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Inter-Annual Variation in Alpha and Beta Diversity of Benthic Macroinvertebrate Communities in Agricultural Ditches

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ABSTRACT

Ditches are essential elements of the agricultural landscape because of their role as habitat or refuge for aquatic species, especially in homogenized and intensively cultivated areas. However, data on the biodiversity associated with agricultural ditches, and its variation over time, are underrepresented in ecological research. This study aimed at quantifying the inter-annual changes in the alpha and beta diversity of benthic macroinvertebrate communities across five agricultural ditches in the lowland, agricultural area of the Novara Province (northwestern Italy). Benthic macroinvertebrates were always sampled in October but in two different years: 2022 and 2023, respectively. These two years differed in the climate and hydrological conditions with lower precipitations and discharge in 2022 than 2023. The alpha diversity metrics, such as taxon richness, total abundance, Ephemeroptera, Plecoptera and Trichoptera richness and abundance, and Shannon index, were significantly higher in 2023 than in 2022. Moreover, these metrics were positively correlated with the water velocity, and negatively correlated with the concentration of phosphates. Benthic macroinvertebrate communities in 2022 showed higher beta diversity compared to 2023, with significant differences in the taxonomic composition. By controlling for the season, the results here obtained demonstrate that ditch macroinvertebrate communities are responsive to temporal variability in hydrological, climate, and environmental conditions with changes in the taxonomic diversity and composition driven by water velocity and nutrient enrichment. As agricultural ditches are still poorly monitored, this study provides science-based information and potential insights for their sustainable management.

1 | Introduction

Artificial and modified watercourses in agricultural landscapes, such as ditches, are now a defining feature of modern agroecosystems (Dollinger et al. 2015). Originally constructed to regulate water levels for irrigation, soil drainage, and flood prevention, ditches form complex hydrological networks across lowland agricultural regions (Clifford et al. 2025). Their continued management to prevent flooding and ensure water availability and transfer has, however, intensified ecological

pressures on former wetlands, particularly in Europe and North America, where natural aquatic habitats have been drastically reduced (EEA 1996; Maltby and Acreman 2011; Verdonshot et al. 2011). As such, these watercourses often represent a significant proportion of the remaining aquatic environments in agricultural areas and, despite their artificial or modified nature, contribute significantly to ecological functions and ecosystems services, including groundwater recharge, pollution filtration, and habitat provision (Herzon and Helenius 2008; Hill et al. 2016). Yet, their biodiversity has

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long been underestimated and agricultural ditches are rarely included in standard environmental monitoring programs (Lagrotteria et al. 2025).

Typically, ditches integrate both lotic and lentic ecosystem features and are subjected to periodic anthropogenic maintenance practices and disturbances. Chemical pollution associated with the usage of pesticides, herbicides, and fertilizers often represents the most recognized cause of alteration for agricultural ditches (Clarke 2015; Rolke et al. 2018). Among other anthropogenic causes of impairment, even dredging and the removal of riparian and in-stream vegetation exert remarkable effects. While necessary for agricultural productivity, these practices can drastically alter biological communities and disrupt habitat continuity and integrity (Herzon and Helenius 2008; Dollinger et al. 2015). In light of this, agricultural ditches are often assumed to support lower taxonomic richness and less diverse biological communities. However, previous studies have shown that these watercourses may harbor unexpectedly high biodiversity because of their role as refuges for aquatic species, including rare and endangered taxa, that are no longer found in larger water bodies (Manhoudt and de Snoo 2003; Verdonschot et al. 2011; Foster et al. 1990; Hill et al. 2016; Simon and Travis 2011).

Among the biological communities of agricultural ditches, most of previous research focused on macroinvertebrates (Leslie and Lamp 2019; Linares et al. 2023). Benthic macroinvertebrates represent a key component of aquatic biodiversity in both natural and artificial water bodies. These organisms, that include different taxa such as Insecta, Crustacea, Mollusca, Hirudinea, and Oligochaeta, occupy every consumer level in the trophic chain of freshwater ecosystems. To this end, they play key roles in food webs and biogeochemical cycling through processes like organic matter decomposition and nutrient transformation (Needelman et al. 2007; Kartikasari 2013). Moreover, the sensitivity to changes in the chemical and physical characteristics of water (Pastorino et al. 2020; Begum et al. 2023) and hydro-morphological conditions (Rempel et al. 2000; Doretto et al. 2022), along with the simplicity of collecting them, make benthic macroinvertebrates the foremost organisms employed in river biomonitoring (Davis et al. 2003; Leslie et al. 2012; Buss et al. 2015; Tornwall et al. 2015). Therefore, benthic macroinvertebrates are excellent candidate bioindicators for improving our knowledge of the ecology, management, and conservation of agricultural ditches as the community-level analysis of this target group provides complementary and multifaceted insights ranging from the taxonomic and functional diversity to ecosystem attributes and biomonitoring applications (Verdonschot et al. 2012; Vallefucio et al. 2023; Chiorino et al. 2024). For example, invertebrate species of notable conservation value have been recorded in the fenland ditches of the UK (Painter 1999), and some drains hosted macroinvertebrate diversity levels comparable to parent rivers, significantly contributing to biodiversity in agricultural landscapes (Gething and Little 2020).

Macroinvertebrate communities in agricultural ditches are hence expected to be shaped by the interplay between different factors, including the magnitude and frequency of human-driven alterations and the environmental control played by ditch features such as water chemistry, detritus subsidies, and shading

from riparian vegetation. Also, the connectivity with surrounding aquatic habitats that may serve as sources of new dispersing taxa and individuals, thus affecting the colonization dynamics over temporal and spatial gradients. While previous studies have mainly examined the changes in the benthic macroinvertebrate communities over different gradients of anthropogenic disturbance and/or connectivity (Iwamoto et al. 2022; Gething et al. 2025), to date, scientific evidence on the temporal variation in the macroinvertebrate richness and diversity in agricultural ditches is still scarce (but see Hill et al. 2016; Krynak et al. 2019).

In highly dynamic and heterogeneous ecosystems like rivers, seasonal and inter-annual changes have strong influence on the occurrence and abundance of riverine macroinvertebrates insofar as several studies (Johnson et al. 2012; Scotti et al. 2019; Rodríguez-Lozano et al. 2023) stress the importance of sampling on different time scales, whether annual, monthly or seasonal, to better elucidate the biological responses. Our understanding of the temporal dynamics of macroinvertebrate assemblages in agricultural ditches, instead, remains limited, thus hindering the development of management strategies that combine biodiversity conservation with agricultural needs.

This study aimed at assessing the inter-annual variation, from 2022 to 2023, in the taxonomic diversity of macroinvertebrate communities in agricultural ditches by controlling for the season (i.e., October—northern hemisphere autumn). Since the two selected years differed in the climate and hydrological conditions, with lower precipitations and discharge in 2022 than 2023, we hypothesised (i) higher macroinvertebrate alpha diversity under high-flow (i.e., 2023) than low-flow (i.e., 2022) conditions. With this respect, we expected that macroinvertebrate richness and abundance were positively affected by the hydraulics conditions (i.e., water velocity) and negatively affected by the amount of nutrients (i.e., nitrates and phosphates). Moreover, we hypothesised (ii) higher dissimilarity in the taxonomic composition (i.e., beta diversity) of macroinvertebrate communities as well as higher differences in the occurrence of macroinvertebrate taxa under low-flow (i.e., 2022) than in high-flow conditions (i.e., 2023).

2 | Materials and Methods

2.1 | Area of Study

The lowland territory which is enclosed between the cities of Pavia, Vercelli, and Novara (northwestern Italy) represents the largest area for rice production in Europe (Arcieri and Ghinassi 2020). Here, rice cultivation dates back to the late Middle Ages (Pelloli 2024) and, today, rice paddies are still the dominant land-use category, along with a dense and complex drainage network including rivers, ditches, and springs. In this study, six sampling sites were selected in the southern part of the Novara Province where the main land use is represented by rice crop (Figure 1). Two sampling sites were identified on the Roggia Cacesca, which is an agricultural ditch directly fed by the Agogna River, with the downstream site (i.e., Cacesca2) that was located 5 Km apart from the upstream one (i.e., Cacesca1). Four sampling sites were chosen in the following ditches: Cavo Dassi, Cavo Cattedrale, Cavo Panizzina, and Fontana Pietta (Figure 1). From a hydrological standpoint, all

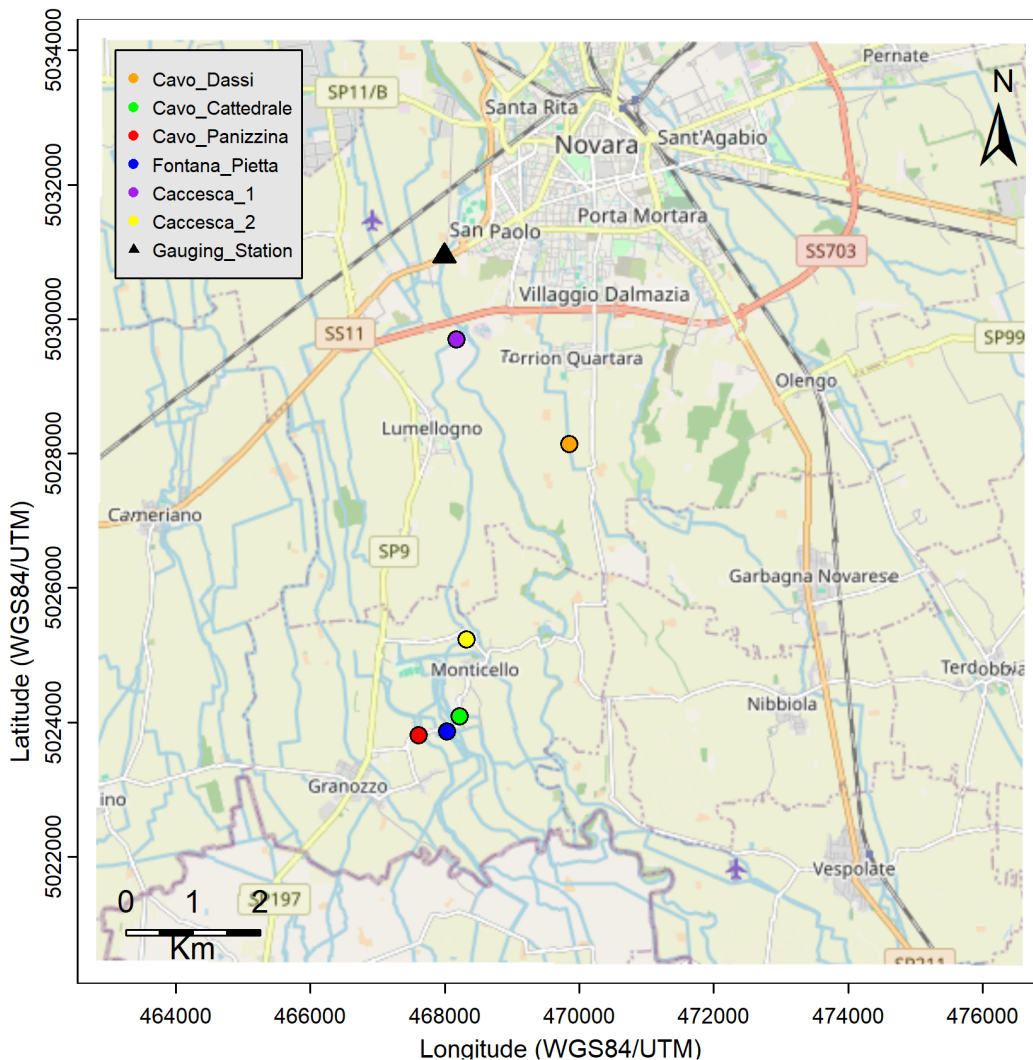


FIGURE 1 | Map of the area of study (southern Novara Province, Italy): Sampling sites are indicated by the colored dots. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

selected sampling sites are perennial, with exception of Cavo Dassi that is usually affected by a drying phase during the winter season (December–March).

The gaging station “Novara – Agogna” of the Local Agency for Environmental Protection (i.e., ARPA Piemonte) is located on the Agogna River (Figure 1). Since there are no gaging stations in the agricultural ditches included in this study, data on the discharge of the Agogna River were used as proxy and representative indicator of the hydrological conditions for all the sampling sites. Available data on the daily discharge (m^3/s) from 1st January 2010 to 31st December 2023 were downloaded from the website of the ARPA Piemonte (Supporting Information Figure S1). On average, the discharge was higher in 2023 (mean = $7.26 \text{ m}^3/\text{s}$; min = $0.65 \text{ m}^3/\text{s}$; max = $41.02 \text{ m}^3/\text{s}$) than 2022 (mean = $3.59 \text{ m}^3/\text{s}$; min = $0.33 \text{ m}^3/\text{s}$; max = $15.58 \text{ m}^3/\text{s}$), thus confirming the differences between these 2 years (Figure 2a). Moreover, the analysis of the discharge from 2010 to 2023 and even at month-level (i.e., October) confirmed that 2022 was characterized by particularly low-flow conditions,

while in 2023 the river discharge was moderately higher (Figure 2b,c).

2.2 | Macroinvertebrate Sampling

Benthic macroinvertebrates were always sampled in autumn but in two consecutive years: 20th and 27th October 2022, and 9th and 10th October 2023, respectively. In each site, a reach of 40 m was selected and six quantitative samples of macroinvertebrates were collected with a Surber net (0.05 m^2 , 250- μm mesh size; Doretto, Bo, et al. 2020). Surber samples were placed at least 5 m apart and were collected across all the available microhabitats, including mineral and biotic substrates (6 Surber samples for each site X 6 sites X 2 years = 72 samples). For each Surber sample, the substrate was perturbed by the same operator for 1 min. Previous research in the same area of study proved that six Surber samples were adequate to obtain a representative macroinvertebrate community in agricultural ditches (Chiorino et al. 2024). Macrophytes

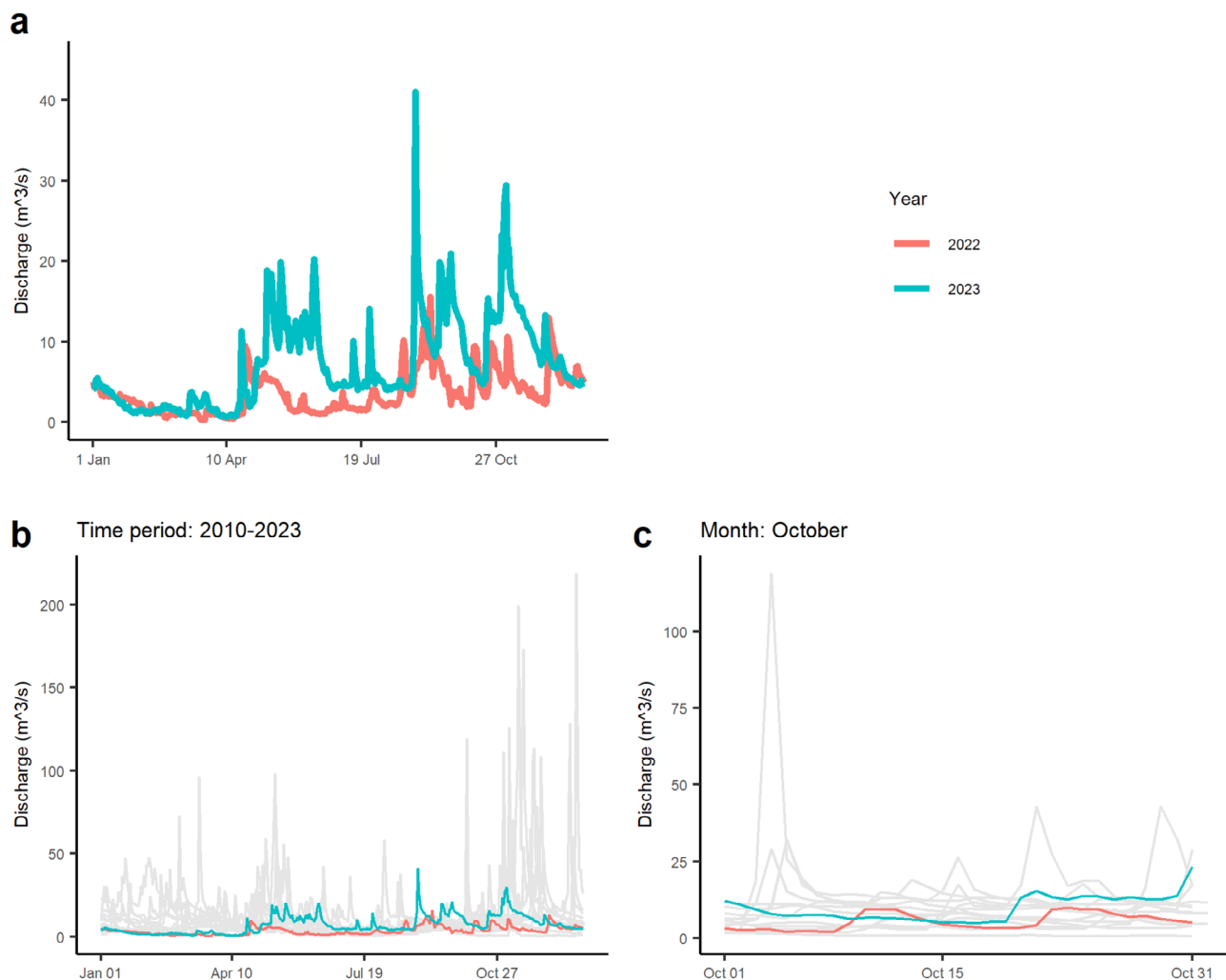


FIGURE 2 | Hydrographs (a–c) showing the daily discharge of the Agogna River measured in the gaging station “Novara – Agogna”. Colors indicate the two different years (2022 and 2023). In (b) and (c) grey lines represent the daily discharge recorded from 2010 to 2021. [Color figure can be viewed at wileyonlinelibrary.com]

and microlithal (diameter: 2–6 cm) were the most frequent microhabitats across all sampling sites, followed by gravel (0.2–2 cm), mesolithal (6–20 cm), fine sediment (<0.2 cm), and organic detritus. Moreover, except for Caccasca1, negligible changes in the sampled microhabitats occurred from 2022 to 2023 (Supporting Information Table S1).

2.3 | Environmental Data Collection

To better characterize the environmental conditions associated with benthic macroinvertebrates, for each sample the water velocity (m/s) and water depth (cm) were recorded with a flowmeter (Scubla, mod. N.01.200). Electrical conductivity ($\mu\text{S}/\text{cm}$), water temperature ($^{\circ}\text{C}$), and pH were instead measured once for each sampling site with a multiprobe (Hanna, mod. HI98194). Moreover, two samples of water (0.5 L) were collected and returned in laboratory where they were analysed with standard spectrophotometric methods for nitrates and phosphates (mg/l). Benthic macroinvertebrates were preserved in plastic tins with ethanol (90%), and delivered to the laboratory where they were sorted, counted,

and taxonomically identified to genus (mostly Plecoptera, Ephemeroptera, and Odonata) or family level by using a stereomicroscope (Leica, mod. EZ4) and the taxonomic keys for the Italian fauna (Campaioli et al. 1994; Campaioli et al. 1999; Sansoni 1988; Tachet et al. 2000; Carchini 2016).

2.4 | Statistical Analyses

All the statistical analyses (significant threshold: $p < 0.05$) were performed with the R software (R Core Team 2025) by using both basic and specific functions as described in the following sections. Paired *t*-test was used to evaluate significant differences in the physical and chemical parameters from 2022 to 2023.

2.4.1 | Differences in the Alpha Diversity

Generalized Linear Mixed Models (GLMMs) and Linear Mixed Models (LMMs) were used to test for differences in the total taxon richness, total abundance, EPT (Ephemeroptera,

TABLE 1 | Mean and standard deviation (SD) of the physical and chemical parameters recorded in 2022 and 2023. Velocity = water velocity, Depth = water depth, Temp = water temperature, Cond = electrical conductivity, NO₃ = concentration of nitrates, PO₄ = concentration of phosphates. Significant results are in bold.

Parameter	2022		2023		t-value	p
	Mean	SD	Mean	SD		
Velocity (m/s)	0.264	0.065	0.299	0.146	0.102	0.920
Depth (cm)	28.639	9.846	26.500	5.114	0.454	0.657
pH	7.818	0.689	7.790	0.697	0.121	0.905
Temp (°C)	16.550	1.143	18.417	1.418	-2.922	0.012
Cond (µS/cm)	228.133	95.828	249.067	58.602	0.744	0.472
NO ₃ (mg/l)	6.883	1.013	6.716	2.497	0.009	0.993
PO ₄ (mg/l)	1.183	0.697	0.572	0.395	1.194	0.260

Plecoptera, and Trichoptera) richness, EPT abundance, and Shannon index among sampling sites and years. To this end, the previously-mentioned community metrics were included as response variables in the regression models with the fixed interacting effects of the factors “site” and “year”, while the item (1|Site:Year) was added to the models as a random intercept. GLMMs with Poisson distribution were applied for count data, while LMMs were used for the Shannon index.

2.4.2 | Relations Between Alpha Diversity and Environmental Variables

Similarly, Generalized Linear Mixed Models (GLMMs) and Linear Mixed Models (LMMs) were applied to evaluate whether the alpha diversity metrics were statistically affected by the environmental variables. Community metrics were included as response variables, while the water velocity, water depth, pH, conductivity, water temperature, and the concentration of nitrates and phosphates were included as independent variables. To account for the spatial and temporal dependency in the data, the item (1|Site:Year) was added to the models as a random intercept. GLMMs with Poisson distribution were applied for count data, while LMMs were used for the Shannon index. According to the approach proposed by Zuur and Ieno (2016), model selection was performed and, based on the AIC (Akaike Information Criterion) value, only the most significant and influential variables were retained.

2.4.3 | Differences in the Beta Diversity

Differences in the taxonomic composition of macroinvertebrate communities among sampling sites, years, and their interaction were visually inspected and statistically tested by means of Non-Metric Multidimensional Scaling (NMDS) and Permutational Analysis of Variance (PERMANOVA), respectively (*mds* and *adonis2* functions, “vegan” R package—Oksanen et al. 2015). To account for the spatial dependency in the data, PERMANOVA was performed by restricting the permutation scheme by site. The Bray–Curtis dissimilarity index was used as distance measure in these multivariate analyses.

Environmental variables (i.e., water velocity, water depth, pH, water temperature, electrical conductivity, and the concentration of nitrates and phosphates) were plotted as arrows in the NMDS ordination graph and PERMANOVA was used to statistically evaluate their effects on the macroinvertebrate community composition.

According to the approach proposed by Baselga (2010), the total beta-diversity and its nestedness (i.e., richness difference) and turnover (i.e., taxa replacement) components were calculated on the total macroinvertebrate community (i.e., by aggregating the Surber samples at site level) with the function *beta.multi* of the “BAT” R package (Cardoso et al. 2022) and using Jaccard’s dissimilarity index. This analysis served to evaluate the contribution of taxa replacement and richness-based differences on the inter-annual variation of the macroinvertebrate communities in each site from 2022 to 2023 as well as the beta diversity and its components among sites in 2022 and 2023, respectively.

In order to identify macroinvertebrate taxa statistically associated with one or more sampling sites, the Indicator Species Analysis (ISA; Dufrene and Legendre 1997) was run separately for the 2 years by using the function *multipatt* of the “indicpecies” R package (De Caceres et al. 2016). Moreover, the Local Contribution to Beta Diversity (LCBD) was calculated for each site and separately for both years ($N=12$) by aggregating all Surber samples at site level (*beta.div* function of the “adespatial” R package—Dray et al. 2018). LCBD is a univariate metric that indicates the “uniqueness” of the biological communities (Sor et al. 2018; Tolonen et al. 2018; Bo et al. 2020): the higher the LCBD value, the higher the occurrence of rare and exclusive taxa in the community of that site. Wilcox test for paired data was used to test for differences in the average LCBD between 2022 and 2023.

3 | Results

Among the environmental parameters, only water temperature significantly differed between years: it was, on average, higher in 2023 than 2022 (Table 1).

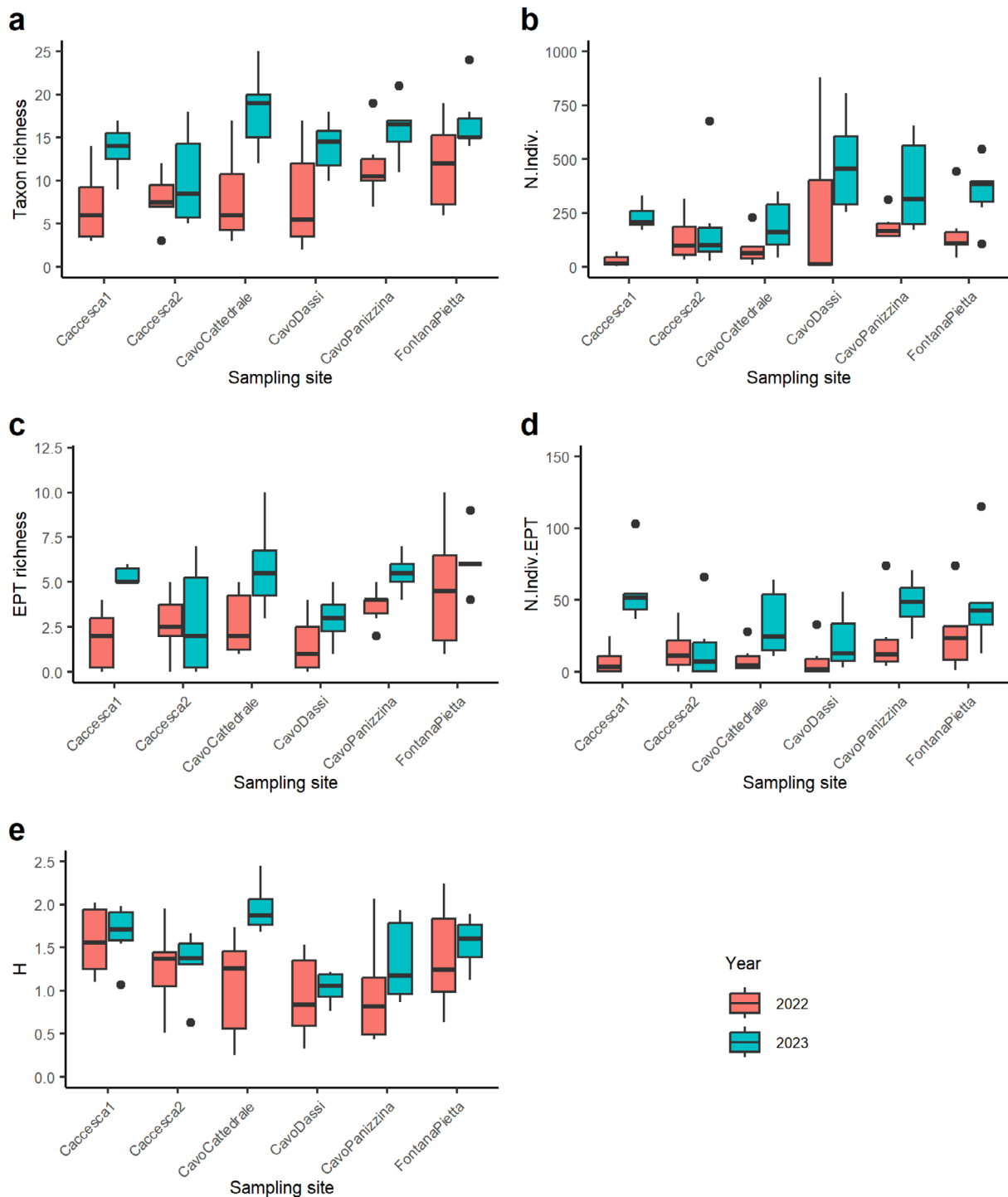


FIGURE 3 | Boxplots illustrating the variation in: (a) taxon richness, (b) total abundance, (c) EPT richness, (d) EPT abundance, and (e) Shannon index in relation to the sampling sites and years. Black horizontal line = median; upper and lower box edges = 3rd and 1st quartile, respectively; vertical lines = whiskers (± 1.5 interquartile distance). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

A total of 15,939 macroinvertebrates belonging to 58 different taxa were collected in this study (Supporting Information Table S2). The number of macroinvertebrate taxa per Surber sample ranged from 2 to 25 (mean = 12 taxa), while the total abundance of macroinvertebrates per Surber sample varied from 2 to 879 (mean = 221 individuals). Chironomidae (48%), *Echinogammarus* (20%), *Baetis* (3%), and *Corbicula* (3%) were the most abundant taxa and together sum up to 74% of the total macroinvertebrates.

3.1 | Patterns in Alpha Diversity

Both the taxon richness and total abundance of macroinvertebrates significantly varied among sampling sites and years with, on average, lower values observed in 2022 than 2023 (Figure 3a,b; Table 2). EPT richness and EPT abundance were significantly higher in 2023 than 2022, while statistical differences among sites were observed only in relation to the EPT richness, with the highest number of EPT taxa recorded in Fontana Pietta and

TABLE 2 | Outputs of the regression models for the community metrics. Chi-square for GLMMs, F-value for LMMs. Significant values are in bold.

Metric	Factor	Chi-square	p
Taxon richness	Site	22.842	<0.001
	Year	47.849	<0.001
	SiteXYear	8.182	0.146
Total abundance	Site	24.830	<0.001
	Year	21.457	<0.001
	SiteXYear	8.252	0.143
EPT richness	Site	20.615	<0.001
	Year	16.157	<0.001
	SiteXYear	6.227	0.285
EPT abundance	Site	9.591	0.088
	Year	16.632	<0.001
	SiteXYear	6.294	0.278
Shannon index	Site	14.050	0.015
	Year	5.866	0.015
	SiteXYear	6.291	0.279

Cavo Panizzina (Figure 3c,d). On average, the Shannon index was significantly higher in 2023 than 2022, and statistically differed among sites with the highest and lowest values recorded in Cavo Cattedrale and Cavo Dassi, respectively (Figure 3e; Table 2).

3.2 | Relationships Between Alpha Diversity and Environmental Variables

Taxon richness was positively affected by water velocity and electrical conductivity, while the concentration of phosphates significantly reduced the taxon richness (Figure 4a). Similarly, the water velocity and water temperature had a positive effect on the total abundance of macroinvertebrates, while the opposite pattern was observed for the concentration of nitrates, despite this relationship not being significant (Figure 4b). In this study, the number of EPT taxa significantly increased with increased water velocity and electrical conductivity, but it was negatively correlated with pH and phosphates (Figure 4c). Similarly, water velocity and electrical conductivity significantly and positively affected the abundance of EPT taxa, while the opposite pattern was observed for water depth and pH (Figure 4d). Shannon diversity index was positively and negatively affected by water velocity and phosphates and water temperature, respectively (Figure 4e).

3.3 | Patterns in Beta Diversity

Multivariate analyses (Figure 5a) resulted in significant shift in the taxonomic composition of macroinvertebrate communities in relation to the “sampling site” ($F_{7,80} = 8.118$; p -value

<0.001), “year” ($F_{1,80} = 6.291$; p -value <0.001), and even the interaction between these two factors ($F_{7,80} = 2.652$; p -value <0.001). Specifically, the taxa that showed the greatest variations were *Baetis* and *Caenis*, with an increase in their abundance in 2023 at every site. Other taxa also showed an increased number of specimens, such as Hydropsychidae, especially in Cacesca 1, Empididae in Cavo Panizzina, and Chironomidae and *Echinogammarus* in all sampling sites. On the contrary, some taxa, showed a reduced abundance in 2023, such as Leptoceridae and *Corbicula* at every site, and *Theodoxus* mainly in Cavo Cattedrale (Supporting Information Table S2).

Among the environmental variables, only water temperature ($F_{1,64} = 3.598$; p -value=0.015) and the concentration of nitrates ($F_{1,64} = 5.328$; p -value=0.043) played a significant role on shaping the macroinvertebrate communities (Supporting Information Table S3). Ditch communities in 2022 were mostly oriented on the left side of the ordination plot and they were characterized by higher water depth and nutrient enrichment. On the contrary, macroinvertebrate communities in the agricultural ditches in 2023 were mostly oriented in right part of the plot and associated with higher water temperature and conductivity (Figure 5a).

The analysis of the beta-diversity and its components highlighted a marked inter-annual variation in the taxonomic composition, which was observed across all sites (Figure 5b), as indicated by the medium to high values of the total beta-diversity (minimum=0.51 in Cavo Dassi, maximum=0.9 in Cacesca1). On average, the difference in the taxon richness (i.e., nestedness scenario) was the main mechanism that explained the inter-annual compositional changes of the macroinvertebrate communities, except for Cacesca2. In this latter site, the percentage contribution of taxa replacement (i.e., turnover scenario) exceeded that of nestedness and it accounted for 56.2% of the total beta-diversity (Figure 5b). When the beta-diversity and its components were calculated among the ditch communities surveyed in the same year, the total beta-diversity was higher in 2022 than 2023 (Figure 5c). Moreover, the percentage contribution of richness differences to the total beta-diversity among ditch communities was higher in 2022 than 2023, while the percentage contribution of turnover increased to 61.3% in 2023 (Figure 5c).

The Indicator Species Analysis identified 7 and 5 taxa statistically associated with one or two sampling sites in 2022 and 2023, respectively (Table 3). In 2022, four taxa out of seven, namely Psychomidae, *Echinogammarus*, *Asellus*, and *Calopteryx*, were significantly associated with the Fontana Pietta site, alone or in combination with other sites. The coleopteran family Elmidae and the snail *Theodoxus*, instead, were indicator taxa of the site Cavo Dassi and Cavo Cattedrale, respectively, while the dipteran family Simuliidae was statistically associated with Cavo Panizzina (Table 3). In 2023, only *Theodoxus* was confirmed as an indicator taxon of Cavo Cattedrale along with Cavo Panizzina, while the other four taxa differed from those observed in 2022. *Lymnaea* was identified as an indicator taxon for Fontana Pietta, while *Micronecta* and Empididae were statically associated with Cavo Dassi and Cavo Panizzina, respectively. The mayfly

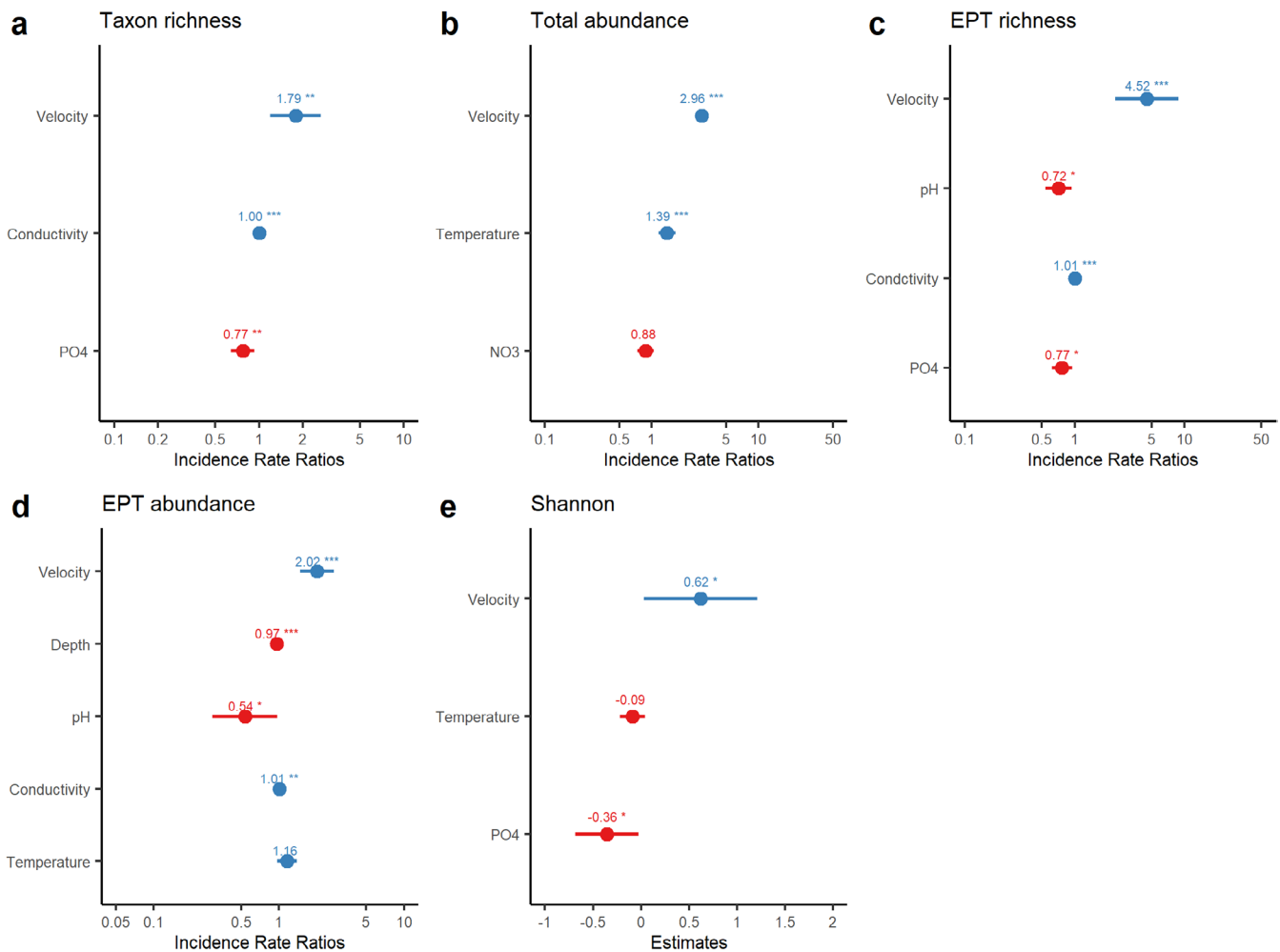


FIGURE 4 | Outputs of the regression models (GLMMs and LMMs) testing the relation between community metrics and environmental parameters retained after model selection. Velocity=water velocity, Depth=water depth, Temperature=water temperature, Conductivity=electrical conductivity, NO3=concentration of nitrates, PO4=concentration of phosphates. Variables having a positive effect are in blue, while variables having a negative effect are in red. Asterisks indicate the statistical significance (*= p -value < 0.05; **= p -value < 0.01; ***= p -value < 0.001). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.com)]

Serratella ignita was an indicator species for Caccasca1 and Cavo Cattedrale (Table 3).

On average, LCBD was higher in 2022 than 2023 (Figure 6a) but this difference was not significant (p -value=0.270). In 2022, Fontana Pietta was the sampling site with the highest LCBD value (0.118), followed by Caccasca1 (0.117); while the lowest LCBD (i.e., 0.041) was observed in Caccasca2 (Figure 6b). On the contrary, in 2023, the highest LCBD was observed in Caccasca1 (0.11), followed by Cavo Dassi (0.086); while Cavo Cattedrale had the lowest LCBD (0.049) (Figure 6b). Overall, all the sampling sites but Caccasca2 and Cavo Dassi showed a reduction in the LCBD value from 2022 to 2023 (Figure 6b).

4 | Discussion

Understanding the causes and mechanisms of temporal variation of biological communities is a primary goal in ecological research (Lehman and Tilman 2000; Collins et al. 2018; Ontiveros et al. 2021). This assumes even more importance for

highly heterogeneous and dynamic ecosystems such as lotic ecosystems (Thorp et al. 2006; Doretto, Piano, and Larson 2020). Indeed, in a theoretical review on the typical features of rivers, Ward (1989) recognized the time as their fourth dimension, along with the longitudinal gradient (first dimension), the lateral connectivity (second dimension), and the vertical connectivity (third dimension). In the last decades, several studies have examined the temporal variation of the riverine macroinvertebrate communities over multi-year periods or seasons (Monk et al. 2008; Mesa 2012; Burgazzi et al. 2020) and across different river typologies (Munné and Prat 2011; Holt et al. 2015; Scotti et al. 2022). Although there is evidence of anthropogenic pressures on the hydro-morphological conditions (Vallefuoco et al. 2023), agricultural ditches usually maintain some common features with rivers so that they are expected to align, at least partially, with the processes of river ecosystems (Needelman et al. 2007). Nevertheless, the temporal dynamics of agricultural ditches have often been neglected and, in turn, ecological information on the macroinvertebrate diversity and its changes over time in this type of freshwater ecosystems is still scarce and limited (Leslie and Lamp 2019; Gething 2021; Chiorino et al. 2024).

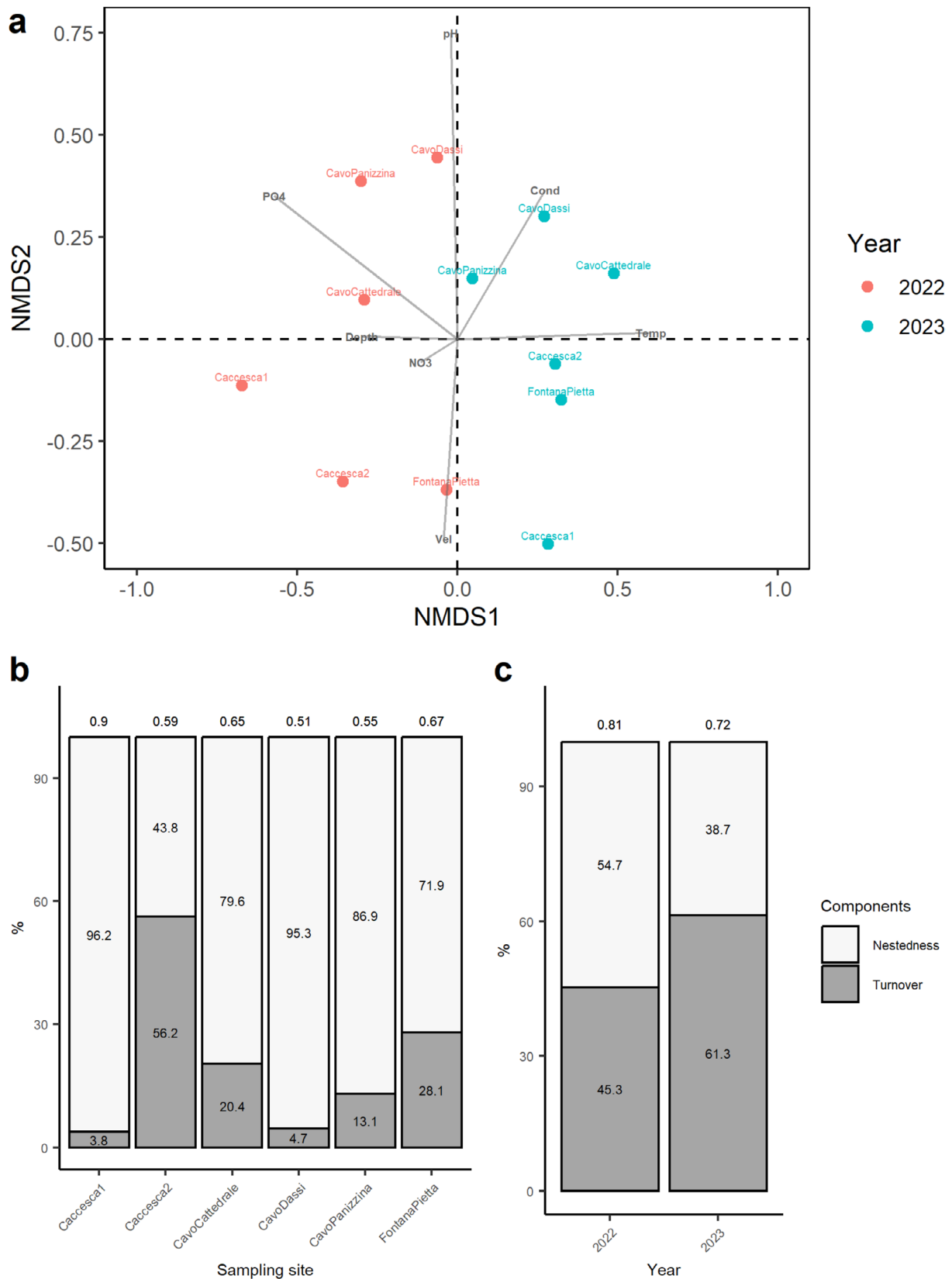


FIGURE 5 | NMDS (stress value=0.161) ordination plot (a): Dots represent the benthic macroinvertebrate communities of each sampling site (i.e., aggregated Surber samples for improving visualization) in the 2years. Arrows represent the environmental variables: Depth=water depth, Vel=water velocity, pH, Cond=electrical conductivity, Temp=water temperature; NO3=concentration of nitrates, PO4=concentration of phosphates. Barplots (b, c) illustrating the percentage contribution of nestedness (i.e., richness difference) and turnover (i.e., taxa replacement) to the total beta-diversity (number above bars) in (b) each site by comparing the macroinvertebrate communities from 2022 to 2023, and (c) among sites surveyed in the same year. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

TABLE 3 | Output of the Indicator Species Analysis (ISA). IndVal= Indicator value. Only significant (in bold) taxa are illustrated.

Year	Sampling site(s)	Taxon	IndVal	<i>p</i>
2022	Cavo Dassi	Elmidae	0.595	<0.001
	Cavo Cattedrale	<i>Theodoxus</i>	0.896	<0.001
	Fontana Pietta	Psychomyiidae	0.718	0.003
	Cavo Panizzina	Simuliidae	0.617	0.049
	Caccasca2 + Fontana Pietta	<i>Echinogammarus</i>	0.986	<0.001
	Caccasca1 + Fontana Pietta	<i>Asellus</i>	0.792	0.004
	Cavo Cattedrale + Fontana Pietta	<i>Calopteryx</i>	0.665	0.005
2023	Cavo Dassi	<i>Micronecta</i>	0.913	<0.001
	Cavo Panizzina	Empididae	0.738	0.035
	Fontana Pietta	<i>Lymnaea</i>	0.840	<0.001
	Caccasca1 + Cavo Cattedrale	<i>Serratella ignita</i>	0.750	0.002
	Cavo Cattedrale + Cavo Panizzina	<i>Theodoxus</i>	0.712	0.009

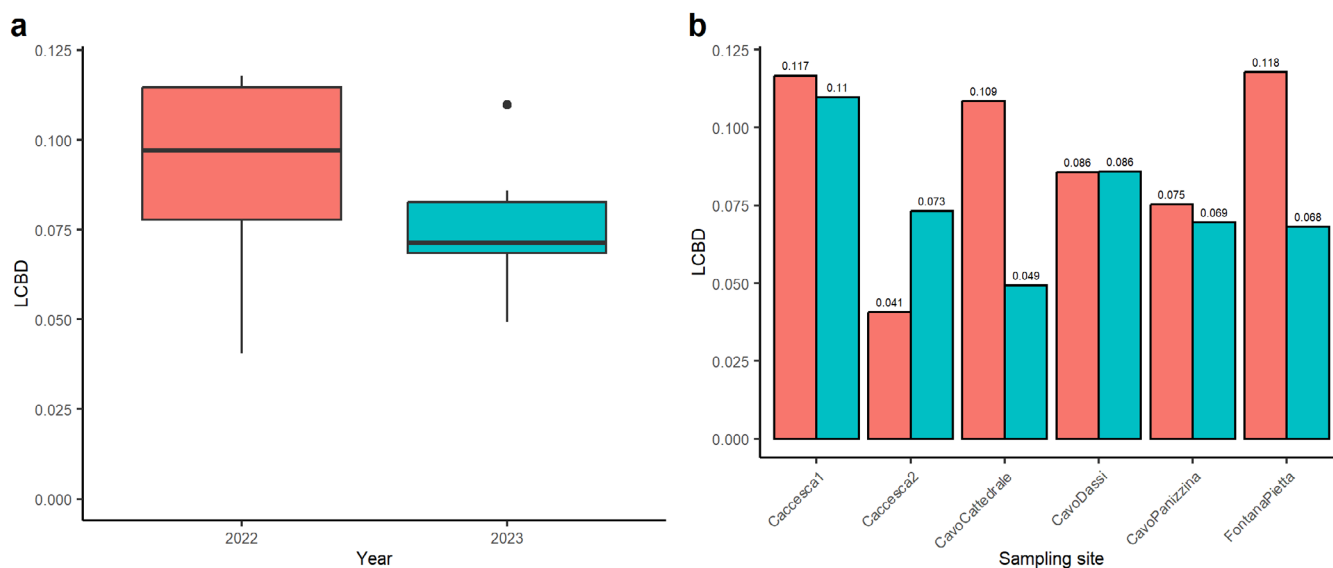


FIGURE 6 | Boxplot (a) illustrating the average variation in LCBD (Local Contribution to Beta Diversity) between 2022 and 2023. Bars (b) show the LCBD values (numbers above the bars) for all sampling sites in both years. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

The main goal of this study was to evaluate the inter-annual variation in the alpha and beta diversity of benthic macroinvertebrate communities in agricultural ditches by comparing 2 years characterized by different flow conditions (i.e., discharge). Our results show that alpha diversity, including richness-based metrics, abundance-based metrics, and Shannon index, was significantly greater in 2023 than 2022. Except for a few cases (e.g., Caccasca2), such an increment in alpha diversity from 2022 to 2023 was coherently observed across sampling sites. Moreover, strong and clear effects of local environmental parameters on benthic macroinvertebrates were found in this study. Water velocity significantly and positively affected all the community metrics; while the amount of nutrients (i.e., phosphates and nitrates) reduced the alpha diversity of macroinvertebrate communities. Among the other physical and chemical parameters, electrical conductivity positively affected the richness and abundance of

macroinvertebrates, especially for the EPT taxa; while the opposite pattern was observed for pH. Water temperature, instead, exerted a positive and negative effect on the macroinvertebrate abundance and Shannon diversity index, respectively. Thus, these findings confirm our first original hypothesis for which inter-annual variation in flow-related conditions would have affected the alpha diversity of local macroinvertebrate communities. In particular, it should be hypothesised that the richness and abundance of ditch macroinvertebrate communities likely benefit from increased discharge and water velocity due to their positive effects on the water chemistry and near-bed, hydraulics conditions. Also, increased discharge and water velocity are expected to enhance the connectivity among ditches and even between them and the parent rivers through the passive dispersal of macroinvertebrates by drift. On the contrary, previous studies (Boulton and Lake 2008; Couto et al. 2023) have demonstrated

that low-flow conditions and lentication process alter the physical and chemical properties of watercourses by increasing the concentration of nutrients and water temperature, and decreasing the dissolved oxygen as well as the habitat for species associated with fast-flowing water. These changes, in turn, reduce the diversity and abundance of benthic macroinvertebrates and alter the taxonomic composition of benthic communities.

Since benthic macroinvertebrates were collected always in the same month (i.e., October) and almost from the same microhabitats in each site, we can likely exclude relevant biases due to the species phenology and taxon-specific preferences for substrates. Therefore, the findings here obtained suggest that hydrological conditions play a strong control in shaping ditch macroinvertebrate communities, with inter-annual changes in discharge and water velocity affecting the richness and abundance of macroinvertebrates along with the local physical and chemical parameters. Our results align with previous research dealing with the response of macroinvertebrate richness and density to the temporal fluctuations in flow and precipitations both in pristine and regulated rivers (Robinson et al. 2004; Huttunen et al. 2012; Fornaroli et al. 2019). For instance, in a large-scale study Fornaroli et al. (2020) examined the spatial and temporal variation in the benthic macroinvertebrate composition and diversity across the Po River Basin (northern Italy), including the area of study of this experiment. Authors found clear altitudinal shifts in the composition of macroinvertebrate communities, thus highlighting how altitude acts as a possible proxy of precipitations and temperature, as well as changes in the taxonomic and functional diversity over time from 2008 to 2018 (Fornaroli et al. 2020). Larsen et al. (2023), instead, found that the warmer and wetted conditions associated with the North Atlantic Oscillation altered the population synchrony and community stability of macroinvertebrates in temperate streams over a four-decade period. Similarly, several authors have recognized the role of stochasticity in shaping the diversity and structure of riverine macroinvertebrate communities (Milner et al. 2006; Ge et al. 2021).

The patterns in alpha diversity were further confirmed by the results on the beta diversity: multivariate analysis showed that the taxonomic composition of benthic macroinvertebrate communities significantly differed among sites and years. Moreover, these compositional differences were statistically driven by water temperature and the concentration of nitrates. While the increased water temperature in 2023 may be explained by the fact that the macroinvertebrate sampling was performed earlier (9th and 10th October) in 2023 than 2022 (20th and 27th October), the concentration on nitrates was slightly higher in 2022 than 2023, thus corroborating the role of nutrient enrichment as a driver of the taxonomic composition of ditch macroinvertebrate communities especially under low-flow conditions. From 2022 to 2023, each sampling site showed relatively high values of total beta diversity associated with the temporal variation in the taxonomic composition of macroinvertebrate communities, with the highest values recorded in Caccasca1 (0.90), while the lowest variation was observed in Cavo Dassi (0.51). In most sites (e.g., Caccasca1, Cavo Dassi, Cavo Panizzina, Cavo Cattedrale, and Fontana Pietta), nestedness was the main component that explained these compositional differences, and it was also coherent with the average increment in taxon richness observed

in this study. By contrast, in Caccasca 2 taxa replacement (i.e., turnover scenario) was the main mechanism that accounted for the compositional variation in the macroinvertebrate communities from 2022 to 2023. When looking at the beta diversity and its components among ditch macroinvertebrate communities surveyed in the same year, we found that in 2022 the total beta diversity was higher than 2023, and both nestedness and turnover contributed almost equally to the compositional differences among sites. On the contrary, increased contribution of taxa replacement was observed in 2023, despite it should be recognized that the total beta diversity among ditch communities was lower than 2022.

In this study, the Indicator Species Analysis (ISA) identified a higher number of taxa (7) in 2022 than in 2023 (5). Among these, only the gastropod *Theodoxus* was confirmed in Cavo Cattedrale from 2022 to 2023, while all the remaining indicator taxa changed from 2022 to 2023 across sampling sites. Except for Psychomyidae and *Serratella ignita*, the indicator taxa were not-EPT macroinvertebrates, thus indicating that ditch communities were mainly composed of relatively tolerant taxa. Nevertheless, these taxa were statistically associated with one or two sampling sites, with a significant variation from 2022 to 2023. It should be hypothesised that these patterns are likely explained by the inter-annual differences in the meta-population dynamics and flow-related dispersal abilities rather than improved water and hydro-morphological water quality of ditches (Johnson et al. 2012). These results corroborate the significant shifts in the taxonomic composition of ditch communities gained from the multivariate analysis and affected the differences here observed in the LCDB, which describes the uniqueness of a sampling site based on the taxa occurrence. Although this indicator did not differ between years, all sites but Caccasca2 and Cavo Dassi had higher LCBD values in 2022 compared to 2023, thus indicating that the presence of exclusive and unique taxa in the sampling sites was generally higher under low flow conditions (i.e., 2022) than under increased discharge (i.e., 2023).

Overall, all the results here obtained on the beta diversity, including its nestedness and turnover components, ISA, and LCBD, confirmed our second hypothesis with respect to inter-annual differences. In 2022, the ditch macroinvertebrate communities were generally poorer in taxa but showed higher site-specific assemblages (i.e., high LCBD value) and among-sites beta diversity, as demonstrated also by the higher number of taxa statistically associated with one or two sampling sites. On the contrary, in 2023 the macroinvertebrate communities displayed pronounced shifts in taxonomic composition that were mainly driven by gains of taxa (i.e., nestedness) coupled with the decrease in site-specific assemblage composition (i.e., lower LCBD values) and indicator taxa. Conversely to our experiment, in a field-study focussed on the beta diversity across agricultural streams in southern Ontario (USA), Krynak et al. (2019) identified species turnover as the primary driver of taxonomic variation in macroinvertebrate communities. However, their study was based on a long-term sampling effort spanning 9 years (i.e., from 2006 to 2015), whereas benthic macroinvertebrates in our study were collected over a shorter time period (i.e., 2022 and 2023), emphasizing the importance of the temporal scale when interpreting beta-diversity patterns. These findings highlight the need for future research explicitly examining temporal variation in

macroinvertebrate diversity in agricultural ditches and streams across multiple time scales, from seasons to multi-year periods, and assessing whether ditches may act as temporary refuge habitats during periods of hydrological stress.

Finally, agricultural ditches are commonly expected to host poor and less-diverse aquatic communities owing to both their modified nature and intensity of anthropogenic maintenance practices and disturbances (Verdonschot et al. 2011, Verdonschot 2012). While previous research has mainly shown that the macroinvertebrate diversity and richness are generally lower in ditches than in other aquatic ecosystems, including rivers and ponds (Williams et al. 2004; Davies et al. 2008; Shaw et al. 2015), differences in the diversity of ditch macroinvertebrate communities over time have been largely ignored. To our knowledge, this study represents one of the few available investigations providing novel insights on the temporal patterns in both the alpha and beta diversity of benthic macroinvertebrates in agricultural ditches. In particular, the results obtained here shed light on the role of flow conditions in shaping ditch benthic macroinvertebrate communities and try to foster further research aimed at integrating the temporal variation in the diversity of macroinvertebrates with the local environmental conditions. For instance, by providing different microhabitats compared to rivers and occupying different locations in the river networks, future studies should examine whether and to what extent agricultural ditches can host a relevant proportion of macroinvertebrate diversity depending on the flow conditions and seasonality. Owing to the homogenization of the intensively cropped agricultural landscape and its pressures on aquatic biodiversity (Hill et al. 2016; Gething 2021), taking into consideration the causes and mechanisms of variation in macroinvertebrate diversity across spatial and temporal gradients associated with flow conditions, water chemistry, and anthropogenic practices assumes a pivotal relevance to improve our understanding of the ecological role of agricultural ditches. This latter aspect, in turn, may serve to better evaluate their contribution to regional biodiversity in homogenized and impacted agricultural landscapes with the ultimate goal of implementing the sustainable management of agricultural ditches and conservation of their associated biodiversity.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Screenshot of the website of the Local Agency for Environmental Protection (ARPA Piemonte) where the daily data on river discharge were downloaded for the station “Agona – Novara” for the period: 1st January 2010 to 31st December 2023. This is the link to the website: https://www.arpa.piemonte.it/rischi_naturali/snippets_arpa_graphs/dati_giornalieri_idro/?staid=PIE-003106-700-2002-01-01¶m=I. **Table S1:** Environmental parameters and microhabitat types in each sampling site and year. Long = longitude (datum = WGS84), Lat = latitude (datum = WGS84), Velocity = mean water velocity, Depth = mean water depth, Temp = water temperature, Cond = electrical conductivity, NO3 = concentration of nitrates, PO4 = concentration of phosphates. For microhabitats: numbers inside cells indicate the number of Surber samples collected in that microhabitat. **Table S2:** TaxaXsite matrix illustrating the occurrence and abundance (i.e., numbers inside cells) of macroinvertebrate taxa in each sampling site. In this table, Surber samples were aggregated at site level by summing all individuals for each taxon. **Table S3:** Output of the PERMANOVA applied to environmental parameters. Significant results are in bold.