

Effect of management strategies and substrate composition on functional and taxonomic macroinvertebrate communities in lowland ditches of Alto Adige/Südtirol.

Abstract

Ditches could represent a tool for biodiversity enhancement and preservation in agricultural environments, also in Alpine regions. Macroinvertebrates and environmental factors describing water quality, substrate composition, hydrology, and geomorphology were collected in 10 ditches located in the Adige Valley, within the Biodiversity Monitoring program of South Tyrol. This study investigates the effects of ditch maintenance strategies operated *in situ* by the consortia (i.e., within an area of none, low and medium intensity) on taxonomic and functional macroinvertebrate diversity. We observed significant differences between management strategies in terms of both biological assemblages and environmental parameters. According to our results, these lowland ditches were mainly characterised by low substrate heterogeneity and dominated by fine organic sediments, with filamentous algae occurring only in managed sites. High concentrations of water nutrients, temperature and conductivity were associated with high intensities and frequencies of ditch maintenance. A significantly higher Shannon evenness index was found in low intensity management sites than in those with no or more intensive management. Furthermore, a decrease in %EPT and functional divergence was observed with increasing management intensity. Therefore, a sustainable management plan for ditch functioning is crucial to secure and improve both agricultural purposes and its biodiversity conservation potential in Alto Adige/Südtirol.

1. Introduction

Agricultural ditches, an integral feature of agricultural landscapes, have played a pivotal role in farming practices especially in North America and Europe where natural wetlands have been dramatically reduced for centuries (EEA 1996, WILLIAMS et al. 2004, DOLLINGER et al. 2015, HILL et al. 2016). They form intricate networks within cultivated catchments, and they contribute to efficiently manage runoff and drainage fluxes, as well as to effectively regulate floods and irrigation (LEVAVASSEUR et al. 2014, DOLLINGER et al. 2015, LEVAVASSEUR et al. 2016). Moreover, ditches affect groundwater hydrology by favoring water infiltration and can alter overland flow paths, therefore supporting agricultural water needs. As linear features characterized by a substantial edge-to-area ratio, ditches experience a significant influx of organic matters, organisms and pollutants from the adjacent terrestrial landscape (HERZON & HELENIUS 2008, DAGÈS et al. 2009). Therefore, they can also act as a buffer between agricultural fields and larger rivers, diluting the pollution coming from surrounding industrial outlets and agricultural runoffs. In this regard, riparian vegetation and substrate composition are main factors for retaining sediment and filtering pollutants, contributing to improved water quality (NEEDELMAN et al. 2007, HERZON & HELENIUS 2008).

Keywords: macrozoobenthos, biodiversity, lowland artificial water bodies, ditches maintenance, agricultural environment

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Further, ditches have an important function as potential habitat for different organisms. Indeed, they contribute to enhance landscape connectivity, sometimes becoming biodiversity hotspots for freshwater species (HERZON & HELENIUS 2008, LESLIE et al. 2012, DOLLINGER et al. 2015). In this regard, ponds and drainage ditches are frequently the sole remaining water refuges in intensive agricultural areas (SAYER et al. 2012, HILL et al. 2016), and many case studies demonstrated their conservation value, with high biodiversity within macroinvertebrate and macrophyte communities recorded even in intensively cultivated and managed agricultural landscapes (ARMITAGE et al. 2003, WILLIAMS et al. 2004, DAVIES et al. 2008, DOROTOVIČOVÁ 2013). However, despite the important contribution of ditches in supporting aquatic biodiversity, the overall environmental quality of drainage networks within intensely managed agricultural landscapes is largely undocumented and understudied (VERDONSCHOT 2012, HILL et al. 2016). In fact, even if they are included in monitoring programs (e.g., water bodies designated as ‘Artificial water bodies’ and ‘Heavily modified water bodies’ need to achieve at least ‘good ecological potential’ according to Water Framework Directive, WFD, 2000/60/EC), their benthic macroinvertebrate communities are not well known (LESLIE et al. 2012, HILL et al. 2016). Specifically, the most recent findings from biological quality analyses conducted by the Provincial Agency for Environment and Climate Protection indicate that the monitored canals in South Tyrol exhibit an ecological status ranging from good to poor (Provincia autonoma di Bolzano – Alto Adige 2023).

To retain their hydrological functions, ditches especially located within intensively managed agricultural landscapes require regular management that may encompass bank vegetation cutting, in-channel vegetation removal, and streambed dredging (HERZON & HELENIUS 2008, CLARKE 2015). Vegetation management (in-stream and on banks) can be achieved through various operations, including controlled burning, chemical herbicide applications, or mowing (NEEDELMAN et al. 2007, LEVAVASSEUR et al. 2014). However, as pointed out by HERZON & HELENIUS (2008), especially in-stream vegetation-clearing maintenance operations can significantly impact stream benthic communities, removing potential habitats and sources of shelter and food. Other more invasive management operations, such as dredging, as highlighted by DOLLINGER et al. (2015), can result in the complete or partial removal of accumulated stream bed sediments, subsequently displacing benthic organisms. However, in cases where the shortage of affordable priced farmland has resulted in restricted land availability for expanding shoreline vegetation strips, dredging can be the only solution for restoring original morphology and capacity of ditches, thus reestablishing their key hydrological functions. It is therefore clear how assessing and proposing an effective management of ditches may pose a complex task. As emphasized by GETTING et al. (2020), advancing successful management strategies and biodiversity support in agricultural landscapes requires a deeper understanding of ditch water quality and their associated benthic communities.

Despite the growing body of research on aquatic ecosystems and their associated biota, a notable gap still exists in our understanding of the intricate relationships between habitat variability, management practices, and the community structure of benthic macroinvertebrates in lowland ditches. The region of Alto Adige/Südtirol makes no exception: although differences in the benthic macroinvertebrate assemblages of lowland ditches in Alto Adige/Südtirol compared to surrounding mountain streams were analysed (VALLEFUOCO et al., in review) with more than 30 taxa that were found only in ditches, this study did not provide any insight concerning the potential influence of specific management on the biodiversity value of ditches. It is indeed extremely important to consider the effects of management strategies at local/patch level to better understand ecological potential of ditches, and link this to their current level of biodiversity (TÖLGYESI et al. 2021). Thus, the aim of this study is to investigate the importance of drainage ditches as potential hotspots for aquatic biodiversity in agricultural environments of Alto Adige/Südtirol, taking into account an increasing gradient of disturbance stemming from different management strategies.

More specifically, in this paper, we intended to: 1) assess changes in environmental conditions – including physico-chemical parameters, substrate cover, substrate com-

position – among three categories of drainage ditch management strategies (i. e., none = absence of management, low intensity = mowing of the embankment and of the bottom just if required, and medium intensity = periodical mowing of the banks and mowing of the streambed); 2) identify the specific macroinvertebrate community related to each category of management strategy and, in particular, quantify their taxonomic and functional diversity of the benthic communities.

We hypothesised that: 1) ditches subjected to medium intensity of management strategy exhibit higher water temperature and organic nutrients due to the removal of riparian and in-stream vegetation, compared to the environmental conditions of the other two management categories. In addition, we expected that substrate heterogeneity could be reduced by bottom cleaning operation, with significant effects on the benthic community structures; 2) ditches with a medium intensity management plan have less biota richness and diversity in comparison to ditches with low or null management. Moreover, referring to the theory of the intermediate disturbance hypothesis by TOWNSEND et al. (1997), we expect that low intensity management host the highest number of taxa.

2. Material and Methods

2.1 Study Area

The studied ditches are located in the Autonomous Province of Bolzano/Bozen (Italy). The ten surveyed artificial or semi-artificial water bodies are located between 208 and 487 m a.s.l., 9 located in the lowland agricultural area of the Etsch/Adige Valley, and 1 site located in the Passeier/Passirio Valley (Fig. 1; Table 1). Although surrounded by the Alps, the southern part of the Province is characterized by subcontinental climate with relatively low annual precipitation (700 –800 mm), low mean annual temperature (Bolzano, 254 m a.s.l.: 6,8°C min.; 18,1°C max.; source: <https://meteo.provincia.bz.it/download-dati.asp>) and high solar radiation (Bolzano annual Global Horizontal Irradiation: 1468 kWh/m²; source: <http://www.solaritaly.enea.it/TabellaRad/TabellaRadEn.php>).

Three provincial agencies are responsible for ditch maintenance in the study area: Land Reclamation Consortium of *Passer-Eisackmündung /Foce Passirio – Foce dell’Isarco*, Land Reclamation Consortium of *Eisackmündung-Gmund/Foce Isarco-Monte*, and Land Reclamation Consortium of *Gmund-Salurn/Monte-Salorno*. These agencies are responsible for the management and mowing of the ditch banks, the cleaning of their bottoms and the restoration of landslide scarps. Another related operation is the maintenance of sluice gates and weirs to regulate the water level of drainage ditches. Since the deterioration of a ditch ecosystem depends on the type and on the frequency of maintenance operations, we considered both for classifying the intensity of local management strategies (BELLENTANI 2022). Management measures were categorized in three classes of maintenance (i. e., none, low and medium intensity; Fig. 1; Table 2, 3). As we investigated macroinvertebrate fauna at local scale, our classification of sites considered the specific conditions of the sampling sites. We collected information on ditch maintenance strategies from the Reclamation Consortium website, maps and/or direct contact.

The agriculture in the Etsch/Adige Valley is dominated by vineyards on smooth slopes and apple orchards in flat areas (Table 3). A specific analysis of land use was carried out within a 2 km buffer upstream of each sampling site through a general GIS analysis using the Corine Land Cover (CLC 2018).

Table 1: Georeferenced data of the ten sampling sites analysed in this study.

Site	Y North (WGS84)	X East (WGS84)	Elevation [m a.s.l.]	Stream	Municipality
1	46.74201	11.204524	487	Schafflerbach/Rio delle Pecore	St. Martin in Passeier/ San Martino in Passiria
2	46.59605	11.193197	257	Burgstallerbach/Rio di Postal	Burgstall/Postal
3	46.57275	11.181392	252	Gießenbach/La Roggia	Lana/Lana
4	46.49289	11.299789	242	Neufeldleege/Fosse di scolo di Camponuovo	Bozen/Bolzano
5	46.34431	11.253421	213	Höllentalbach/Rio di Val di Inferno	Tramin/Termenò
6	46.36994	11.304832	222	Uhlgraben/ Fossa Uhl	Auer/Ora
7	46.45477	11.311546	228	Leiferergraben/ Fossa di Laives	Bozen/Bolzano
8	46.26199	11.209739	208	Großer Kalterergraben	Salurn/Salorno
9	46.33037	11.243374	231	Feldgraben/Fossa del Campo	Salurn/Salorno
10	46.34665	11.273215	214	Tillgraben/Fossa Till	Kaltern/Caldaro

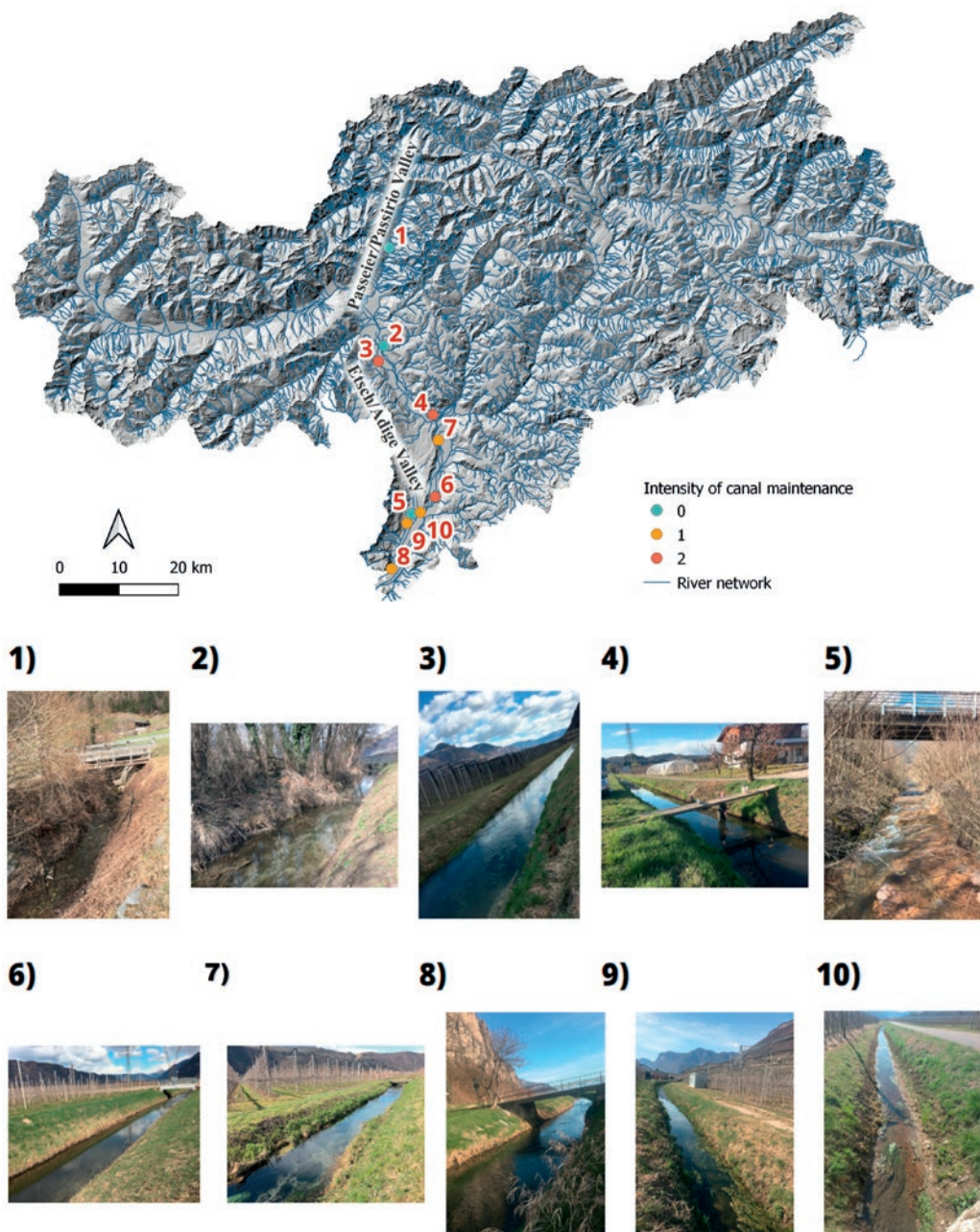


Fig. 1: Map of the Autonomous Province of Bolzano-South Tyrol (Italy) with the pictures of the sampling sites and locations.

Table 2: Description of ditch management classification based on three different activity intensities and frequencies.

Category of management strategy	Management type	Period and frequency	Sites
0 (null)	Absent	/	1; 2; 5
1 (low)	Mowing of the embankment	1–2 times/yr (June/July) or if required	7; 8*; 9; 10
	Bottom if required	/	
2 (medium)	Mowing of the banks with packers	2–3 times/yr (May-July-November)	3; 4; 6
	Mowing of the bed stream with motorboat and/or cutter bar	1–2 times/yr (June/July)	

* Site 8 is located downstream of a sewage treatment plant and is characterised by intensive maintenance along most of the ditch, but it exhibited a good bottom quality at the time of sampling. Therefore, it was classified as affected by a low intensity management strategy.

Table 3: Site identification number, substrate type and number of different substrates sampled, type of management in each stream and the three main land use types analysed 2 km upstream. For type management description see table 2. The main land use type reports the three most common land use types and their percentage of coverage within a 2 km buffer upstream of each sampling site, calculated according to the Corine Land Cover (CLC 2018).

Site	No. of different substrates	Substrate type	Category of management strategy	Main land use type
1	6	big mineral sediments fine sediments gravel CPOM sand mobile blocks	0	agricultural area with natural vegetation (51%) mixed forest (31%) fruit stock (18%)
2	7	big mineral sediments fine sediments gravel helophytes sand mobile blocks hydrophytes	0	fruit stock (65%) discontinuous urban fabric (18%) broad-leaved forest (17%)
3	6	algae gravel hydrophytes big mineral sediments fine sediments mobile blocks	2	fruit stock (90%) broad-leaved forest (8%) mixed forest (1%)
4	6	hydrophytes algae fine sediments helophytes natural & artificial surfaces CPOM	2	fruit stock (71%) transitional woodland-shrub (12%) vineyards (6%)
5	7	sand big mineral sediments fine sediments natural & artificial surfaces moss mobile blocks CPOM	0	vineyards (55%) discontinuous urban fabric (35%) broad-leaved forest (7%)
6	7	hydrophytes helophytes fine sediments mobile blocks gravel big mineral sediments sand	2	fruit stock (93%) transitional woodland-shrub (6%) broad-leaved forest (1%)
7	6	gravel sand big mineral sediments hydrophytes helophytes algae	1	fruit stock (88%) road and rail networks (7%) airport (3%)

Site	No. of different substrates	Substrate type	Category of management strategy	Main land use type
8	6	hydrophytes mobile blocks big mineral sediments gravel sand helophytes	1	vineyard (46%) transitional woodland-shrub (26%) fruit stock (16%)
9	6	hydrophytes algae sand helophytes gravel fine sediments	1	fruit stock (76%) vineyards (21%) discontinuous urban fabric (3%)
10	7	mobile blocks algae sand big mineral sediments gravel hydrophytes fine sediments	1	fruit stock (83%) broad-leaved forest (17%)

2.2 Sampling and processing

Biological and environmental data were collected in 10 lowland ditches across the Etsch/Adige Valley and in the Passeier/Passirio Valley (see table 1), within the first year of the aquatic Biodiversity Monitoring program South Tyrol (BMS; HILPOLD et al. 2023). All 10 sites were sampled during March 2023. Stream benthic macroinvertebrates and a set of environmental factors describing water quality, stream/river geomorphology and in-stream habitat characteristics were surveyed and analysed according to the methodology described in SCOTTI et al. (2022). Benthic macroinvertebrates were sampled using a kick-net (mesh-size 500 µm) according to the Swiss IBCH method (modular level concept for macroinvertebrates, level F, STUCKI et al. 2019). In each site, 8 subsamples were taken based on the available substrate, maximizing substrate heterogeneity, and prioritizing the most habitable substrates for macroinvertebrates such as mobile blocks (size > 250 cm) and bryophytes (DUAN et al. 2008, STUCKI et al. 2019). Concurrently, water depth, bottom current velocity and Froude number were measured. In addition, the geomorphological parameters stream width, streambed width, wetted perimeter, and the three sub-indices of the Pfankuch Stability Index (PSI) – an indicator for channel stability and sensitivity to disturbance of the streambed, lower and upper banks – were evaluated at each site (PFANKUCH 1975). The percentage of the different substrate types in the sampling area was estimated (i. e., % substrate cover). Finally, the water parameters pH, water temperature, dissolved oxygen, oxidation-reduction potential, conductivity and turbidity were measured in the field whereas water nutrients (i. e., total nitrogen, total phosphorus, orthophosphate, ammonium, nitrate, and nitrite) were analysed in the laboratory, within the same day of collection, using a portable spectrophotometer (Hach Lange DR1900) and the specific protocols and reagents produced by Hach Lange. Benthic macroinvertebrates were stored in 75 % ethanol and identified to the lowest possible taxonomic level (SCOTTI et al. 2022).

2.3 Data analysis

To assess the evenness of substrates at each sampling sites, we calculated the Inverse Simpson's diversity index on the % substrate cover data converted to proportions, to measure how evenly the % cover of different substrates is distributed within the sampling sites, using diversity function in *vegan* package (OKSANEN et al. 2019). Higher values of the index (*Sub-index*) indicated greater evenness, meaning that the % cover of different substrates was more evenly distributed, while low values indicated the dominance of one substrate over the others. Afterwards, the influence and the relative correlation of environmental variables, including management intensity and the *Sub-index*, were assessed using a Principal Component Analysis (PCA) on the data aggregated by site (i. e., most of the physico-chemical parameters included were single

values per site, while the average per site was calculated for water column depth, flow velocity and Froude number parameters, which were measured at each subsample and thus, averaged).

Concerning fauna analysis, macroinvertebrate density data were also aggregated by sites by averaging the 8 subsamples collected at each site, in order to compare the community structure and diversity among three different management strategies. A non-metric multidimensional scaling (nMDS) ordination of sampling units based on Bray–Curtis dissimilarities of log-transformed macroinvertebrate densities (85 Taxa) from the 10 lowland ditches was conducted to characterise sites on the basis of taxa composition. To assess which taxa were driving the patterns observed in the nMDS ordination space, a posteriori projection of significant taxa, associated with the respective sites, was performed using the *envfit* function with 999 permutations for statistical assessment (i.e., explaining the variation in sites distribution with respect to taxa densities through least squares regression analysis). The relationships between taxa and sites were interpreted by proximity. Furthermore, a non-parametric permutational multivariate analysis of variance (PERMANOVA) was used to assess differences in community composition among ditch management strategies (ANDERSON 2001), using the *adonis2* function implemented in the *vegan* package (OKSANEN et al. 2019) and using a nested approach. The condition of homogeneity of the multivariate dispersions of variances among groups with respect to management types was tested with the *betadis* function (ANDERSON 2006), accounting for 999 permutations with Bray–Curtis distances. This indicates that the PERMANOVA and the homogeneity test were conducted using the Bray–Curtis dissimilarity matrix derived from the densities of 80 subsamples (8 subsamples multiplied by 10 sites), with the factor of management types nested within sites. An empirical pseudo-F distribution and p-values were calculated from 999 permutations. In addition, taxa and their exclusive and/or ubiquitous presences in relation to management strategies were visually represented by a Venn diagram.

To investigate the variation of alpha diversity among different intensities of management strategies, we calculated for each site several biological indices such as total density, taxonomic richness, % Ephemeroptera–Plecoptera–Trichoptera (%EPT) and Shannon evenness (Hill's ratio), using diversity function in the R *vegan* package. For the functional indices we used the *dbFD* (i.e., Distance-Based Functional Diversity Indices diversity) function in the *FD* package (LALIBERTÉ et al. 2014) to calculate functional richness (FRic, i.e., the volume of the functional space occupied by the assemblages), functional evenness (FE, i.e., how regularly species abundances are distributed in the functional space), functional divergence parameter (FDiv, i.e., divergences in abundance distributions within this functional space) and functional dispersion (FDis, i.e. the distribution of taxa in functional space) from the community-weighted means of trait categories matrix. Therefore, we selected specific traits from <https://freshwater-ecology.info> (SCHMIDT-KLOIBER & HERING 2015) linked to the particular physico-chemical properties of drainage ditches and to macroinvertebrates habitat preferences and “life & body” parameters to explore whether they were influenced by a gradient of management intensity. In detail, the functional structure of the benthic communities was described using seven biological traits and a total of 33 categories related to micro-habitat/substrate preference, temperature range preference, trophic status, feeding habits, locomotion types, respiration strategy and the sensitivity to pesticide effects and contamination (i.e., SPEAR pesticides index) (Table 4). Statistical differences in both biological and functional metrics, between substrates and management intensities, were tested separately with non-parametric Kruskal-Wallis's test, followed by the BENJAMINI & HOCHBERG'S (1995) false discovery rate (*fdr*) p-values correction for multiple comparisons ($\alpha=0.05$).

All the analyses were performed in R statistical software (R Development Core Team version 4.3.0) with the packages *vegan* (OKSANEN et al. 2018), *agricolae* (DE MENDIBURU & SIMON 2015), *ade4* (DRAY & DUFOUR 2007), *VennDiagram* (CHEN & BOUTROS 2011) and *ggplot2* (WICKHAM et al. 2016).

Table 4: The seven functional traits and 33 categories explored in the analysis, selected from <https://freshwaterecology.info> (SCHMIDT-KLOIBER & HERING 2015). 1= MOOG et al. (1999); 2= TACHET et al. (2010); 3= LIESS et al. (2005); 4= SCHMIDT-KLOIBER & HERING (2015).

Trait	Category name	Code	Explanation
Microhabitat/ substrate preference (8 categories) ¹	argyllal	arg	silt, loam, clay (grain size < 0.063 mm)
	pelal	pel	mud (grain size < 0.063 mm)
	psammal	psa	sand (grain size 0.063–2 mm)
	akal	aka	fine to medium-sized gravel (grain size 0.2–2 cm)
	lithal	lit	coarse gravel, stones, cobbles, boulders, bedrock (grain size > 2 cm)
	phytal	phy	algae, mosses, macrophytes
	pom	pom	coarse and fine particulate organic matter
	other	oth	other substrates
Temperature range preference ⁴	cold stenotherm	cos	preference for a small cold temperature range (below 10 °C)
	warm stenotherm	was	preference for a small warm temperature range (above 18 °C)
	eurytherm	eut	no specific preference; wide temperature range
Trophic status (preferendum) ²	oligotrophic	oli	low nutrient availability, low biological productivity
	mesotrophic	meso	low nutrient availability, intermediate biological productivity
	eutrophic	eu	high nutrient availability, high biological productivity
feeding type ¹	grazers/scrapers	gra	feed on endolithic and epilithic algal tissues, biofilm, partially POM, partially tissues of living plants
	miners	min	feed on leaves of aquatic plants, algae and cells of aquatic plants
	shredders	shr	feed on fallen leaves, plant tissue, CPOM
	gatherers/collectors	gat	feed on sedimented FPOM
	active filter feeders	aff	feed on suspended FPOM, CPOM; micro prey is whirled; food is actively filtered from the water column
	passive filter feeders	pff	feed on suspended FPOM, CPOM, prey; food is filtered from running water, e.g., by nets or specialised mouthparts
	predators	pre	feed on prey
	parasites	par	feed on host
	other feeding types	oth	use other food sources not meeting the above categories
	locomotion type ⁴	swimming/skating	sws
swimming/diving		swd	swimming or active diving
burrowing/boring		bub	burrowing in soft substrates or boring in hard substrates
sprawling/walking		spw	sprawling or walking actively with legs, pseudopods or on a mucus
(semi)sessil		ses	tightening to hard substrates, plants or other animals
other locomotion type		oth	other locomotion type like flying or jumping (mainly outside the water)
SPEAR pesticide ³	yes	1	species at risk
	no	0	species not at risk
	tegument	teg	respiration through the body surface
Respiration ²	gill	gil	respiration using special respiration organs

4. Results

In total, 17,686 individuals belonging to 85 different aquatic invertebrate taxa were identified in all investigated lowland ditches. The most abundant order was Diptera (dominated by Chironomidae and *Simulium* sp.), contributing to the total abundance with 52.2%, followed by oligochaetes (Oligochaeta, 18.9%, composed mainly by Naididae, Mermithidae and Lumbriculidae taxa), mayflies (Ephemeroptera, 10.8%, where *Baetis rhodani* was the most abundant taxa) and crustaceans (Crustacea, 6.9%, composed mainly by *Asellus aquaticus*). The joint contribution of all other groups such as snails (Gastropods), bivalves (Bivalvia), beetles (Coleoptera), caddisflies (Trichoptera), leeches (Hirudinea), stoneflies (Plecoptera), flatworms (Turbellaria), and dragonflies (Odonata), was less than 12% of the total abundance.

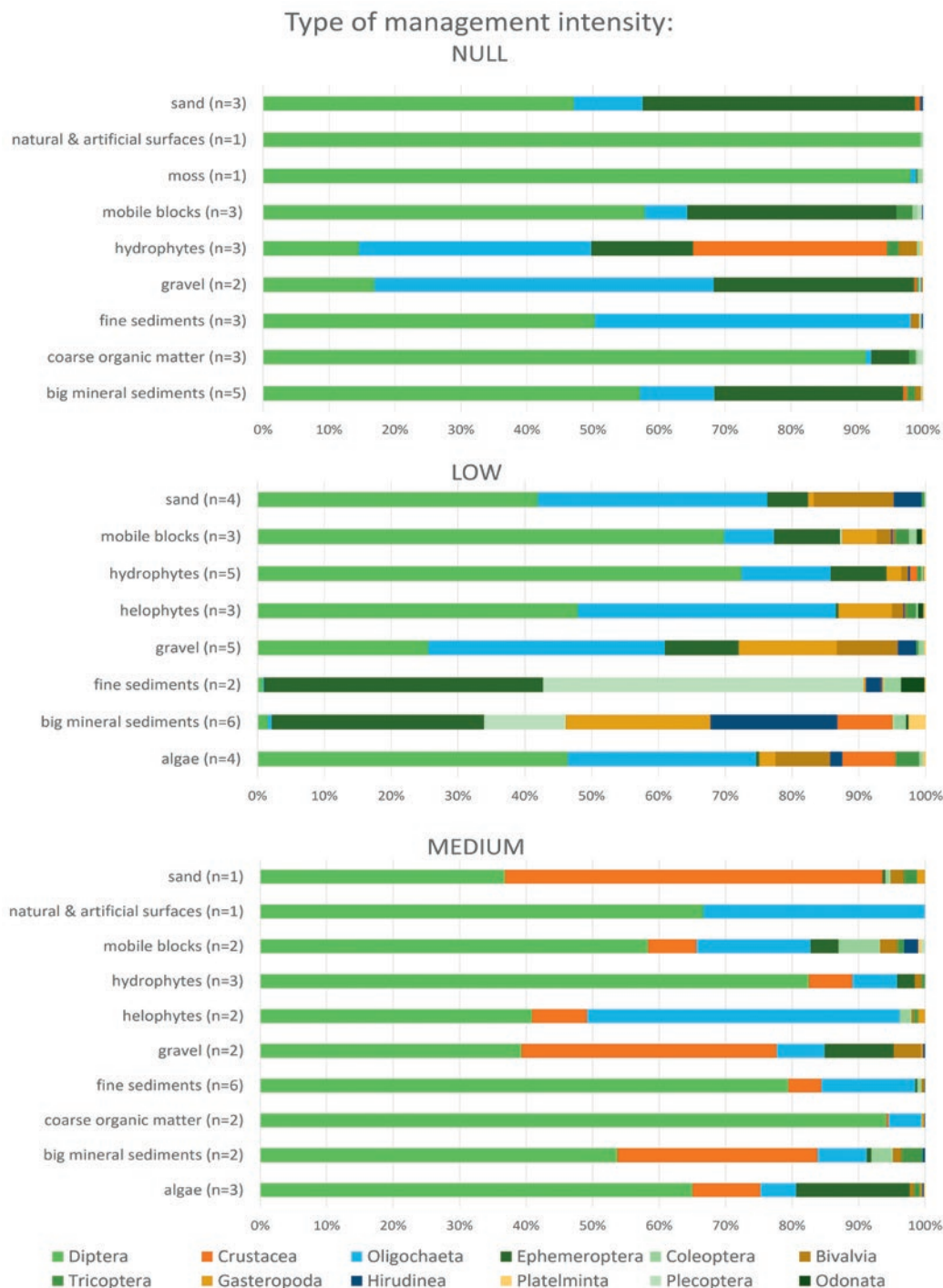


Fig. 2: Macroinvertebrate taxonomic composition grouped by substrate types and the three intensities of management strategy.

Despite the different number of observations sampled for each substrate type, we observed differences both in substrate diversity and colonisation, in terms of taxa assemblages, between the three categories of management strategy (Fig. 2). Diptera was the most dominant order in all three categories of management strategy, followed by Oligochaeta and Ephemeroptera in the null and low intensity management category, and followed by Crustacea and Oligochaeta in the medium intensity category. In detail, Diptera represented more than 90% of the community composition in bedrock and moss in unmanaged sites, and in CPOM in sites with both null and medium intensity management. On the contrary, they represented less than 25% of the community in hydrophytes and gravel sediments (i. e., size 2,5–25 mm) in unmanaged sites, and in fine organic sediments and big mineral sediments in sites with low management intensity. Moreover, Helophytes and algae were only found in managed ditches. In unmanaged ditches, the highest %EPT, composed mainly by Ephemeroptera, were found in sand (41.3%), gravel (30,7%), big mineral sediment (i. e., size 25–250 mm; 30%) and mobile blocks (i. e., size > 250mm; 34.8%). In sites of the low intensity managed category, EPTs contributed with about 90% to the total taxa pool in fine sediments, where Plecoptera were dominant, and almost 45% in big mineral sediments. Finally, in the medium intensity management a low %EPT (ca. 10–20%) was observed exclusively in gravel and algae.

In addition, high evenness of Sub-index was observed in sites 1 and 8 (*Sub-index* = 0.83 and 0.75, respectively), as shown in fig. 3. Low values of *Sub-index* were reported in sites 4, 5 and 9 (*Sub-index* = 0.33; 0,28 and 0.34, respectively), which were dominated by fine organic sediments, bedrocks and algae, respectively.

The PCA summarised the environmental differences between the sampled lowland ditches, with 41.6% of the variation explained by the first two principal components (Fig. 3). The first axis explained 22.6% of the total variance and was positively corre-

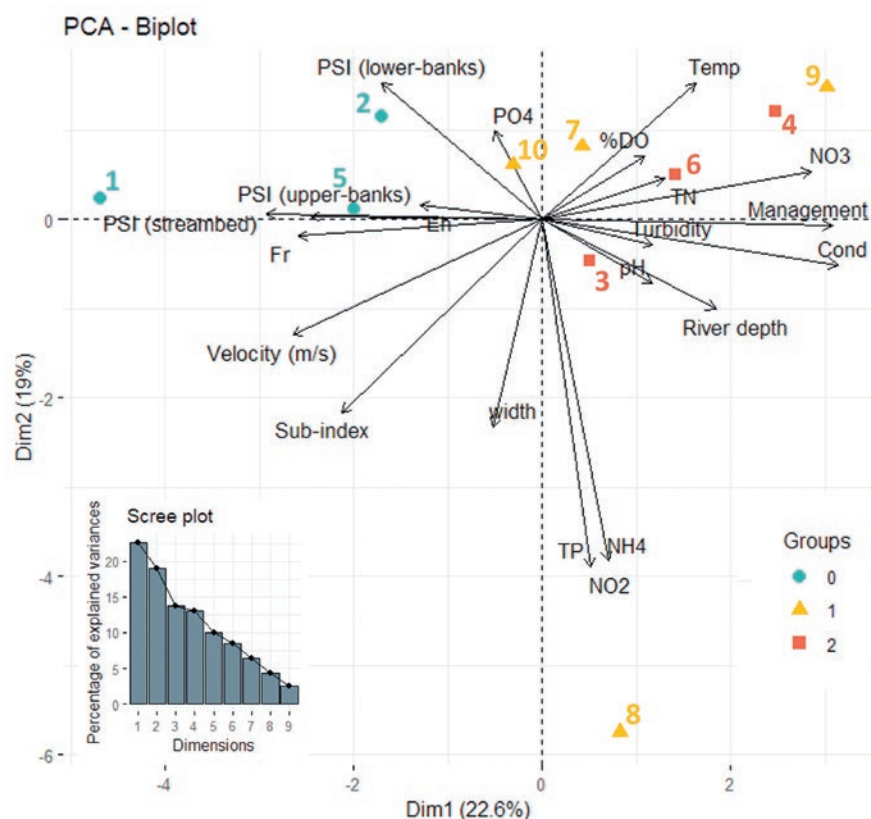


Fig. 3: Biplot of the first two axes of the Principal Component Analysis (PCA) of the environmental, representing the associations between the environmental variables on the sampling sites. All subsamples' data were aggregated by site (i. e., abundancies sum). Arrows represent continuous variables, including the substrate dominance index (i. e., *Sub_index*) and the management intensity data; groups represent site location in the bidimensional space grouped by management strategy (i. e., 0/cyan dots = area of none, 1/yellow triangles = low and 2/red squares = medium intensity of ditch maintenance).

lated with management intensity, conductivity, nitrate (NO₃), temperature and river depth, and negatively correlated with low velocity (m/s) and to the streambed component of the Pfankuch index. The first axis showed a clear gradient related to the management strategies, with an increase of management intensity in the positive direction. The second axis, which explained 19% of the variance, was negatively related to nitrite (NO₂), ammonium (NH₄) and total phosphorus (TP) and was interpreted as an increase in nutrient and pollutant levels, with higher values in site 8 compared to the other ditches.

The nMDS analysis based on the Bray–Curtis dissimilarity matrix provides a good representation of the taxa composition in reduced dimensions (stress = 0.11). Although some overlaps, a pattern of differences between the assemblages from different ditch maintenance intensities can be discerned (Fig. 4). The *envfit* function identified 12 significant taxa associated with the site scores observed in the first two axes of nMDS ordination space (Fig. 4; Table 5). A clustering of sites according to the management intensities was observed along the NMDS1 axis. In particular, Psychodidae, *Rhypholophus* sp. (Limonidae) and *Nemoura mortoni* were significantly correlated with no managed conditions (i.e., negative NMDS1 direction), whereas *Radix* sp., Tanypodinae and *Pisidium* sp. were significantly associated with the low management intensity in the opposite direction (Fig. 4). However, sites classified as medium intensity of maintenance did not clearly differ in fauna composition compared to those with null and low intensity management activity. Indeed, only 5 taxa exclusively occurred in this maintenance classification, and none significantly, while mostly were in common with the other two classification types (Fig. 5, Table 5).

The nested PERMANOVA ($R^2 = 37.1\%$; $F = 4.588$; $p.value = 0.001$; $N.perm. = 999$) suggested a significant difference of community composition in the different maintenance classification types. However, the significant beta-dispersion analysis ($F = 3.285$; $p.value = 0.002$; $N.perm. = 999$) showed that the taxa within each management strategy group were differently dispersed and/or variable. In particular, the Tukey multiple comparison of means test indicated that the variance of site number 5, classified as an unmanaged site, was significantly different from site 1 ($p.value = 0.042$), site 3 ($p.value = 0.009$), and site 7 ($p.value = 0.008$).

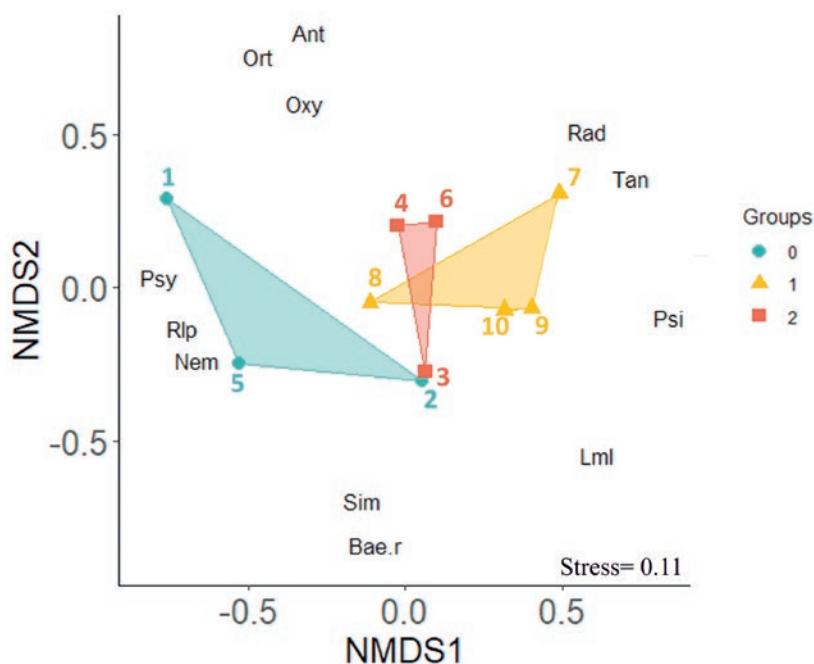


Fig. 4: 3D non-metric multidimensional scaling (nMDS) ordination of sampling units based on Bray–Curtis dissimilarities of log-transformed macroinvertebrate densities (85 Taxa) from 80 samples (8 subsamples x 10 lowland ditch sites). Sites were coloured according to the management classification (i.e., 0/cyan colour = area of none, 1/yellow colour = low and 2/red colour = medium intensity of ditch maintenance). Significant envfit vectors overlaid on the first two axes of the original NMDS plot. For full taxa name refer to table 5.

Category of management strategy

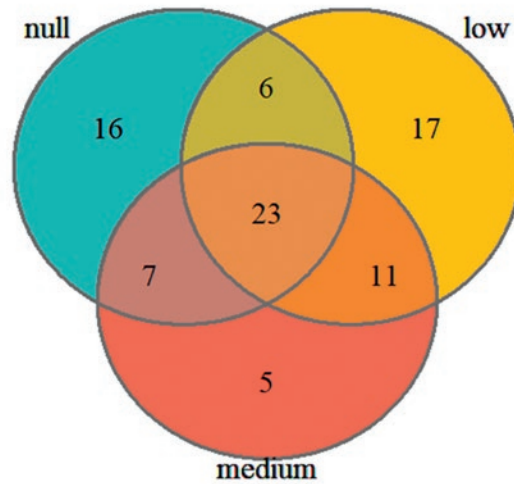


Fig. 5: Venn diagram plot representing the taxa in common between different classes of ditch maintenance intensities and taxa that exclusively occurred in one group (refers to table 5 for taxa presence in each management type).

Table 5: Macroinvertebrate taxa list and *envfit* outputs scaled by their correlation with the first two nMDS axis (i.e., r^2 value). MN = classes of ditch management intensity; x = taxa presence, bold indicates significantly exclusive taxa of one type of management; $Pr(>r)$ bold = level of significance p value < 0.01 (**); p value < 0.05 (*).

Taxa code	Taxa (extended name)	MN			Envfit outputs			
		0	1	2	NMDS1	NMDS2	r2	Pr(>r)
Psi	<i>Pisidium</i> sp.	x	x	x	0.982	-0.187	0.695	0.011 *
Dys	Dytiscidae	x	x	x	0.670	-0.742	0.091	0.764
Elm	<i>Elmis</i> sp.	x	x	x	0.380	0.925	0.072	0.758
Lin	<i>Limnius</i> sp.	x	x	x	-0.969	0.245	0.230	0.431
Hal	<i>Haliplus</i> sp.		x	x	0.588	0.809	0.203	0.484
Hdp	Hydrophilidae	x			-0.613	-0.790	0.359	0.315
Not	Noteridae	x			-0.613	-0.790	0.359	0.315
Ase	<i>Asellus aquaticus</i>	x	x	x	0.409	-0.912	0.265	0.345
Gam	Gammaridae		x	x	0.825	0.566	0.279	0.339
Gar	<i>Gammarus</i> sp.		x		0.504	0.864	0.399	0.216
Ech	<i>Echinogammarus stammeri</i>		x	x	0.844	0.536	0.048	0.838
Ant	Anthomyiidae	x		x	-0.444	0.896	0.775	0.007 **
Ath	<i>Atherix</i> sp.	x	x	x	0.992	0.126	0.109	0.687
Cer	Ceratopogonidae	x	x	x	0.864	-0.504	0.110	0.652
Ort	Orthocladiinae	x	x	x	-0.511	0.859	0.651	0.019 *
Chi	Chironominae	x	x	x	0.139	0.990	0.029	0.902
Tan	Tanypodinae		x	x	0.849	0.528	0.614	0.034 *
Emp	Empididae	x	x		-0.600	-0.800	0.119	0.62
Rlp	<i>Rhypholophus</i> sp.	x			-0.971	-0.240	0.626	0.044 *
Rbd	<i>Rhabdomastix</i> sp.			x	-0.044	0.999	0.091	0.791
Pil	<i>Pilaria</i> sp.		x		0.903	-0.431	0.140	0.603
Lis	<i>Lispe</i> sp.	x		x	0.055	0.998	0.002	1
Dic	<i>Dicranota</i> sp.	x		x	-0.309	-0.951	0.432	0.171
Ped	<i>Pedicia</i> sp.	x			-0.613	-0.790	0.359	0.315
Psy	Psychodidae	x			-1.000	-0.027	0.711	0.021 *
Prm	<i>Prosimulium</i> sp.	x	x	x	-0.379	-0.925	0.310	0.273
Sim	<i>Simulium</i> sp.	x	x	x	-0.255	-0.967	0.600	0.042 *
Oxy	<i>Oxycera</i> sp.	x		x	-0.498	0.867	0.576	0.039 *
Tab	Tabanidae	x			-0.613	-0.790	0.359	0.315

Taxa code	Taxa (extended name)	MN			Envfit outputs			
		0	1	2	NMDS1	NMDS2	r2	Pr(>r)
Tip	Tipulidae	x	x		0.996	-0.084	0.046	0.83
Bae	<i>Baetis</i> sp. (juv.)		x	x	-0.054	-0.999	0.001	0.993
Bae.r	<i>Baetis rhodani</i>	x	x	x	-0.168	-0.986	0.827	0.002 **
Bae.a	<i>Baetis alpinus</i>	x	x		-0.899	-0.437	0.564	0.06 .
Bae.b	<i>Baetis buceratus</i>		x		0.756	-0.654	0.139	0.602
Bae.m	<i>Baetis muticus</i>		x		0.404	0.915	0.022	0.896
Cae	<i>Caenis</i> sp.		x		-0.639	-0.769	0.015	1
Srt	<i>Serratella ignita</i>		x		0.504	0.864	0.399	0.216
Hep	Heptageniidae		x		0.504	0.864	0.399	0.216
Ecd	<i>Ecdyonurus</i> sp.	x			-0.613	-0.790	0.359	0.315
Ep.as	<i>Epeorus assimilis</i>	x			-0.613	-0.790	0.359	0.315
Rhi	<i>Rhithrogena</i> sp.			x	0.084	-0.996	0.166	0.485
Bit	<i>Bithynia</i> sp.		x		0.886	-0.465	0.253	0.366
Rad	<i>Radix</i> sp.		x	x	0.816	0.578	0.613	0.039 *
Phn	Physidae			x	-0.044	0.999	0.091	0.791
Phs	<i>Physa</i> sp.		x		0.504	0.864	0.399	0.216
Phy	<i>Physella</i> sp.		x	x	-0.848	-0.531	0.012	0.95
Anc	<i>Ancylus fluviatilis</i>	x	x	x	0.644	0.765	0.190	0.47
Val	<i>Valvata</i> sp.		x		0.851	-0.525	0.091	0.911
Erp	Erpobdellidae	x	x	x	0.410	-0.912	0.459	0.121
Glo	Glossiphoniidae	x	x	x	1.000	-0.003	0.297	0.312
Pis	Piscicolidae			x	0.084	-0.996	0.166	0.485
Sia	<i>Sialis</i> sp.			x	0.163	0.987	0.110	0.711
Cal	Calopterygidae		x		-0.639	-0.769	0.015	1
Coe	Coenagrionidae		x		-0.639	-0.769	0.015	1
Cor	Corduliidae		x		0.244	-0.970	0.014	0.924
Plt	Platycnemididae		x		-0.639	-0.769	0.015	1
Enc	Enchytraeidae	x	x	x	-0.284	-0.959	0.363	0.226
Hap	<i>Haplotaxis</i> sp.	x	x	x	0.467	-0.884	0.349	0.203
Lmb	Lumbricidae	x	x	x	-0.825	-0.565	0.270	0.319
Lml	Lumbriculidae	x	x	x	0.679	-0.734	0.672	0.027 *
Mer	Mermithidae	x	x	x	-0.320	-0.948	0.151	0.542
Nai	Naididae	x	x	x	0.853	-0.521	0.514	0.094 .
Leu	<i>Leuctra</i> sp.	x		x	-0.309	-0.951	0.432	0.171
Amp	<i>Amphinemura</i> sp.		x		-0.639	-0.769	0.015	1
Pro	<i>Protonemura</i> sp.	x			-0.613	-0.790	0.359	0.315
Nem	<i>Nemoura mortoni</i>	x	x		-0.946	-0.323	0.646	0.03 *
Hyd	<i>Hydropsyche</i> sp.	x		x	-0.345	-0.939	0.531	0.08 .
Hyd.a	<i>Hydropsyche angustipennis</i>		x	x	0.243	-0.970	0.237	0.333
Hyd.f	<i>Hydropsyche fulvipes</i>	x			-0.687	0.726	0.644	0.1 .
Hyd.i	<i>Hydropsyche instabilis</i>	x	x		-0.616	-0.788	0.365	0.24
Hdt	<i>Hydroptila</i> sp.		x	x	0.492	0.871	0.337	0.234
Lmp	Limnephilidae (juv.)	x	x	x	0.380	-0.925	0.276	0.318
Acr	<i>Acrophylax zerberus</i>	x			-0.687	0.726	0.644	0.1 .
All.a	<i>Allogamus auricollis</i>	x			0.063	-0.998	0.208	0.42
Cha.f	<i>Chaetopteryx fusca</i>		x	x	0.635	0.773	0.314	0.264
Lim.l	<i>Limnephilus lunatus</i>	x	x		0.567	0.824	0.297	0.286

Taxa code	Taxa (extended name)	MN			Envfit outputs			
		0	1	2	NMDS1	NMDS2	r2	Pr(>r)
Mcp	<i>Micropterna</i> sp.		x		-0.639	-0.769	0.015	1
Pot.c	<i>Potamophylax cingulatus</i>	x			-0.613	-0.790	0.359	0.315
Odo	<i>Odontocerum albicorne</i>		x	x	0.695	0.719	0.276	0.316
Rhy	<i>Rhyacophila</i> sp.	x			-0.613	-0.790	0.359	0.315
Rhy.n	<i>Rhyacophila nubila/vulgaris</i>	x		x	-0.478	-0.878	0.442	0.196
Rhy.p	<i>Sericostoma personatum</i>	x			-0.613	-0.790	0.359	0.315
Ser	<i>Sericostoma</i> sp.	x			-0.613	-0.790	0.359	0.315
Cre	<i>Crenobia alpina</i>	x	x	x	0.434	-0.901	0.352	0.205
Pol	<i>Polycelis</i> sp.		x		0.947	0.321	0.507	0.122

Regarding the biological metrics comparison between different management strategies and intensities, we observed only significant differences for Shannon evenness (Kruskal-Wallis $\chi^2 = 6.5636$; $df = 2$; p -value = 0.037; Fig. 6). In detail, we observed a significantly higher diversity in low managed sites compared to sites with no or medium management intensity (pairwise comparison p -value adjusted with “*fdr*” correction > 0.05). Moreover, a higher number of exclusive taxa (i. e., $n = 17$, see Fig. 5) and a higher total number of taxa, although not significant, were found in ditches with low intensity of management compared to the other two categories. A clear but not significant decline in %EPT was observed as management intensity was higher. In terms of functional diversity, no significant differences in functional indices were found between management intensities. Nevertheless, a decrease in functional divergence, indicating a greater differentiation of functional traits among taxa in the unmanaged sites compared to each type of management activity was observed (Fig. 6).

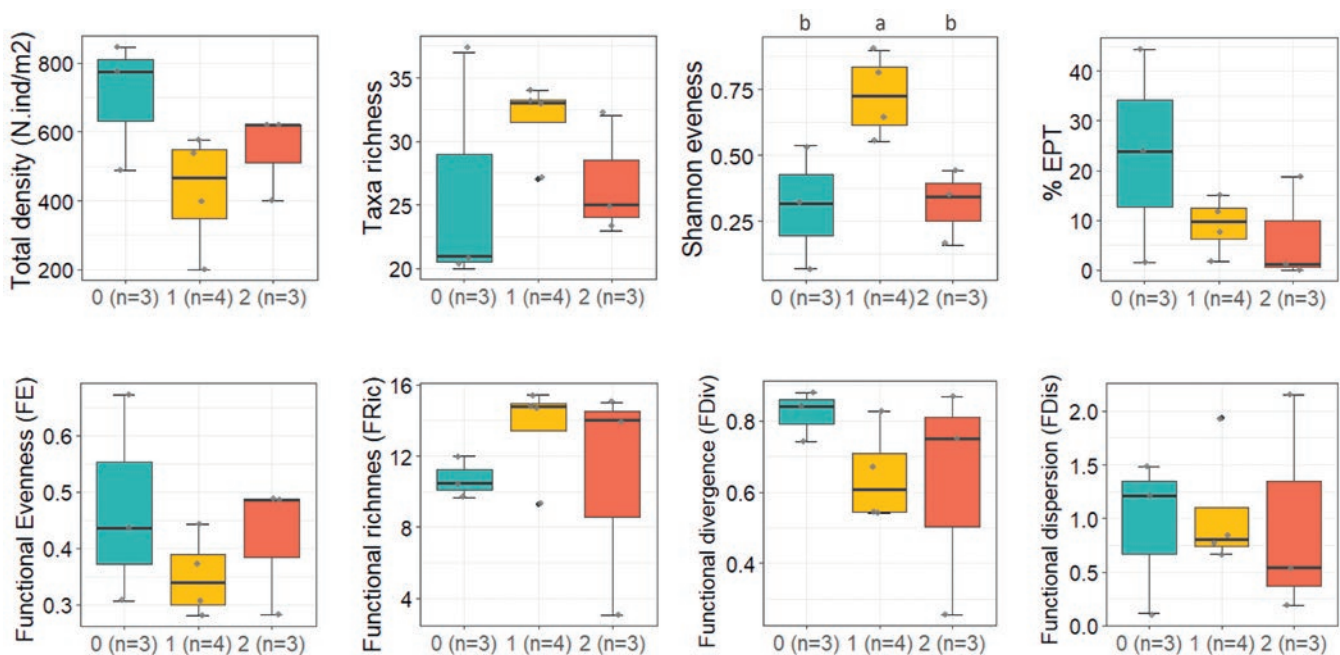


Fig. 6: Comparisons of biological and functional diversity metrics using the average data per site among the three different management intensities (i. e., 0/cyan colour = area of none, 1/yellow colour = low and 2/red colour = medium intensity of ditch maintenance). Different letters above the boxplot describe significant pairwise Kruskal-Wallis Test differences at $\alpha = 0,05$; p . adjusted with “*fdr*” correction.

5. Discussion

This study constituted an initial effort in assessing the importance of drainage ditches as hotspots for macroinvertebrate biodiversity in agricultural landscapes of Alto Adige/Südtirol (Italy). Although the benthic community was dominated by Diptera and Oligochaeta, and mainly composed by lower average of %EPTs compared to the surrounding mountain streams (VALLEFUOCO et al., in review), we found that lowland ditches exhibited a wide range of taxa richness.

The importance of these small artificial water bodies in many lowland agricultural landscapes as corridors between rivers and their associated floodplain habitats (AMOROS & BORNETTE 2002, CLARKE 2015) and as reservoirs of species richness, and therefore as potential refuges for macroinvertebrates and other species from environmental disturbances such as flood events (WILLIAMS et al. 2004), has been widely documented in the literature (ARMITAGE et al. 2003, HERZON & HELENIUS 2008, VERDONSCHOT 2012, HILL et al. 2016). However, in addition to generic freshwater issues such as eutrophication and invasive species, ditches are affected by a wide range of pressures related to their ongoing management strategies (HERZON & HELENIUS 2008, DOLLINGER et al. 2015).

In this study, we investigated the effects of different ditch maintenance activities on macroinvertebrate communities in 10 lowland ditches located mainly along the Etsch/Adige Valley. Despite the constraints related to the limited availability of information about management operations and the classification of ditches in the defined categories, this study constitutes a further resource that help elucidating the changes in benthic invertebrate communities associated with different ditch management intensities. As hypothesised, we found differences in benthic communities between ditches subjected to different strategies of management. Indeed, our results suggest several differences between management strategies in terms of both environmental parameters and biological assemblages. In detail, high concentrations of nitrate, high river depth, water temperature and conductivity were associated with high intensities and frequencies of ditch maintenance. Although all water nutrients detected in these artificial ditches were not considered as polluted (2020/2184/EC, 91/676/EEC), high concentrations of nitrate, commonly used as fertilisers in agriculture, and conductivity may be related to the impact of anthropogenic activities (NAGANNA et al. 2017). Indeed, the main surrounding land use type of all the sampling sites located along the Adige valley, is fruit stock and orchards (except for the single site located in Passeier/Passirio Valley). It is also important to recognize that our analysis only considered management impacts on local environmental and biological conditions, but this may be a proxy for the variation of other relevant environmental factors. Certainly, lowland ditches, which are located downstream of the catchment, urbanisation area and/or sewage plants, could be indirectly influenced by their geographical location and altitudinal gradient, which could potentially contribute to reduced water quality.

However, ditch management activities aiming at the removal of in-channel vegetation and dredging of the streambed can affect the ability of the riparian vegetation to retain organic and suspended solids and reduce the heterogeneity of substrate composition, contributing to habitat type reduction and water quality deterioration (HERZON & HELENIUS 2008, CLARKE 2015). In addition, alteration of riparian and bottom vegetation influence in-channel water temperature and biogeochemical processes (POOLE & BERMAN 2001). Indeed, constructed wetlands and vegetated ditches are promising techniques used to mitigate agricultural pollution from diffuse sources in agricultural landscapes (KUMWIMBA et al. 2018, VYMAZAL et al. 2018). According to our results, these lowland ditches were mainly characterised by homogeneous substrates, dominated by fine organic sediments. Hydrophytes were most abundant in low intensity management, while filamentous algae were only present in managed ditches. Therefore, contrary to our assumption, the similar number of substrates in all the sampled ditches suggests that the substrate heterogeneity is not strictly related to the investigated management strategies. However, within sampling sites, the coverage of the different substrates was not always evenly distributed (e.g., in site 5 bedrocks and artificial substrates dominated, accounting for 70% of substrate cover). Although it is well known

that a varied substrate mosaic and a high level of patchiness provides a greater number of invertebrate niches, enhancing taxa diversity and richness (BEISEL & USSEGLIO-POLATERA 2000, GETHING et al. 2020), the dominance of one substrate over the others could also be a limiting factor in diversifying taxonomic composition. Moreover, as expected, we observed different taxa associations between different substrates, which provide different suitable living spaces for macroinvertebrates, influencing colonisation and distribution dynamics. For example, EPT taxa were mostly found in gravel, big mineral sediment (i.e., size 25–250 mm) and mobile blocks (i.e., size > 250mm). Despite the wide tolerance of the Baetidae, Caenidae and Hydropsychidae families to multiple stressors, EPTs are the most pollution-sensitive macroinvertebrate taxa and are commonly used as indicators for ecological quality in aquatic ecosystems (CONSIGLIO 1980, BELFIORE 1983). For example, species of the Heptageniidae family are characteristic of environments with fast currents and rocky substrates (BELFIORE 1983). Some species of the genus *Ecdyonourus*, and in rare cases *Rhitrogena*, may be less sensitive to certain types of pollution. However, the genus *Epeorus*, found only in unmanaged sites, is a good indicator of environmental quality (BELFIORE 1983). Trichoptera taxa such as *Acrophylax* sp., *Potamophylax* sp. and *Sericostoma* sp., which also occur exclusively in unmanaged sites, are predominantly xylophagous and feed on fallen leaves and plant tissues (MORETTI 1983), and may be affected by riverbank and bottom mowing, which reduce CPOM input. Furthermore, exclusive taxa found in low intensity management sites such as *Bythinia* sp. and *Valvata* sp. are mostly associated with slow current and standing waters (SANSONI 1988).

Although not supported by statistical significance, the high values of FDiv exhibited by the sampling sites located in unmanaged ditches, revealed remarkable niche differentiation, thus reducing competition and the efficient use of resources (SCOTTI et al. 2020). On the contrary, low values of FRic in unmanaged sites indicated low levels of resource exploitation and productivity compared to low-intensity managed ditches. Moreover, concerning differences in taxa and functional richness between management strategies, we observed higher significant Shannon evenness in sites with low-intensity maintenance type. High evenness values are associated with a more even distribution of individuals among different species, and such communities are generally considered to be more diverse and resilient to disturbance. Additionally, there was a higher, although not significant, number of taxa and functional variability based on their functional characteristics and habitat preferences in sites with low-intensity management type rather than none or medium, matching only partially our second hypothesis. In fact, the relationship between disturbance and environmental change on diversity may be more complex than the intermediate disturbance theory, simultaneously affecting different ecological mechanisms and population dynamics (FOX 2013). These results suggest that managing artificial drainage ditches by establishing guidelines to reduce disturbance and considering habitat characteristics may help to support community heterogeneity and taxa richness (GETHING et al. 2020). Based on our findings, promoting a low intensity ditch maintenance rather than more intensive management is recommended. Sustainable management activities may include mowing ditch banks and cleaning ditch bottoms when necessary to ensure surface water drainage, recognizing the important role of riparian vegetation in nutrient dilution, and providing diversified streambed substrates, such as different grain sizes of pebbles, cobbles and boulders, to enhance the taxonomic and functional biodiversity of aquatic biota (DUAN et al. 2008). A properly implemented management, considering both intensity and frequency of disturbance, could improve both agricultural purposes and its biodiversity conservation potential. In conclusion, as our findings are based on single sampling events, to comprehensively understand the effects of different management strategies on aquatic fauna, further seasonal samplings would be advisable. Furthermore, given that Diptera is the dominant order in these environments, an appropriate taxonomic resolution within this specific insect order is advisable for future assessments. Indeed, these habitats should be included in future monitoring programs, requiring further research to investigate the expanding role of drainage ditches as biodiversity hotspots and to identify optimal preservation management activities.

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Data availability statement: The original data that support the findings of this study are available on PANGAEA® Data Publisher at <https://doi.org/10.1594/PANGAEA.941501> and <https://doi.org/10.1594/PANGAEA.941321>.

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