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The microbiota of *Idaea inquinata* developing on dry herbs

Giulia Magoga[1,2] | **Chiara Piombo**[3] | **Daria Patrizia Locatelli**[3] | **Lidia Limonta**[3] | **Matteo Montagna**[2,4]

[1]Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy

[2]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Italy

[3]Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Milano, Italy

[4]Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology (BAT Center), University of Naples Federico II, Via Università 100, 80055 Portici, Italy

Correspondence
Matteo Montagna, Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Italy.
Email: matteo.montagna@unina.it

Giulia Magoga, Department of Agricultural and Environmental Sciences, Universita degli Studi di Milano, via Celoria 2, 20133 Milano, Italy.
Email: giulia.magoga@unimi.it

Abstract

Idaea inquinata (Scopoli) (Lepidoptera: Geometridae, Idaeini) is a potential pest of stored food, mainly dry herbs. In this study, the role of diet in the shaping of the *I. inquinata*-associated bacterial community was investigated and its impact on insect performance (i.e., proportion of adult emergence and duration of postembryonic development). Larvae were reared on three diets with different nutritional compositions: (1) *Matricaria chamomilla* L. flowers, (2) *Angelica archangelica* L. roots, and (3) artificial diet. A DNA metabarcoding approach targeting V1-V2 and V4 regions of the bacterial 16S rRNA was adopted to characterize the bacterial communities associated with adults and larvae reared on different diets, and estimate their composition and diversity. The core microbiota of this species was found to include some bacterial genera commonly associated with Lepidoptera. When a coverage-based integration of rarefaction and extrapolation of Hill numbers was used to compare groups of samples, the microbial diversity (estimated as phylogenetic diversity) differed among individuals reared on different diets, and also between larvae vs. adults. The lowest taxon diversity was found associated with individuals reared on *M. chamomilla*. Larvae fed with this fiber-rich diet had also a significantly slower development. The composition of the microbial community varied among individuals with different diets, but not between adults vs. larvae. This study highlights the important role of diet in shaping *I. inquinata* microbiota, but also suggests that the microbiota of non-feeding adult moths could be partially inherited from larvae.

KEYWORDS
16S rRNA, bacterial community, diet, dry herbs, Geometridae, *Idaea inquinate*, insect performance, Lepidoptera, metagenomics, microbiome, microbiota, stored product pests

INTRODUCTION

The rusty wave moth, *Idaea inquinata* (Scopoli) (Lepidoptera: Geometridae, Idaeini), is known to develop on hay and dried herbs (Candura, 1931a,b). Moreover, it is reported as a pest of stored food characterized by high fibre content, such as dried medicinal plants (Locatelli et al., 2005; Limonta & Locatelli, 2015). The ability of *I. inquinata* to develop on nutrient-poor substrates poses the question of whether the associated microbiota support the host with nutrients such as essential amino acids (as in the case of other insect species; Ayayee et al., 2016; Xia et al., 2017; Di Lelio et al., 2023). Enhancing this knowledge for an economically important insect group such as Lepidoptera may provide novel insights for improving pest control using innovative strategies (Visôtto et al., 2009; Tang et al., 2012; Chen et al., 2016; Paniagua Voirol et al., 2018). In some Lepidoptera species, the role of the microbiota in providing digestive enzymes and supporting the detoxification of harmful plant secondary metabolites has been hypothesised (Pinto-Tomás et al., 2007; Mason & Raffa, 2014; Mason et al., 2015, 2019; Paniagua Voirol et al., 2018; MsangoSoko et al., 2021). The microbiota of Lepidoptera, that mostly consists of the gut microbiota, has been found in some

studies to be of lower abundance compared to those of vertebrates or insects of other orders (Hammer et al., 2017), whereas in others it was defined as proportionate or even abundant in respect to the body size (Mason et al., 2020).

In Lepidoptera, the important determinants of the gut microbiota abundance are gut alkalinity and rapid food throughput, but also O_2 content and host immune response, which contribute to shaping the bacterial communities associated with the digestive tract (Funke et al., 2008; Anand et al., 2010; Hammer et al., 2017; Shao et al., 2017; Mason et al., 2020). Besides abundance, a high variability in terms of microbial taxa composition among species, and sometimes even within species, has been observed (Gayatri Priya et al., 2012; Hammer et al., 2017; Paniagua Voirol et al., 2018). This variability could be dependent on different factors, the living environment in particular (Staudacher et al., 2016; Hannula et al., 2019; Gomes et al., 2020). In the case of polyphagous species, diet has been demonstrated to have an important role in shaping microbiota diversity (Broderick et al., 2004; Gayatri Priya et al., 2012; Montagna et al., 2016; Staudacher et al., 2016; Paniagua Voirol et al., 2018; Mereghetti et al., 2019). Moreover, in some studies the bacterial communities associated with Lepidoptera have been found to differ consistently between life stages of the same species (Staudacher et al., 2016; Xia et al., 2017), with a higher diversity and abundance of microbial taxa associated with larvae than with pupae and adults (Lin et al., 2015; Xia et al., 2017), as has also been observed in other insect orders (Chouaia et al., 2019). These differences may be the consequence of the morphological and physiological changes resulting from the metamorphosis (Hammer et al., 2014; Paniagua Voirol et al., 2018), but also of the variability in food sources between life stages (Hammer et al., 2014; Xia et al., 2017).

Despite this high microbial diversity among developmental stages and the various factors promoting it, some taxa exist that persist throughout the entire life cycle of individuals of the same species (Hammer et al., 2014), whereas the presence of a core microbiota (or entomotype sensu Montagna et al., 2016) has been outlined in some cases (Broderick et al., 2004; Pinto-Tomás et al., 2011; Paniagua Voirol et al., 2018; Di Lelio et al., 2023). So far, the most commonly detected bacterial phyla among lepidopteran species have been Proteobacteria and Firmicutes, in particular taxa belonging to the families Enterobacteriaceae, Enterococcaceae, Bacillaceae, and Pseudomonadaceae (Paniagua Voirol et al., 2018; Mereghetti et al., 2019; Xia et al., 2020) and, more specifically, to the genera *Pseudomonas*, *Bacillus*, *Staphylococcus*, *Enterobacter*, and *Enterococcus* (Xia et al., 2013; Hammer et al., 2014; Staudacher et al., 2016; Mereghetti et al., 2019; MsangoSoko et al., 2020; Di Lelio et al., 2023). It has also been hypothesized that some of these microbial taxa are involved in fundamental metabolic functions in Lepidoptera (Montagna et al., 2016; Vilanova et al., 2016; Huff et al., 2020; Mason et al., 2022) – and recently it was demonstrated through ad hoc manipulative experiments in case study of *Enterococcus casseliflavus* and its host *Spodoptera littoralis* (Di Lelio et al., 2023).

In this study, a DNA-metabarcoding approach, targeting V1-V2 and V4 regions of 16S rRNA, was used for characterizing the bacterial community associated with adults and larvae of *I. inquinata* reared on each of three diets in order to investigate: (1) whether the diet is a factor shaping the microbiota diversity and composition; (2) the existence of substantial differences in the bacterial diversity associated with larvae and adults of this species; and (3) the presence or absence of a core microbiota preserved across the life stages of the species.

MATERIALS AND METHODS

Idaea inquinata rearing

Idaea inquinata was collected on dried *Hypericum perforatum* L. (Hypericaceae) in a warehouse in Milano (Italy), and reared for 15 years on an artificial diet in a thermostatic chamber (CFT 600; Piardi Tecnologie per il Freddo, Castenedolo, Italy) at 26 ± 1 °C, 70 ± 5% r.h., and L16:D8 photoperiod. The artificial diet was composed of 62 g bran, 8 g maize flour, 7 g wheat flour, 4 g wheat germ, 3 g dried yeast, 9 g glycerol, and 7 g honey (Limonta & Locatelli, 2013). Some newly emerged adults of this rearing were put in a glass jar (10 cm diameter, 19 cm high) closed with tulle, turned upside down on a Petri dish with filter paper to collect their eggs. The eggs were then reared on the three diets, thus some of them were placed on dried *Matricaria chamomilla* L. (Asteraceae) flowers, others on *Angelica archangelica* L. (Apiaceae) roots, and yet others on the artificial diet described above. These new rearings were maintained for a further 2 years before starting the following experiments.

Rearing and diet analyses

Eggs were collected from the three rearing sources and incubated at 26 ± 1 °C and 70 ± 5% r.h. First instars of 1–24 h old, obtained from the eggs of each rearing, were transferred with a fine paint brush into Petri dishes (15 cm diameter) with 10 g of the respective diet. Five Petri dishes of 50 larvae each were set up for each diet type and maintained at 27 ± 1 °C, 70 ± 5% r.h., and L16:D8 photoperiod. The number of adults emerged was recorded daily.

Differences in the post-embryonic developmental time among individuals fed on different diets were assessed through a Kruskal–Wallis test performed using the R v.3.6.2 software (R Core Team, 2019). In the model, the dependent variable was the time passed (expressed in days) from egg hatching to adult emergence, and the diet type was the independent variable. The post-hoc analysis was performed through a Dunn test using the R library FSA (Ogle et al., 2021).

The influence of diet on the number of emerged adults was tested by using a Poisson regression analysis, where

the count of emerged adults from each replicate (Petri dish) per diet type was used as dependent variable in the model; the diet type was the independent variable. The last analysis was performed using R library stats (R Core Team, 2019), and the model was tested for overdispersion using the library AER (Kleiber & Zeileis, 2008). Proximate analyses were performed on 50 g of the artificial diet, *M. chamomilla* flower, and *A. archangelica* roots, to determine their nutritional value (two replicates). Fiber content was analysed according to Prosky et al. (1988), carbohydrates were determined with the Rocklin & Pohl (1983) method, and methods of the Association of Analytical Communities and American Association for Clinical Chemistry were performed to measure proteins (1995), fats (1996), and ashes (AACC 08–01.01).

DNA extraction, library preparation and sequencing

Adults (both males and females) and larvae (third instar) fed on the different diets were collected from the rearing and conserved in absolute ethanol. Larvae at the third instar were selected for the experiments as they were in the middle of the larval stage (usually this species has five instars), whereas adults of this species do not feed. Soon before DNA extraction, the specimens were sterilized to remove bacteria possibly present on the external surface of the body, by performing three washes of 1 min in 10% NaClO, followed by three washes of 1 min in sterile water, as previously described (Montagna et al., 2015). DNA was extracted from pools of individuals fed on the same diet type (as the study target was the microbiota of different diet-associated populations, pooling allowed maximising the number of processed individuals per condition) and from the entire specimen body. Specifically, DNA was extracted from pools of 10 individuals fed on the same diet, for a total of three pools of 10 larvae and three pools of 10 newly emerged adults for each diet type (for a total of 18 samples). Prior to the DNA extraction, sterile glass beads (0.1 mm diameter) were added to the samples, and then they were homogenized using the Tissue Lyser II (Qiagen, Hilden, Germany) for 2 min at 25 Hz. The DNA extraction was performed by the CTAB method, modified as described in Mereghetti et al. (2019). For each batch of DNA extraction samples, a blank replicate (negative control) was performed to monitor environmental and reagents contaminations. Polymerase chain reaction amplification of V1-V2 and V4 regions of the 16S rRNA gene, library preparation, and sequencing were performed at the Life Sciences Department of Trieste University (Italy), as described in detail in previous works (Chouaia et al., 2019; Brunetti et al., 2022). Polymerase chain reaction primers 27FYM (Frank et al., 2008) and 338R (Amann et al., 1990) were used to amplify V1-V2 region, whereas 515F (Caporaso et al., 2011), 802R (Claesson et al., 2009), and 806R (Caporaso et al., 2011) primers were used for the V4 region. Raw reads were deposited on the NCBI SRA database under the project accession PRJNA800637. DNA extraction blanks were checked for bacterial contamination through PCR amplification of the V1-V2 and V4 regions (using the same primer pairs mentioned before). No amplicons were obtained for any of them (PCR amplification results visualized by 1.5% agarose gel electrophoresis). As a further check, the extraction blanks were pooled to form a single sample and attempts were made to prepare the libraries from it (both for V1-V2 and V4 regions of 16S rRNA gene), but no result was obtained.

Bioinformatic analyses

Raw data analyses were performed using the QIIME2 platform (Bolyen et al., 2019). The obtained reads of V1-V2 and V4 16S rRNA gene regions were denoised and taxonomically annotated separately. The DADA2 algorithm (Callahan et al., 2016) was used for denoising and estimating the amplicon sequence variants (ASVs) present in each sample. After training the naïve Bayes classifiers on SILVA reference sequences (SILVA release 138; Quast et al., 2012), trimmed for the amplified region, the fit-classifier-sklearn method (Pedregosa et al., 2011; Bokulich et al., 2018) was employed for the taxonomic annotation of the ASVs (confidence = 0.95). Subsequently, the ASVs assigned to mitochondria and chloroplasts were excluded (considering altogether V1-V2 and V4 regions of all samples, a total of 290 reads assigned to mitochondria and 1324 reads assigned to chloroplasts were removed) and then the ASVs of the V1-V2 and V4 regions merged (merging species-level taxonomy). Subsequently, the SATé-enabled phylogenetic placement (SEPP; Mirarab et al., 2012) method was used for placing ASVs from both regions within a common phylogeny and the resulting tree was used for estimating the phylogenetically informed diversity metrics: Faith's phylogenetic diversity (PD; Faith, 1992) and unweighted UniFrac (Lozupone & Knight, 2005). A Kruskal-Wallis test was performed for comparing the phylogenetic diversity among groups of samples (grouped by diet types and life stages). Non-metric multidimentional scaling (NMDS) analysis was performed on the unweighted UniFrac distance matrix using the R package vegan v.2.5-6 (Oksanen et al., 2019). Moreover, the iNextPD R package was used to compute the phylogenetic diversity estimates and the associated 95% confidence intervals from coverage-based rarefaction and extrapolation of the Hill numbers starting using sample-based incidence data (Chao et al., 2015; Hsieh et al., 2016). PERMANOVA analyses on the unweighted UniFrac distance matrix were performed using QIIME2 (Bolyen et al., 2019) for examining differences in the community composition between diet types and life stages.

RESULTS

Impact of diet on adult emergence and post-embryonic development

The number of adults emerged from pupae of individuals reared on *M. chamomilla* flowers, *A. archangelica* roots, and artificial diet was not different (Kruskal-Wallis $\chi^2 = 0.054$, d.f. = 2,

TABLE 1 Influence of diet type on mean (± SE) number of *Idaea inquinata* emerged adults (starting from 50 newly hatched larvae per diet) and post embryonic developmental time (days)

Diet	No. adults	Egg to adult emergence (days)
Matricaria chamomilla flowers	46.4 ± 0.51	64.7 ± 0.78a
Angelica archangelica roots	46.8 ± 0.58	58.4 ± 0.90b
Artificial diet	47.4 ± 0.75	52.2 ± 0.73c

Means followed by different letters are significantly different (Dunn test: $P < 0.001$).

TABLE 2 Faith's phylogenetic diversity (PD) of microbiota estimated for samples of *Idaea inquinate* adults and larvae reared on each of three diet types: *Matricaria chamomilla* flowers, *Angelica archangelica* roots, and artificial diet

Diet type	Stage	PD
M. chamomilla	Adult	32.32
		29.44
		40.33
	Larva	22.73
		28.98
		21.81
A. archangelica	Adult	35.92
		21.07
		28.94
	Larva	15.46
		34.93
		44.68
Artificial diet	Adult	86.36
		45.84
		27.08
	Larva	33.57
		27.12
		37.32

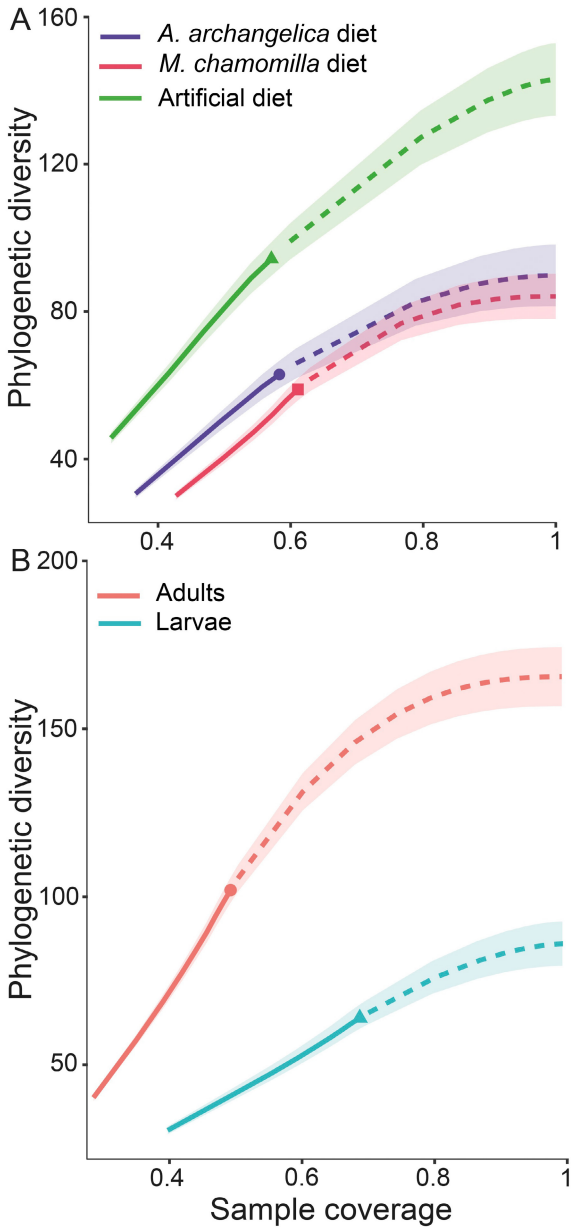

FIGURE 1 Coverage-based rarefaction (solid line segment) and extrapolation (dashed line segment) sampling curves with 95% confidence intervals (shaded areas) for *Idaea inquinata* groups of individuals developed for the diversity order q = 1 (phylogenetic entropy). The solid shapes represent the reference samples. (A) Comparison among groups of individuals reared on different diets; (B) comparison between larvae and adults.

P = 0.97; Table 1). However, the postembryonic developmental time was affected by diet type (Kruskal-Wallis $\chi^2 = 163.66$, d.f. = 2, $P < 0.001$). Larvae developed slowest when reared on *M. chamomilla* and fastest on artificial diet (Tables 1 and S1).

Diet analysis

The nutritional characterization of the three diets indicated that the protein content in the artificial diet was highest, whereas it was lowest in *M. chamomilla* flowers (13.9 vs. 9.7% dry matter; Table S2). Lipid content varied from 2.9% (artificial diet) to 3.7% (*M. chamomilla*), starch content from 1.3% (*M. chamomilla*) to 22.0% (artificial diet), and fibre content from 27.6% (artificial diet) to 52.1% (*M. chamomilla*) (Table S2).

Microbiota diversity and composition

From the 18 samples, consisting of pools of 10 *I. inquinata* individuals fed on the same diet type (three pools of larvae and three pools of adults), in total 69465 raw reads were obtained from the sequencing of the 16S rRNA V1-V2 regions (mean per sample = 3859.2) and 740 387 from the sequencing of the 16S rRNA V4 regions (mean per sample = 41132.6).

Considering both regions together, the highest phylogenetic diversity was associated with adults reared on the artificial diet (mean Faith's PD = 53.09), whereas the lowest diversity was recovered for larvae reared on *M. chamomilla* (mean Faith's PD = 24.51) (Table 2). Faith's PD did not differ between developmental stages (Kruskal-Wallis H = 0.86, P = 0.35) nor among diet types (Kruskal-Wallis H = 1.73, P = 0.42). If the coverage-based integration of rarefaction and extrapolation of Hill numbers was used to compare phylogenetic diversity estimates, differences were observed when grouping samples based on diet type as well as developmental stage. Specifically, regardless of the q value considered, the diversity associated with individuals fed on the artificial diet was higher than that of individuals fed *M. chamomilla* or *A. archangelica*; the latter were found to be comparable (i.e., curves overlapped; Figure 1). Differences were also observed between life stages, with adults being associated with a higher diversity than larvae (Figure 1). The taxon richness for the various sample groups is reported in Table S3.

PERMANOVA pairwise results suggest that the composition of the microbiota associated with *I. inquinata* differed among diet types (artificial diet vs. *A. archangelica*: pseudo-F = 2.88, P = 0.032; artificial diet vs. *M. chamomilla*: pseudo-F = 4.01, P = 0.006), but not between *A. archangelica* vs. *M. chamomilla* (pseudo-F = 0.47, P = 0.79). The same analysis suggests no differences between the bacterial communities associated with larvae vs. adults (pseudo-F = 1.68, P = 0.11) (Figure 2).

Microbial taxa associated with *Idaea inquinata*

The microbiota of almost all *I. inquinata* individuals, independent of the rearing diet, was dominated by Proteobacteria of the genus *Pseudomonas* (median relative abundance of 74.8%), with the only exception represented by one larval pool reared on artificial diet (*Pseudomonas* relative abundance of 11.8%) that, instead, was dominated by Firmicutes of the genus *Bacillus* (74.1% relative abundance) (Figure 3A). The second most abundant taxon (4.1%) was *Sphingobacterium*. Interestingly, 16S rRNA reads assigned to *Neisseria* were recovered in all samples and accounted for an overall median relative abundance of 1.4% (Figure 3A).

Approximately 39% of the taxa (i.e., 22 over a total of 56 in common among at least four samples of each diet group) were shared among the three diet groups (Figure 3B). Individuals reared on *M. chamomilla* and *A. archangelica* harboured only a few unique taxa (four and one, respectively), whereas 23 taxa were uniquely associated with individuals reared on the artificial diet. The core microbiota of the *I. inquinata* individuals (taxa recovered in all samples with a median relative abundance >1%) consisted of seven bacterial taxa: *Pseudomonas* 74.8%, *Sphingobacterium* 5.1%, an unidentified genus belonging to the family Oxalobacteraceae 2.4%, *Acinetobacter* 2.2%, *Neisseria* 1.4%, *Duganella* 1.1%, and *Sphingomonas* 1.1%.

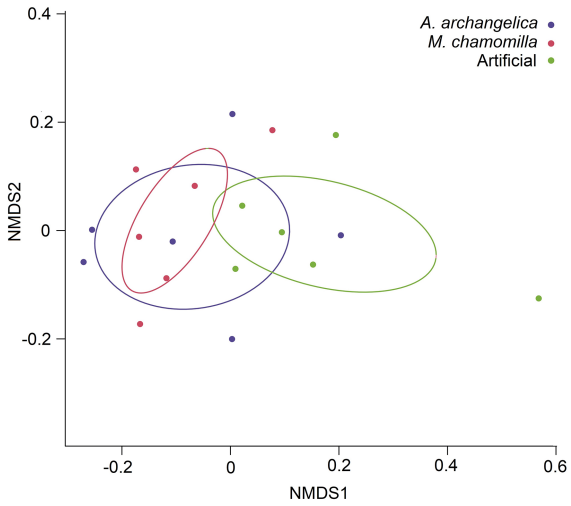

FIGURE 2 Biplot of two-dimensional non-metric multidimensional scaling (NMDS) representing the structure of the microbiota associated with *Idaea inquinata* individuals reared on one of three diets: *Angelica archangelica* roots, *Matricaria chamomilla* flowers, or artificial diet. The dots indicate the 18 samples of this study. Standard error ellipses represent the 95% confidence area around the mean of the various diets.

DISCUSSION

Idaea inquinata microbiota

In this study, the composition and diversity of the microbiota of the rusty wave moth were investigated, with a special focus on the influence of diet in shaping them. Overall, it is necessary to consider that the microbial community composition and diversity were estimated from samples consisting of pools of individuals (potentially hiding the individual variation) and from a limited sample size (potentially biasing the statistical analysis results), thus the obtained results should be confirmed by further, more comprehensive studies. The microbiota of the *I. inquinata* individuals were simpler than those of other non-lepidopteran insects (e.g., Coleoptera; Montagna et al., 2015; Chouaia et al., 2019; Kolasa et al., 2019; Brunetti et al., 2022), as has been found in previous studies on other lepidopteran species (Robinson et al., 2010; Pinto-Tomás et al., 2011; Hammer et al., 2017; Paniagua Voirol et al., 2018; Wang Y et al., 2020; Näsvall et al., 2021). Specifically, 23 taxa only were found with a relative abundance >1% in at least one sample. Among them, seven taxa were identified as the core microbiota shared among all the analysed individuals. This core microbiota was mainly composed of taxa frequently found within the gut of Lepidoptera, such as *Pseudomonas*, *Acinetobacter*, *Sphingomonas*, *Sphingobacterium*, and Oxalobacteraceae (Robinson et al., 2010; Pinto-Tomás et al., 2011; Hammer et al., 2014; Montagna et al., 2016; Chen et al., 2018; Paniagua Voirol et al., 2018; Jones et al., 2019; Mereghetti et al., 2019; González-Serrano et al., 2020; Yang et al., 2020).

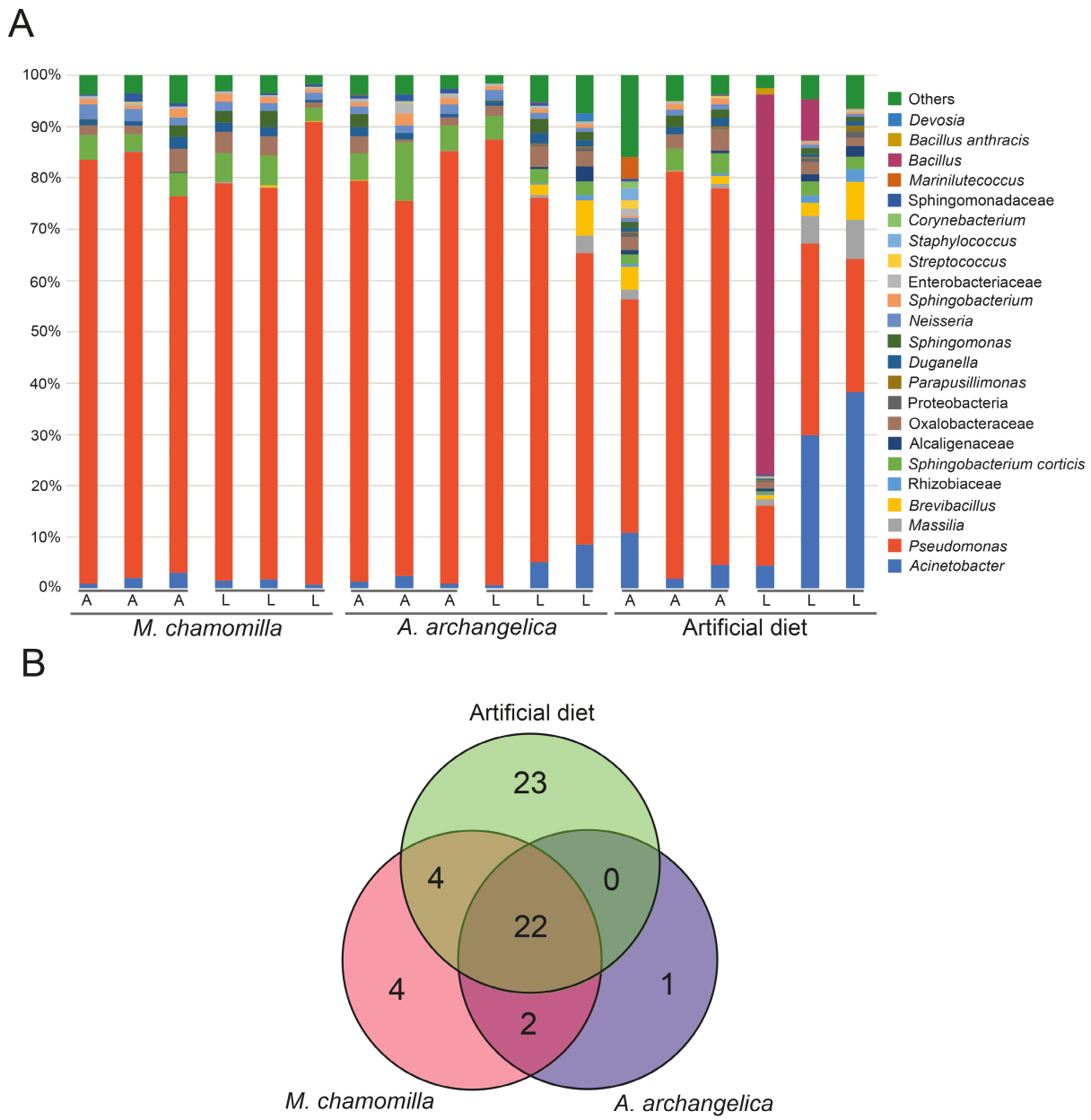

FIGURE 3 Taxonomic composition and core microbiota of *Idaea inquinata* individuals reared on one of three diets: *Angelica archangelica* roots, *Matricaria chamomilla* flowers, or artificial diet. (A) Histogram representing the taxonomic assignment of the bacterial amplicon sequence variants (ASVs) having a median relative abundance ≥1% at least in one sample. (B) Venn diagram showing the bacterial ASVs shared among individuals reared on each of the three diets (only ASVs present in at least four out of the six replicates of each group were considered).

The other two taxa composing the core microbiota in a low relative abundance, i.e., *Neisseria* and *Duganella* (mean relative abundance within the core 1.4 and 1.1%, respectively), are not commonly associated with Lepidoptera. *Neisseria* includes many species: some of them are well known human pathogens, some others are non-pathogenic, and they can also be associated with non-human hosts or be free-living (e.g., in soil) (Liu et al., 2015). Although not common, this is not the first time that *Neisseria* species have been found to be associated with insects; in fact, some *Neisseria* species were isolated from Diptera (Liu et al., 2015) and a new Neisseriaceae-related symbiont was also detected in Phthiraptera (Říhová et al., 2021). A BLAST analysis of the ASV identified as *Neisseria* in this study indicates that it likely belongs to the species *Neisseria oralis* (100% identity; e-value <0.0001), a well-known commensal inhabiting the mucosae of humans and other animals. In any case, due to the short length of 16S rRNA gene segments analysed, the taxonomic assignment to species level should be considered uncertain. *Neisseria* was slightly less abundant in individuals fed with the artificial diet than in the others.

Regarding *Duganella*, a bacterial genus including species typically found in soil and well-known for their antifungal effects (Haack et al., 2016), in previous studies it was found associated with larvae of the moth *Agapeta*

zoegana (L.) (Frederick & Caesar, 2000), but also with the wasp *Asobara tabida* (Nees) (Zouache et al., 2009). The outlined core taxa could represent the resident microbiota of the *I. inquinata*, but the influence of the long-term rearing under laboratory conditions should also be considered as a factor that could have shaped the bacterial community associated (Staudacher et al., 2016).

Diet impact on microbiota diversity and composition

The lowest diversity (based on mean Faith's PD estimates, rarefaction and extrapolation of Hill numbers, and observed taxon richness) was associated with individuals fed on *M. chamomilla*. Regarding the nutritional characterization of the *M. chamomilla* diet, it was the one with the highest fibre content (52%) among those used for feeding *I. inquinata* larvae. Feeding on fibre-rich diets is known to influence the microbiota composition of insect species, since highly specialized microbial taxa are required to sustain them (Tang et al., 2012; Huang et al., 2021). Some bacterial taxa specifically associated with *M. chamomilla* feeding individuals of this study were found, which may be involved in the degradation of fibres. Yet, their very low relative abundance (mean <0.8%) points towards excluding a key role in supporting *M. chamomilla* feeding individuals. Other bacterial taxa found in higher relative abundance in all individuals independent from the diet source are more likely to be involved in fibre degradation (e.g., *Pseudomonas* and *Bacillus* that are reputed to have cellulolytic activity; MsangoSoko et al., 2021). Nevertheless, the significantly longer developmental time recorded for individuals fed on *M. chamomilla* suggests that this diet could be the least suitable one for the healthy development of *I. inquinata*. It is indeed known that slowing development is a compensatory action accomplished by lepidopteran larvae subjected to a poorer nutritional source (Da Silva et al., 2021).

Individuals reared on the artificial diet showed the highest microbiota diversity. They harboured a higher abundance of *Acinetobacter* than individuals fed on the other diets. The bacteria of this genus are considered ubiquitous – they are found associated with water, soil, and animals, but their presence within the gut of adults and larvae of Lepidoptera is widely documented too (Jones et al., 2019; Ugwu et al., 2020; Ma et al., 2021; Saikia et al., 2022), as well as on the surface and within lepidopteran eggs (Chen et al., 2016; Ma et al., 2021). A recent study showed that *Acinetobacter* abundance within the gut of various *Spodoptera frugiperda* (Smith) individuals varies with diet source (Jones et al., 2019), as was found in the current study, but whether these bacteria have a specific role in food metabolism is not well understood.

A significant difference in the composition of the bacterial communities of individuals reared on different diets was found (based on PERMANOVA results), confirming the role of diet in shaping the gut microbiota of Lepidoptera.

Microbiota variation across life stages

Although adults were found to host a higher bacterial diversity than larvae, the bacterial community composition was found not to differ significantly between life stages. This result is in agreement with what was observed for *Plodia interpunctella* (Mereghetti et al. 2019), another species in which adults do not feed, like *I. inquinata*, but it is in contrast with other studies (Lin et al., 2015; Staudacher et al., 2016; Xia et al., 2017; Wang X et al., 2020), and in particular with the notion that physiological changes occurring during the Lepidoptera metamorphosis lead also to reshaping of the microbiota associated with them (Paniagua Voirol et al., 2018). Possibly, major changes in the microbiota after metamorphosis may occur in species in which morphological re-arrangement is followed by a shift in food sources (i.e., larvae and adults require different diets), thus allowing the substitution of resident microbes with those acquired by ingesting different food or from the new environment in which they are in contact, but this could not pertain to species in which adults do not feed. In this scenario, it is likely that the microbiota of *I. inquinata* adults is mainly inherited from the larval stage, and thus that the large part of the larvae-associated bacterial community survives past the metamorphosis.

In conclusion, this study sheds light on *I. inquinata*-associated microbiota, which so far had never been investigated, but also represents a further step towards the understanding of Lepidoptera–bacteria relations and of the main drivers shaping them.

AUTHOR CONTRIBUTIONS

Giulia Magoga: Conceptualization (supporting); data curation (lead); formal analysis (lead); methodology (equal); software (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Chiara Piombo:** Investigation (supporting); methodology (supporting); writing – review and editing (supporting). **Daria Patrizia Locatelli:** Conceptualization (equal); funding acquisition (lead); investigation (supporting); methodology (equal); supervision (equal); writing – original draft (supporting); writing – review and editing (equal). **Lidia Limonta:** Conceptualization (equal); funding acquisition (lead); investigation (supporting); methodology (supporting); supervision (equal); writing – original draft (supporting); writing – review and editing (equal). **Matteo Montagna:** Conceptualization (lead); methodology (lead); supervision (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The 16S rRNA gene raw data generated in this study framework are available in on the NCBI SRA database under the project accession PRJNA800637. Other data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Giulia Magoga https://orcid.org/0000-0002-0662-5840
Matteo Montagna https://orcid.org/0000-0003-4465-7146

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SUPPORTING INFORMATION

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

Table S1. Dunn test for multiple comparisons used for assessing differences in the postembryonic developmental time between the groups of *Idaea inquinata* individuals fed with different diets: *Angelica archangelica* roots, *Matricaria chamomilla* flowers, or artificial diet

Table S2. Nutritional characterization of *Idaea inquinata* diets (*Angelica archangelica* roots, *Matricaria chamomilla* flowers, or artificial diet). Values are expressed as dry matter percentage (mean of two replicates ± SD)

Table S3. Observed taxon richness of the group of samples analyzed in this study (values calculated using iNEXTpd R package).

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