modulate the composition of honey bee gut microbiota, field-based studies assessing other environmental cofactors, are lacking. This knowledge gap constrains both disease management strategies and policy development for honey bee protection. This study examined the effects of *Nosema*, arable land use, seasonality and latitude on honey bee gut microbiota under natural conditions across Great Britain. Using worker bees collected via a citizen science beekeeper network, we extracted DNA from digestive tissues. This was analysed using qPCR to detect *Nosema ceranae* and *Nosema apis*, alongside 16S rRNA metabarcoding to characterise microbiome composition. Approximately 40% of colonies contained the parasite *N. ceranae*, while *N. apis* was not detected. Increases in the amount of arable land cover surrounding hives significantly reduced *N. ceranae* gene copy number. Although *N. ceranae* caused significant shifts in microbiome diversity indices, environmental fitting (envfit) permutation tests revealed this was not a significant factor in influencing the gut composition in comparison to other factors such as seasonal variation. Both seasonality and latitude indicated that climate and honey bee diet shapes the honey bee gut microbiome. Multivariate analyses identified shifts in both core and low-abundance taxa in arable landscapes, highlighting the influence of dietary differences and increased pesticide exposure upon the gut microbiota. Collectively, these findings improve our understanding of factors associated with declining honey bee health and colony collapse.

Introduction

The gut microbiota of animals has been shown to play an integral role in food metabolism, growth and development, and can play an important role in a host's immune system [1]. This is also true of the microbial gut community within the Western Honey bee (*Apis mellifera* L.), a globally important pollinator with significant economic value, largely due to their role in honey production and crop pollination [2, 3]. Approximately 90–95% of this insect's gut microbiome is comprised of 12 core species of bacteria belonging to 8 genera [4]. This core bacterial group consist of species belonging to the genera; *Gilliamella*, *Snodgrassella*, *Lactobacillus*, *Bombilactobacillus*, *Commensalibacter*, *Bartonella*, *Frischella* and *Bifidobacterium* [5]. Whilst there is a degree of functional overlap across these microbes, each genus occupies a partially specialised niche [4]. For instance, both *Gilliamella* and *Bifidobacterium* contribute to the digestion of sugars, but *Bifidobacterium* has also been discovered to aid in the detoxification of pesticides [6].

As well as the core microbiota, there are numerous beneficial genera that are detected at lower abundances and frequencies within honey bees which include *Apibacter*, *Apilactobacillus*, *Bombella* and *Zymobacter* [4]. These bacteria play a role in detoxification of secondary metabolites in nectar and chemicals in pesticide sprays, as well as assisting in immune defence through production of antimicrobial substances [7]. Plant pathogenic taxa are also frequently discovered within the gut microbiota of these insects, such as *Arsenophonus* and *Pantoea* [8, 9]. Further, although this typically considered a plant pathogen there is evidence that *Arsenophonus* could negatively affect honey bees, as these microbes are regularly identified within collapsing or failed colonies [10].

In addition to commensal microbes, opportunistic pathogens are frequently present at low abundances within the honey bee gut microbiome [4]. These include bacterial genera such as; *Pseudomonas* and *Serratia* [11, 12]. Dysbiosis, i.e disruption of the gut and its associated microbial community facilitates the infections of these opportunistic taxa [4]. Such dysbiosis is caused by multiple stressors [2], including infection by the microsporidian parasite *Nosema* [13]. Symptomatic infection of *Nosema* (Nosematosis) is found in over 40% of hives worldwide and identified as a major cause of hive collapse [14, 15]. In the UK, there are two species of *Nosema* (*apis* and *ceranae*), both *Nosema* species produce spores that disrupt the alimentary canal in honey bees but differ in clinical presentation [12].

Over the past 60 years, *N. ceranae* case abundance has significantly increased following a host shift after the ranges of the Western Honey bee and the Asian Honey bee (*Apis cerana* L.) began to overlap [16]. This pathogen's subtle symptoms (reduction in vitality and lifespan) avoid beekeeper detection, enabling continued transmission [17]. In contrast, *N. apis*, causes noticeable dysentery prompting containment and decontamination, restricting this parasite's prevalence [18]. Infections of *N. apis* appear to be seasonally distributed, however, whether comparable patterns exist for *N. ceranae* have not yet been established [15, 19].

Laboratory studies that have examined the effects of these pathogens on the gut microbiome, particularly *N. ceranae* [20], suggest a minimal impact on the overall gut microbial diversity [21, 22]. However, indications suggest it can influence key symbiotic genera,

such as *Gilliamella*, *Snodgrassella* and *Lactobacillus* [23, 24]. Braglia et al. demonstrated how this parasite decreases the levels of a few species of *Serratia* within the gut [13].

Since the early 2000s, high rates of colony losses have highlighted the need for further investigation into honey bee health [25]. For example, agricultural intensification can indirectly influence insect gut microbiota [26], through reductions in the floral diversity consumed by these organisms [27]. If plant species fed on by insects affect gut microbiota, then changes in plant species composition temporally (i.e. within season) and spatially (i.e. latitudinal gradients) may affect gut microbiota [28–30]. Poor nutrition and elevated pesticide exposure in agricultural systems negatively alters the honey bee gut microbial community [29, 31]. Despite this, research into how environmental stressors affect both the core and opportunistic gut microbiota, has been limited. Additionally, current studies investigating impacts on gut microbiomes are predominantly conducted in laboratory-controlled conditions, which limits translation to natural environments where multiple factors interact simultaneously [32].

In response, we assessed how individual honey bee gut microbiomes responded correlatively to both disease and environmental factors. We hypothesise that variation in pathogen prevalence, land use, seasonality and latitude, would cause significant shifts of microbiome genera abundance levels. To assess this, we used in situ samples of honey bee specimens collected from operating hives, from which gut microbial data was obtained. These samples were screened for *N. ceranae* and *N. apis*. We analysed the pathogenic data in relation to land use, seasonality and latitude to determine whether these abiotic factors influence these microsporidian parasites. We discuss the results in the context of previous laboratory-based studies, with a focus on how the earlier projects compare to observations from natural environments.

Materials and Methods

Bee Sampling

In 2024, 79 beekeepers participating in the UK National Honey Monitoring Scheme (NHMS) [33], provided euthanized honey bees, from all nine ITL-1 (International Territorial Level) regions within England (North East, North West, Yorkshire and the Humber, East Midlands, West Midlands, East of England, London, South East, and South West) as well as locations in Scotland (Fig. 1a). From each beekeeper 3–5 euthanized adult worker bees were collected and deposited in 2 ml of DNA/RNA shield preservation buffer (Zymo Research) and subsequently placed in -20°C storage on receipt after 2–3 days of transit (See supplementary methods for protocols on collection and euthanasia). Hives from which honey bees were sampled were selected from landscapes with at least 10% of arable land within 2 km of the hives based on the 2023 UKCEH land cover map [34] (Arable land use percentage of samples was between 10.1–82.3% and a mean average of 38.7% with a standard error of +/- 2%). Samples were collected at multiple time points in 2024 (May = 1, June = 10, July = 16, August = 28, September = 12 and October = 12). Variation is due to when beekeepers were available for sampling. Additionally, beekeepers provided information of the health of the colony, including known disease symptoms.

DNA extraction, quantification and normalisation

The specimens' digestive tissue was excised using the protocol outlined in the supplementary methods section. These samples were placed in a 2 ml disruption tube containing 0.3 g lysis matrix A (MP Biomedical) with 1 ml of lysis buffer, consisting of 995 µl DNA/RNA shield and 5 µl of 20 mg/ml proteinase K. Disruption tubes were incubated in a shaking incubator set at 56°C at a speed of 100 rpm for 3 hours. DNA was extracted from the lysed samples using the Zymo Research Soil/Fecal 96 kit as per the manufacturer's protocol for tissue samples. Resultant DNA concentration was measured on a Qubit 4 fluorometer using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Based on these concentrations, samples were diluted to 1 ng/µL to minimise bias in downstream processing arising from differences in template concentration.

Nosema qPCR detection

Samples from each hive were pooled to estimate colony-level *Nosema* abundance. *Nosema* prevalence was quantified using quantitative polymerase chain reaction (qPCR) with primers and probes from previous *Nosema* studies (Table 1), and following the methodology described in Bennett et al. [19], and detailed in the supplementary methods section. Standard curves were generated,

using synthetic oligonucleotide DNA standards (gBlocks) (IDT), in a dilution series ranging from 1 ng/μL to 1 × 10⁻⁷ ng/μL, from which quantification cycle (Cq) values were obtained. The gBlock sequences were obtained from GenBank [35], and the corresponding sequences and accession numbers are provided in the supplementary methods. Samples with Cq values greater than 35 were considered false positives [19, 36]. Gene copy number was calculated from Cq values using the standard curve efficiency, providing an estimate of in-hive pathogen abundance [37].

Table 1
Sequences for primers and probes utilised in the qPCRs in this study on the honey bee gut extracts.

Assay	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe (5'-3')	Reference
<i>Nosema ceranae</i>	CGGATAAAAGAGTCCGTTACC	TGAGCAGGGTTCTAGGGAT	6FAM_5'- CGTTACCCCTTCGGGGAATCTTC	[38]
<i>Nosema Apis</i>	CCATTGCCGGATAAGAGAGT	CCACCAAAAACTCCCAAGAG	5HEX_5' ATAGTGAGGCTCTATCACTCCGCTG	

Bacterial Metabarcoding

Metabarcoding methodology was outlined in Newbold et al. (see also supplementary methods) [39]. In summary, bacterial gut communities were selected through the amplification of the gene encoding for 16S small subunit ribosomal RNA (16S) [39]. A two-step amplification approach was used, whereby universal 16S rRNA gene primers 515F and 806R were modified to include Illumina Nextera adapter sequences (Table 2). The final library was sequenced at a concentration of 7 pm via a V3 chemistry and 10% Phix, onto an Illumina Miseq platform.

Table 2
Sequences of primers used in this study for 16S metabarcoding on the honey bee gut extracts.

Primer Name	Sequence (5'-3')	Reference
515F	GTGYCAGCMGCCGCGGTAA	[40]
806R	GGACTACNVGGGTWTCTAAT	

Sequencing analysis

All reads were processed using the software R (v 4.4.0) and the DADA2 pipeline (v1.30.0) [41, 42]. This involved joining and filtering paired end sequences, as well as removing chimeras from the dataset. Both forward and reverse 16S amplicon reads were trimmed to 250 bases. Filtering parameters were set to maxN = 0 and maxEE = c(2, 2), corresponding to a maximum of two expected errors for both forward and reverse reads. Primer sequences were removed by trimming the first 20 bases from both forward and reverse reads using trimLeft = c(20, 20). The resultant reads were dereplicated and amplicon sequence variants (ASVs) were inferred using the DADA2 core error-correction algorithm. Forward and reverse reads were merged using mergePairs with default parameters. A sequence table was then constructed from merged reads. Chimeric sequences were identified and removed using removeBimeraDenovo with the consensus method. Taxonomy was assigned to resultant ASVs using assigntaxonomy and the SILVA SSU rRNA database release 138 (SILVA v138) [43], and a taxa table was produced. Prior to rarefaction, a sample metadata table was constructed, incorporating pathogen gene copy number, arable land use percentage, collection date and latitude for hive location for each sample. This was combined with both the ASV and taxa tables to make a Phyloseq (v1.46.0) dataset to allow for rarefaction [44].

Proceeding all the filtering stages, 1,990,831 bacterial sequences were retained for downstream analyses. Samples with fewer than 3,000 sequences were excluded from downstream analyses to minimise potential biases associated with low sequencing depth. This threshold was selected based on rarefaction curves, which approached an asymptote at approximately 3000 sequences per sample, as assessed using the Phyloseq and Vegan (v2.6-6) packages [44, 45]. In total, 315 samples from 78 hives remained post rarefaction. The dataset was converted into a MICROeco (v1.6.0) compatible format and subsequently this package was used to

filter and to remove non-target sequences (mitochondrial and chloroplast) [46], resulting in 1,901,084 sequences with 88 taxa spanning 68 genera.

Statistical analysis

Statistical analyses and plots were conducted and produced using R (v4.4.0) statistical environment [41]. To determine which factors influenced *Nosema* intensity, a generalized additive model (GAM) with a log link function and Gaussian error distribution was fitted using the mgcv package (v1.9-0) [47]. Only hives testing positive for *Nosema* were included in the analysis. Log₁₀-transformed *Nosema* gene copy number was used as the response variable, while arable land cover percentage, sampling day, and latitude were included as explanatory variables. The same model format and error term was applied to explore the factors impact on bacterial species composition, by Shannon's diversity index as the response and all the other variables, including log₁₀ *Nosema* gene copy number, as the fixed effects. Smoothing terms were applied for potential non-linear relationships between the predictors, seasonality and latitude, and the response variables in both models.

Beta diversity of the whole gut microbial community was assessed by constructing a Bray–Curtis dissimilarity matrix, followed by permutational multivariate analysis of variance (PERMANOVA) to test for differences in community composition. This was assessed comparing against categorical groupings of disease and environmental covariates (*Nosema* copy levels: 0–21, 22–10,000, and > 10,000 gene copies; arable land cover: low (10.1–26%), medium (> 26–49%), high (> 49%); seasonality: separate seasons (Early-Late), Latitude; split by distinct regions in the UK South (50–<53 °N), Midlands (53–<55 °N) and (≥ 55 °N), see supplementary methods for details). The *Nosema* thresholds were selected based on the distribution of the data and informed by previous qPCR studies investigating these pathogens (Figure A1) [48]. Arable land use, seasonal variation and latitude were categorised to facilitate interpretation of broad-scales patterns in gut microbiome composition. All analyses were conducted using the MICROeco package as well as other packages described [46]. In addition, environmental and biological variables were fitted onto the Bray-Curtis-based distance-based Redundancy analysis (dbRDA) ordination using the *envfit* function in the vegan package. Permutation tests (999 permutations) were used to assess the significance and strength of associations between explanatory variables and the major gradients of microbial community composition observed in the NMDS ordination.

To identify how the bacterial ASVs discovered in the gut microbiome are influenced by biological and environmental stressors, differential abundance analysis was performed using the multivariate ANCOMBC with Benjamini-Hochberg correction for multiple testing, using the ANCOMBC package (v2.12.1) [49]. Predictor variables were assessed in the same format as the GAMs, with the exception of seasonality. This was treated as a categorical variable comprising an early (May-July) and late (August-October) season to identify major shifts in ASV abundances associated with seasonal changes. Prior to analysis, the dataset was converted to a Phyloseq object combining all sequencing and metadata together based on hive origination. Only ASVs present in at least 10% of hives were retained to improve the robustness of downstream statistical analyses [44, 50]. Volcano plots were used to visualise changes in ASV abundance in relation to each explanatory variable. These figures display the log fold change of individual ASVs alongside their significance, as determined through the ANCOMBC testing, thereby facilitating the identification of taxa that were significantly enriched or depleted across conditions.

Results

Nosema prevalence and effects of abiotic factors

Nosema ceranae was detected in 37.5% of hives tested, while *N. apis* was entirely absent (Fig. 1b). Whilst one hive reported *N. apis* symptoms, when tested through qPCR, this colony was negative for both *N. apis* and *N. ceranae*, suggesting a misdiagnosis of Nosematosis. No symptoms of *N. ceranae* were reported by any beekeepers involved.

The generalized additive model (GAM) that was used to assess the effects of the environmental factors on log₁₀ *Nosema* gene copy, explained 27.3% of the variance of log pathogen gene copy and had an adjusted R² value of 0.186. This model revealed that as the percentage of arable land cover around hives increased, log₁₀ *N. ceranae* gene copy number significantly decreased ($\beta = -0.01 \pm 0.005$, $t = -2.45$, $p < 0.05$) (Fig. 2). However, the model suggested there was no significant association between the microsporidian parasite and either seasonality or latitude (Table A1 & Figure A2a-b).

Alterations in the whole gut microbial community

Gut microbiome Shannon diversity was significantly and positively correlated to $\log_{10}$ *N. ceranae* gene copy number ($\beta = 0.01 \pm 0.004$, $t = 2.30$, $p < 0.05$) with this GAM explaining 16.7% of model variance (Fig. 3). No other tested covariate had such impact on the Shannon values (Table A2 & Figure A3a-c). Statistical analyses of beta diversity using PERMANOVA revealed both *N. ceranae* copy level and season sampled had significant influence on the gut microbial community ($F_{4,312} = 2.18$, $R^2 = 0.03$, $p < 0.005$) ($F_{2, 314} = 1.62$, $R^2 = 0.01$, $p < 0.005$). Pairwise PERMANOVA indicated a significant difference in gut microbiome beta diversity between bees with $> 10,000$ *Nosema* gene copies and other copy levels. Arable land cover and latitude had no significant effect on beta diversity within the bacterial community of the gut ($F_{2, 314} = 1.13$, $R^2 < 0.001$, $p > 0.05$), ($F_{2, 314} = 0.87$, $R^2 < 0.001$, $p > 0.05$).

Based upon the envfit analysis, seasonal variation (day of sampling) was the strongest correlate of gut microbiome composition ($r^2 = 0.10$, $p < 0.05$), followed by latitude ($r^2 = 0.038$, $p < 0.05$). Whereas *N. ceranae* copy number and arable land cover were not identified as significant predictors ($r^2 < 0.013$, $p > 0.05$), ($r^2 < 0.005$, $p > 0.05$) (Fig. 4).

Differences of genera influenced by factors

Thirty six ASVs that were identified in at least 10% of hives and subsequently included in the multivariate analyses (Table A4-A7). Of these, only nine ASVs had significant differential abundance according to the ANCOMBC analysis (Fig. 6a-c). $\log_{10}$ *N. ceranae* gene copy number significantly influenced the abundance of only two ASVs, belonging to *Bombella* sp. and *Pantoea* sp., respectively. The *Bombella* sp. ASV showed a positive association with increasing *Nosema* gene copy number, whereas the *Pantoea* sp. ASV showed a negative association (Fig. 6a). Seasonality exerted the strongest overall effect on bacterial abundance. We also found a single *Gilliamella* sp. ASV was significantly more abundant during the late season. The single ASVs belonging to *Arsenophonus* sp. and *Serratia fonticola* also increased in abundance later in the year. In contrast, the *Pantoea* sp. ASV decreased in abundance in the late season (Fig. 6b). Arable land cover had comparatively modest effects on log fold change, although there were six ASVs that were significantly associated with this variable. Increasing arable land cover was negatively associated with ASVs belonging to *Apibacter* sp., *Apilactobacillus* sp., *Bombella* sp., *Commensalibacter* sp. and *Pseudomonas* sp. The same *Gilliamella* sp. ASV that increased during the late season was also positively associated with arable land cover, although the magnitude of this effect was smaller (Fig. 6c). Latitude did not have any significant influence on any ASVs (Figure A7).

Discussion

This study demonstrates that both biological and environmental factors significantly influence the composition of the honey bee gut microbiome. Our findings indicate that *Nosema ceranae* prevalence is increasing and may be spreading rapidly through apiaries as a result of current management practices. Although significant alterations in the gut microbiome were associated with *N. ceranae*, these effects were relatively minor compared with those of other environmental stressors. In particular, seasonality and latitude emerged as key drivers of gut microbial community composition. While arable land cover did not significantly affect overall microbiome structure, it was associated with changes in the abundance of specific taxa.

Prevalence and influences on *N. ceranae*

Nosema ceranae was first confirmed in the UK in 2007 as a newly colonised pathogen to the western honey bee [51]. Our results suggest that *N. ceranae*, which was detected in 37.5% of tested hives, is outcompeting *N. apis*, that was completely absent in the same examined colonies. Similar patterns of competition have been revealed across Europe [16, 22]. One explanation for this is *N. ceranae*'s lack of clinical symptoms allows this pathogen to avoid detection and subsequent decontamination by beekeepers [18]. Previously, *N. apis* was present in 8% of hives across the UK in 2022, suggesting it is on the decline [19]. Generally, *N. apis* is contracted in the summer and becomes symptomatic in the winter months [52]. However, the summer of 2024 experienced more than double the rainfall recorded in the summer of 2022 [53, 54], potentially resulting in a reduction in potential for the honey bees to encounter this pathogen whilst foraging in the natural environment [19]. Therefore, the lack of *N. apis* detection is consistent with current expectations about its climatic sensitivity. Alternatively, *N. ceranae* does not appear to have significant seasonal restrictions [15] (Figure A2a).

Although, we found that *Nosema ceranae* abundance within bees was reduced in high-arable landscapes, this may be explained by differences in colony density with such landscapes [55]. Apiaries in predominantly arable areas are usually sparsely distributed than those in urban environments, leading to reduced interaction between colonies [56]. A key transmission route for honey bee pathogens is through forager behaviours such as robbing, where workers collect resources (e.g. honey) from neighbouring colonies that are contaminated with *Nosema* spores, subsequently introducing the pathogen into their own hive [57]. Therefore, lower colony density in arable landscapes would reduce opportunities for robbing behaviour and pathogen transfer, resulting in lower *N. ceranae* copy number in these areas.

Nosema ceranae effect on the gut microbiome

Consistent with previous laboratory studies, we showed that *N. ceranae* caused significant changes of both alpha and beta diversity of the gut microbiome of honey bees. Both Rubanov et al. and Lau et al. experimentally examined *N. ceranae* infections influence of the gut microbiota and demonstrated the same changes in microbiome indices [21, 22]. These patterns are potentially being caused by *N. ceranae* facilitating the proliferation of less abundant taxa, as a possible consequence of this parasite's disruption within the honey bee's digestive tract [58]. The increase in alpha diversity caused by *N. ceranae*, correlates to the increase of relative abundance of taxa such as *Pseudomonas* and *Zymobacter* (Figure A6a). Parasitic disruption can cause nutrient depletion that could result in a small reduction in relative abundance of core genera, causing a competition release for less abundant taxa such as *Pseudomonas* and *Zymobacter* [59, 60].

Although there were significant alterations caused by *N. ceranae* within the gut microbiome, there was no significant changes to relative abundance of core microbiome genera (Figure A5a), subtle effects that have been noted in previous literature [21, 22, 61]. The core microbiome of honey bees has a relatively low taxonomic diversity but exhibits a great level of stability [4]. If any major shifts in this community did occur there would be noticeable physical symptoms displayed by the bees [62]. Such clinical representations are not demonstrated by individuals with *N. ceranae* [18]. This is further substantiated by the results of the envfit analysis revealing this pathogen was not a significantly influential variable on the community diversity of the gut microbiome.

Changes in the gut microbiota associated to *N. ceranae* may partly reflect age structure of the sampled bees rather than this pathogen's disturbance alone. Beekeepers in this project provided specimens that were potentially of multiple ages. Older forager bees have been shown to be more susceptible to infection of this parasite [63, 64]. These individuals predominantly contain higher levels of *Zymobacter* and lower levels of *Tatumella*, a bacterial genus that is frequently found within nurse bees [65]. *Tatumella* was detected only in *Nosema* free bees, corroborating the possibility that age could be a confounding factor influencing the relationship between *N. ceranae* and gut microbial composition (Figure A6a). Although, *Bombella* abundance increased with *N. ceranae*, despite this genus generally being rare in forager bees [4, 65]. *Bombella* through research has been identified as containing antifungal properties [66], subsequently this genus could have some resistance to the disruption that *N. ceranae* causes. Further supporting the hypothesis that age variation is a confounding factor in its influence of the gut microbiome, and it is not solely responsible for the alterations observed at high *N. ceranae* copy levels in this study.

Other factors impact on the gut microbiome

Seasonal variation was the most important variable in influencing the overall community composition of the gut microbiome within this study. This is probably linked to alterations in the honey bee's diet throughout the year [29, 33]. Seasonality's influence on bees' microbiota has previously been examined across numerous investigations, though usually a comparison of winter and summer bees, but within these studies diet is highlighted as a possible reason for significant changes in the gut microbiome [61, 65, 67]. Reduction in floral diversity is observed as the year progresses in the UK [28, 68], this can impair the nutritional uptake of the bees and disrupt the gut microbiota which could be why majority of ASVs were most abundant in the earlier season [29]. Diet being a key associative factor in these shifts is further supported by latitude being revealed as a significant factor in shaping gut microbiome composition as floral species richness declines from south to north [30].

This project only sampled honey bees across one year and this may not be the usual seasonal pattern. As mentioned earlier, there were high levels of rain in the summer months and possibly lower foraging rates in this period [54]. This means that rather than foraging, the bees could have consumed the hive stores of pollen, nectar and honey at a significantly higher than average rate [69]. Consequently, the increase in *Gilliamella* later in the year could reflect such shifts of feeding behaviour, given the important role of this genus in processing and metabolising dietary carbohydrates within the honey bee gut [70, 71]. Furthermore, the increase in

consummation of the food stores would put additional strain on the hive which could be the reason for observing higher abundances of *Arsenophonus* and *Serratia* later in the year. These taxa are associated with negative consequences such as hive collapse and disease [10, 12]. Consequently, this data could provide potential evidence of the detrimental impact climate change causing extreme weather patterns, has on this important pollinator.

Although arable land use did not significantly alter community indices, this variable still significantly influenced core and less abundant taxa within the microbiome, possibly due to the lower diversity diet of bees in these landscapes [72]. Fields are generally monoculture landscapes in highly arable areas, this significantly reduces floral species that are available for bees to feed on, lowering the amounts of vital nutrients for beneficial bacteria [29, 73].

However, another reason for observed alterations in taxa within arable areas, could be due to an increase in pesticide exposure [68]. Many studies have revealed the detrimental impact these agrochemicals have on taxa such as *Apilactobacillus* and *Bombella* [31, 74]. While *Gilliamella* has previously demonstrated resistance and detoxifying effects to these compounds, this causes an increase in abundance of these bacteria [6, 31], as displayed in this study. Though there is currently limited evidence regarding the relationships between pesticide exposure and the honey bee associated genera *Commensalibacter*, *Apibacter* and *Pseudomonas*. Consequently, further research is required to determine the relative contributions of reduced floral diversity and increased pesticide use to changes in the honey bee gut microbiome within arable landscapes. This highlights the need for additional investigation of honey bees in the natural environment of factors that could have more direct effect on the gut microbiome, such as diet, pesticide use and climate.

Conclusions

Laboratory studies on pollinators such as western honey bees are essential for testing environmental factors under controlled conditions; however, it is equally important to verify that these findings translate to natural systems. Our findings highlight *N. ceranae* as potentially influencing the gut microbiomes in honey bees, through results suggesting a significant impact of the composition of this microbial community. Though *N. ceranae* was revealed to have a relatively weak influence on the gut microbiota in comparison to other factors such as seasonal variation, which is substantiated by previous literature. Analysis of other environmental stressors has emphasized the need for further examination of key influences of the honey bee gut microbiome, including diet, pesticide use and climate, within the natural environment. Future multifactorial analyses measuring these factors could demonstrate how these variables interact and identify key factors influencing honey bee gut microbiota. This information could supply key data for conservation policies, such as furthering the promotion of hedgerows and field margins, and probiotic implementation in order to help a key pollinator that has been showing declines in many populations around the world.

Declarations

Author Contribution

The contributions of each author are as follows: All authors contributed to project conception and design. MB contributed to the acquisition of the data. Data was analysed by MB, LN and BW. MB, LN and BW wrote the first draft, which was reviewed, edited, and approved by all co-authors. MB, LN and BW obtained funding for this research.

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Data Availability

All qPCR, land use and seasonal based data can be obtained in the supplementary. Due to data protection I can disclose latitudinal data of hive locations. Metabarcoding data was deposited on NCBI database with the accession number PRJNA1498048 and the link <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1498048>.

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Figures

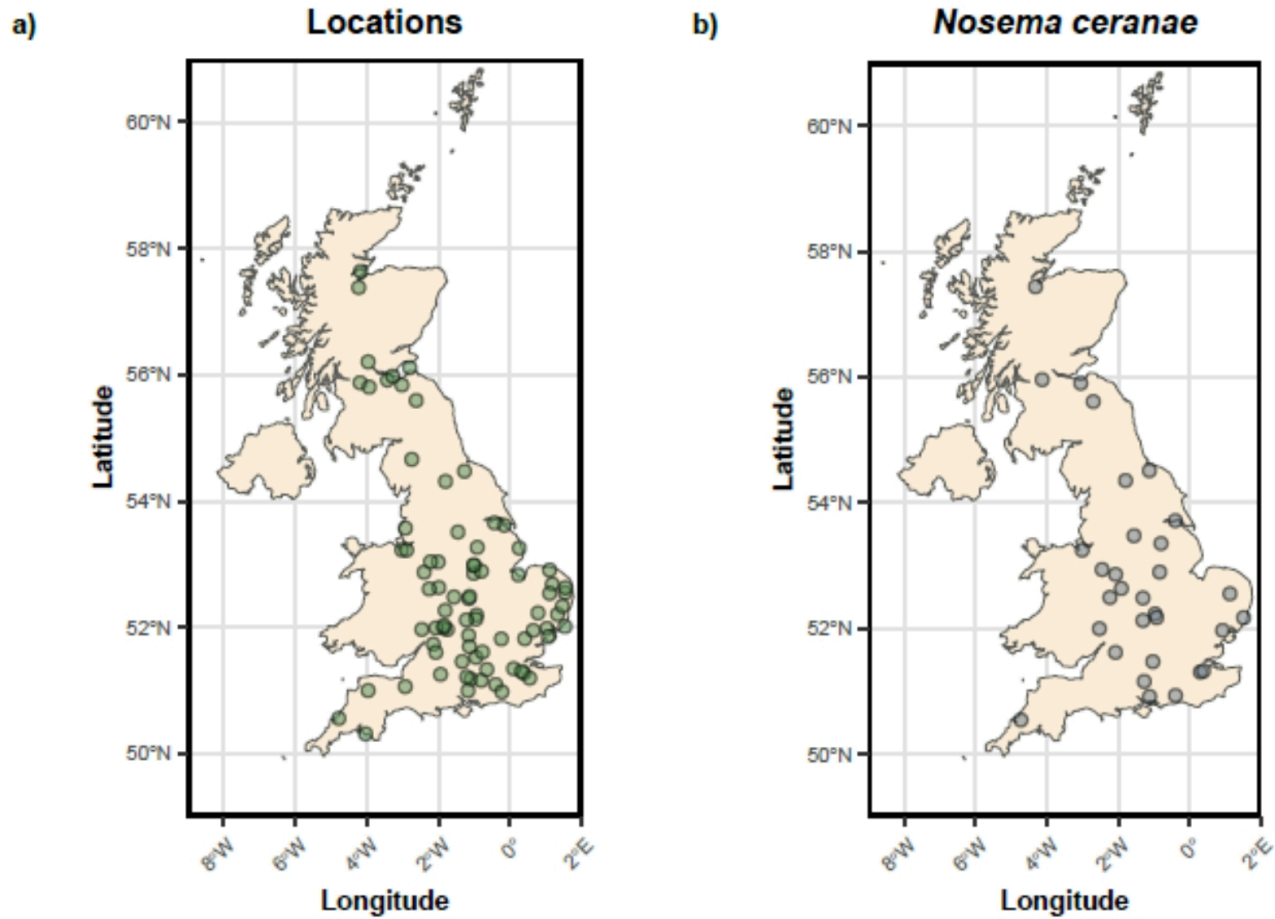


Figure 1

a) Locations of hives that were involved in the project represented by green points. b) Hives with positive *N. ceranae* detections are displayed in grey. Note *N. apis* was entirely absent.

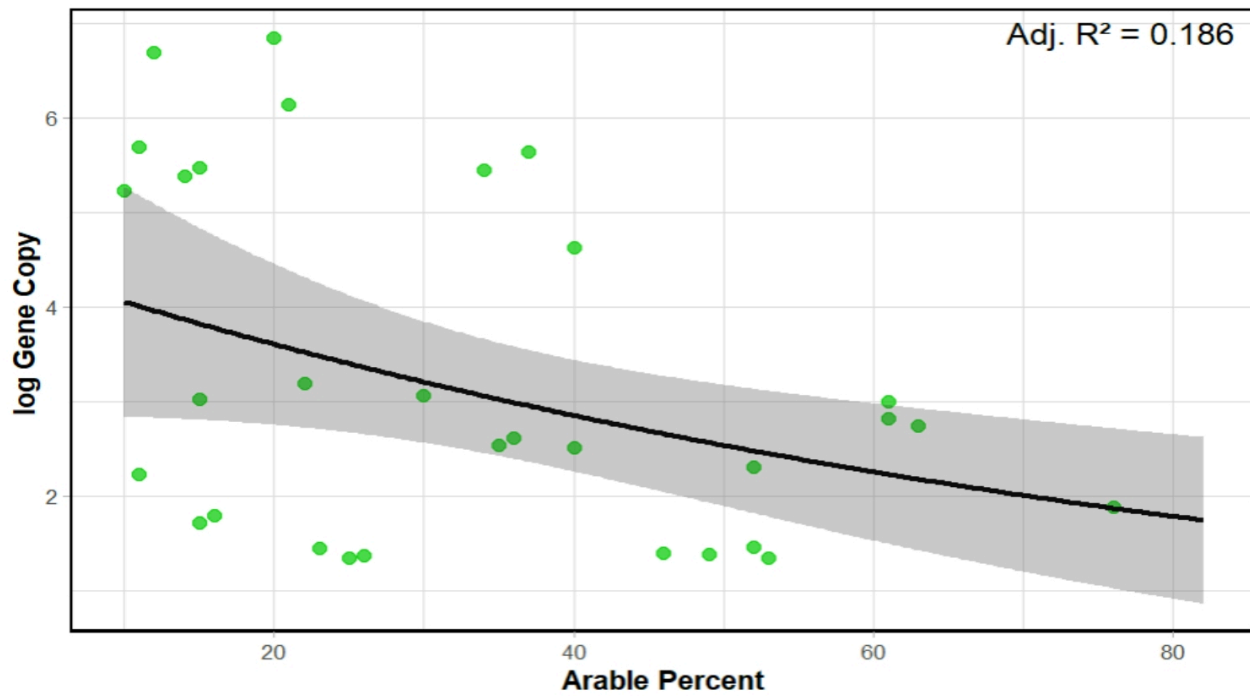


Figure 2

Scatter plot displaying the effect arable land use has on *N. ceranae* intensity.

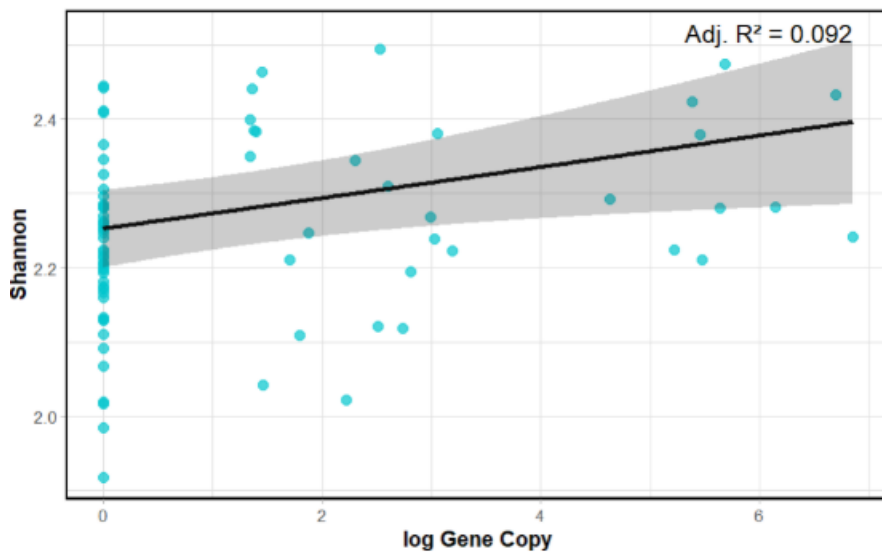


Figure 3

Scatter plot demonstrating how log *N. ceranae* gene copy influences the Shannon diversity index within the gut microbiome of honey bees.

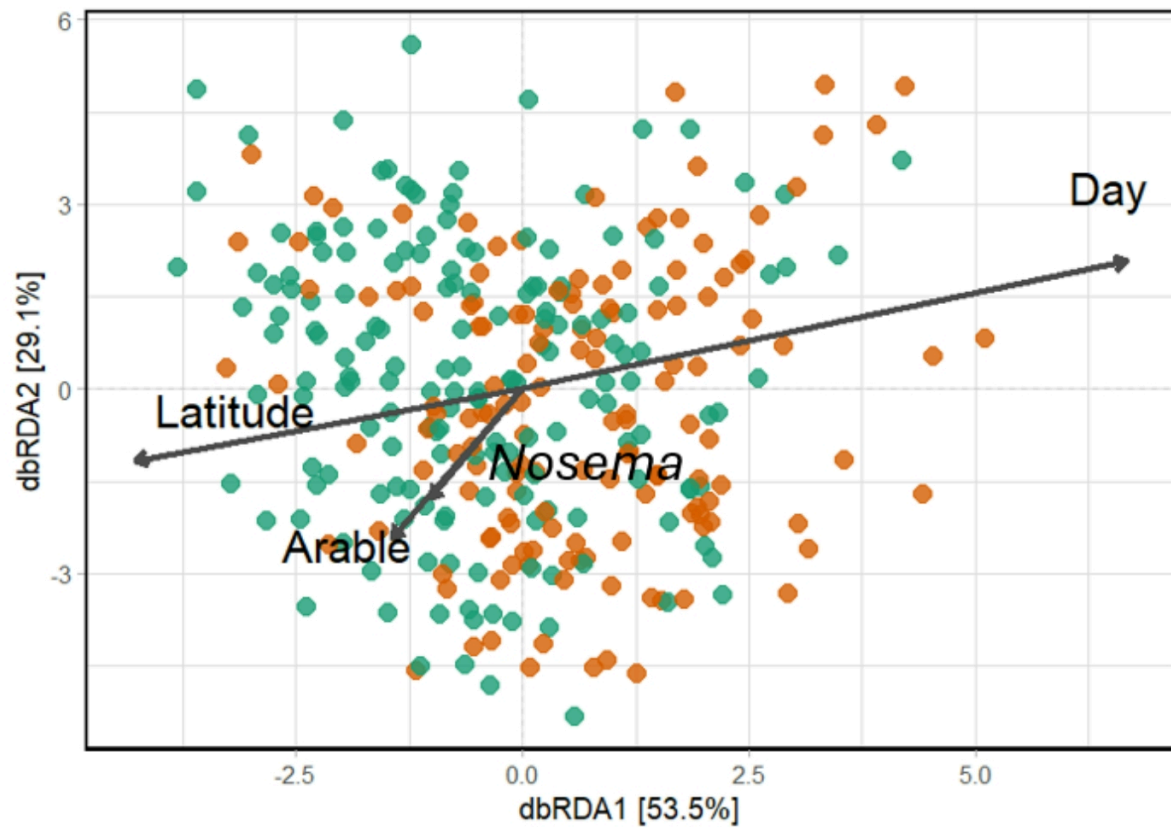


Figure 4

Distance-based redundancy analysis (dbRDA) of gut microbiome community composition based on Bray-Curtis dissimilarities. Samples are coloured by season, with samples collected between May and July being displayed in green and the specimens obtained between August and October shown in orange. The environmental vectors, *Nosema* copy number (*Nosema*), arable land use (Arable), sampling day (Day) and latitude (Latitude) were fitted using envfit.

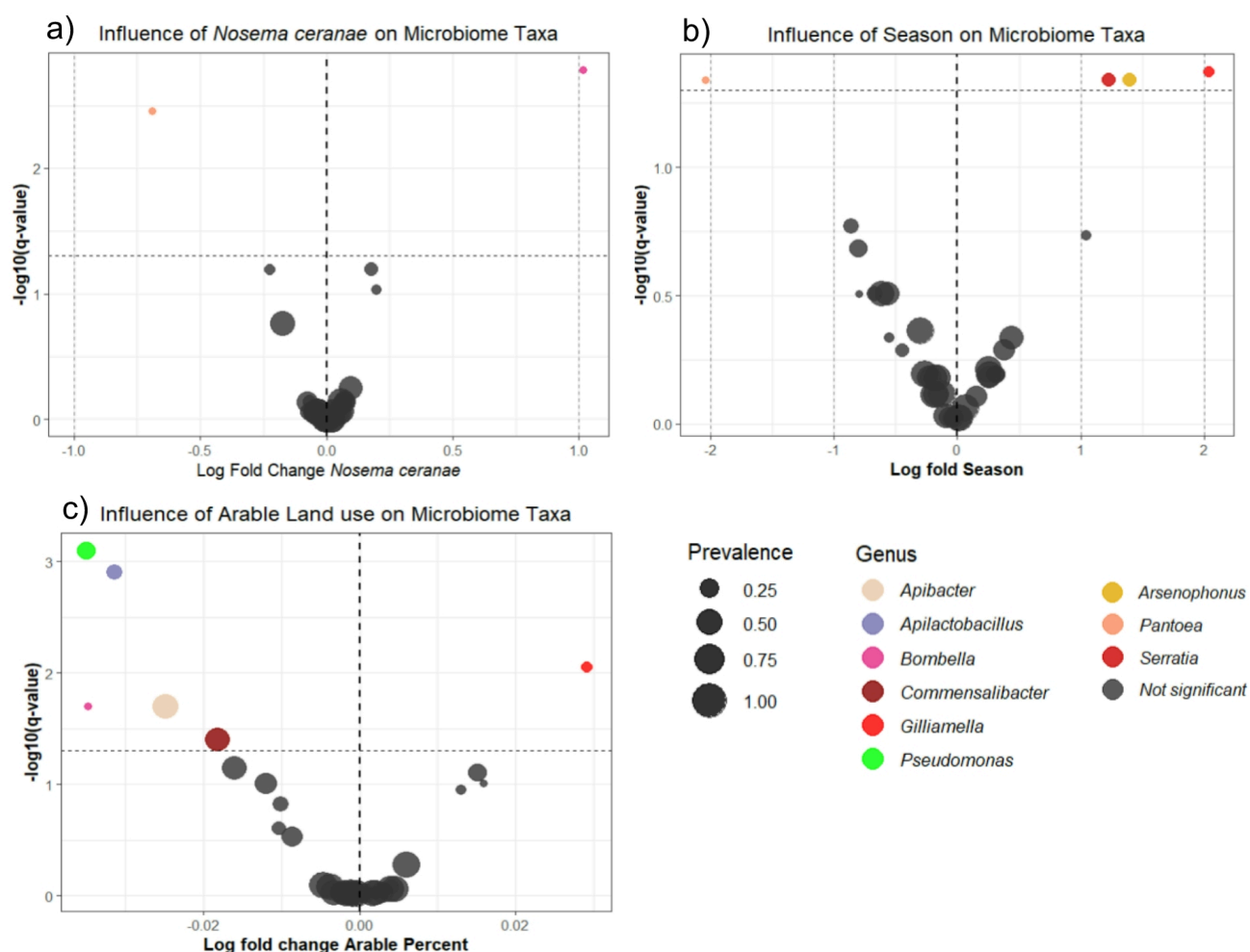


Figure 5

Volcano plots displaying differentially abundant bacterial ASVs identified as being influenced by the tested factors through ANCOMBC analysis. Each point represents an individual ASV, with colours denoting the genus of each ASV. Point size is proportional to the prevalence of each ASV across all 78 hives. The horizontal dashed line indicates the significance threshold ($q = 0.05$), while the vertical dashed line at log fold change = 0 separates positive and negative associations. The vertical dashed line at log fold change = 1 and 2, is to display differences in scales of the x axis between the plots. Each graph displays the results of each factor; a) $\log_{10}$ *Nosema ceranae* gene copy number. b) Season of sampling in the year, with negative log fold change representing ASVs that are detected at higher abundance in the early period, and positive log fold change displaying the variants discovered at higher levels later in the year. c) Arable land use percentage.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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