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From Field to Guts: Hidden Gastropod Diversity in Gardens and in the Diet of the Invasive Flatworm *Obama nungara*

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Abstract: Urban green spaces host diverse assemblages of terrestrial gastropods, including native, synanthropic, and non-native species, whose diversity and composition are shaped by multiple abiotic and biotic factors. Among these, predation may represent an important but poorly characterized driver. Standard field surveys can underestimate local diversity by overlooking small, cryptic, or low-abundance taxa. Here, we assessed gastropod diversity and the influence of predation by the invasive terrestrial flatworm *Obama nungara* across urban sites in mainland France using complementary field surveys and molecular approaches. Previously published COI and 16S barcoding data were used for gastropod identification and diversity analyses, while new metabarcoding data were generated from predator gut contents to characterize their diet. Gastropod assemblages showed strong variation in species richness and composition among sites. The barcoding data revealed intraspecific structure within several taxa, suggesting cryptic diversity and highlighting the limits of morphology-based identification. Assemblage structure was only weakly associated with the abiotic variables considered, including soil characteristics and contamination levels. Analysis of predator gut contents revealed a spectrum of gastropod prey, including taxa not detected through field surveys, thereby increasing the estimate of diversity. Diet composition was dominated by slugs, and prey diversity per predator was positively related to site-level gastropod richness. Introduced slug species were detected more frequently in predator gut contents than native ones, while larger slug species were detected less frequently. Together, these results demonstrate the complementarity of field surveys and predator-based metabarcoding for assessing urban gastropod diversity and provide new insights into invasive flatworms as generalist predators.

Keywords: urban biodiversity; terrestrial gastropods; DNA barcoding; DNA metabarcoding; *Obama nungara*

1. Introduction

Urbanization is a major driver of environmental change, profoundly altering habitat structure, microclimatic conditions, and species interactions [1,2]. Although urbanization is often associated with biodiversity loss, urban environments do not necessarily exhibit a uniform decline in species richness. In particular, peri-urban areas, parks, and gardens can sustain relatively high local diversity due to their fine-grained mosaic of heterogeneous habitats [3,4]. However, this apparent maintenance of species richness often masks substantial shifts in community composition.



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Anthropized green spaces typically harbour assemblages dominated by synanthropic and non-native taxa, while many habitat-specialist or less tolerant species are excluded. Beyond reflecting environmental filtering, these compositional changes are also reinforced by biotic pressures associated with biological introductions, including competition with non-native species and the establishment of introduced predators facilitated by human-mediated transport [5]. Together, these processes lead to pronounced spatial turnover among sites. At broader spatial scales, they also result in biotic homogenization driven by the widespread distribution of a limited set of human-associated species [6,7].

Among the soil macrofauna inhabiting urban green spaces, terrestrial gastropods are particularly abundant and conspicuous organisms. They occur in nearly all terrestrial ecosystems and play key functional roles, notably through litter decomposition, nutrient recycling, soil formation, and calcium transfer within food webs [8–10]. Their low mobility and bioaccumulation capacity also make them valuable indicators of environmental conditions [11,12]. Owing to their generally limited active dispersal ability, but high potential for passive transport via human-mediated vectors (e.g., Ref. [13]), and their strong dependence on fine-scale habitat features such as soil properties, calcium availability, vegetation cover, and the presence of shelters including stones, litter, or built structures, terrestrial gastropods are particularly sensitive to urban-induced environmental changes [14–16]. As a result, urban gradients strongly influence gastropod community composition, with factors such as distance from the city center, habitat heterogeneity, vegetation cover, and microhabitat availability playing a key role in structuring assemblages [17–19]. Thus, while relatively high gastropod richness may persist locally in heterogeneous urban green spaces, this diversity is often driven by a subset of generalist or introduced species rather than by the full complement of regional fauna.

Beyond these abiotic and structural drivers, biotic interactions such as predation and competition are also expected to influence the composition and dynamics of gastropod communities in anthropized environments [20]. Terrestrial gastropods occupy a central position in terrestrial food webs and are preyed upon by a wide range of vertebrate and invertebrate predators. Their soft body and calcium-rich shell make them a valuable resource for numerous taxa, including insects, amphibians, reptiles, birds, and mammals [14,21,22]. Anthropized landscapes are also characterized by frequent introductions of non-native gastropods, many of which establish viable populations through human-mediated transport. These introduced species may alternative assemblages through competitive interactions, shifts in resource use, or differential tolerance to disturbed conditions, thereby contributing to community restructuring in urban environments [20,23]. In addition to these competitive effects, predation pressure may be further modified by the introduction of non-native predators, which represents an additional anthropogenic driver affecting land snail populations [7].

Among invertebrate predators, terrestrial flatworms represent a distinctive and often overlooked group. They are mostly carnivorous and prey on a broad range of soil invertebrates, including earthworms, arthropods, other planarians, and terrestrial gastropods [24,25]. Several terrestrial flatworms have attracted attention due to their invasive dynamics and their documented impacts on native gastropod faunas, as other geoplanid species are increasingly reported outside their native ranges, largely through human-mediated transport associated with horticultural trade [26–29]. These introductions predominantly occur in anthropized environments such as gardens, parks and other managed green spaces, which act as entry points and suitable habitats for invasive terrestrial flatworms.

Within the invasive terrestrial flatworms reported in Europe, *Obama nungara* (Platyhelminthes: Geoplanidae) has emerged as a particularly successful species. Native to South America, its introduction into Europe has been traced to human-mediated transport associated with the trade of plants and soil [29,30]. In metropolitan France, *O. nungara* is currently the most abundant invasive terrestrial flatworm, having colonized more than three quarters of the territory and being especially frequent in gardens, parks and other anthropized habitats [29,31]. Laboratory experiments and field observations indicate that *O. nungara* is a generalist predator of soil invertebrates, feeding on earthworms, gastropods (land snails and slugs) and other terrestrial flatworms [25,31,32]. Terrestrial gastropods are recurrently included in its diet, particularly small slugs and snails, consistent with the widespread malacophagy reported in geoplanid flatworms and suggesting a possible influence of prey characteristics on consumption patterns [24,25,33]. However, despite the recognized consumption of gastropods by *O. nungara*, data on its realized diet in invaded environments remain scarce and have so far been largely restricted to earthworm-targeted molecular analyses [34,35], using DNA metabarcoding to identify prey species from digestive samples through high-throughput sequencing [36–38]. The diversity of gastropod prey was then largely under-explored (but see [39] for preliminary results). Consequently, the potential effects of this widespread predator on the diversity and structure of terrestrial gastropod communities in urban environments remain poorly understood, despite its high abundance and frequent occurrence in anthropized habitats where it may act as an important biotic constraint.

Accurately characterizing communities and predator-prey interactions involving terrestrial gastropods is further complicated by the high diversity and complex taxonomy of this group. In France alone, 421 terrestrial

gastropod species are assessed in the Red List of Continental Molluscs of France (https://uicn.fr/liste-rouge-mollusques-continentaux/, accessed on 10 March 2026). However, species identification can be challenging in terrestrial gastropods due to pronounced cryptic diversity and complex species boundaries. Therefore, DNA barcoding enables reliable species identification using standardized genetic markers, complementing morphological identification and allowing access to cryptic diversity that may remain undetected based on external characters alone [40]. Many widespread terrestrial gastropod species, including those commonly occurring in urban and anthropized habitats, belong to taxonomic complexes composed of genetically divergent lineages, raising questions about species delimitation and ecological differentiation within nominal taxa. This has been documented, for example, in the genus *Arion*, with cases involving *A. subfuscus* mitochondrial lineages [41], or *A. vulgaris* and *A. ater* sensu lato (i.e., *A. ater* sensu stricto and the two morphotypes of *A. rufus* described by [42]), in which species introductions and introgression processes further blur species boundaries.

In this study, we investigated the diversity of terrestrial gastropod communities in French urban green spaces invaded by the terrestrial flatworm *O. nungara*. The study specifically examined predator-prey interactions in order to evaluate the impact of predation on terrestrial gastropod communities, as well as the potential of dietary analysis in revealing overlooked diversity. To achieve this, we combined extensive field surveys and DNA barcoding of gastropods with DNA metabarcoding of predator gut contents in ten sampling sites for three years, allowing a detailed comparison between local prey availability and the realized diet of this predator. Firstly, we sought to assess terrestrial gastropod diversity across multiple sites by sequencing COI and 16S rRNA genes (barcoding) and metabarcoding (assessing the potential of *O. nungara*'s diet to reveal hidden gastropod diversity). Secondly, we aimed to evaluate the influence of several environmental factors such as soil type and calcium content, which were predicted to shape local gastropod diversity. Finally, we explored the predatory behaviour of *O. nungara* and examined whether gastropod species status and broad morphological traits influence their frequency of detection in predator gut contents.

2. Methods

2.1. Sampling of Flatworms and Gastropods

Specimens of *Obama nungara* and terrestrial gastropods were sampled twice per year, in autumn and spring, from autumn 2021 (A21) to spring 2024 (P24), across ten sites distributed in four regions of mainland France (Paris region, La Rochelle, Bordeaux region, and Brittany) (Figure 1). These sites were identified through a citizen science program coordinated by the Muséum national d'Histoire naturelle (MNHN), which aimed to document the national distribution of invasive flatworm species [29]. Participants who reported *O. nungara* at their location and who agreed to further investigations provided us access to their properties or organisations. These included private gardens and a municipal greenhouse.

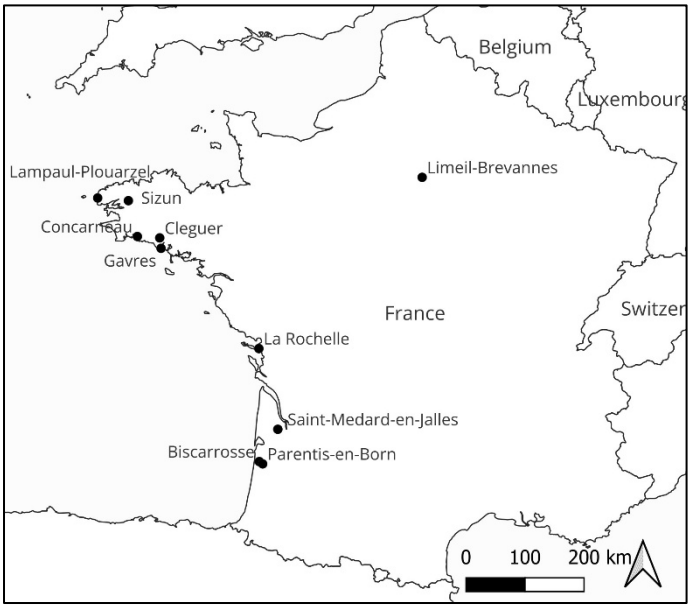


Figure 1. Map of mainland France showing the sampling sites included in the study, represented by points within regions: Limeil-Brévannes in Paris region; La Rochelle; Biscarrosse, Parentis-en-Born and Saint-Médard-en-Jalles in Bordeaux; and the five remaining sites in Brittany. The map is oriented northwards.

For all sites and campaigns, two people conducted a one-hour standardized search within a 100 m^2 area. During this time, both flatworms and gastropods were actively searched for in all favorable microsites, such as on and under vegetation, litter, pieces of wood, stones, and containers. All collected *O. nungara* individuals were preserved in absolute ethanol for subsequent analyses. For gastropods, one individual of each new morphotype encountered was collected and preserved in absolute ethanol (except in a few cases, such as when only juveniles were present or when the species was easily recognizable), and a rough estimate of the abundance of each morphotype within each site was also recorded.

2.2. Identification of the Collected Gastropods

Where possible, morphospecies were preliminary identified based on their external morphological characteristics, such as size, color, and patterns on their bodies and shells, using taxonomic keys [43–46].

Mitochondrial COI and 16S rRNA gene sequences were obtained in previous works (Supplementary Table S1), the aim of which was to create a molecular database for validating metabarcoding primers that target gastropods specifically [39,47]. All sequences were submitted to GenBank and to the Barcoding of Life Database (BOLD) as part of the "MOLPW-Terrestrial molluscs from Platworm sites (France)" project (BOLD dataset: http://dx.doi.org/10.5883/DS-MOLPWP1, accessed on 18 February 2026).

We used Automatic Barcode Gap Discovery (ABGD) [48] and Assemble Species by Automatic Partitioning (ASAP) [49] tools, which are implemented into the Spart explorer web platform (https://spartexplorer.mnhn.fr/, accessed on 3 March 2026), to propose Molecular Operational Taxonomic Units (MOTUs) from the COI sequence dataset. ABGD uses a distance threshold estimated from the distribution of genetic distances between specimens from the same species (intraspecific diversity) and from different species (interspecific diversity). ASAP is based on a hierarchical clustering algorithm that uses only pairwise genetic distances. It proposes species partitions that are ranked by a scoring system that uses no biological prior insight of intraspecific diversity. The analysis was run using Jukes-Cantor (JC69) distances, and the other parameters were set to their default values. The monophyly of the MOTUs was verified in COI and 16S Maximum Likelihood (ML) trees reconstructed using PhyML [50] with 1000 bootstrap replicates and the model of nucleotide substitution selected by Smart Model Selection (SMS, [51]). The trees were rooted with sequences of *Pomatias elegans* (Caenogastropoda, Pomatiidae).

In order to affiliate MOTUs to species, the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 4 March 2026) was used to query GenBank (NCBI). The resulting identifications were then systematically checked against the Barcode of Life Data System (BOLD) Identification Engine [52] using the animal species-level library and the Rapid Species Search operating mode, which consistently confirmed the BLAST assignments without providing additional information.

2.3. Gastropod Diversity Analyses

All diversity analyses were performed in R (version 4.4.2; [53]), primarily using the package vegan (version 2.6-8; [54]). Terrestrial gastropod communities were analyzed based on presence/absence data collected at the ten sites over six sampling campaigns. For diversity analyses, we used the best level of taxonomic information obtained from the combination of morphotypes on the field and ABGD results to calculate species richness and overall community composition at the end of the sampling period. Indeed, since the individuals observed were not systematically collected and sequenced, intraspecific lineages (e.g., *Arion ater rufus* L1, L2 and L3) were not identified at some sites and/or campaigns. When several mitochondrial lineages of the same nominal species were revealed through molecular analysis, these MOTUs were grouped under the corresponding nominal species for the diversity analyses, as they could not be distinguished morphologically in the field. Furthermore, some species could not be distinguished without dissecting their genital anatomy i.e., *Ambigolimax parvipenis* and *A. valentianus*, *Arion hortensis* and *A. distinctus*, juvenile *Cepaea nemoralis* and *C. hortensis*, and *Deroceras invadens* and *D. panormitanum*. When some individuals of these pairs of species were observed and not sequenced, we either conserved the level of morphospecies for the analyses (i.e., *Cepaea nemoralis*/*hortensis* and *Ambigolimax parvipenis*/*valentianus*) or differentiate the species when one of them was restricted to a single site (i.e., *A. distinctus* was found only in Cléguer and *D. panormitanum*, only in La Rochelle). To standardize analyses and reporting, we hereafter refer to these units as "consensus species". Each consensus species thus corresponds to the taxonomic or morphological unit identifiable in the field, including cases where several morphotypes or cryptic lineages could not be fully resolved. This terminology will be used consistently throughout the diversity analyses and subsequent results.

Consensus species richness (i.e., alpha diversity) was calculated for each sampling campaign as the total number of consensus species recorded for each site. In addition, cumulative consensus species richness was

computed by progressively pooling campaigns in chronological order to evaluate the rate of species accumulation and the contribution of each campaign to the final species pool per site. Beta diversity was assessed using the Jaccard dissimilarity index, which is appropriate for presence/absence data. Pairwise Jaccard dissimilarities were calculated based on the final species-by-site matrix, pooling all sampling campaigns. These dissimilarities were used to explore patterns of community composition among sites through hierarchical clustering using the unweighted pair-group method with arithmetic mean (UPGMA) [55].

The diversity and composition of consensus species were subsequently tested against various abiotic parameters. Measurements of these parameters were obtained from [56], in a study that focused on the dynamics of *O. nungara* invasion in France and included the same sites as in the present study (see data in Supplementary Table S2). The influence of soil type, soil calcium content, and concentrations of two heavy metals (lead and chromium, previously shown to affect terrestrial gastropods through toxicity and bioaccumulation processes, e.g., Refs. [57,58], on consensus species diversity and community composition was assessed at the site scale, considering all sampling campaigns together. Other environmental variables (e.g., pH, nitrogen or cadmium concentration) measured in [56] exhibited little variation across the sampled sites and were therefore not included in the analyses. Correlation and ANOVA tests were performed to examine relationships between diversity metrics and continuous (e.g., soil calcium, heavy metal concentrations) or categorical (soil type) environmental variables, while non-metric multidimensional scaling (NMDS) was used to examine patterns in species composition across sites. Differences in species composition among environmental conditions were further assessed using PERMANOVA. Indicator species analyses were performed using the indicspecies package in R (version 1.8; [59]), using the IndVal.g method with 999 permutations.

2.4. DNA Extraction of Obama nungara's Gut Contents

To capture diversity of local trophic interactions, *O. nungara* individuals were selected for DNA extraction as follows: for each sampling campaign (total of six, from A21 to P24) and at each site (ten sites), flatworms were collected from 1 to 10 microhabitats. Three distinct microhabitats were then chosen, with three individuals selected from each, when available. This yielded a total of 337 individuals, with a maximum of nine individuals selected per site and per campaign. DNA from about 20 mg of gut content was extracted from the region corresponding to the opening of the mouth of *O. nungara* according to [34]. DNA extraction was performed using a cetyltrimethylammonium bromide (CTAB) buffer-based method adapted from [60]. Samples were immersed in CTAB extraction buffer and incubated overnight at 57 °C. DNA was then extracted twice using an equal volume of chloroform:isoamyl alcohol (24:1).

2.5. PCR Amplification and High-Throughput Sequencing

A 124 bp fragment of the mitochondrial 16S rDNA was amplified using the primer pair VRF (5′-AYRGCCGCAGTACHYTGACT-3′) and VRR (5′-AGGGTCTTCTCGTCTWWT-3′) developed by [39], a primer set specifically designed to target terrestrial gastropod DNA. The mitochondrial 16S rRNA gene was chosen for gastropod metabarcoding, as COI primers are known to show taxon-specific biases that can lead to underrepresentation or non-detection of some species [61,62]. This rationale is supported by previous studies indicating that 16S is a robust alternative marker for metabarcoding in diverse organisms [63–65]. PCR reactions were performed in 50 µL volumes, including 6 µL of DNA extract. The reaction mixture contained 4 U of GoTaq® G2 DNA Polymerase (Promega, France), 1X Colorless GoTaq® Reaction Buffer, 2 mM $MgCl_2$, 0.5 mM dNTPs, 0.2 µM of each primer, and 0.25 mg/mL bovine serum albumin. Thermal cycling conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 51 °C for 30 s, and 72 °C for 60 s, with a final extension at 72 °C for 10 min.

Out of the 337 field-collected gut samples, 215 produced a positive PCR amplification for terrestrial gastropod DNA (i.e., 63.8% of the samples). Among these, 114 samples (including four juveniles) showing strong amplification signals were selected for high-throughput sequencing (https://www.genoscreen.fr/en/, accessed on 9 April 2026) (see Supplementary Table S3 for metadata). For each sample, a separate library was prepared with a target sequencing depth of 10,000 reads. Each library was assigned a unique index combination, following GenoScreen's protocol for multiplexing. Sequencing was performed on the Illumina MiSeq platform (2 × 150 bp paired-end chemistry) with 15% PhiX.

2.6. Bioinformatic Analyses

Merging of pair-end reads was realized by Genoscreen NGS team using FLASH tool [66] with the following parameters: searching and removing PCR primers (100% nucleotide identity), trimming bad quality reads (Phred quality score Q < 30 bp coverage zone and 97% nucleotide identity).

The Migale platform (https://galaxy.migale.inrae.fr/, accessed on 12 February 2026) was used to filter and cluster sequences. Reads containing ambiguous nucleotides and whose length was not comprised between 119 and 135 bp were discarded and sequences dereplicated using FROGS PRE-PROCESS program. Clustering was then performed using FROGS CLUSTERING SWARM program with d = 1 (a small local linking threshold, representing the maximum number of differences between sequences in each aggregation swarm step) and the fastidious option selected. Clustered sequences will be referred to and identified hereafter as Operational Taxonomic Units (OTUs) in this study. Sequences identified as chimera in all samples were discarded using FROGS REMOVE CHIMERA program. Finally, clusters were filtered using the FROGS CLUSTER FILTERS program: following the detailed analyses from [39], a filtering threshold of 0.4% was applied. This threshold was calibrated in that study using the VRF-VRR primer pair, mock communities, and field replicates to ensure accurate detection of terrestrial gastropods while minimizing false positives.

For the taxonomic assignment of OTUs, a double BLAST approach was used: first with the BLASTn program from NCBI and second, with the reference database described in [39]. Only BLAST matches with at least 95% identity and 95% sequence coverage were used to assign a gastropod species to each OTU. Unassigned OTUs, including those under the threshold and those unknown from any reference database, were then discarded from the analysis to keep clean assignments and real detections. To further validate the taxonomic assignments, a Neighbor-Joining phylogenetic tree was reconstructed including OTU sequences with the corresponding reference sequences. This tree was then inspected to confirm that OTUs assigned to the same species were clustered together and that the reference sequences of each species were included within the same clade.

2.7. Diet Analyses

Sequencing results were analyzed in R 4.4.2 [53] with the phyloseq 1.46.0 R package [67]. Dietary diversity of the predator *O. nungara* was first assessed using the frequency of occurrence (%FOO, i.e., the percent of samples that contain a given food item) and the relative percent of occurrence (POO, i.e., %FOO rescaled so that the sum across all food items is 100%) of prey taxa detected in gut contents [68].

To allow comparisons between dietary diversity results and prey gastropod communities sampled in the field, molecular data were aggregated at the consensus species level. The consistency between field and molecular data in terms of available and consumed prey communities was first assessed using a Jaccard index for each sampled site. This index allowed the proportion of species detected exclusively in the field, exclusively in gut contents, and by both methods to be quantified. To investigate the feeding breadth of *O. nungara* and to evaluate potential changes in feeding behavior with the predator abundance (data extracted from [56]), we analyzed the relationship between the mean number of prey species detected per predator at each site and two variables: the number of prey species available in the field and flatworm density. This approach allowed us to assess (i) whether gut-content richness simply reflects the diversity of available prey, and (ii) whether increasing predator population size is associated with broader or narrower individual feeding niches (i.e., whether changes in predation patterns arise from individual dietary shifts or from cumulative community-level effects). Predator abundance data were obtained during the same sampling periods as the gut-content sampling and were estimated using the same field sampling approach as that used for terrestrial gastropods.

Finally, the frequency of detection of a gastropod species in predator gut contents was modeled using a set of relevant traits: the presence or absence of a protective shell (i.e., snail vs. slug); for snails, the mean shell diameter reported for the species; and for slugs, the mean body length reported for the species and the status (i.e., native or introduced in France) for each species. These data were obtained from Delachaux's Guide to the Identification of European Snails and Slugs [69] and from [70] (see Supplementary Table S4). Using a generalized linear mixed model (GLMM) with a binomial error distribution, fitted with the R package lme4 [71], the response variable was defined as the number of predator gut content samples in which a given gastropod species was detected, relative to the total number of gut content samples analysed for the corresponding site and sampling campaign. The nesting of *O. nungara* individuals within sites was not included in the GLMM, as doing so would alter the model's assumptions and restrict the set of predictors that could be tested. Crossed random intercepts were included for site and gastropod species to account for non-independence among observations within the same site and intrinsic variations between prey species. Species that were detected solely through DNA metabarcoding in predator gut contents but not observed in the field were excluded.

3. Results

3.1. Species Delimitation and Diversity Analyses

At total, 400 gastropod individuals representing 29 morphotypes encountered at the ten sites and during the six campaigns were recorded (Supplementary Table S5). Previously published COI and 16S sequences were available for the gastropod taxa recorded across the ten sites [39], comprising a total of 304 COI and 94 16S sequences (Accession numbers are available in Supplementary Table S1).

After trimming, the COI alignment was 615 bp and 317 nucleotide positions were variable. The distribution of genetic distances showed two modes separated by a barcode gap ranging from 0.02 to 0.12 (Supplementary Figure S1). Species delimitation analysis using the ABGD method produced a set of 43 MOTUs (Supplementary Table S1) based on initial partitioning over all prior values for maximum intraspecific divergence (P), except for extreme values of P. The initial partitioning results were kept consistent, as this approach has proven to be more stable across different settings and consistent with other species delimitation methods [48,72]. Species delimitation was also assessed using ASAP. However, the best-scoring partitions resulted either in the grouping of distinct nominal species or in further fragmentation of nominal species compared with the ABGD partition. Therefore, ASAP results were not retained for subsequent analyses, and the ABGD partition with 43 MOTUs was used.

MOTUs proposed with ABGD were then examined to check if they were represented by supported clades in the ML trees reconstructed using the mitochondrial COI and 16S genes. All MOTUs represented by more than one sequence were monophyletic in the COI tree and supported by bootstrap values >70 except MOTU 23 (*Deroceras reticulatum*) and MOTU 19 (*Cornu aspersum* L2) (Supplementary Figure S2). Most of the MOTUs represented by more than a 16S sequence were distinct and highly supported clades in the ML tree. Exceptions were MOTU 21 (*Deroceras invadens*), MOTU 27 (*Hygromia cinctella* L3) and MOTU 39 (*Theba pisana* L1) that were not recovered monophyletic (Supplementary Figure S3). In two-thirds of cases, the morphotype used to distinguish individuals on the field corresponded to a single MOTU proposed by ABGD and could be affiliated to a known species in France using BLAST and BOLD ID (Supplementary Table S1). In 10 cases the morphotype corresponded to two or more MOTUs, most often reflecting the presence of distinct genetic lineages within the morphological species. It was this level of detail in the delineation of taxonomic units that was chosen for the subsequent analysis of prey present in the digestive contents using metabarcoding.

For the analysis of gastropod diversity on the field, we retained the concept of consensus species (i.e., combining information from morphological and molecular identification) and a total of 32 terrestrial gastropod consensus species were detected across the ten sampled sites and six sampling campaigns (Figure 2). Consensus species occurrences varied widely among sites. *Cornu aspersum* was recorded in all sites. Several consensus species were detected in most sites, including *Ambigolimax parvipenis/valentianus* (nine sites) and *Deroceras invadens* (eight sites), as well as *Oxychilus draparnaudi* and *Cepaea hortensis/nemoralis* (each detected in eight sites). In contrast, several consensus species were restricted to a single site. For example, *Milax nigricans*, *Milax gagates*, *Cochlicella barbara*, and *Theba pisana* were detected exclusively at Lampaul-Plouarzel. *Deroceras panormitanum* and *Limacus flavus* were recorded only at La Rochelle, while *Helix lucorum* and *Arion vulgaris* were detected exclusively at Limeil-Brévannes (Figure 2).

3.2. Influence of Environmental Factors on Gastropod Communities

No significant difference in observed consensus species richness was detected between sampling campaigns or between autumn and spring (Figure 3A,B). When comparing richness values across sites over all six campaigns, significant differences were observed (one-way ANOVA, $F = 8.218$, $p < 0.001$) (Figure 3C). Lampaul-Plouarzel consistently exhibited the highest richness per campaign, ranging from 8 to 11 consensus species. In contrast, Sizun and Gâvres were the least species-rich sites, with campaign-specific richness values ranging from 1 to 3 consensus species at Sizun and 1 to 5 consensus species at Gâvres.

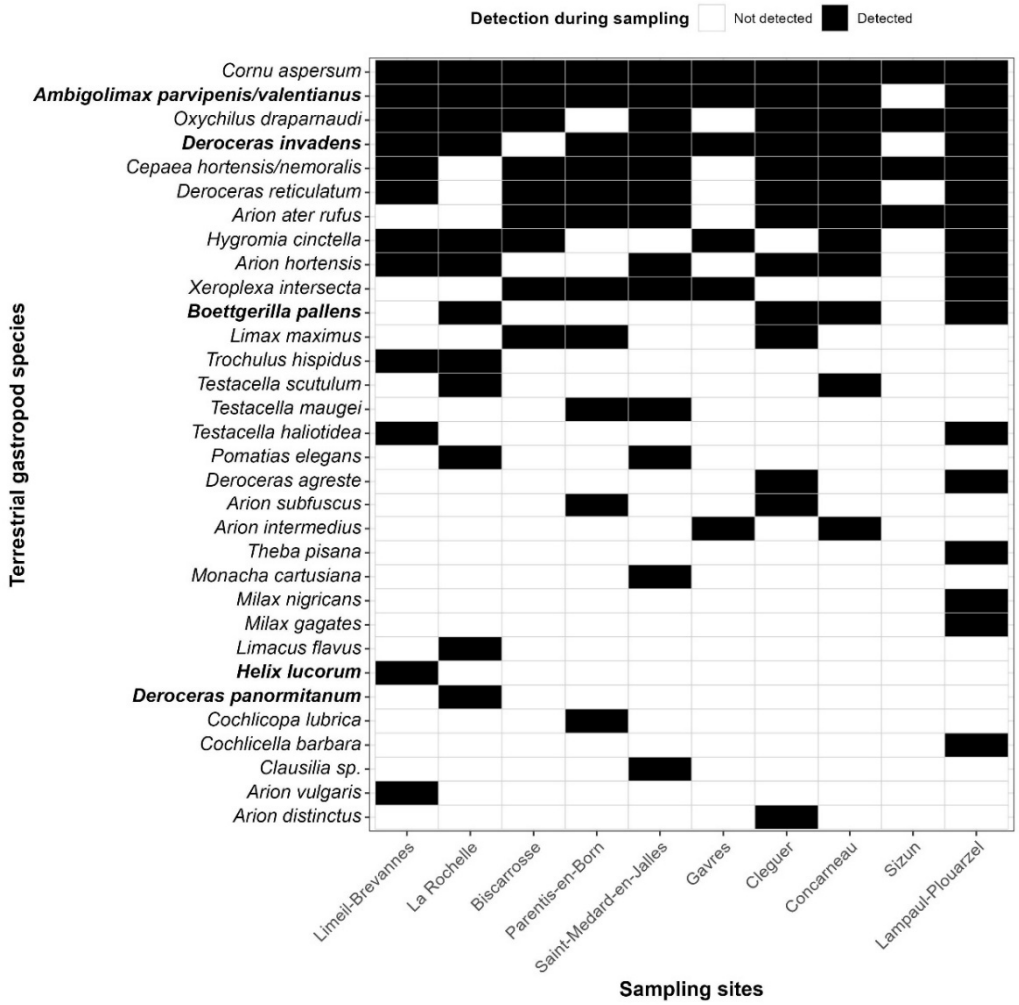


Figure 2. Diversity of terrestrial gastropod consensus species from the field detected across the different sampling sites. Introduced gastropod species are marked in bold.

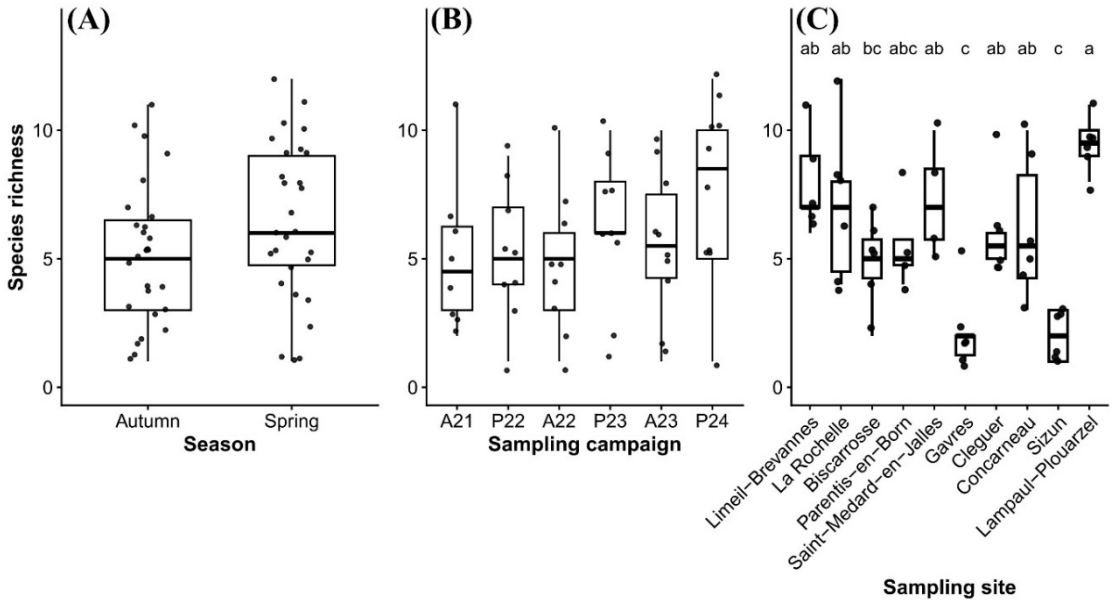


Figure 3. Variation of consensus species richness according to (**A**) sampling season, (**B**) sampling campaign, and (**C**) sampling site over campaigns. Different letters indicate significant differences between sampling sites (post-hoc pairwise comparisons, $p < 0.05$).

Consensus species composition varied among sites (Jaccard dissimilarity), but region did not significantly account for these differences (PERMANOVA, F = 1.25, R^2 = 0.38, p = 0.22). Excluding the two most dissimilar sites with low diversity (Sizun and Gâvres) resulted in a significant effect of region on species composition (PERMANOVA, F = 2.12, R^2 = 0.61, p = 0.006). However, dispersion differed significantly among regions (betadisper, F = 25.87, p = 0.004), indicating that this result may be influenced by heterogeneity in within-region variability rather than solely by differences in species composition. Finally, no significant relationship was detected between consensus species richness and the abiotic parameters tested. Neither total gastropod richness nor richness of snails or slugs separately was significantly associated with soil calcium content or soil type. Similarly, no significant effect of soil heavy metal concentrations (lead and chromium) was observed on total gastropod consensus species richness. Non-metric multidimensional scaling (NMDS) ordinations did not reveal a clear structuring of consensus species assemblages according to soil type, soil calcium content, or heavy metal concentrations. PERMANOVA analyses indicated no significant differences in species composition associated with either soil type and calcium content (PERMANOVA, F = 1.43, R^2 = 0.42, p = 0.06) or with heavy metal concentrations (PERMANOVA, F = 1.46, R^2 = 0.29, p = 0.08), although both tests showed marginal trends. In addition, no indicator species were identified for any soil type, calcium level, or heavy metal concentration.

3.3. Diet Analyses

High-throughput sequencing resulted in 2,337,784 paired-end reads for the 114 samples of *O. nungara* digestive contents. Raw reads have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB107164 (https://www.ebi.ac.uk/ena/browser/view/PRJEB107164, accessed on 20 April 2026). The initial filtering step, which aimed at removing reads outside the expected size range (119–135 bp), retained 2,310,331 reads. Clustering yielded 1764 OTUs, of which 669 were identified as chimeric and removed, corresponding to only 7641 sequences. Finally, applying an OTU abundance filtering threshold of 0.4% of the total sequences per sample resulted in the retention of 63 OTUs, representing 2,265,062 reads.

Among these 63 OTUs, 54 were taxonomically assigned to terrestrial gastropod putative species, meeting the arbitrary assignment thresholds of 95% nucleotide coverage and 95% sequence identity (Supplementary Table S6). Several species were represented by multiple OTUs, ranging from one to five OTUs per species, such as *Cornu aspersum* L1 (4 OTUs) and L2 (5 OTUs), or *Arion hortensis* L2 (4 OTUs). In contrast, eight OTUs could not be assigned taxonomically (i.e., failing to meet the assignment thresholds or returning "*no significant similarity*" in BLASTn searches) and were therefore excluded from downstream analyses. Finally, one OTU was assigned to a trematode species (*Tanaisia zarudnyi*); however, as this primer pair was not designed to target this taxon, and as this species may occur as parasite in terrestrial gastropods [73], this detection likely reflects parasite DNA associated with ingested prey rather than direct consumption and was therefore excluded from subsequent diet analyses.

Within the diet of *O. nungara*, 33 putative species of terrestrial gastropods belonging to 15 different families were detected, including cryptic mitochondrial lineages within nominal species and taxa that could not be distinguished morphologically at the species level (Table 1). Among these taxa, one species was particularly prevalent: *Deroceras invadens*, which was detected in more than half of the gut contents and accounted for over 20% of the diet of *O. nungara* (POO = 20.97, which provides a convenient view as each food taxon contributes a percentage of total diet). At the family level, *Agriolimacidae* (slug species of the genus *Deroceras*) represented nearly 25% of the diet of *O. nungara*, largely driven by the high contribution of *Deroceras invadens*. Other families were variably represented, notably slugs of the family *Arionidae* (7 putative species, 23% of the diet) and snails of the family *Helicidae* (3 putative species, 14.51% of the diet). No specific dietary pattern was observed for juveniles, whose prey did not differ markedly in composition from those of adults.

Across the ten sampled sites, a total of 42 terrestrial gastropod consensus species were detected either during field surveys only, in gut-content analyses only, or in both datasets (Figure 4, Supplementary Figure S4). The mean Jaccard similarity index between field and gut-content data was 44.4%, ranging from 33.3% at La Rochelle to 58.3% at Parentis-en-Born, with values of 35% and 53.3% observed at Cléguer and Concarneau, respectively. Across sites, between 0 and 7 species were detected exclusively in gut contents, 1 to 9 species exclusively during field sampling, and 3 to 8 species by both approaches. Overall, between 6 and 20 species were detected per site when both approaches were considered (Figure 4).

Table 1. Dietary diversity detected in 114 gut contents of *O. nungara* individuals. Gastropod prey items are ordered by family then by taxa. Species status is mentioned. %FOO is the frequency of occurrence and POO is the relative percentage of occurrence for each prey taxa detected.

Prey Taxonomy			Metric	
Family	**Taxa**	**Status**	**%FOO**	**POO**
Agriolimacidae	*Deroceras invadens*	Introduced	57.02	20.97
Agriolimacidae	*Deroceras laeve*	Native	1.75	0.64
Agriolimacidae	*Deroceras panormitanum*	Introduced	0.88	0.32
Agriolimacidae	*Deroceras reticulatum*	Native	7.02	2.58
Arionidae	*Arion ater rufus L1*	Native	4.39	1.61
Arionidae	*Arion distinctus*	Native	9.65	3.55
Arionidae	*Arion hortensis L1*	Native	24.56	9.03
Arionidae	*Arion hortensis L2*	Native	3.51	1.29
Arionidae	*Arion intermedius*	Native	13.16	4.84
Arionidae	*Arion subfuscus S1*	Native	4.39	1.61
Arionidae	*Arion vulgaris*	Native	2.63	0.97
Boettgerillidae	*Boettgerilla pallens*	Introduced	5.26	1.93
Cochlicopidae	*Cochlicopa lubrica*	Native	7.89	2.9
Geomitridae	*Cochlicella acuta*	Native	1.75	0.64
Geomitridae	*Cochlicella barbara*	Native	7.02	2.58
Geomitridae	*Xeroplexa intersecta*	Native	8.77	3.23
Geomitridae	*Xerotricha conspurcata*	Native	1.75	0.64
Helicidae	*Cepaea nemoralis*	Native	0.88	0.32
Helicidae	*Cornu aspersum L1*	Native	21.05	7.74
Helicidae	*Cornu aspersum L2*	Native	17.54	6.45
Hygromiidae	*Hygromia limbata*	Native	0.88	0.32
Lauriidae	*Lauria cylindracea*	Native	14.91	5.48
Limacidae	*Ambigolimax parvipenis/valentianus*	Introduced	16.67	6.13
Limacidae	*Limax maximus*	Native	2.63	0.97
Milacidae	*Milax gagates*	Native	6.14	2.26
Milacidae	*Milax nigricans*	Native	3.51	1.29
Milacidae	*Tandonia* cf. *sowerbyi*	Native	0.88	0.32
Oxychilidae	*Oxychilus draparnaudi*	Native	14.04	5.16
Punctidae	*Paralaoma servilis/morti*	Introduced	6.14	2.26
Punctidae	*Punctum pygmaeum*	Native	0.88	0.32
Pupillidae	*Pupilla muscorum*	Native	0.88	0.32
Succineidae	*Oxyloma elegans*	Native	1.75	0.64
Testacellidae	*Testacella scutulum L1*	Native	1.75	0.64

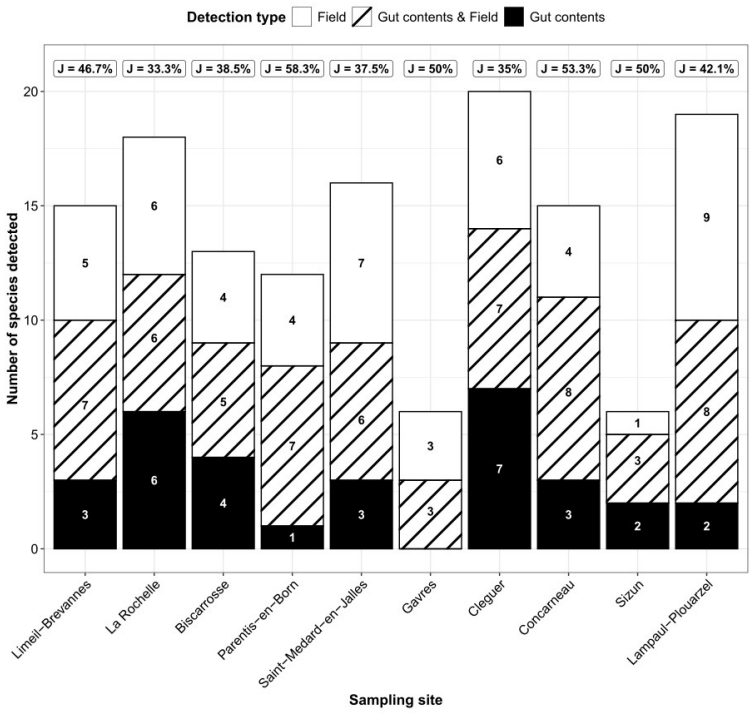


Figure 4. Comparison of gastropod species detected by field sampling and flatworm gut-content metabarcoding across the ten sampling sites. Bars show the number of species detected exclusively in gut contents (black), exclusively during field sampling (white), or by both approaches (hatched). Numbers within each bar segment indicate the number of species in each category. Jaccard similarity coefficients (J) between field and gut-content species assemblages are indicated above each sampling site.

A significant and strong positive relationship was observed between the mean number of prey species per predator gut content and site-level gastropod species richness (R = 0.91, $p < 0.001$; Figure 5). Sites with low species richness, such as Sizun (four species) and Gâvres (six species), showed mean values below two prey species per gut content, whereas Lampaul-Plouarzel, the most species-rich site (17 species), showed a mean close to 3.5 prey species per gut content. No significant relationship was detected between the mean number of prey species per gut content and predator abundance at the site level, although a negative trend was observed (R = −0.57, $p = 0.086$; Figure 5).

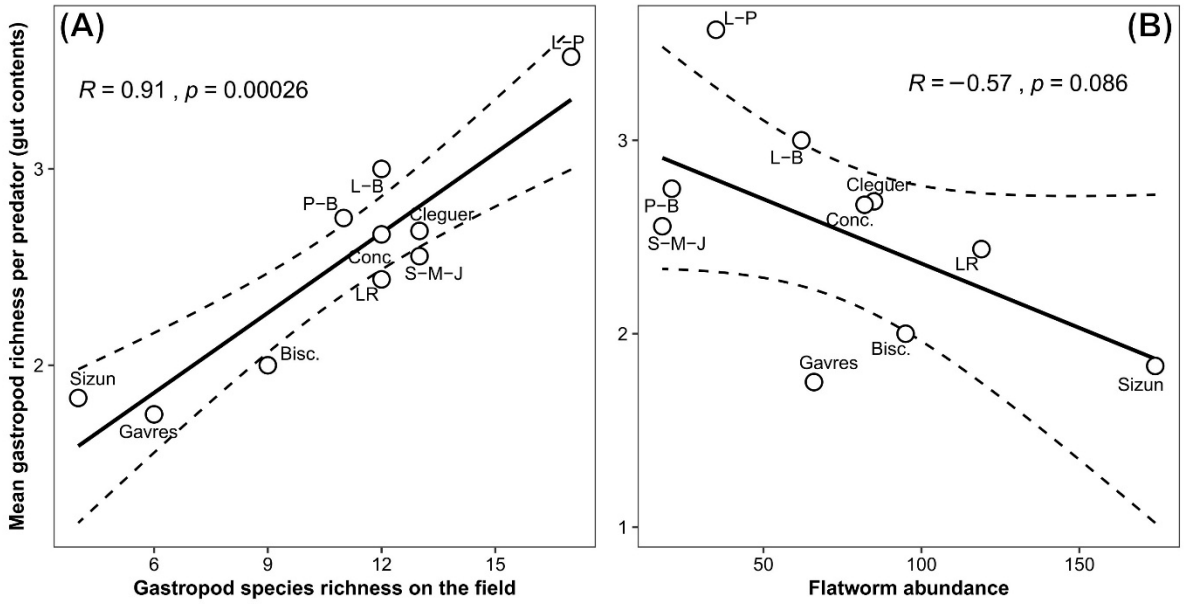


Figure 5. Relationship between the mean number of different prey species detected per predator in gut contents and (**A**) the number of prey species and (**B**) flatworm abundance observed at each site. Each point represents one site (n = 10). (**B**) The linear regression is shown for illustrative purposes, although the Pearson correlation was not significant (R = −0.57; $p = 0.086$). Abbreviations: Bisc.: Biscarrosse; Conc.: Concarneau; L-B: Limeil-Brévannes; LR: La Rochelle; P-B: Parentis-en-Born; S-M-J: Saint-Médard-en-Jalles.

Generalized linear mixed models were used to assess whether gastropod morphological traits were associated with their frequency of detection in *O. nungara* gut contents (Supplementary Figure S5). Snails were detected significantly less frequently than slugs (Estimate = −0.433 ± 0.184, z = −2.351, $p = 0.019$). No significant relationship was detected between detection frequency and mean adult shell diameter in snails. Among slugs, detection frequency decreased significantly with increasing mean adult body length (Estimate = −0.919 ± 0.373, z = −2.465, $p = 0.014$), indicating that larger slug species were detected less frequently in gut contents. Finally, introduced slug species were detected significantly more frequently than native slug species (Estimate = −0.625 ± 0.249, z = −2.512, $p = 0.012$) (Supplementary Figure S5).

Finally, when detection patterns were examined at the family level (Supplementary Figure S6), most slug families showed relatively high proportions of detected species relative to those expected from field observations (i.e., a species recorded in a site is expected to be detected in at least one gut content from this site), with the exception of *Testacellidae*, for which one detection was recorded out of six expected. *Arionidae*, *Agriolimacidae* and *Limacidae* showed higher detection levels, averaging approximately 66% of expected species detected. Among snails, detection levels varied strongly among families: *Oxychilidae* and *Geomitridae* showed relatively high detection rates (seven out of eight and four out of six expected detections, respectively), whereas *Helicidae* and *Hygromiidae* were at low detection, with nine out of twenty and zero out of nine expected detections, respectively (Supplementary Figure S6).

4. Discussion

In this study, terrestrial gastropod diversity in urban green spaces in mainland France was assessed in the context of invasion by the terrestrial flatworm *O. nungara*. By combining field-based inventories with DNA barcoding and metabarcoding approaches, we aimed to characterize gastropod communities and to examine how a widespread generalist predator may contribute to shaping their diversity through trophic interactions. This

integrative framework allows us to discuss both the composition of gastropod assemblages across sites and the potential role of predation by *O. nungara* as a biotic factor influencing these communities.

DNA barcoding provides a useful tool for exploring biodiversity by revealing genetically distinct lineages that may not be distinguishable based on morphology alone. Species delimitation and identification in terrestrial gastropods can be complicated by morphological convergence, intraspecific variability, and cryptic diversity [74–76]. Several cases of cryptic diversity were revealed within morphotypes, including taxa previously reported to be morphologically indistinguishable, as well as distinct mitochondrial lineages within nominal species [47,77–79]. Secondly, morphotypes were found to comprise mitochondrial lineages within nominal species. These results are consistent with the findings of numerous studies indicating that mitochondrial markers could reveal deep genetic structuring within gastropod nominal species. This structuring sometimes corresponds to distinct evolutionary lineages or to ancient phylogeographic patterns, but not necessarily to currently recognized taxonomic units [74,77]. These results highlight the complexity of gastropod species delimitation and provide a basis for further investigation of the evolutionary and taxonomic status of the lineages identified here. The observed mitochondrial lineages show clear genetic divergence, which will be further investigated and refined through ongoing work incorporating nuclear markers and more detailed analyses of divergence patterns.

Terrestrial gastropod richness varied markedly among the ten sampled urban green spaces, ranging from four to 17 consensus species per site, with a total of 32 consensus species detected across all sites and sampling campaigns. Of the 32 consensus species recorded, 27 are native to France [70], and the assemblages were dominated by widespread synanthropic species that are typical of disturbed and human-modified habitats. As examples, *Cornu aspersum* was recorded at all sites, while *Oxychilus draparnaudi* and *Cepaea hortensis/nemoralis* were each recorded at eight out of ten sites. The frequent occurrence of these taxa is consistent with their well-documented association with anthropogenic environments, high dispersal capacity, and long history of human-mediated transport [17,80–83]. Such species are characteristic components of urban gastropod communities and often contribute to buffering overall richness along gradients of habitat degradation through replacement of more anthropophobic taxa, that is, species restricted to natural and semi-natural habitats [3].

In addition to these native synanthropic species, five non-native gastropod species were detected across the ten study sites, comprising four slug and one snail species. These included *Ambigolimax parvipenis*/*valentianus*, recorded at nine sites, *Deroceras invadens* at eight sites, *Boettgerilla pallens* at four sites, and *Helix lucorum* and *Deroceras panormitanum*, each detected at a single site. The occurrence of multiple introduced gastropods is not unexpected in urban environments, where horticultural activities, plant trade, soil movement, and other human-mediated pathways strongly facilitate species introductions and secondary spread [3,19,84]. Urban gastropod assemblages are therefore frequently characterized by a mixture of native and alien taxa. The presence of these non-native gastropods is consistent with numerous studies showing that urbanization promotes the decline of habitat-specialist native species and the spread of generalist and alien taxa, leading to biotic homogenization of terrestrial mollusc communities [3,85]. However, the marked differences in community composition observed among the sites indicate that, in the present study system, the limited number and uneven distribution of introduced species are not sufficient to override local environmental conditions and site-specific management effects.

By applying metabarcoding to the gut contents of a predator occurring in the same urban green spaces, ten additional gastropod species that were not recorded during direct field surveys were detected. This represents an indirect form of biodiversity detection, as the environment itself was not sampled, but rather a potential carrier of prey DNA reflecting local prey availability when the predator exploits a broad spectrum of prey. Nevertheless, these results illustrate how molecular approaches, and high-throughput sequencing in particular, can substantially complement traditional visual inventories and improve estimates of local species pools, especially when combined with conventional sampling methods [86–88]. In terrestrial gastropods, visual surveys can underestimate diversity by failing to detect small, cryptic, or low-abundance taxa, and are further influenced by factors such as weather conditions, sampling timing, and observer experience. In contrast, DNA metabarcoding can detect these overlooked species, including micro-gastropods, thereby complementing traditional field inventories [17,39].

Notably, five of the ten additional species detected by metabarcoding were micro-snails (<5 mm), including *Lauria cylindracea*, which was detected in six sites. Micro-snails are known to be especially sensitive to disturbance and microhabitat conditions. Although their active dispersal capacities are limited, they may be passively transported over long distances by various vectors, making them both ecologically informative and particularly easy to overlook in standard field protocols [22,89]. Among the additional micro-snails detected, the *Paralaoma servilis/morti* complex represents an introduced taxon not recorded during field surveys, likely due to its small size, low detection probability, and the difficulty of visually identifying such taxa. Given the currently known distributions of these species, the detection most likely corresponds to *P. servilis*, which is widely introduced in Europe, whereas *P. morti* is considered endemic to Australia [22,90,91]. Its detection at four sites is

therefore consistent with its high invasive potential and further illustrates the propensity of urban environments to harbor cryptic, widely distributed non-native micro-gastropods that are unlikely to be detected through visual sampling alone.

As shown in numerous previous studies, the pronounced spatial heterogeneity observed among our sites could be expected to reflect the influence of both abiotic and biotic factors (including vegetation cover, habitat heterogeneity, soil properties as soil type, calcium content and pH, microclimatic conditions, and the degree and type of urbanization, and local management practices such as the use of molluscicides in private gardens) on urban gastropod diversity [14,16–19]. In particular, urban gardens are widely recognized as fine-grained mosaics of microhabitats resulting from heterogeneous management practices and structural features, which can sustain high local biodiversity despite their small size [4,18]. However, in this study, analyses of environmental variables that showed sufficient variation among sites to be tested, such as soil type, calcium content, and heavy metal concentrations (lead and chromium), revealed no clear relationship with gastropod richness or community composition. It should also be noted that terrestrial gastropod communities are highly variable over time. Temporal fluctuations in species abundances, as well as in microhabitat conditions and resource availability, can further affect species occurrence within sites [23,92], making such analyses particularly challenging and context dependent. Finally, biotic factors, such as the widespread occurrence of the flatworm *Obama nungara*, may represent a potentially important biotic factor shaping gastropod assemblages, adding an additional layer of complexity to community structure.

Terrestrial flatworms are widely known to exploit a broad range of prey, and invasive species in particular are frequently characterized by flexible feeding strategies [29,93,94]. In this study, 33 gastropod species were detected in the digestive contents of *Obama nungara*, 23 of which were also recorded during field surveys out of a total of 32 consensus species. This high diversity of consumed prey across sites supports the hypothesis, based on previous experimental and preliminary metabarcoding studies at four sites [25,31,39], that *O. nungara* behaves as a generalist predator, consuming a broad range of terrestrial gastropods. The ability to exploit a wide prey spectrum is widely recognized as a key mechanism underlying invasion success, as generalist feeding strategies confer trophic flexibility and facilitate persistence across heterogeneous environments through shifts toward locally available resources [95–97], thereby enhancing establishment and resilience under fluctuating resource availability [93]. In addition, a significant positive relationship was observed between the mean number of prey species detected per digestive content and local gastropod species richness, indicating that a greater diversity of prey is consumed where more prey species are available. Although the absence of quantitative abundance data precludes formal inference on prey selectivity, this pattern is consistent with an opportunistic foraging strategy and suggests a capacity for rapid diet shifts in response to local prey pools.

A significant result toward a higher consumption of slugs relative to shelled snails was observed. This is consistent with several ecological and behavioral mechanisms that may bias predation toward slugs in urban environments. First, slugs are generally more abundant and more consistently present in urban gardens than snails, as repeatedly reported for synanthropic mollusc assemblages [3,7,23]. Given the opportunistic foraging behavior of *O. nungara*, a higher encounter rate with slugs would be expected to translate into higher consumption rates. Second, shelled snails may benefit from more effective anti-predator strategies, including withdrawal into the shell, less active behavior and frequent climbing behavior, which can reduce accessibility to ground-active predators [31,98]. Third, *O. nungara* does not appear highly specialized for snail predation. It captures snails using a simple “capping” strategy (covering or immobilizing the prey) which is less effective against shelled gastropods than the more specialized techniques of some congeners, which may thus further reduce predation efficiency on snails relative to slugs [99]. In addition, personal field observations from authors suggest that slugs may leave more abundant mucus trails than snails, potentially facilitating prey detection and tracking by *O. nungara*, as demonstrated for other terrestrial flatworms such as *Platydemus manokwari* [28].

At a broader taxonomic scale, most slug families showed relatively high detection frequencies in predator gut contents, with the notable exception of *Testacellidae*. This low representation is most likely related to their predominantly subterranean lifestyle, as species of *Testacella* are known to live and forage mainly below ground, where they prey primarily on earthworms [100], which likely reduces both their detectability during field surveys and their encounter probability by *O. nungara*. Among shelled snails, the low detection of *Helicidae* is largely driven by the poor representation of *Cepaea hortensis/nemoralis*, which may reflect both their patchy distribution in gardens and the climbing behavior often described for this genus [98,101]. For *Hygromiidae* and *Pomatiidae*, the low detection frequencies may partly reflect technical biases associated with primer-template mismatches, as in silico and experimental evaluations of the primer pair used in this study have shown reduced amplification efficiency for several species belonging to these families, including *Hygromia cinctella*, *Trochulus hispidus*, and *Pomatias elegans* (along with a thick operculum), due to mismatches near the 3′ end of the reverse primer [39].

These observations underscore the inherent limitations of amplicon-based diet analyses, where the presence and amplification of one taxon's DNA can be affected by the simultaneous presence of others [102], a challenge that is intensified in complex samples such as gut contents, where can occur DNA degradation, inhibitors, and preferential amplification [103].

Beyond these differences, the identity and origin of prey species can further shape predation patterns. The significant difference in predation between native and non-native slugs, with introduced species being consumed more frequently, may reflect broader urban biodiversity trends in which non-native gastropods often become dominant components of communities. Although abundance was not assessed in the present study, previous studies have shown that introduced species frequently reach higher abundances and can progressively replace native taxa, contributing to the biotic homogenization of urban mollusc communities [3,6,7]. In garden habitats in particular, slug assemblages are often dominated by introduced taxa, largely due to repeated introductions via horticultural trade, the high invasibility of these environments, and life-history traits such as high reproductive rates and by competitive or predatory interactions with other gastropods [23,104]. Under an opportunistic foraging strategy, a higher encounter rate with non-native slugs would therefore be expected to translate into higher consumption rates. This pattern is especially well illustrated by *Deroceras invadens,* which was detected in all sites where it was expected and additionally at one site where it had not been recorded in field surveys, and was found in more than half of the analyzed predators. This species is known to reach high abundances in urban and horticultural settings and is frequently among the most common slugs in gardens, sometimes rivaling or exceeding native congeners [23,105].

5. Conclusions

This study illustrates how the combined use of DNA barcoding and metabarcoding enhances the understanding of terrestrial gastropod diversity and predator-prey interactions in urban green spaces. Barcoding revealed cryptic mitochondrial lineages within several nominal gastropod species, refining species identification beyond morphology alone, while predator gut metabarcoding detected ten additional species not recorded in field surveys, highlighting overlooked taxa and the limitations of visual sampling. Gastropod richness and community composition varied among sites, but environmental factors such as soil type, calcium content, and heavy metal concentrations showed no clear effect, suggesting that other drivers, including microhabitat variability, vegetation structure, biotic interactions, and local management practices, likely play a stronger role. Finally, *Obama nungara* was confirmed as a generalist predator that consumes a wide variety of gastropods. This suggests that it could be a factor in altering communities in certain ecological contexts. Although slugs tended to be more frequently consumed than snails, prey traits did not produce clear patterns, suggesting the need for behavioral studies to further explore selective predation. Future studies could expand upon these findings by integrating predator-derived metabarcoding with environmental DNA (eDNA) from soil or litter, which would provide a more direct and standardized assessment of local gastropod diversity. Investigating interspecific interactions among prey, including recently acquired data on earthworm predation, could further clarify community-level dynamics and potential indirect effects of *O. nungara*'s predation. Behavioral assays to test prey selection and the functional consequences of slug versus snail traits will also help refine our understanding of predator-prey interactions in heterogeneous urban habitats.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/26110214 30242077/SE-26060040-SI.zip. Figure S1: Distribution of genetic distances indicating two modes separated by a barcode gap ranging from 0.02 to 0.12. Species delimitation analysis using the ABGD method; Figure S2: Maximum likelihood (ML) tree reconstructed from the COI sequences obtained in this study. The tree is rooted with the COI sequence of *Pomatias elegans*. The numbers indicated at the nodes correspond to bootstrap values greater than 70. At the end of each sample name, an abbreviation indicates the site of origin: LB: Limeil-Brévannes; LR: La Rochelle; BISC: Biscarrosse; PB: Parentis-en-Born; SMJ: Saint-Médard-en-Jalles; GAV: Gâvres; CLG: Cléguer; CONC: Concarneau; SIZ: Sizun; LP: Lampaul-Plouarzel. MOTU numbers refer to the Automatic Barcode Gap Discovery (ABGD) analysis; Figure S3: Maximum likelihood (ML) tree reconstructed from the 16S sequences obtained in this study. The tree is rooted with the 16S sequence of *Pomatias elegans*. The numbers indicated at the nodes correspond to bootstrap values greater than 70. At the end of each sample name, an abbreviation indicates the site of origin: LB: Limeil-Brévannes; LR: La Rochelle; BISC: Biscarrosse; PB: Parentis-en-Born; SMJ: Saint-Médard-en-Jalles; GAV: Gâvres; CLG: Cléguer; CONC: Concarneau; SIZ: Sizun; LP: Lampaul-Plouarzel. MOTU numbers refer to the Automatic Barcode Gap Discovery (ABGD) analysis; Figure S4: Detection level of terrestrial gastropod consensus species across the ten sampled sites based on field surveys and

flatworm gut content analyses. Columns represent sampling sites and rows represent gastropod species. Hatching denotes species detected only during field surveys, circles indicate species detected only in gut contents, and filled black indicates species detected by both approaches. Jaccard similarity indices (%) between field and molecular detections are shown above each site. Introduced gastropod species are marked in bold; Figure S5: Predicted frequency of detection of gastropod in predator gut contents as a function of (**A**) gastropod category (slug or snail), (**B**) slug species status (native vs. introduced), and (**C**) for slugs, the average body length (mm). Curves represent predicted probabilities from the final generalized linear mixed models (GLMM). Areas indicate 95% confidence intervals, and *p*-values shown are derived from likelihood-ratio test (type II ANOVA) assessing the overall effect of the variables; Figure S6: Observed and expected detections of gastropod prey across families, shown separately for slugs and snails. For each family, bars represent the total number of detections summed across sites. Black portions indicate observed detections in gut contents, while white portions indicate expected detections based on field presence but not recovered in gut contents. Expected detections correspond to the sum of site-level presences of all species within a family (e.g., a species present at multiple sites contributes multiple expected detections); Table S1: Morphological and molecular identification of terrestrial gastropod specimens collected across sampling sites and campaigns. For each specimen, COI and 16S accession numbers, field-based morphospecies identifications, MOTU assignments obtained through ABGD-K2P species delimitation, and taxonomic assignments based on BOLD/BLAST comparisons are reported. For specimens assigned to reference lineages, the corresponding COI and 16S reference clades, closest reference accession numbers, query coverage, percentage identity, and associated references are provided; Table S2: Soil characteristics of the ten sampling sites, including soil type and concentrations of chromium (Cr), lead (Pb), and calcium oxide (CaO); Table S3: Metadata and molecular processing information for terrestrial flatworm samples. Each row corresponds to an individual specimen, identified by its sample code, and includes the sampling campaign, year, season, region and site, PCR amplification outcome, sequencing status, and juvenile status; Table S4: List of terrestrial gastropod taxa recorded in the study, classified by category (snail or slug) and biogeographic status (native or introduced), with their average shell diameter (snails) or average body length (slugs), expressed in millimeters; Table S5: Number of terrestrial gastropod individuals recorded for each taxon and, where applicable, lineage, at each sampling site during each sampling campaign. Counts are reported separately according to the identification method: S, molecular identification based on COI or 16S sequences; M, identification based on morphology only. TOT indicates the total number of individuals recorded at each site across all sampling campaigns; Table S6: Operational taxonomic unit (OTU) table generated from DNA metabarcoding of terrestrial flatworm digestive contents. For each OTU, the representative seed sequence, the total number of observations across all samples, and the number of observations in each individual sample are reported.

Author Contributions

M.V.: Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing—original draft, writing—review and editing, visualization; N.M.: Investigation, writing—review and editing; X.C.: Investigation, writing—review and editing; S.N.: Investigation, writing—review and editing; L.D.: Investigation, resources, writing—review and editing, project administration, funding acquisition; V.R.: Conceptualization, methodology, validation, formal analysis, investigation, writing—review and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Ethical review and approval were waived for this study because it involved only terrestrial invertebrates (gastropods and terrestrial flatworms), which are not covered by the scope of Directive 2010/63/EU on the protection of animals used for scientific purposes.

Informed Consent Statement

Not applicable.

Data Availability Statement

All data supporting the findings of this study are available in the Supplementary Materials or through publicly accessible databases and online repositories, as cited in the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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