



The impact of *Triticum aestivum* L. cultivation management on genomic polymorphism of *Harpalus rufipes* and *Silpha obscura* assessed by RAPD markers

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Abstract

Beetle genetics is becoming increasingly important in research on agricultural ecosystems, not only from a basic biological perspective but also for practical applications in pest management, biodiversity, and agroecosystem sustainability. Our study analyzed the genetic variability of beetle populations (*Harpalus rufipes* and *Silpha obscura*) under two types of agricultural management systems—organic and conventional—using RAPD markers. Out of six tested markers (OPB 5, OPB 8, OPB 11, OPB 12, OPB 14, and OPB 18), three markers (OPB 11, OPB 14, and OPB 18) demonstrated clear genetic differentiation between beetle samples collected from organically and conventionally cultivated wheat. The results of DNA fingerprinting and t-SNE analysis confirmed the formation of two genetic clusters corresponding to the management type. Jaccard similarity coefficient values indicated moderate to strong genetic similarity within individual management systems, while similarity between systems was weaker. These findings suggest that agronomic practices influence the genetic structure of beetle populations, likely due to ecological and anthropogenic factors such as pesticide use and landscape modification. The study emphasizes the importance of molecular markers in assessing population-level responses to agroecosystem management and their contribution to sustainable agriculture.

Keywords Coleoptera · Agroecosystem · Crop management · Wheat · DNA analysis

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Introduction

Genetic variability of insect populations represents a key aspect in understanding their adaptations to changing agroecosystems. The development of molecular approaches, from RAPD to SNP and genome sequencing, has fundamentally expanded the possibilities for detecting genetic differentiation, gene flow, and evolutionary responses to management interventions (Williams et al. 1990; Welsh and McClelland 1990; Behura 2006; Webster et al. 2023; Pita et al. 2025).

Genomic syntheses in Coleoptera indicate that beetle diversity is linked to a long evolutionary history and adaptations to angiosperms, which frame the expected patterns of variation even in agricultural environments (McKenna et al. 2019). In an applied context, population genetics of pests plays a crucial role, for example, in documenting and managing resistance to transgenic crops or insecticides. A notable example is the field-confirmed resistance of the western

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corn rootworm to corn, followed by cross-resistance among multiple Bt traits (Gassmann et al. 2011, 2014, 2019).

Alongside Bt approaches, RNAi strategies and new biopesticides are rapidly developing, specifically targeting molecular resistance pathways particularly in *Leptinotarsa decemlineata* and complementing IRM recommendations (Sparks and Nauen 2015; Balaško et al. 2020; Timani et al. 2023). At the same time, genomic resources for invasive species such as *Harmonia axyridis* provide opportunities for detailed testing of invasion routes and changes in genetic structure across landscapes (Chen et al. 2021; Kajtoch et al. 2022). Further studies build on these findings through methodological frameworks of DNA fingerprinting and similarity quantification, aiming to link molecular indicators with practical implications for sustainable agroecosystem management (Denholm and Rowland 1992; Pumariño et al. 2011; Sun et al. 2023).

Harpalus rufipes and *Silpha obscura* are suitable model species for genetic studies in agricultural landscapes due to their high abundance, ecological relevance, sensitivity to management practices, and suitability for molecular marker analyses. Their occurrence in arable fields enables the assessment of genetic differentiation in response to farming systems and environmental pressures. In agroecosystems, *Harpalus rufipes* is particularly valuable because carabid beetles can deliver an important regulating ecosystem service via weed seed predation; recent syntheses and field studies show that higher carabid activity-density is associated with stronger seed removal and that management (e.g., tillage regime) can alter ground-beetle communities and their potential contribution to weed control (Charalabidis et al. 2025). *Silpha obscura* adds complementary functional relevance: carrion-associated beetles contribute to decomposition and nutrient/energy cycling through scavenging, and *S. obscura* is also reported from agricultural areas (e.g., collected in wheat fields) and has been discussed as an agricultural pest, making it informative for linking landscape management, population genetics, and ecosystem functioning (Guseva and Koval 2024).

The aim of our research is to test the hypothesis of whether there are differences in the genetic structures on the level of DNA polymorphism of the species *Harpalus rufipes* and *Silpha obscura* between two types of management systems (organic and conventional).

Materials and methods

In 2022, we performed DNA analysis on 10 individuals of *Harpalus rufipes* and 10 individuals of *Silpha obscura*. Specimens were collected in a *Triticum aestivum* field cultivated under both organic and conventional management.

The individuals were obtained using pitfall traps on each study plot, five pitfall traps were placed in a line, with a distance of 10 m between each trap. As a fixative, 4% formalin was used. Sampling was conducted at monthly intervals from April to July. Individuals of both species originated from the spring generation. The specimens were identified to species level, and nomenclature was adjusted according to Zahradník (2017).

Under conventional soil management, spraying was carried out using Delmetros 100 SC, Lambada, Karate Zeon 5 CS, Karate Zeon 050 CS. Delmetros 100 SC is an pesticides containing the active substance deltamethrin, a synthetic pyrethroid. It acts on voltage-gated sodium channels in the nervous system of insects, causing paralysis and subsequent death after contact or ingestion. Lambada, Karate Zeon 5 CS, Karate Zeon 050 CS they contain lambda-cyhalothrin, a synthetic pyrethroid widely used in crop protection. It acts on voltage-gated sodium channels in the insect nervous system, leading to hyperexcitation, paralysis, and subsequent death after contact or ingestion.

The study areas are located in the cadastral territory of Nitra (geographical coordinates: 48°17'19.6" N, 18°06'48.1" E). The area lies at an altitude of 130 m a.s.l., the soil type is cambisol, and the climate is warm with mild winters.

DNA isolation

Genomic DNA was isolated from the samples (insect elytron) of *Harpalus rufipes* and *Silpha obscura* using the commercially available ISOLATE II Plant DNA Kit (Meridian Bioscience®), according to the manufacturer's guidelines. DNA was purified by a sodium acetate-based protocol. The concentration and purity of the extracted DNA were determined using a NanoPhotometer P360 spectrophotometer (IMPLEN).

RAPD assay

To generate distinct genetic fingerprints, the Random Amplified Polymorphic DNA (RAPD) method was applied. The following primers and their reverse sequences (5'–3') were used: OPB_05 (TGCGCCCTTC), OPB_08 (GTCCA CACGG), OPB_11 (GTAGACCCGT), OPB_12 (CCTTG ACGCA), OPB_14 (TCCGCTCTGG), and OPB_18 (CC ACAGCAGT). The amplification mix comprised 2× Eli-Zyme HS Robust MIX Red, 0.6 µmol·dm⁻³ of the selected primer, DNA template, and ultra-pure H₂O to a final reaction volume of 10 µL. PCR amplification was conducted on a C1000™ Thermal Cycler (BIO-RAD) using the following cycling conditions: initial denaturation at 94 °C for 5 min, followed by 45 cycles of denaturation at 94 °C for 1 min,

annealing at 36 °C for 1 min, and extension at 72 °C for 2 min. A final extension step was performed at 72 °C for 7 min. Reactions were performed in duplicates.

Amplified products were separated by electrophoresis on a 1.5% agarose gel, stained with GelRed™ Nucleic Acid Stain (Biotium), and visualized using a G-Box imaging system (SYNGENE).

Data analysis

The banding patterns were interpreted using GeneTools software (SYNGENE), which enables automatic detection of distinct amplicons based on signal intensity. Presence or absence of individual bands was encoded into a binary format to generate matrices, which were subsequently analyzed in the R environment (R Core Team 2020), using dplyr, stats, pheatmap, and RColorBrewer packages (RStudio Team 2020). These binary matrices were converted into pairwise distance matrices using the Jaccard similarity coefficient. The derived distance metrics were then utilized to construct dendrograms and heatmaps via the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering algorithm.

All RAPD profiles were consolidated into a single binary matrix. Pairwise distances were recalculated using the Jaccard similarity coefficient to quantify fragment presence/absence. For dimensionality reduction and visualization, t-distributed Stochastic Neighbor Embedding (t-SNE) analysis was performed in Python 3.12 using the scikit-learn library with the following parameters: perplexity=13, learning_rate=200, and random_state=42. The resulting t-SNE plots were visualized in Python using the matplotlib library, providing a complementary view of the genetic relationships identified through clustering.

Results

Our results show that three of six tested RAPD markers (OPB 11, OPB 14, and OPB 18) confirmed genetic difference between beetle samples from conventionally grown wheat monoculture and those from organically managed monoculture. This result suggests that the type of agronomic management had, in this case, an influence on the genetic differentiation of populations, which may be a consequence of ecological, geographical, and anthropogenic factors (such as the use of insecticides and pesticides), as well as the mobility of the studied species.

The graphical processing of DNA fingerprinting results illustrates the clustering of analyzed samples, with individual groups showing clear differentiation according to the type of cultivation system for the RAPD markers (OPB 11,

OPB 14, and OPB 18). From the perspective of ecogenetic evaluation of populations, this observed similarity is an important indicator of the instability of genetic structure in agricultural habitats (Fig. 1).

Heatmaps expressed the strength of similarity between beetle species and management type. The Jaccard similarity coefficient was 0.5 for the *Harpalus rufipes* and *Silpha obscura* species recorded in wheat grown conventionally at marker OPB_11. For wheat grown organically, this coefficient was 0.56. Thus, the similarity was moderately strong. For marker OPB 14, we confirmed a Jaccard similarity coefficient of 0.75 for species found in organic farming which indicates a very strong similarity. In conventional farming, this index was 0.2. Marker OPB 18 confirmed similarity at the level of coefficient 0.86 for species in conventional farming, which indicates a moderately similarity. For organic farming is 0.40, which indicates a strong similarity. Thus, these markers are suitable for identifying the genetic similarity of species based on management type.

For markers OBP_5, OBP_8 and OBP_12, these coefficients mostly had a value of 1, which indicates a lower similarity and therefore they are not suitable for indicating the genetic similarity of species bordering on organic and conventional farming (Fig. 2).

The results of the t-SNE analysis indicate 2 clusters. The first cluster is between the species *Harpalus rufipes* and *Silpha obscura* in conventional management, and the second cluster is between the species in organic management (Fig. 3). Below we present a table with primary data on the occurrence of loci of the species *Silpha obscura* and *Harpalus rufipes* in conventional and organic farming (Table 1).

Discussion

The results of our study showed that in the species *Harpalus rufipes* and *Silpha obscura*, genetic differentiation exists depending on the type of management (organic vs. conventional), suggesting that agronomic practices may influence the genetic structure of beetle populations in agroecosystems. This conclusion corresponds to the broader context of insect population genetics research in agricultural environments. For instance, studies have shown that the genetic structure of insects is shaped not only by geographical barriers and gene flow but also by ecological practices and management interventions (Cordeiro et al. 2019; Calla et al. 2020).

Interpretation of population genetic patterns in beetles must consider species-specific dispersal ability. Highly mobile species can maintain substantial gene flow across fragmented agricultural landscapes, which reduces genetic differentiation even at large spatial scales (Schauer et al.

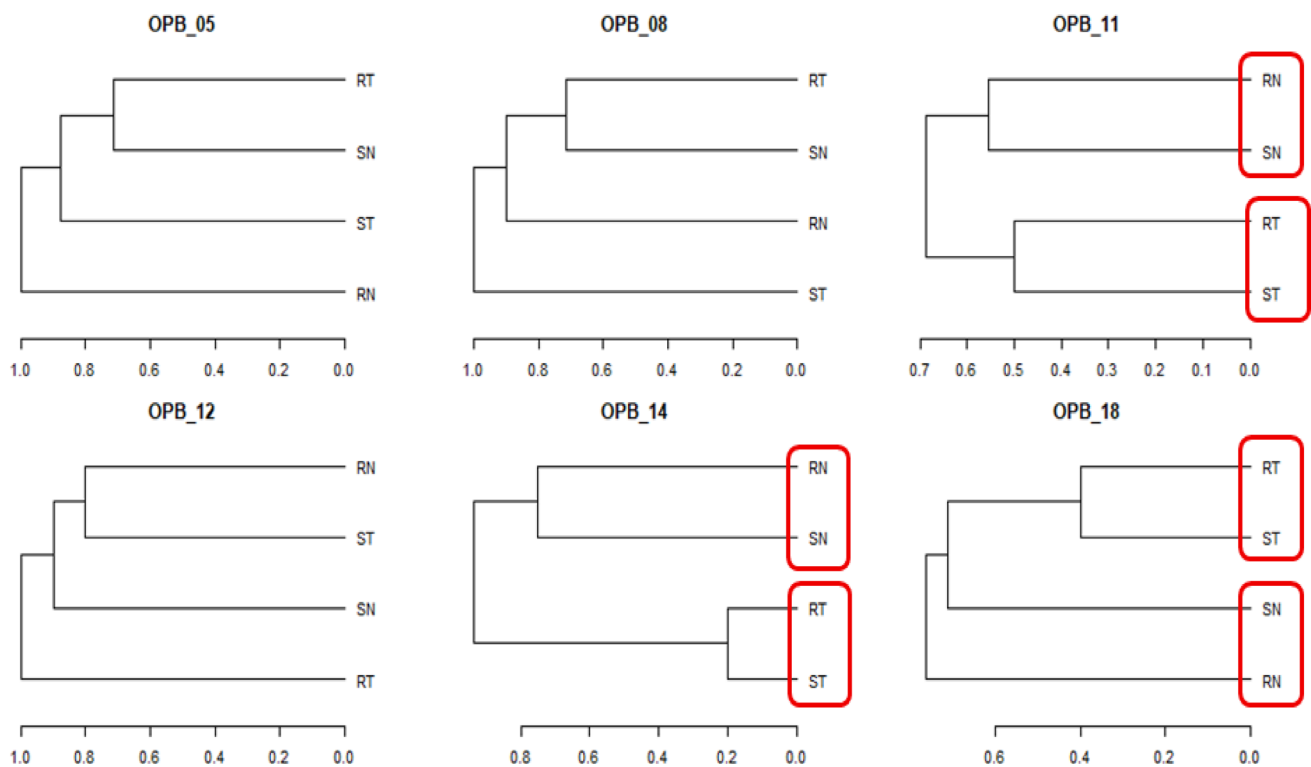


Fig. 1 Cluster analysis (dendrograms) based on Jaccard similarity coefficient of treated (T) and nontreated (N) samples of *Harpalus rufipes* (R) and *Silpha obscura* (S). The coefficient values range from

0 to 1, with low values indicating genetic similarity at the level of mapped DNA polymorphisms and values close to 1 indicating genetic dissimilarity

2025). In contrast, species with limited dispersal are more prone to genetic drift, local bottlenecks, and gradual erosion of genetic diversity when landscape connectivity is reduced (Suárez et al. 2022).

Our identification of three RAPD markers (OPB 11, OPB 14, and OPB 18) that showed clear differentiation between management systems is consistent with previous studies employing RAPD and similar marker-based approaches in beetles, for example, in species differentiation using RAPD within beetle groups (Corrêa et al. 2019; Humadi et al. 2024; Sun et al. 2025). The use of RAPD in the context of agricultural population studies continues to yield relevant results. The literature emphasizes that in population structure research, it is desirable to combine both neutral and adaptive genetic variability, as adaptive markers can reveal differences that neutral markers may not capture (Choi et al. 2010; Batista et al. 2016; Pálesová et al. 2025; Yadav et al. 2025). In our case, the OPB 11, OPB 14, and OPB 18 markers may be considered indicators of variation associated with the type of management, suggesting that the observed differences reflect not only neutral variability but also variation linked to selection or adaptation to different conditions (e.g., pesticide impact, nutrient composition, vegetation cover).

In addition to landscape fragmentation, pesticide use in winter wheat systems may indirectly shape population genetic structure through repeated reductions in abundance and recolonization events. Pyrethroid insecticides commonly applied in cereals, such as lambda-cyhalothrin and deltamethrin, have been shown to negatively affect non-target arthropods including spiders and parasitoids in wheat fields (Wick and Freier 2000). Herbicides used in cereal production, including glyphosate-based formulations, can also influence soil invertebrates such as earthworms and alter soil ecological functions (Brandmaier et al. 2023). Together, limited dispersal and recurrent chemical disturbance may reduce effective population size and accelerate genetic erosion, while simultaneously affecting broader non-target invertebrate communities that contribute to agroecosystem functioning.

The values of the Jaccard similarity coefficient (indicating moderately strong to strong similarity within management types and weaker similarity between them) confirm the existence of genetically distinct groups according to the management regime. The t-SNE analysis, which identified two genetic clusters, corresponds with this differentiation. Such results can be interpreted in the context of more limited gene flow between systems or different selective pressures in organic versus conventional fields. For example, in

Fig. 2 Heatmaps expressing the similarity between markers in the studied species and different management practices. Explanations: treated (T) and nontreated (N) samples of *Harpalus rufipes* (R) and *Silpha obscura* (S)

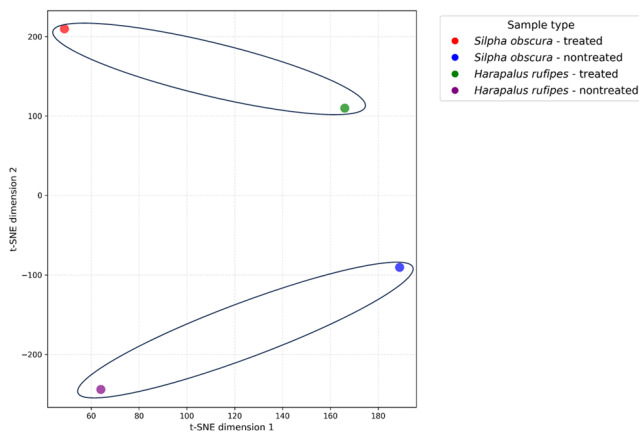
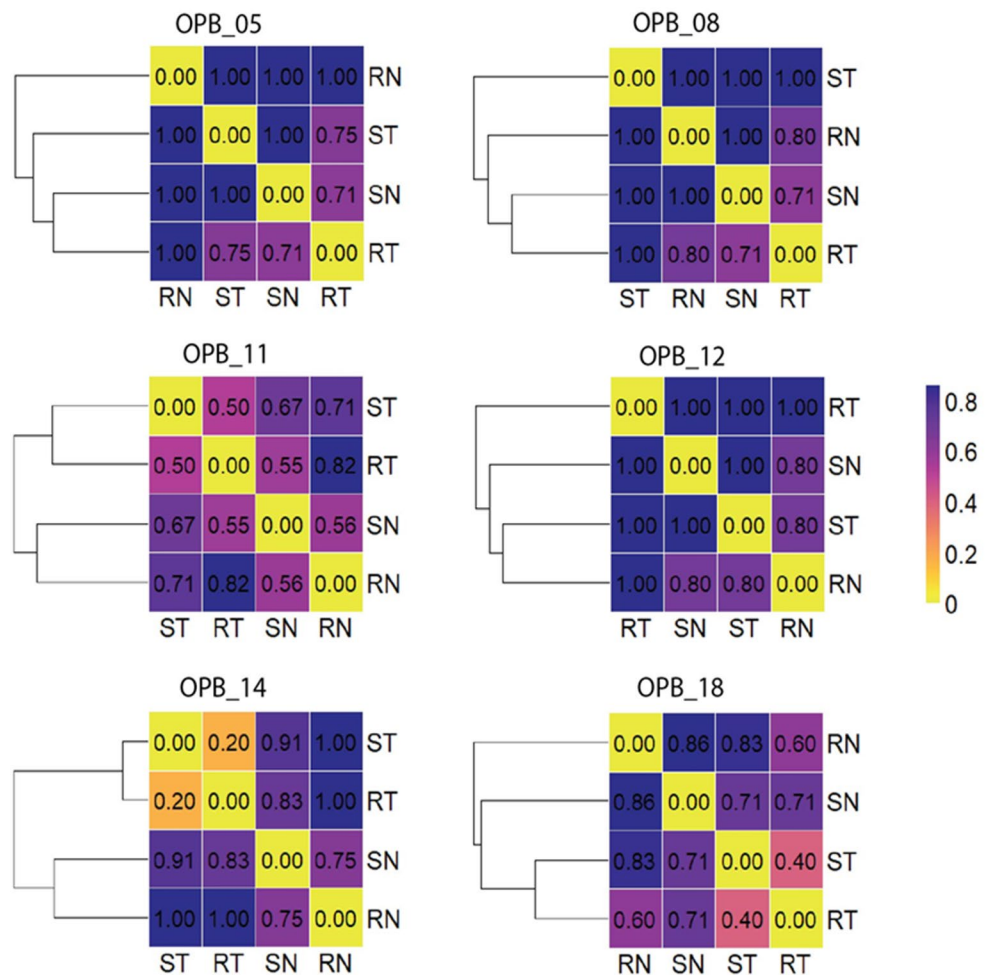


Fig. 3 t-SNE plot of the genetic relationships of treated—conventional management samples (ST, RT) and nontreated—ecological management samples (SN, RN) identified through clustering

insects within agroecosystems, it has been suggested that intensive management interventions can promote rapid evolutionary responses and population differentiation (Alyokhin et al. 2008; Brevik et al. 2018).

In the context of management type, it is important to discuss which mechanisms may lead to the observed differences. In organic systems, chemical treatments are typically applied less intensively, vegetation structure is more complex, host plant diversity is higher, and there are potentially better opportunities for movement and exchange of individuals between fields and the surrounding landscape. In contrast, conventional systems, characterized by more intensive pesticide use, reduced landscape heterogeneity, frequent soil disturbance, and monocultures, may limit gene flow, act as selective pressures, and lead to population differentiation. These mechanisms have also been described in invasive insects as major genetic shifts associated with adaptation to cultivated environments (Crispo et al. 2011; Allio et al. 2017; Péliissié et al. 2022). In our case, it could be assumed that beetle populations in organically managed systems may be genetically richer or less modified than populations in conventional systems; however, our data do not unequivocally show higher variability in the organic system, but rather indicate a different population structure.

Another factor that requires attention is the spatial aspect, although our sampling sites were in relatively close

Table 1 The binary data on the loci occurrence of *Silpha obscura* and *Harpalus rufipes* tested samples

RAPD primer/sample	ST	SN	RT	RN
OPB_05	0	0	1	0
	1	0	1	0
	0	1	0	0
	0	1	0	0
	0	0	0	1
	0	1	1	0
	0	1	1	0
	0	1	0	0
OPB_08	0	0	1	0
	0	0	1	0
	0	1	1	0
	0	1	1	0
	0	0	1	1
	0	1	0	0
	0	1	0	0
	0	1	1	0
OPB_11	0	1	1	0
	0	1	1	0
	0	1	0	0
	0	0	1	0
	0	1	0	1
	1	1	1	0
	0	0	0	1
	1	1	1	1
OPB_12	1	0	1	0
	0	0	1	0
	1	0	0	1
	0	0	0	1
	0	0	0	1
	0	1	0	1
	0	0	0	1
	0	0	1	0
OPB_14	1	0	1	0
	0	1	0	0
	1	0	1	0
	1	0	1	0
	1	0	1	0
	1	1	1	0
	0	1	1	0
	1	0	1	0
	0	0	1	0
	0	1	0	1
	1	0	1	0
	1	0	1	0

Table 1 (continued)

RAPD primer/sample	ST	SN	RT	RN
OPB_18	0	1	0	0
	0	1	0	0
	0	1	0	0
	0	0	1	1
	1	1	1	0
	1	0	1	0
	0	0	0	1
	1	0	0	0
	1	1	1	1

ST, *Silpha obscura* conventional; SN, *Silpha obscura* organic; RT, *Harpalus rufipes* conventional; RN, *Harpalus rufipes* organic

proximity and within the same agroecosystem, the type of management appeared to be a differentiating factor. This suggests that the management regime may exert a stronger influence than mere geographical distance. This trend aligns with the literature on the impact of environmental versus geographic isolation on genetic differentiation (Bradburd et al. 2013).

From a practical perspective, our findings suggest that agroecosystem management may have genetic implications for beetle populations. This can be relevant for sustainable pest management, biodiversity preservation, and the resilience of population units against, for example, chemical interventions. As noted by several studies, pest control strategies based on an understanding of population genetics can be more effective (Hendrichs et al. 2007; Beaurepaire et al. 2024). In our case, it could be proposed that populations in organic systems may develop higher genetic diversity or distinct population structures, potentially leading to better adaptive capacity or resistance. Conversely, differentiation observed in conventional systems may indicate population isolation, which could affect their evolutionary dynamics.

Conclusions for future biology

The future relevance of the obtained results lies primarily in their contribution to a deeper understanding of the impact of different agricultural management systems on the genetic structure of soil invertebrate populations in agroecosystems. The analysis of genetic variability in beetle populations (*Harpalus rufipes* and *Silpha obscura*) using RAPD markers suggests that the type of agricultural management may significantly influence the level of genomic polymorphism and genetic differentiation among populations. The identification of markers (OPB 11, OPB 14, and OPB 18) that show clear differentiation between populations originating from organically and conventionally managed fields highlights the potential of molecular markers as sensitive indicators of environmental and anthropogenic influences. In the

future, these findings may contribute to a better understanding of evolutionary processes occurring within agricultural landscapes and provide insights into the long-term effects of agronomic practices, including pesticide use and landscape management, on the genetic diversity of populations. Furthermore, the results indicate that the application of molecular genetic approaches can represent an important tool for biodiversity monitoring and for the development of strategies aimed at the sustainable management of agroecosystems. Such approaches may support the preservation of genetic variability and the ecological stability of organism populations associated with agricultural landscapes.

Author contributions Author Contributions: Conceptualization, V.L., K.R.; methodology, V.L., K.R., S.Č., M.K.; software, K.R., S.Č., M.K.; validation, V.L., K.P.; formal analysis, V.L., K.R., K.P.; resources, V.L., K.P.; data curation, K.R., S.Č., M.K.; writing—original draft preparation, V.L., K.R.; writing—review and editing, V.L., K.P.; visualization, K.R., M.K.; supervision, V.L.; project administration, V.L., K.P.; funding acquisition, V.L., K.P.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interest.

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