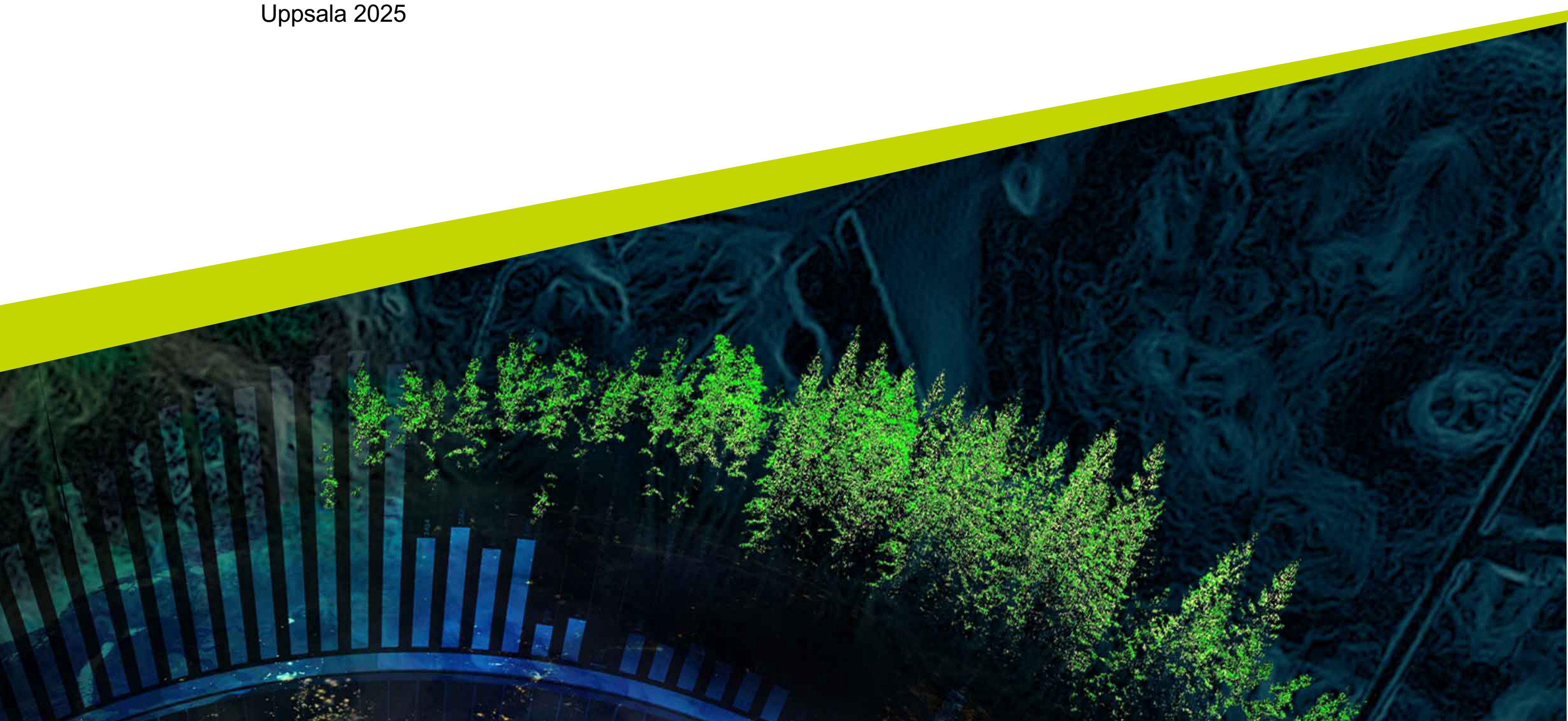
Ecological Analysis of Macroinvertebrates in Remediated Ditches Based on Their Functional Traits

Valeri Bornaz

Master´s Thesis • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Soil and Environment
Soil, Water and Environment – Master's programme
Examensarbeten / Institutionen för mark och miljö, SLU
Partnumber 2025:11
Uppsala 2025



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Supervisor:	**Magdalena Bieroza, Swedish University of Agricultural Sciences, Department of Soil and Environment**
Assistant supervisor:	John Livsey, Swedish University of Agricultural Sciences, Department of Soil and Environment
Examiner:	Eva Krab, Swedish University of Agricultural Sciences, Department of Soil and Environment

Credits:	30 credits
Level:	Advance A2E
Course title:	Master thesis in Environmental science, A2E
Course code:	EX0897
Programme/education:	Soil, Water and Environment
Course coordinating dept:	Department of Soil and Environment
Place of publication:	Uppsala
Year of publication:	2025
Partnumber:	2025:11
Series title:	Examensarbeten / Institutionen för mark och miljö, SLU
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	two-stage ditch, functional traits, ecological assessment, aquatic macroinvertebrates

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Soil and Environment

Abstract

This study evaluated the effect of agricultural ditch rehabilitation using a two-stage design on water quality and the composition of functional traits in aquatic macroinvertebrate communities. Nineteen paired sites in southern and central Sweden were analysed, each with a remediated downstream section and an unremediated upstream section.

No statistically significant differences were found in the physicochemical parameters analysed, although the remediated sections had lower maximum values and less dispersion for several pollutants. Likewise, traditional taxonomic indicators such as richness, total abundance and metrics based on Ephemeroptera, Plecoptera and Trichoptera (EPT) also showed no clear differences between conditions.

However, functional analysis identified nine traits with a high contribution to functional dissimilarity. Intermediate body size showed a positive relationship with total phosphorus and a negative relationship with suspended solids. Significant associations were also observed between life cycle duration traits (short and long) and turbidity, suggesting reproductive responses to conditions of increased sedimentation.

These results suggest that, although no overall improvements in water quality and taxonomy were detected, the analysis of functional traits highlighted ecological signals that conventional metrics overlooked in this dataset. Therefore, incorporating this complementary approach into monitoring programmes could improve the ecological assessment of restoration interventions in agricultural landscapes.

Keywords: two-stage ditch, functional traits, ecological assessment, aquatic macroinvertebrates

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Abbreviations

EPT	Ephemeroptera, Plecoptera and Trichoptera
ASPT	Average Score Per Taxon
WFD	Water Framework Directive
EQR	Ecological Quality Ratio
CWM	Community Weighted Mean
NMDS	Non-Metric Multidimensional Scaling
PERMANOVA	Permutational Multivariate Analysis of Variance
SIMPER	Similarity Percentage
VIF	Variance Inflation Factors
CV	Coefficient of Variation
BACI	Before-After-Control-Impact

1. Introduction

1.1 Background

In agricultural landscapes, drainage ditches play a crucial role in managing water flow, nutrient and sediment transport. Morover, they significantly influence the biodiversity of local aquatic ecosystems. Agricultural ditches constitute a particular type of lotic ecosystem, with characteristics of both streams and wetlands, whose primary function is the drainage of excess water. However, they also act as pathways for the unintentional transport of pollutants from crop fields to receiving water bodies (Chiorino et al., 2024; Needelman et al., 2007).

In recent years, there has been growing interest in the ecological benefits that agricultural ditches could provide, if properly managed, in terms of improving water quality, providing habitat, and promoting biodiversity (Chiorino et al., 2024; Dollinger et al., 2015).

Agriculture occupies almost half of the EU territory and is responsible for nearly 50% of total water consumption in the region (Bieroza et al., 2021). Reducing diffuse pollution from agriculture, including nutrient and pesticide losses, is a major objective of current European policies. This is reflected in key directives such as the Water Framework Directive, the Groundwater Directive, the Nitrates Directive, and the Sustainable Use of Pesticides Directive (Bieroza et al., 2021). Despite these efforts, a recent report indicated that only 37% of Europe's surface water bodies achieve good or high ecological status, and only 29% meet good chemical status, highlighting the continued pressure, especially from the agricultural sector, and the persistence of pollutants (EEA, 2024).

The impact of multiple stressors on aquatic ecosystems is complex, often nonlinear, and can exhibit delayed or buffered responses from biological communities (Davis et al., 2018). This complexity makes it difficult to establish direct causal relationships between observed ecological impacts and specific sources of pollution (Birk et al., 2020; Glendell et al., 2019). Therefore, holistic and integrative approaches to ecosystem monitoring and management are required, capable of capturing the interactions between various pressures and their ecological responses (Bieroza et al., 2021).

In this context, aquatic macroinvertebrates such as insects, molluscs and crustaceans are considered key bioindicators due to their sensitivity to changes in habitat and water quality. In particular, the functional approach, based on the ecological traits of these communities, allows the identification of response patterns that are not always evident when analysing only the taxonomic composition or physicochemical parameters, thus providing a complementary perspective focused on ecological processes (Birk et al., 2020; Glendell et al., 2019; Hill et al., 2016; Laini et al., 2019). Their functional traits, including respiration mechanisms, feeding strategies, reproductive modes, and mobility patterns, allow for the evaluation of ecological aspects that are not always evident through conventional physicochemical analyses (Poff, 1997; Edegbene et al., 2021). Therefore, the examination of these traits allows us to infer ecosystem processes and assess the ecological integrity of the system (Ieromina et al., 2016).

In this project, existing data from 19 paired sites in southern and central Sweden were analysed. Each ditch included an upstream section preserved as a traditional ditch (unremediated) and a downstream section remediated (remediated) using a two-stage ditch design, which incorporates side terraces to improve nutrient and sediment retention and promote greater habitat diversity (Powell and Bouchard, 2010; Västilä et al., 2021). Although a control consisting of completely traditional (unremediated) ditches was not included, it was assumed that the differences observed between sections are mainly due to the remediation intervention, since both sectors belong to the same system, are spatially close, and were sampled under the same environmental, temporal, and methodological conditions.

Previous studies in remediated agricultural ditches, similar to those evaluated in this study, have reported improvements in water quality after remediation (Needelman et al., 2007; Mahl et al., 2015; Hodaj et al., 2017; Kindervater & Steinman, 2019; Speir et al., 2020; Västilä et al., 2021; Huttunen et al., 2024). In this project, previously collected raw data, which had not been analysed in terms of physicochemical quality or functional composition, were analysed to determine whether, at these specific sites, remediation is associated with improvements in water quality and whether these effects are reflected in the functional composition of macroinvertebrate communities. Therefore, the objective of this thesis was to evaluate whether ditch rehabilitation influences the diversity and composition of the functional traits of macroinvertebrates and to determine whether these traits allow for the detection of ecological changes that complement or reinforce the information obtained through traditional physicochemical parameters and taxonomic analyses.

By focusing on functional traits of macroinvertebrate communities, including respiration mechanisms, feeding strategies, reproductive modes and habitat preferences, the project aims to detect patterns that may reveal ecological benefits of remediation practices beyond conventional water quality measurements (Lavorel et al., 2007).

Rather than focusing only on on species identity, the study provides information on how remediated ditches can foster functional diversity and resilience, crucial for ecosystem health in agricultural areas (Sargac et al., 2021). The project's findings have the potential to highlight long-term ecological changes and improvements in water quality that may not be captured through sporadic chemical analyses alone, as aquatic macroinvertebrates integrate the cumulative effects of environmental conditions over time into their presence and composition (Berger et al., 2017).

1.2 Aim of the study

The overall objective of this study was to assess how the rehabilitation of agricultural ditches influences the composition of functional traits of aquatic macroinvertebrates, and how these changes relate to water quality conditions.

Specific objectives were set as:

- Review the links between aquatic macroinvertebrate functional traits and water quality through a literature review.
- Evaluate whether ditch rehabilitation influences the taxonomic functional composition and diversity of macroinvertebrate communities.
- Identify specific functional traits that respond to water quality conditions and analyze whether these responses can be attributed to the effects of remediation.

The hypotheses were:

- H1: The functional traits of aquatic macroinvertebrates show patterns of association consistent with physicochemical parameters of water, such as suspended solids, phosphorus, and dissolved oxygen.
- H2: Macroinvertebrate communities in remediated and unremediated ditches are expected to differ functionally from each other.
- H3: Certain functional traits of macroinvertebrates, particularly those related to morphology and life history are expected to show measurable responses to variations in water quality.

2. Literature review

2.1 Two-stage remediate ditches

In intensive agricultural landscapes, drainage has facilitated production, but has also generated significant ecological impacts. Wetland conversion and channelisation of natural waterways have altered hydrology and degraded aquatic habitats, promoting the input of nutrients, sediment and agricultural pollutants into water bodies (Blann et al., 2009; Västilä et al., 2021; Pierce et al., 2012).
Conventional ditches, of trapezoidal design and deep bottom, tend to be ecologically poor due to their uniform shape, accumulation of fine sediments and frequent maintenance such as dredging, which reduce heterogeneity and organic matter input (Jamie et al., 2006; D'Ambrosio et al., 2015; Krider et al., 2017; Needelman et al., 2007; Dollinger et al., 2015).

Two-stage ditches have been proposed as a nature-based solution to restore ecological functionality in agricultural landscapes. Recent studies have shown that this type of intervention can significantly reduce nitrogen and phosphorus concentrations, increase vegetation cover, and improve the richness and diversity of aquatic macroinvertebrates (Huttunen et al., 2024; Powell and Bouchard, 2010). Furthermore, by incorporating stable terraces (Figure 1), a more heterogeneous and resilient structure is promoted in the face of hydrological fluctuations (Västilä et al., 2021).

In agricultural settings where natural aquatic habitats are scarce, these ditches can act as refuges and ecological corridors, although their relevance is not yet fully reflected in agricultural policies. However, there is growing interest in promoting sustainable practices within the framework of the Common Agricultural Policy and the European Green Pact (Clarke, 2014; Gething & Little, 2020; Huttunen et al., 2024).

Figure 1. A two-stage ditch in field, located in Sweden. Photo taken by the autor (2025).

2.2 Aquatic macroinvertebrates as bioindicators

A bio-indicator is understood as an organism (or part of one) or a community of organisms, which contain information on long-term or short-term interactions of various environmental conditions visualised by a reaction to a sudden change of important factors that alter their behaviour (Li et al., 2010).

An ideal bioindicator should have the following characteristics: reliable identification, occurrence in multiple geographic regions, wide or cosmopolitan distribution, low mobility (local indication), known ecological characteristics, abundance, easy experimental manipulation in laboratories and sensitivity to environmental stressors (Markert et al., 2003).

In this context, aquatic macroinvertebrates fulfil many of these characteristics, making them a valuable tool for assessing the ecological status of water bodies, especially in altered agricultural landscapes.

2.3 Functional traits

Functional traits are characteristics related to the form, chemistry, physiology, structure, development, or behaviour of an organism that are visible in its physical features. These traits are important because they help explain how organisms respond to their environment and how they influence ecosystem processes. To be useful, these traits must be measurable in individual organisms and under different environmental conditions (Violle et al., 2007). They should represent the ability of organisms to obtain resources, grow and avoid being negatively affected by factors such as water flow, sediment accumulation or predation (Margalef, 1968; Caponi et al., 2020; Violle et al., 2007).

This idea is often described in two parts: response traits and effect traits (Lavorel and Garnier, 2002). Effect traits describe how a species changes its environment or provides benefits to the ecosystem, even if those traits do not directly help the individual. Response traits, on the other hand, describe how a species reacts to its environment and how it manages to survive when conditions change (Lavorel and Garnier, 2002).

This study focused mainly on response traits, as the objective was to evaluate how macroinvertebrate communities respond to environmental changes caused by remediation. Furthermore, the traits used (such as body size, type of respiration, or habitat preference) are mostly soft traits, i.e., inferred from bibliographic information or databases, and not measured directly in individuals (Statzner et al., 2008). This approach is common in functional bioindication studies, where the focus is on community trends rather than specific physiological measurements.

3. Method and materials

3.1 Literature review

As part of this study, a structured literature review was conducted to collect and synthesise relevant information on the effects of agricultural ditch remediation on the functional traits of aquatic macroinvertebrates and their relationship to water quality. This structured literature review was based on institutional methodological guidelines for systematic reviews adapted to academic contexts (Karolinska Institutet University Library, 2023).

First, the key concepts and objectives of the review were defined. Then, scientific databases were searched using keyword combinations related to agricultural drainage, remediation, functional traits and water quality. Empirical studies addressing the relationship between aquatic macroinvertebrate functional traits and environmental variables, especially in European agricultural systems, were prioritised.
The selected studies were assessed for relevance and quality, and the most relevant findings were extracted to contextualise and support the focus of the present study.

3.2 Study area

This project analysed data collected from 19 paired sites in southern and central Sweden, where each pair consists of a traditional upstream ditch and a remediated downstream ditch (Figure 2).

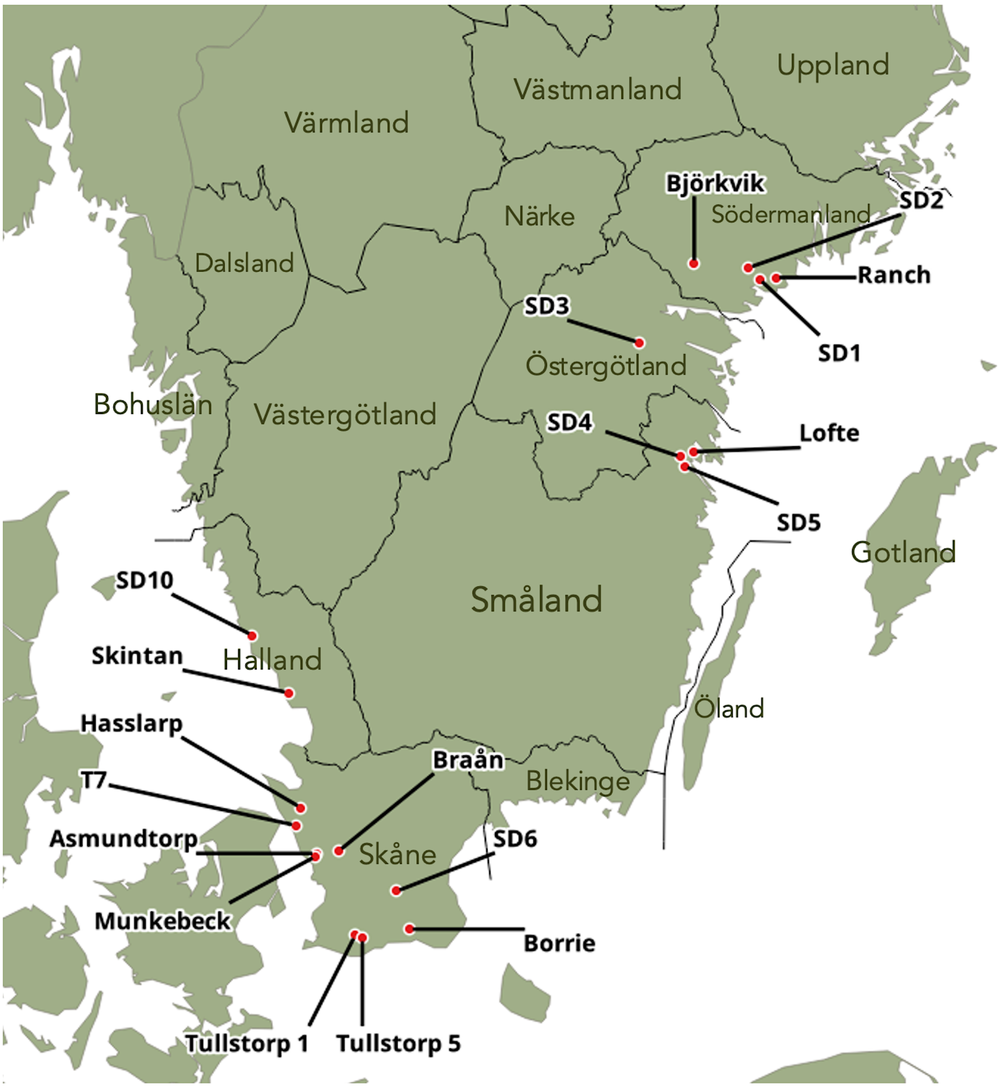


Figure 2. Location of 19 paired agricultural ditches in southern and central Sweden. Each study site is composed of a traditional (unremediated) agricultural ditch located upstream and a two-stage (remediated) ditch located downstream.

3.3 Data processing

3.3.1 Water quality parameters

A total of 12 water quality parameters were analysed: nitrous oxide (N_2O), methane (CH_4), total phosphorus, suspended solids, dissolved oxygen, specific conductivity (SPC), pH, turbidity, phosphate (PO_4^{3}-), nitrate (NO_3^-), water temperature and flow rate. These parameters were selected for their ecological relevance and their ability to reflect agricultural impacts on aquatic systems, especially related to eutrophication, oxygen depletion and changes in microbial metabolism (Allan, 2004; Dodds & Smith, 2016). The units and abbreviations used for each variable are summarised in Table 1.

Table 1. List of water quality parameters used to assess the difference between remediated and unremediated ditches.

Parameter	Abbreviation	Unit
Nitrous oxide	N_2O	µg/L
Methane	CH_4	µg/L
Total phosphorus	Total P	µg/L
Suspended solids	SS	mg/L
Dissolved oxygen	DO	mg/L
Specific conductivity	Conductivity	µS/cm
pH	pH	-
Turbidity	Turbidity	NTU
Phosphate	PO_4^{3-}	mg/L
Nitrate	NO_3^-	mg/L
Water temperature	Temperature	°C
Flow	Q	m^3/s

Initial data processing included a thorough review of missing data and outlier detection, using scatter plots and box plots as recommended exploratory tools to assess data quality and distribution (Zuur et al., 2010). Subsequently, transformations were applied to variables with markedly skewed distributions in order to approximate their distribution to normality, reduce skewness and stabilize variance, as suggested in numerical ecology studies (Legendre & Legendre, 2012).

Specifically, log(x) type logarithmic transformation was used for continuous variables that presented only positive values and strong skewness towards high values, such as N_2O, CH_4, total phosphorus (Total P), suspended solids (SS), conductivity (SPC), turbidity, phosphate and nitrate. This transformation reduced the influence of extreme values and improves comparability between variables. For the flow rate (Q), which contained zeros, the log(x + 1) transformation was applied. This transformation is used to avoid the mathematical problem of calculating logarithms of zero, while maintaining the relative structure of small values (Borcard et al,. 2018).

Finally, all transformed environmental variables were standardised by focusing on the mean of each variable and scaling to the standard deviation prior to multivariate analyses, in order to avoid the disproportionate influence of variables with different scales or units of measurement, as recommended in multivariate ecological data analyses (Borcard et al., 2018).

3.3.2 Invertebrate composition and abundance

Identification of organisms was primarily to family level, with some observations down to genus or species level where possible using standard identification guides (SIS, 2012). This procedure had already been carried out previously, and in this project the pending identifications were verified and completed to the most accurate taxonomic level possible.

Raw abundance data were used to calculate traditional ecological metrics. These included taxonomic richness and total number of individuals, as well as indicators based on sensitive groups, such as Ephemeroptera, Plecoptera, and Trichoptera (EPT). These groups are commonly used for rapid assessments of water quality and ecosystem health (Barbour et al., 1999). The Average Score Per Taxon (ASPT), a standard measure of water quality based on taxa's tolerance to pollution, was also calculated (Armitage et al., 1983; Hering et al., 2006).
In addition, several diversity indices were applied to describe community structure. These included the Margalef richness index, the Shannon and Simpson diversity indices, the dominance index, and the evenness index. Specific metrics for EPTs, such as their richness, abundance, and percentage of total individuals, were also incorporated to gain a more detailed view of the ecological status of the assessed sites.

The ASPT biological index, widely used in Europe and used in the assessment of the Water Framework Directive (WFD) in Sweden to evaluate the overall degradation of water quality in lakes and streams, based on the sensitivity of macroinvertebrates to organic pollution and other types of impairment (SEPA, 2007).
A sensitivity score is assigned to each taxon at the family level, on a scale from 1 (high tolerance) to 10 (high sensitivity). The ASPT (Average Score Per Taxon) index is calculated as the average of these scores for the families present in a sample, as shown in equation (1):

$$ASPT = \frac{\sum_{i=1}^{n}(I_i)}{n} \tag{1}$$

Where:
- n = the number of family;
- I = the indicator value for family i;
  Indicator value from 1 to 10 (most sensitive)

Subsequently, the Ecological Quality Ratio (EQR) is calculated as the ratio between the observed ASPT and the regional reference value corresponding to the ecoregion where the sampling sites are located.

For the classification of benthic macroinvertebrate assemblages, Swedish lakes are divided into three types based on Illies ecoregions: The Central Plains (14), Fenno-Scandian Shield (22) and Borealic Uplands (20), each ecoregion has a corresponding reference value that can be found in the Handbook 2007:4. Status, potential and quality requirements for lakes, watercourses, coastal and transitional waters (SEPA, 2007).

The samples for this study were taken in southern and central Sweden corresponding to the Illies Ecoregion 14 - Central Plains: reference ASPT = 5.37. The ecological quality ratio (EQR) is calculated as follows in equation (2):

$$EQR = \frac{Calculated\ ASPT}{Reference\ value} \qquad (2)$$

Table 2. Reference values and class boundaries for classification of the ASPT established by Naturvårdsverket (2007).

Type	Status	ASPT Ecological quality ratio (EQR)
Illies Ecoregion 14 Central Plains	Reference Value	5.37
	Uncertainty (SD of EQR)	0.075
	High	≥0.90
	Good	≥0.70 and <0.90
	Moderate	≥0.45 and <0.70
	Poor	≥0.25 and <0.45
	Bad	<0.25

As part of the data processing, absolute abundance matrices were transformed to relative abundances (proportions) per site, to reduce the influence of differences in the total number of individuals between samples and to facilitate functional comparison between communities. This transformation was necessary for the calculation of trait-based metrics, such as community-weighted means (CWM), following the approach recommended by Poff et al. (2006) and Villléger et al., (2008).

3.3.3 Functional traits of invertebrate species

Macroinvertebrate functional traits were obtained from the database of Tachet et al. (2010), which includes up to 21 biological traits classified into more than 60 modalities. Each taxon (species, genus, or family, depending on data availability) can be associated with more than one modality per trait, using fuzzy affinity coding on a scale from 0 (no affinity) to 5 (maximum affinity). Fuzzy coding also allows a given species to belong to more than one trait state simultaneously to account for trait plasticity, with trait scores weighted individually for each species (Lavorel et al., 2007; Sargac et al., 2021). In this study, trait values are assigned to the lowest available taxonomic level (species, genus, or family).

From the complete database, 41 modalities were selected, grouped into 9 functional categories, based on their ecological relevance to the environmental gradients assessed in this study (see Appendix 1). In cases where no trait information existed for a taxon, a value of zero was assigned to all corresponding modalities.
The trait matrix was normalized using fractional weighting, dividing each affinity value by the total sum of the trait's affinities in that taxon. This generated a standardized affinity matrix, where the sum of each trait per taxon equals 1.

Subsequently, community weighted means (CWM) were calculated by combining the trait matrix with the relative abundance data for each taxon at the sampled sites. CWMs represent the weighted average of trait values in the community, reflecting the functional dominance of certain traits and allowing functional patterns to be linked to environmental conditions (Lavorel et al., 2007). This metric was calculated using equation (3):

$$CWM = \sum_{i=1}^{s} WiXi \qquad (3)$$

Where:

- Wi = Relative abundance of the i species
- Xi = Value of the trait in the i species
- S = Total number of species

Finally, standardised CWM values were used in multivariate analyses to explore functional differences between remediated and unremediated ditches, as well as in regression models to evaluate associations between functional traits and water quality parameters.

3.4 Statistical analysis

Different statistical analyses were carried out to assess structural and functional differences in macroinvertebrate communities between remediated (downstream) and unremediated (upstream) sites, as well as to explore relationships between environmental conditions and functional traits. Univariate, multivariate and linear regression modelling approaches were applied, according to ecological analysis standards (Legendre & Legendre, 2012; Quinn & Keough, 2002). All statistical analyses were performed using R in RStudio (version 2024.09.1+394; Posit Software, PBC, 2024).

3.4.1 Comparison between remediated and unremediated ditches

To detect differences between remediated and unremediated sites, parametric and non-parametric statistical tests were used, selected according to the distribution of the data. Normality was assessed using the Shapiro-Wilk test, while homogeneity of variances was examined graphically and by Levene's test where necessary (Zuur et al., 2007).

The paired t-test was applied to variables that met the assumptions of normality and homogeneity based on log-transformed values (CH_4, Total P, SS, Conductivity, pH, turbidity, phosphate, and nitrate). In cases where the data did not follow a normal distribution (such as N_2O, DO, water temperature and flow), the Wilcoxon test for paired samples was used (Quinn & Keough, 2002).

In the case of ecological indices, the unpaired t-test was applied to those metrics that met the assumptions of normality in both groups (richness, dominance, evenness and Margalef). For the rest of the metrics, such as total abundance, Shannon and Simpson indices, and EPT metrics (abundance, richness and percentage), the unpaired Wilcoxon test was used.

Tests were performed in R using the t.test() and wilcox.test() functions from the stats package (base R), and results with p-values less than 0.05 were considered significant.

3.4.2 Multivariate analysis

Non-Metric Multidimensional Scaling Analysis (NMDS)

The NMDS was used to assess possible differences in taxonomic and functional composition between remediated and unremediated sites, visualising their clustering in a reduced dimension space. The main objective was to determine whether remediation has generated distinct communities, which could be reflected in a differentiated clustering pattern between sites. Visualisation of such clustering allows interpretation of whether the functional or taxonomic composition differs between conditions, thus providing evidence of the ecological effect of the remediation actions (Clarke, 1993).

Two independent NMDS analyses were performed:

- NMDS of taxonomic composition that was applied to a macroinvertebrate relative abundance matrix.
- NMDS of functional structure was applied to the Community Weighted Means (CWM) matrix of functional traits.

In both cases the Bray-Curtis distance was used, and the number of dimensions was set at two (k = 2). Results were considered interpretable if the stress value was less than 0.2, as recommended by Clarke & Warwick (2001).
To facilitate visual interpretation, the resulting graphs included coded points per group, confidence ellipsoids (95%) and centroids of each group, along with repellency-readable labels to avoid overlap. In addition, a Permutational Multivariate Analysis of Variance test (PERMANOVA) was conducted using the function adonis2 to statistically evaluate whether significant differences in composition existed between remediated and unremediated sites (Oksanen et al., 2001). This test allowed us to complement the graphical interpretation with formal statistical evidence.
All analyses and visualizations were performed in the R environment, using the vegan, ggplot2, dplyr and ggrepel packages.

SIMPER (Similarity Percentage)

It was applied to identify the species or functional traits that contribute most to the observed differences between groups of sites. This technique decomposes the Bray-Curtis dissimilarity and allows the relative contribution of each taxon or trait to the total dissimilarity to be quantified (Clarke, 1993). The analysis was implemented using the simper() function from the vegan package in R.

3.4.3 Relationship between water quality parameters and traits

To assess whether water quality influences the functional composition of macroinvertebrate communities, multiple linear regression models were applied, where the dependent variable was the weighted mean value of a functional trait (CWM), and the independent variables were water quality parameters.

The selected functional traits (n = 9) were chosen based on SIMPER analysis (Clarke, 1993), prioritising those with the largest individual contribution to functional differences between remediated and unremediated sites. This selection focused on the most influential traits without exceeding a number of traits that would compromise the robustness of the model, in order to avoid overfitting and improve its interpretation, due the limited number of sampling sites.

Six parameters commonly associated with macroinvertebrate community structure in agricultural environments were included as predictor environmental variables, according to previous studies (Hill et al., 2016; Chiorino et al., 2024): dissolved oxygen (DO), total phosphorus, suspended solids (SS), conductivity, flow rate (Q) and dissolved methane (CH_4).

Each model was structured according to the following general form (Zuur et al., 2009):

$$Y_{trait} = \beta_0 + \beta_1.DO + \beta_2.Tot\_P + \beta_3.SS + \beta_4.Conductivity + \beta_5.Q + \beta_6.CH_4 + \varepsilon$$

Where:

- Y_{trait} = functional trait value (CWM)
- B_i = coefficients estimated by the model, which indicate the influence of each environmental variable.
- $\mathcal{E}$ = Random error

Nine independent models were adjusted, one for each functional trait, using the lm( ) function of the statistical package. The results were extracted using the tidy( ) function of the broom package. The overall significance of each model was assessed using the p-value and the coefficient of determination (R^2).

Variance inflation factors (VIF) were also estimated using the car package (Fox and Weisberg, 2019), in order to rule out high collinearity between predictors (VIF < 5 was considered acceptable).

Finally, scatter plots between observed and predicted values were generated to visualise the fit of each model, using the ggplot() function of the ggplot2 package.

4. Results

4.1 Literature Review

To frame the functional approach used in this study, previous studies investigating the relationship between functional traits of aquatic macroinvertebrates and water quality parameters were reviewed. This review reveals that traits such as feeding modes, mobility, respiration, pollutant tolerance, and reproductive strategies have been commonly associated with variables such as dissolved oxygen, nutrients, turbidity, and temperature. Table 3 summarizes the main findings of these studies, including the associated traits, the environmental parameters considered, and the geographic regions where they were conducted.

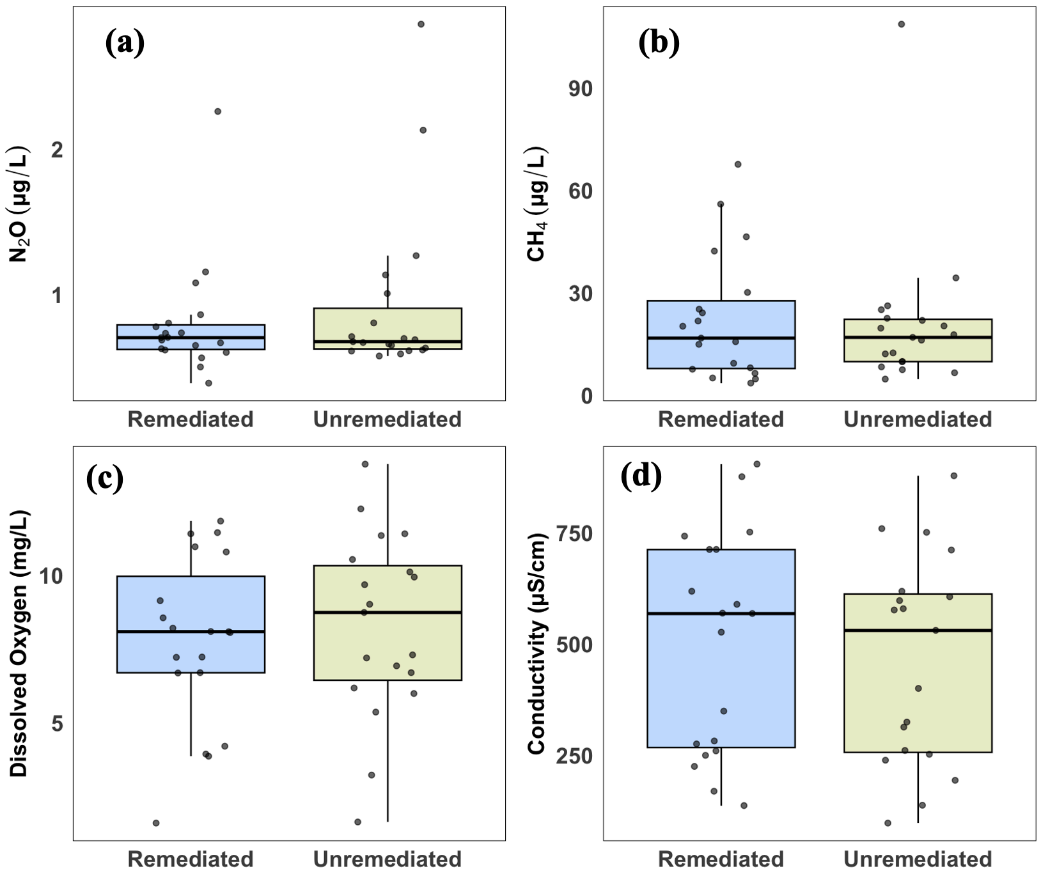
Table 3. Relationship between functional traits and water quality parameters in previous studies

Author(s)	Year	Linked functional traits	Related water quality parameters	Region
Townsend & Hildrew	1994	Feeding, size, reproduction modes	General water quality, eutrophication	Europe
Rosenberg & Resh	1993	Life cycle, feeding, mobility	Pollution, physical and chemical quality of water	United States (North America)
Sargac et al.	2021	Preference for gravel substrates, active aerial dispersal, gill respiration, scraping and filter feeding	Dissolved oxygen, turbidity, sediment load, habitat quality	Sweden (Europe)
Dolédec et al.	1999	Feeding modes, breathing patterns, mobility	General physical and chemical parameters	Europe
Usseglio-Polatera et al.	2000	Morphological features, feeding modes	General water quality and hydromorphological disturbance	France
Townsend et al.	1998	Reproductive strategies, dispersal, feeding	Water quality and disturbances	New Zealand
Hill et al.	2016	Functional diversity (feeding patterns, mobility, body size)	Local physical-chemical variables such as dissolved oxygen, nutrients, turbidity	United Kingdom
Ma et al.,	2024	Functional, taxonomic and phylogenetic diversity	Agricultural land use, nutrient loads, hydromorphological disturbance	China
Statzner et al.,	2004	Contamination tolerance, feeding strategies	Chemical pollution, oxygen, nutrients	Europe

4.2 Variability in water quality between remediated and unremediated ditches

Differences in water quality between remediated and unremediated ditches were explored using box plots (Figure 3), descriptive statistics (Table 4), and paired t-tests (Table 5). Overall, no statistically significant differences were observed in the mean values of the parameters evaluated, according to the t-test ($p > 0.05$ in all cases).

The box plots (Figure 3) show the distribution of each water quality parameter under both conditions. For CH_4 (Figure 3b), total phosphorus (Figure 3e), suspended solids (Figure 3f), turbidity (Figure 3h), and phosphate (PO_4^{3-}) (Figure 3i), a narrower interquartile range and fewer extreme values are observed in the remediated ditches compared to the unremediated ones. However, nitrate (Figure 3j), showed extreme values at both sites. For other variables such as dissolved oxygen (Figure 3c), conductivity (Figure 3d), and flow rate (Q) (Figure 3l), the distributions were more similar between both groups.



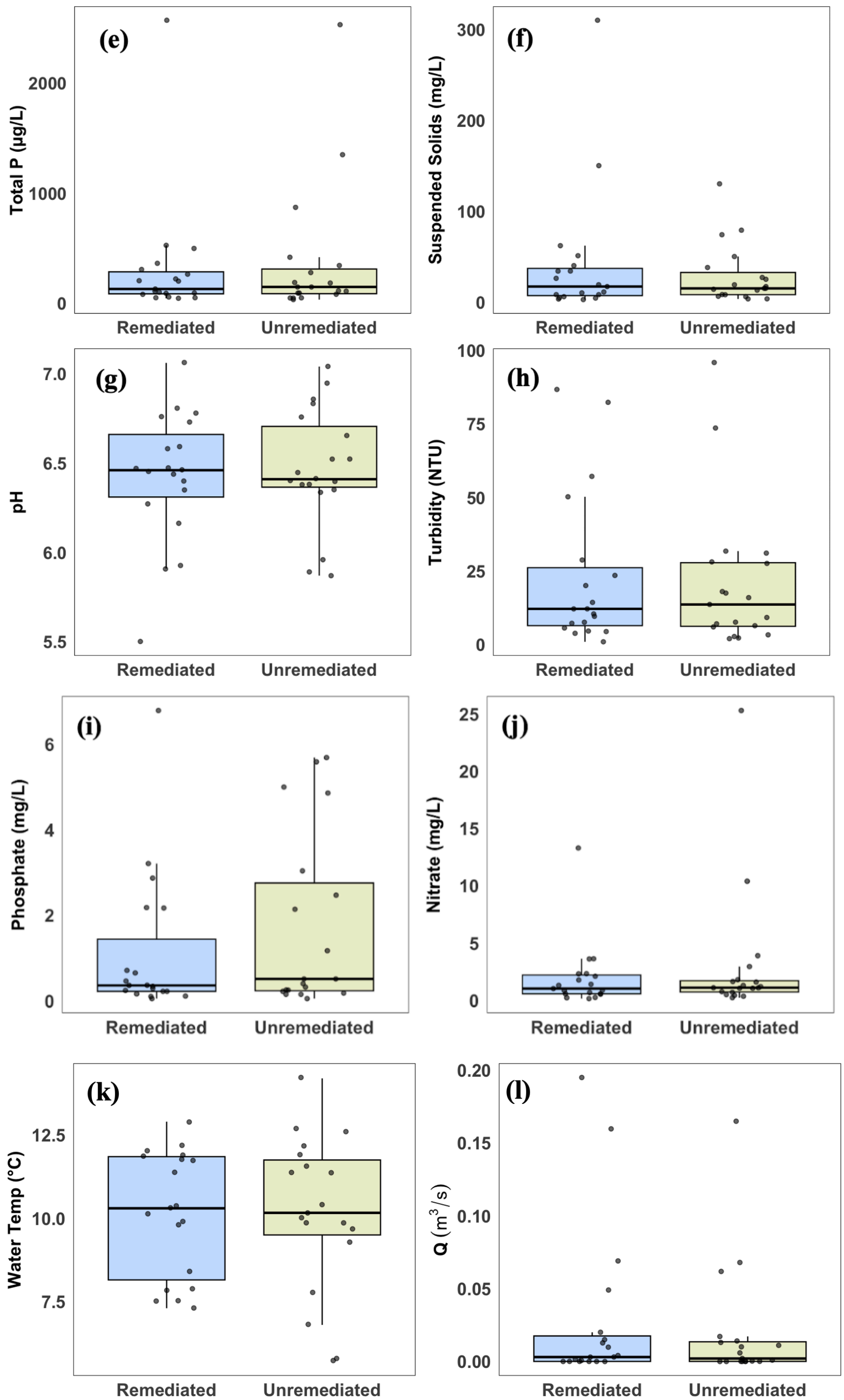


Figure 3. Box plots comparing the distribution of water quality parameters between remediated and unremediated ditches.

Table 4 presents the mean, maximum, minimum, and coefficient of variation (CV) values for each variable. In most cases, the means between remediated and unremediated sites were comparable. For example, CH_4 showed a mean of 22.5 µg/L in remediated ditches and 21.2 µg/L in unremediated ditches; Dissolved oxygen presented values of 7.8 mg/L and 8.3 mg/L, respectively. Several parameters had high CV values (>100%), including CH_4, total phosphorus, and suspended solids, indicating considerable variability between sites. Statistically, a high CV suggests high relative dispersion from the mean, which may affect the ability to detect differences between groups.

Table 4. Descriptive statistics of water quality parameters in remediated and unremediated ditches.

Water Quality Parameter	**Ditch Condition**	**Mean**	**Maximum**	**Minimum**	**Coefficient of Variation (%)**
N_2O (µg/L)	Remediated	0.8	2.3	0.4	50%
	Unremediated	0.9	2.9	0.6	64%
CH_4 (µg/L)	Remediated	22.5	67.7	3.6	82%
	Unremediated	21.2	108.7	4.8	107%
Total P (µg/L)	Remediated	314.9	2570.0	46.2	180%
	Unremediated	376.2	2530.0	34.8	164%
SS (mg/L)	Remediated	42.2	310.0	2.7	174%
	Unremediated	29.0	130.0	3.4	114%
DO (mg/L)	Remediated	7.8	11.8	1.6	37%
	Unremediated	8.3	13.8	1.6	37%
Conductivity (µS/cm)	Remediated	501.5	905.0	137.0	49%
	Unremediated	465.2	879.0	98.1	50%
pH	Remediated	6.4	7.1	5.5	25%
	Unremediated	6.5	7.0	5.9	25%
Turbidity (NTU)	Remediated	23.3	86.7	1.0	113%
	Unremediated	21.1	95.9	2.0	118%
PO_4^{3-} (mg/L)	Remediated	1.1	6.8	0.1	150%
	Unremediated	1.7	5.7	0.1	120%
NO_3^- (mg/L)	Remediated	2.0	13.3	0.2	148%
	Unremediated	3.0	25.3	0.2	194%
Temperature (°C)	Remediated	10.2	12.9	7.3	19%
	Unremediated	10.2	14.2	5.7	23%
Q (m^3/s)	Remediated	0.1	0.2	0.0	112%
	Unremediated	0.0	0.0	0.0	173%

Table 5 summarizes the results of the paired t-tests performed for each water quality parameter. No comparison was statistically significant ($p > 0.05$). This suggests that, based on the available data, no consistent differences in parameter means were detected between remediated and unremediated ditches.

Table 5. Paired t-test results for comparing water quality parameters between remediated and unremediated ditches

Water Quality Parameter	*t*-ratio	*p*-value
N_2O (µg/L)	187	0.86
CH_4 (µg/L)	-0.03	0.98
Total P (µg/L)	0.14	0.89
SS (mg/L)	-0.02	0.99
DO (mg/L)	192	0.75
Conductivity (µS/cm)	-0.45	0.65
pH	0.4	0.69
Turbidity (NTU)	-0.22	0.83
PO_4^{3-} (mg/L)	0.85	0.4
NO_3^- (mg/L)	0.59	0.56
Temperature (°C)	180	1
Q (m^3/s)	167	0.7

4.3 Community and functional composition

4.3.1 Comparison of biological metrics and ecological status

Biodiversity indices and functional metrics

Biological metrics were compared between remediated and unremediated sites. The results are summarized in Table 6 and visualized in Figure 4, which shows the distribution of each metric through box plots labeled (a) to (j).

The mean values for several metrics were slightly higher at the remediated sites (Table 6). Taxonomic richness (Figure 4b) and total abundance (Figure 4a) had means of 12.63 and 255.58 individuals at remediated sites, compared to 10.53 and 149.63 at unremediated sites, respectively. However, these differences were not statistically significant ($p > 0.05$). This indicates that there is insufficient statistical evidence to establish a difference in mean richness or abundance between the two groups.

Similarly, diversity indices, such as Shannon (Figure 4e), Simpson (Figure 4f), and Margalef (Figure 4g), also showed slightly higher mean values at the remediated sites. Dominance (Figure 4c) and evenness (Figure 4d) metrics were comparable across conditions. Statistically, these results do not allow us to reject the null hypothesis of equality across conditions for any of the diversity indices.

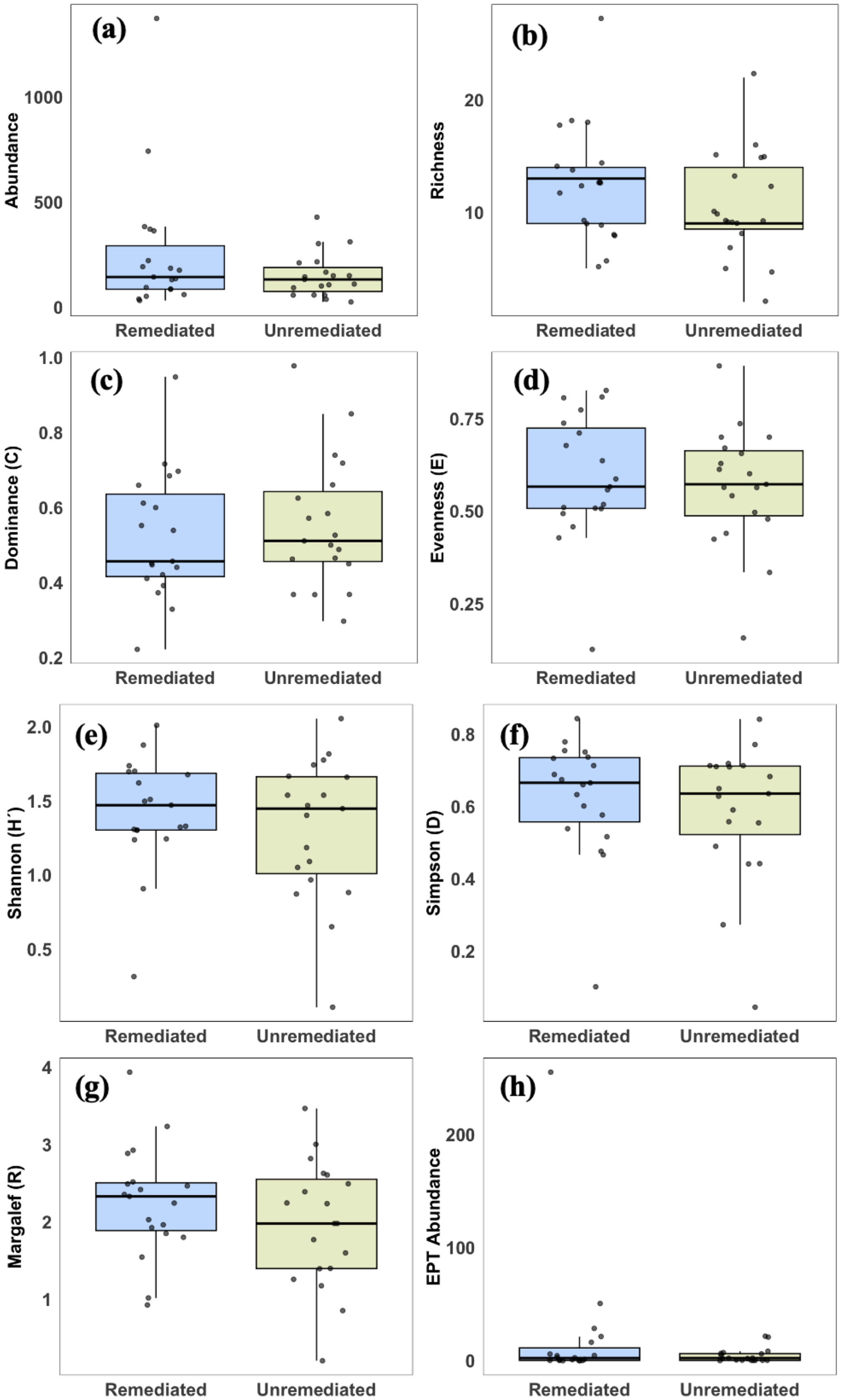
Regarding the sensitive macroinvertebrates in the EPT group, mean values for EPT richness (Figure 4i), EPT abundance (Figure 4h), and EPT percentage (Figure 4j) were also slightly higher at the remediated sites.

However, as with the other metrics, none of the comparisons were statistically significant according to the paired t-test ($p > 0.05$; Table 6). This means that, statistically, no consistent differences were detected in the metrics associated with the EPT group between remediated and unremediated ditches.

The box plots (Figure 4) show the dispersion of the data in both conditions, indicating that, although values tended to be higher at remediated sites for some metrics, internal variability between sites was considerable in both groups.
This high dispersion suggests significant heterogeneity within each group, which may reduce the statistical power to detect differences between means.

Table 6. Descriptive statistics and inference by paired t-test for biodiversity indices and EPT metrics between remediated and unremediated sites.

Indicators	**Remediated**		**Unremediated**		***t*-ratio**	***p*-value**
	Mean	**Std. Dev**	**Mean**	**Std. Dev**		
Richness	12.63	5.19	10.53	4.68	-1.31	0.20
Abundance	255.58	320.69	149.63	105.16	156	0.48
Margalef	2.26	0.72	1.97	0.81	-1.14	0.26
Shannon	1.42	0.38	1.31	0.48	158	0.52
Simpson	0.63	0.16	0.59	0.19	152	0.41
Dominance	0.52	0.17	0.55	0.18	0.54	0.59
Evenness	0.59	0.17	0.57	0.16	-0.45	0.65
EPT Richness	1.21	1.18	1.05	1.08	169	0.74
EPT Abundance	20.53	58.25	4.32	6.47	167	0.69
EPT %	7.90	7.44	9.15	9.87	181	1.00



(a)
Abundance
0
500
1000
Remediated
Unremediated
(b)
Richness
10
20
Remediated
Unremediated
(c)
Dominance (C)
0.2
0.4
0.6
0.8
1.0
Remediated
Unremediated
(d)
Evenness (E)
0.25
0.50
0.75
Remediated
Unremediated
(e)
Shannon (H')
0.5
1.0
1.5
2.0
Remediated
Unremediated
(f)
Simpson (D)
0.2
0.4
0.6
0.8
Remediated
Unremediated
(g)
Margalef (R)
1
2
3
4
Remediated
Unremediated
(h)
EPT Abundance
0
100
200
Remediated
Unremediated

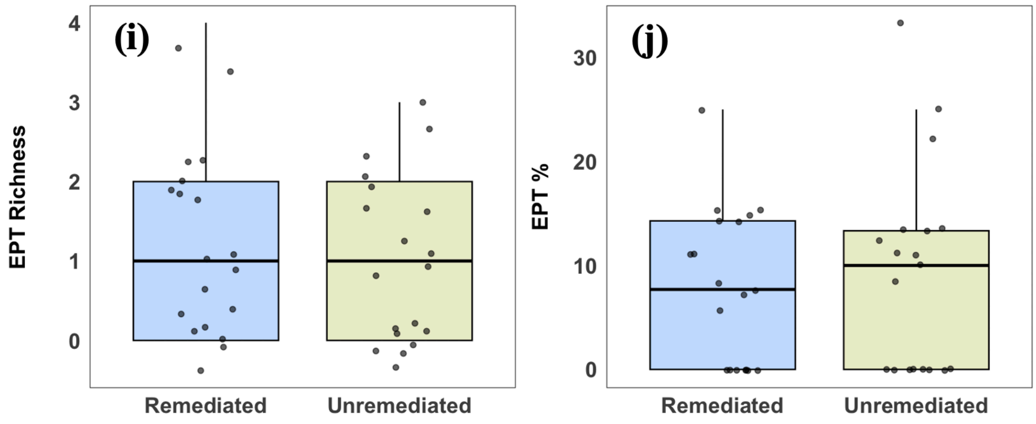


Figure 4. Comparison of biodiversity indices and sensitivity metrics between remediated and unremediated sites. Box plots show the distribution of total abundance, taxonomic richness, diversity (Shannon, Simpson, Margalef), dominance, evenness and EPT metrics (richness, abundance and relative percentage).

Relative composition of EPT groups

The proportional composition of the EPT orders also varied between site types (Figure 5). At remediated sites, approximately 90% of individuals were Ephemeroptera, while Plecoptera and Trichoptera together accounted for 10%. In contrast, in the unremediated sites, the proportion of Ephemeroptera decreased to 72%, while Plecoptera and Trichoptera accounted for 21% and 7%, respectively. This variation could be associated with differences in habitat quality, as certain groups within EPT respond differently to disturbed conditions.

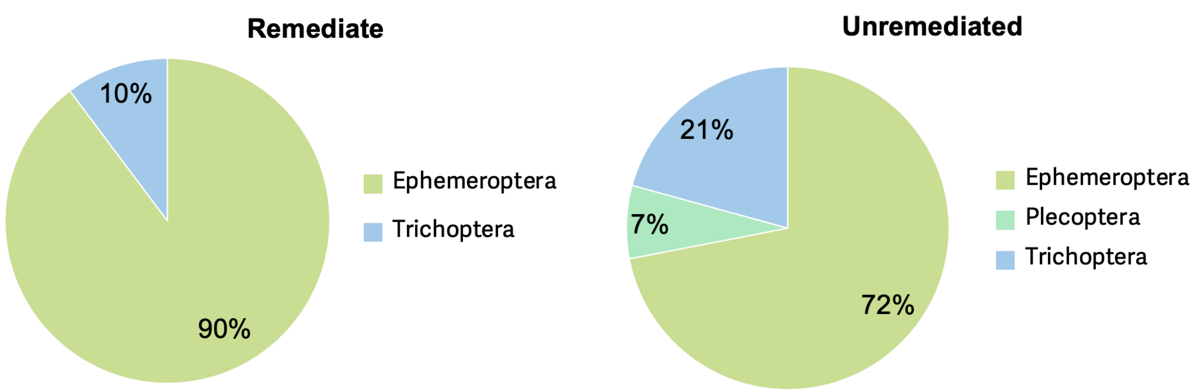


Figure 5. Relative composition of Ephemeroptera, Plecoptera and Trichoptera orders (EPT) in remediated and unremediated sites. Pie charts indicate the proportion of individuals of each order within the EPT assemblage.

Ecological status according to the ASPT index

The ecological status of each site was assessed using the ASPT index, which represents the average sensitivity of the taxa present to contamination. The standardized index values (EQR) and their corresponding ecological status classification (High, Good, Moderate, Poor) are presented in Table 7 for each pair of remediated and unremediated sites.

In four cases, the remediated section showed a higher category than its unremediated site, while in six other cases the opposite occurred.
These differences occurred occasionally between pairs and do not follow a consistent pattern. From a descriptive statistical perspective, no consistent trend in the direction of ecological status associated with remediation status is observed.

Table 7. ASPT index and ecological status classification of remediated and unremediated sites. Remediated sites where the ecological status was higher than in the corresponding unremediated site are indicated with an (*).

Site_ID	**ASPT Ecological quality ratio (EQR)**		**Status**	
	Unremediated	**Remediated**	**Unremediated**	**Remediated**
Asmundtorp	0.88	0.77	Good	Good
Bjorkvik*	0.88	0.92	Good	High
Borrie	0.85	0.83	Good	Good
Braan	0.77	0.78	Good	Good
Hasslarp	0.76	0.82	Good	Good
Lofte	0.89	0.85	Good	Good
Munkebeck	1.01	0.86	High	Good
Ranch	0.93	0.96	High	High
SD 1	1.01	0.71	High	Good
SD 10	0.79	0.62	Good	Moderate
SD 2*	0.79	0.95	Good	High
SD 3	0.82	0.64	Good	Moderate
SD 5	0.96	0.87	High	Good
SD 6	0.91	0.91	High	High
SD4*	0.37	0.87	Poor	Good
Skintan*	0.65	0.84	Moderate	Good
T7	0.82	0.87	Good	Good
Tullstorp 1	0.72	0.72	Good	Good
Tullstorp 5	0.93	0.85	High	Good

4.3.2 Differences in community and functional structure

Multivariate analysis of the taxonomic composition of macroinvertebrates

An NMDS analysis was applied to explore differences in macroinvertebrate taxonomic composition between remediated and unremediated sites, considering their relative abundance proportions. The result shows a high overlap between both groups, with no clear separation in ordination space. Although a slight distinct scatter is observed, most sites are clustered in a mixed manner around the origin of the plot, with no consistent clustering patterns (Figure 6).

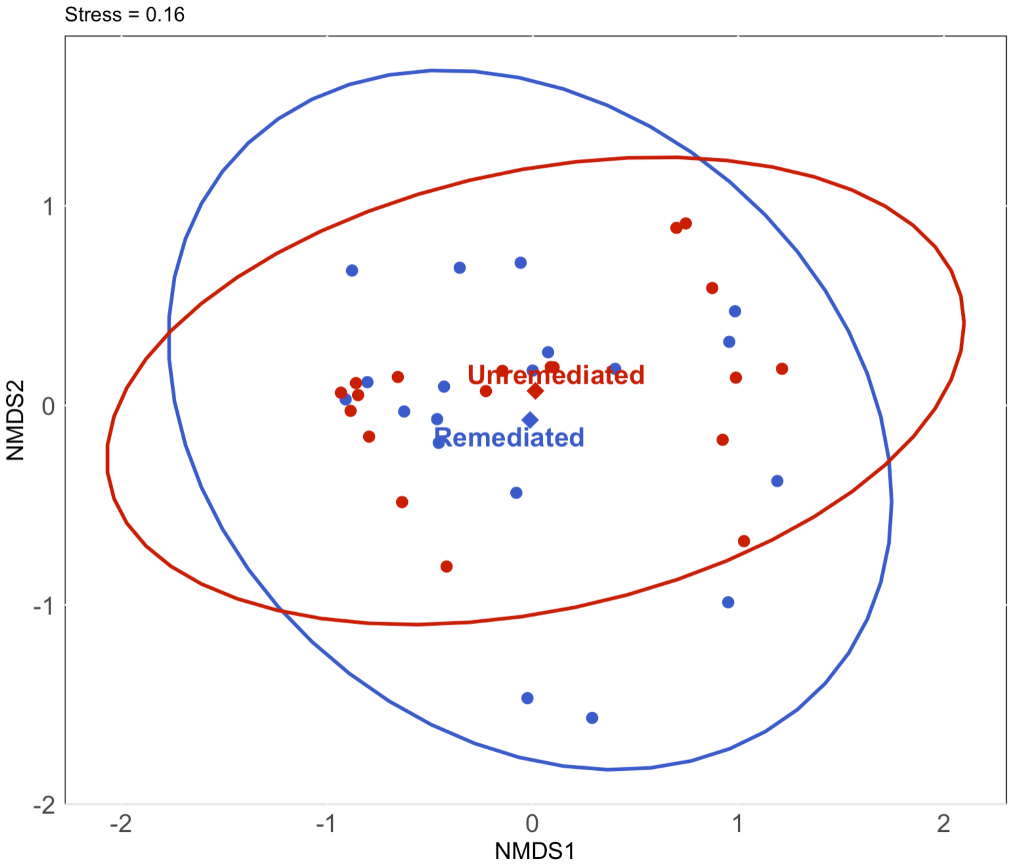


Figure 6. NMDS ordination base on relative abundance of macroinvertebrates in remediated and unremediated sites. Dots represent individual sites, colored according to group. Ellipses indicate dispersion (95% confidence interval) for each group, and diamonds mark group centroids. Stress = 0.160.

The stress value of the model was 0.160, indicating an acceptable representation of taxonomic dissimilarities in two dimensions. However, the PERMANOVA test did not detect statistically significant differences in taxa composition between groups (Table 8), with a value of $R^2 = 0.01$, $F = 0.33$ and $p = 0.96$, indicating that remediation was not associated with evident changes in the taxonomic structure of macroinvertebrate communities.

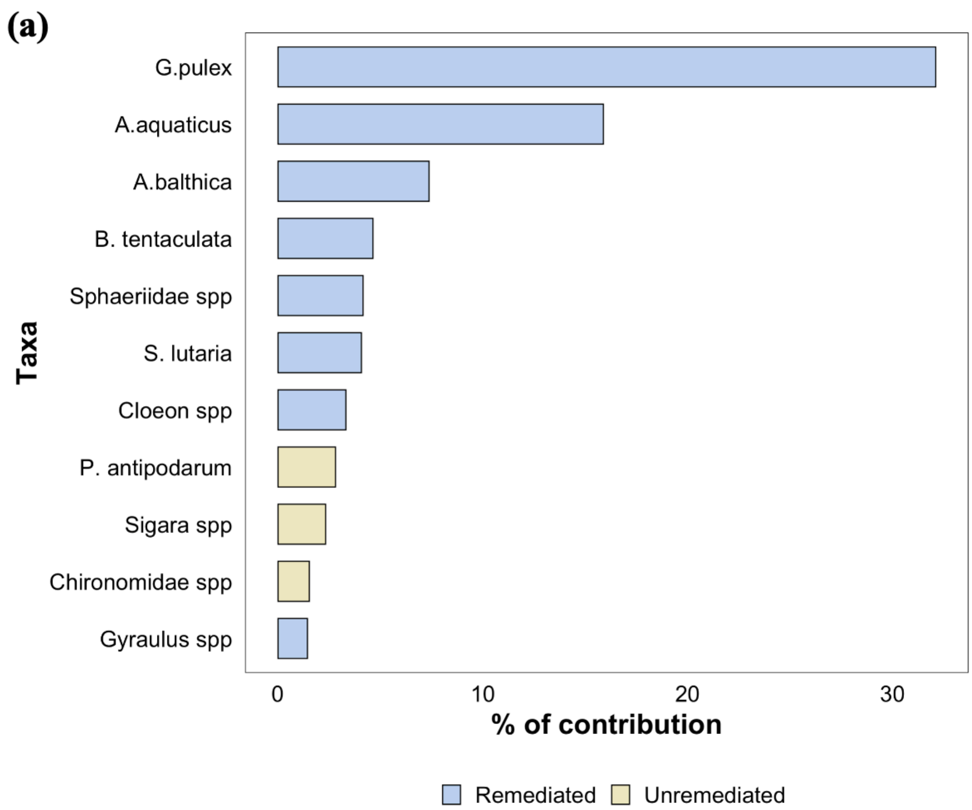
Table 8. PERMANOVA test results for macroinvertebrate taxonomic composition. Based on Bray-Curtis distance and 999 permutations. The p-value indicates that no significant differences were detected between groups.

	Df	SumOfSqs	R2	F	*p*-value
Model	1	0.09	0.01	0.33	0.96
Residual	36	9.89	0.99	-	-
Total	37	9.98	1.00	-	-

SIMPER analysis: identification of taxa responsible for dissimilarity

SIMPER analysis identified which taxa contributed most to the average dissimilarity between the two site types. This selection avoids interpreting noise from taxa that are not very abundant or informative.
Figure 7a. summarises the percentage of dissimilarity explained by each taxon. Gammarus pulex and Asellus aquaticus were the taxa that explained the highest proportion of dissimilarity between groups, with higher average abundance at remediated sites.

Complementarily, the Figure 7b. shows the average abundance of the selected taxa for each group. This graph shows directly in which group each taxon is most dominant.



(b)

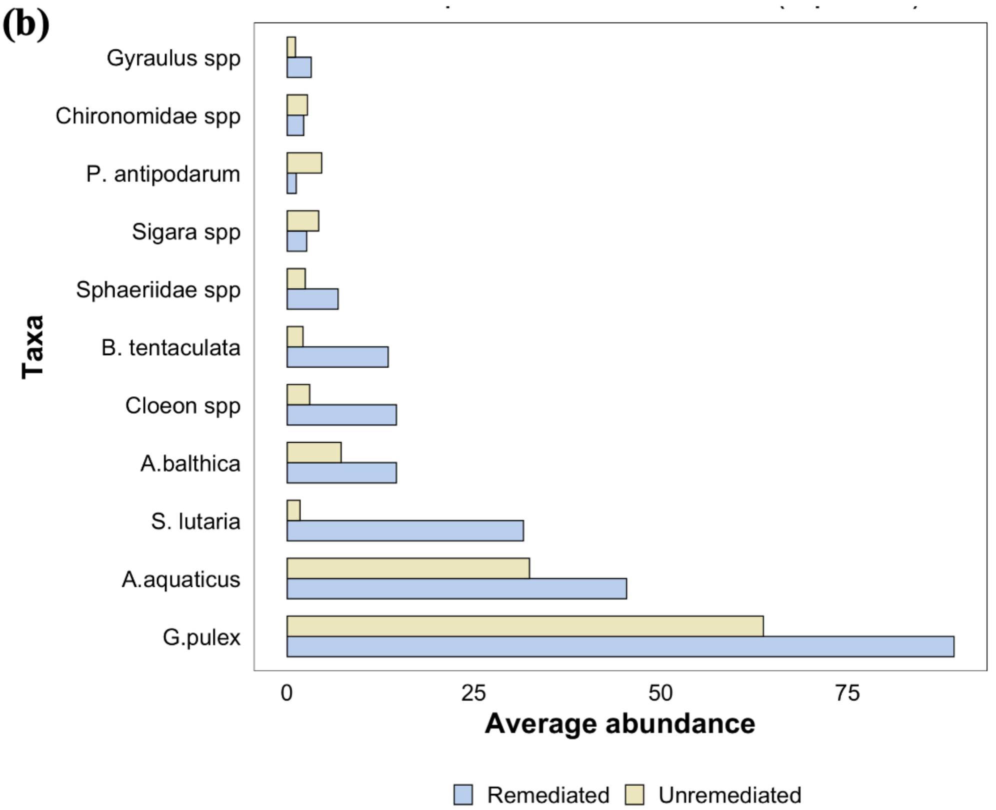


Figure 7. SIMPER analysis results of macroinvertebrate functional traits. (a) percentage contribution of each taxon to the dissimilarity between remediated (blue) and unremediated (yellow) sites. (b) average abundances of taxa in each group.

Multivariate analysis of functional structure of the macroinvertebrate community

To examine differences in the functional structure of macroinvertebrate communities between remediated and unremediated sites, an NMDS analysis based on a matrix of weighted means of functional traits (CWM) was applied. The resulting graph (Figure 8) shows a remarkable overlap between the two groups, with no clear visual evidence of separation. Although the centroids of the groups are slightly offset, most samples are dispersedly clustered in the ordination space, with broad overlap between remediated (blue) and unremediated (red).

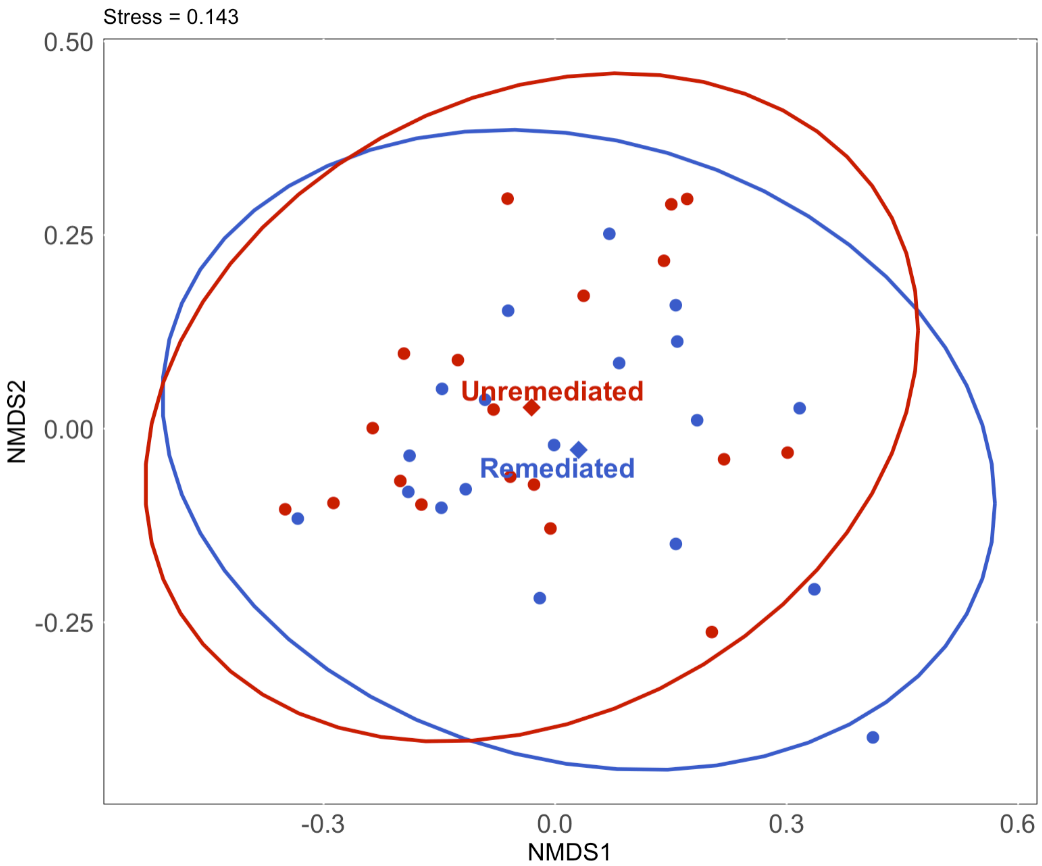


Figure 8. NMDS ordination of functional traits (CWM) of macroinvertebrates in remediated and unremediated sites. Dots represent individual sites, colored according to group. Ellipses indicate dispersion (95% confidence interval) for each group, and diamonds mark group centroids. Stress = 0.143.

The stress value obtained was 0.143 (Figure 8), indicating that the two-dimensional ordination model provides an adequate representation of the functional dissimilarities between communities. However, the PERMANOVA statistical test detected no significant differences in functional structure between groups (Table 9), with a value of $R^2 = 0.01$, $F = 0.51$ and $p = 0.75$. This suggests that remediation, in this data set, was not associated with relevant changes in the functional composition of the macroinvertebrates community.

Table 9. PERMANOVA test results on the functional structure of macroinvertebrates. Based on Bray-Curtis distance and 999 permutations. The p-value indicates that no significant differences were detected between groups.

	Df	**SumOfSqs**	**R^2**	**F**	***p*-value**
Model	1.00	0.01	0.01	0.51	0.75
Residual	36.00	1.02	0.99	-	-
Total	37.00	1.03	1.00	-	-

SIMPER analysis: identification of functional traits responsible for dissimilarity

The results indicate that the main differences between groups are associated with a set of specific traits, both in terms of their percentage contribution and their average abundance (Figure 9).

The traits with the highest contribution to the pattern of dissimilarity (Figure 9a) were the number of life cycles per year (one or more than one), gill respiration, shredder feeding and large body size. These traits explain more than 20% of the total dissimilarity between groups, and are mostly associated with organisms with more complex and specialised life strategies.

In terms of abundance (Figure 9b), these same traits are also among the most represented in both types of sites, although with subtle differences in their distribution. Traits such as gill respiration and shredder feeding were more frequent at unremediated sites, which could indicate that these strategies allow certain organisms to persist under more disturbed or less structured conditions. For example, shredders could take advantage of particulate matter from agricultural runoff, while gill respiration could be common in species resistant to variations in dissolve oxygen. Also, species with more than one life cycle per year shows a predominance in unremediated sites, which could reflect a dominance of opportunistic species with short life cycles, capable of rapidly recolonising more disturbe or less structured habitats.

On the other hand, traits such as respiration through spiracles showed slightly higher values in unremediated sites, while active aerial dispersal and filtration feeding were more frequent in remediated sites, although all remained at low overall abundance (Figure 9b). These characteristics are often associated with more opportunistic species tolerant to adverse environmental conditions, such as low oxygen levels or high turbidity (Poff et al., 2006; Statzner & Bêche, 2010).

Overall, these results suggest that, although there is high functional overlap between sites, certain functional modalities tend to be differentially associated with remediation status, reflecting specific functional responses to habitat quality.

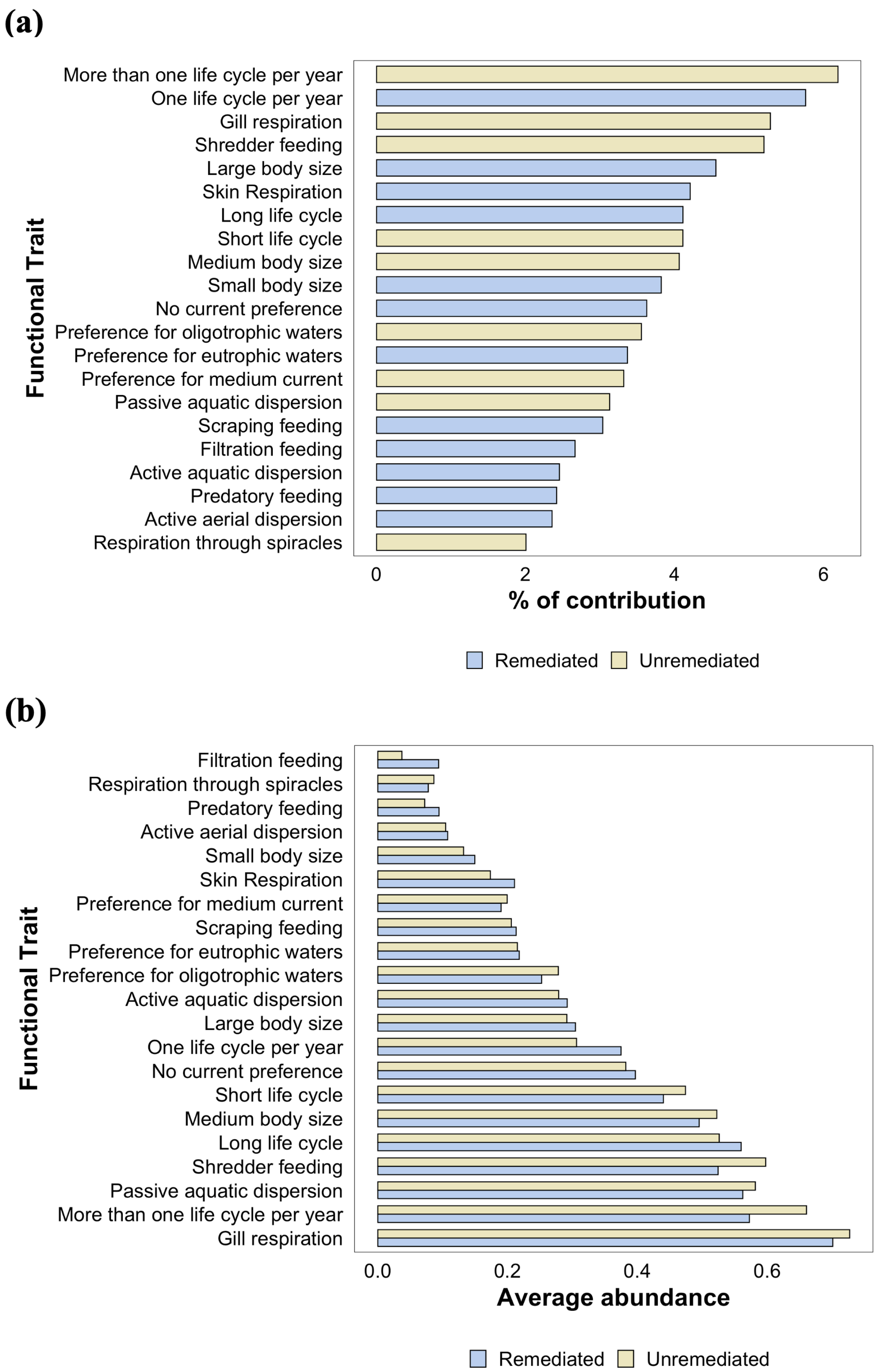


Figure 9. SIMPER analysis results of macroinvertebrate functional traits. (a) percentage contribution of each trait to the dissimilarity between remediated (blue) and unremediated (yellow) sites. (b) average abundances of traits in each group.

4.3.3 Influence of water quality parameters on functional traits

Multiple linear regression models revealed specific associations between water parameters and certain macroinvertebrate functional traits, although overall the relationships were moderate and of limited significance. Of the nine traits evaluated for their high differential functional contribution according to the SIMPER analysis (Figure 9a), only one showed a significant association with environmental predictors.

The results indicated that the medium body size trait was the only one significantly explained by the environmental variables, with an $R^2 = 0.36$ and $p = 0.0225$ (Figure 10h). In this case, total phosphorus ($p = 0.010$) and suspended solids ($p = 0.030$) showed significant effects (positive and negative, respectively) (Table 10).

Statistically significant relationships were also detected between suspended solids (SS) and the traits long life cycle ($p = 0.007$), short life cycle ($p = 0.007$) and large body size ($p = 0.011$), although the full models were not statistically significant ($p > 0.05$), suggesting that these associations should be interpreted with caution (Table 10, Figures 10b, 10f, 10a).

None of the other models were significant overall. However, some traits showed moderate R^2 values, such as gill respiration ($R^2 = 0.30$, $p = 0.072$) (Figure 10d) and long life cycle ($R^2 = 0.26$, $p = 0.132$) (Figure 10b), indicating some trend, although without conclusive statistical evidence under the classical threshold ($p < 0.05$). Other traits such as skin respiration, shredder feeding, number of life cycles per year (one and more than one), showed no relevant associations (Figures 10c, 10i, 10g, 10e).

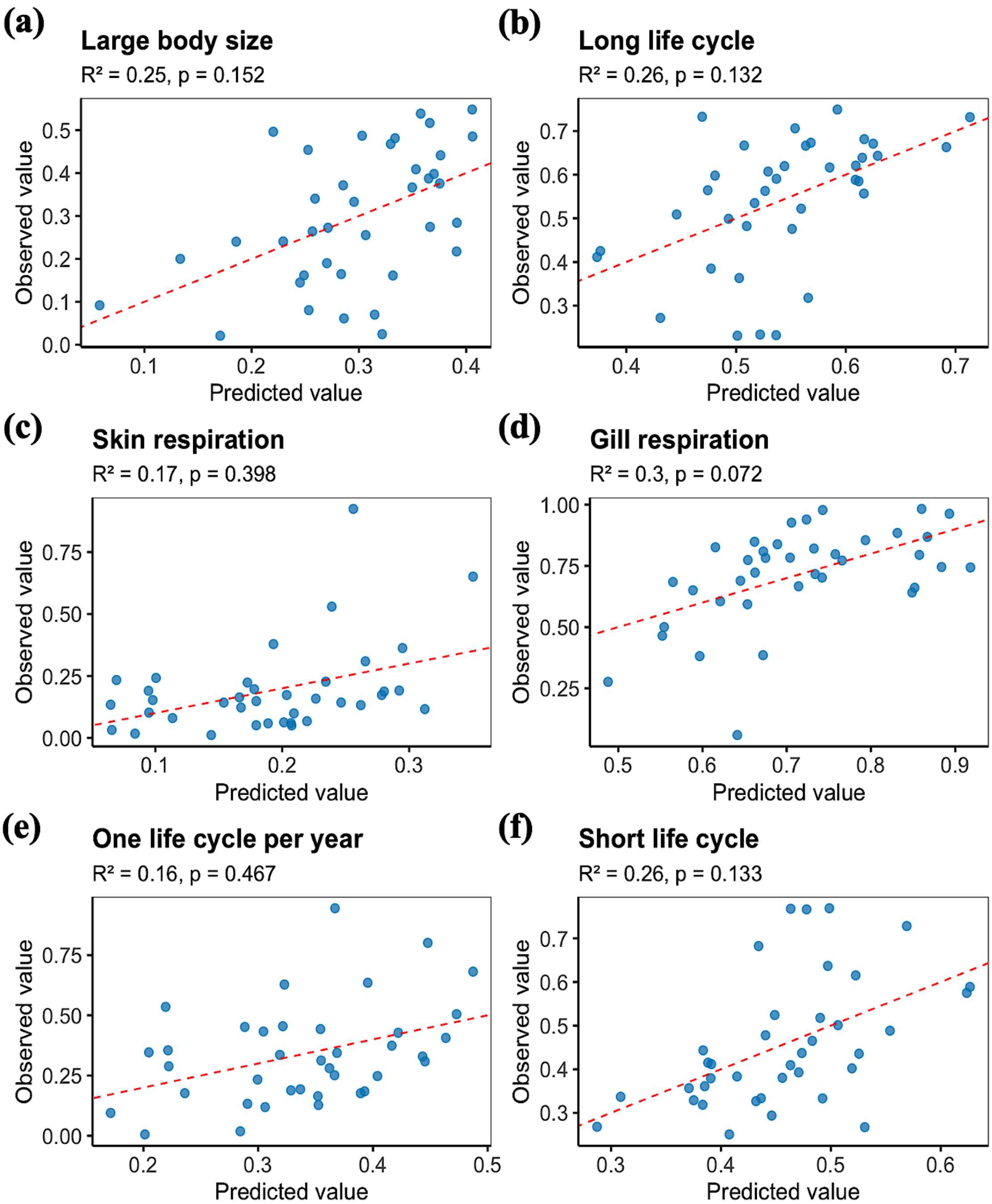
All models presented variance inflation factors (VIF) below 2 (Appendix 2), indicating low multicollinearity among predictors, which reinforces the reliability of the obtained estimates.

Table 10. Results of the multiple linear regression models for each functional trait. Estimated coefficients (β), p-values, coefficient of determination (R^2). Significant effects ($p < 0.05$) are marked by (*).

Functional trait	**Environmental variable**	**Estimated coefficient (β)**	***p*-value**	R^2
Medium body size	CH_4	0.025	0.252	0.361
	Total P	0.067	0.010*	0.361
	SS	-0.057	0.030*	0.361
	DO	-0.008	0.731	0.361
	Conductivity	-0.019	0.418	0.361
	Q	-0.045	0.039*	0.361
Long life cycle	CH_4	0.029	0.226	0.258
	Total P	-0.032	0.243	0.258
	SS	0.079	0.007*	0.258
	DO	0.005	0.844	0.258
	Conductivity	0.035	0.189	0.258
	Q	0.000	0.984	0.258
Short life cycle	CH_4	-0.029	0.228	0.258
	Total P	0.032	0.243	0.258
	SS	-0.079	0.007*	0.258
	DO	-0.005	0.847	0.258
	Conductivity	-0.035	0.189	0.258
	Q	0.000	0.983	0.258
Large body size	CH_4	0.017	0.500	0.249
	Total P	-0.058	0.050	0.249
	SS	0.079	0.011*	0.249
	DO	0.002	0.939	0.249
	Conductivity	0.051	0.073	0.249
	Q	0.014	0.569	0.249
Shredder feeding	CH_4	-0.016	0.600	0.208
	Total P	0.071	0.043*	0.208
	SS	-0.066	0.066	0.208
	DO	-0.028	0.367	0.208
	Conductivity	-0.047	0.160	0.208
	Q	-0.006	0.848	0.208
Skin Respiration	CH_4	-0.026	0.397	0.172
	Total P	-0.032	0.364	0.172
	SS	0.042	0.242	0.172
	DO	0.037	0.244	0.172
	Conductivity	0.049	0.152	0.172
	Q	0.024	0.420	0.172
Gill respiration	CH_4	0.046	0.146	0.297
	Total P	0.045	0.213	0.297
	SS	-0.072	0.054	0.297
	DO	-0.058	0.075	0.297
	Conductivity	-0.062	0.078	0.297
	Q	-0.016	0.613	0.297
One life cycle per year	CH_4	-0.047	0.189	0.157
	Total P	-0.005	0.904	0.157
	SS	0.043	0.307	0.157
	DO	0.042	0.257	0.157
	Conductivity	0.044	0.264	0.157
	Q	0.005	0.891	0.157
More than one life cycle per year	CH_4	0.036	0.345	0.147
	Total P	0.013	0.764	0.147
	SS	-0.047	0.289	0.147
	DO	-0.054	0.167	0.147

Functional trait	Environmental variable	Estimated coefficient (β)	*p*-value	R^2
	Conductivity	-0.051	0.214	0.147
	Q	0.005	0.882	0.147

The scatter plots (Figure 10) between predicted and observed values show generally low patterns of fit, except for the medium body size model, which stands out visually for greater linear consistency.



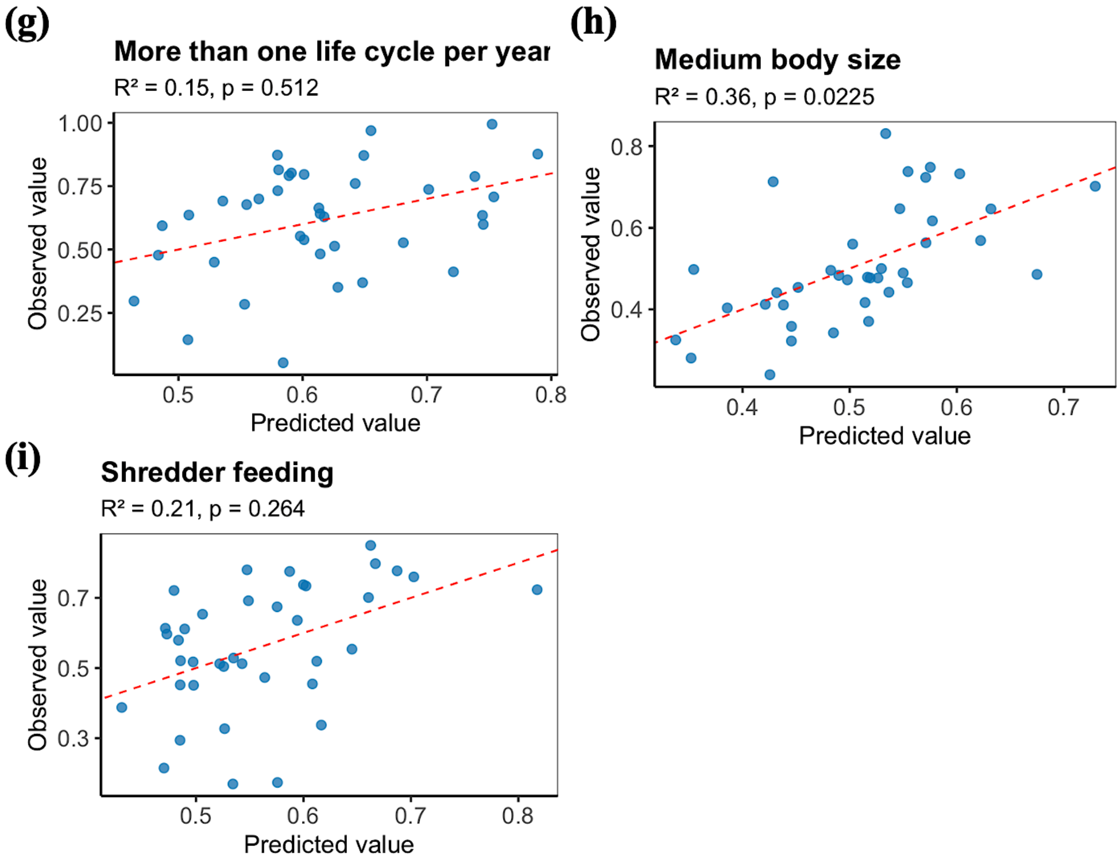


Figure 10. Scatter plots between the values predicted by the multiple linear regression models (X-axis) and the observed values of the functional traits (Y-axis), for each of the nine traits analysed. The coefficient of determination (R²) and the p-value of the corresponding model are indicated.

5. Discussion

5.1 Objetive 1 - Theoretical review

As a first objective, the links between the functional traits of macroinvertebrates and water quality were reviewed according to the scientific literature. This study was based on the functional analysis of aquatic macroinvertebrate communities by calculating community weighted means (CWM) for a set of biological traits, in order to assess their relationship with the physicochemical parameters of water in remediated and unremediated agricultural ditches. Although the design only included one sampling per site and a single measurement per variable, the functional approach used is considered to provide ecological and methodological value within an exploratory context.

CWMs have become a widely recognised tool in functional ecology for detecting the responses of biological communities to environmental gradients, by integrating both the abundance and ecological characteristics of species (Villéger et al., 2008; Laliberté & Legendre, 2010). However, their application in studies with reduced sampling effort, such as when there is only one sample per site, requires caution in interpreting the results. In this study, some CWM estimates were based on the presence of one or a few individuals for certain taxa, which can lead to instability in the values and bias the result towards dominant or unrepresentative traits. This situation is recognised in the literature as a frequent limitation in functional studies without temporal replication (Baird & Van den Brink, 2006; Martini et al., 2020).

5.2 Objetive 2 - Effect of remediation

The statistical tests applied (paired t-test) did not reveal significant differences between remediated and unremediated sites for any of the physicochemical parameters analysed ($p > 0.05$). This suggests that, at least at the time of sampling, no systematic changes in the mean water quality values attributable to remediation were identified.

Although the remediated sites showed lower maximum values and less dispersion for certain parameters, such as methane (CH_4), total phosphorus or nitrate, this difference cannot be conclusively attributed to an effect of remediation. Given that both parameters showed high variability in both conditions (CV > 100%) and multiple extreme values, it is more likely that the differences observed reflect specific events or local conditions at the time of sampling, rather than a systematic pattern of improvement.

Methane (CH_4), total phosphorus and suspended solids are highly dynamic parameters that respond to complex non-linear and biogeochemical processes, such as methanogenesis, nutrient release from sediments and diffuse runoff.

Their concentration can vary dramatically depending on local conditions such as land use, temperature, dissolved oxygen, or vegetation cover (Groffman et al., 2006; Stanfield and Kilgour, 2006; Gücker et al., 2006). In particular, in heterogeneous agricultural landscapes, where water quality can fluctuate significantly at short spatial and temporal scales, a single point measurement may not accurately reflect the average state or represent the actual dynamics of the system (Groffman et al., 2006; Hering et al., 2010). This limitation reduces the ability of conventional statistical tests to detect significant differences, even when ecologically relevant trends may exist (Dodds and Welch, 2000).

Regarding biological indicators, although differences in EPT indices were not statistically significant between remediated and unremediated ditches, ecological patterns consistent with positive restoration effects were observed. In particular, greater EPT richness and absolute abundance were recorded at the remediated sites, supporting the idea that greater vegetation cover, less physical disturbance and greater habitat heterogeneity could favour these sensitive groups (Bonada et al., 2005; Bêche and Resh, 2007).

Similarly, NMDS ordination analysis based on macroinvertebrate abundance revealed a partial separation between communities at remediated and unremediated sites. Although there is considerable overlap, the slight differentiation between centroids suggests some degree of divergence. This differentiation was reinforced by the results of the SIMPER analysis, which identified species such as Gammarus pulex, Asellus aquaticus, Bithynia tentaculata and Sphaeriidae spp. as important contributors to the dissimilarity between groups. These species, commonly associated with structured habitats and aquatic vegetation, were more abundant at remediated sites (Meyer et al., 2005; MacNeil et al., 1996; Sargac et al., 2021). Their higher abundance in remediated sites may reflect the positive effects of increased habitat complexity and reduced disturbance, as expected from restored environments. The results suggest that some rehabilitation projects can make a place safer and more habitable for macroinvertebrates that depend on vegetation cover and physical structure for refuge and feeding.

5.3 Objetive 3 - Trait response

Hypothesis 3 proposed that certain functional traits, especially those related to morphology and life cycle, would respond quantifiably to variations in water quality. However, the results obtained only partially supported the hypothesis. The results of the multiple regression model showed that the relationship between the functional traits of macroinvertebrates and the physicochemical parameters of the water was, in general, limited, although some associations were statistically significant and ecologically reasonable. The medium body size trait was the only complete model with overall significance ($p = 0.0225$; $R^2 = 0.36$). In this model, total phosphorus showed a significant positive association ($p = 0.010$) and suspended solids a negative association ($p = 0.030$), indicating that intermediate-sized organisms may be particularly sensitive to these common stressors in agricultural environments (Hill et al., 2016; Chiorino et al., 2024). One possible explanation is that this functional group includes organisms with moderate growth rates and specific trophic requirements, which are favoured by certain nutrient enrichment (such as phosphorus), but are susceptible to obstruction of their habitats or respiratory tracts by suspended solids, affecting their development or reproduction.

In addition, life cycle-related traits, such as long life cycle and short life cycle, showed significant associations with suspended solids ($p = 0.007$ in both cases), although the overall models were not significant ($p > 0.05$). Even so, these relationships could indicate reproductive adaptations to environments with higher particle loads (Poff et al., 2006; Bonada et al., 2007). These relationships could also reflect different adaptation strategies in response to turbid conditions. On one side, short cycle species can take advantage of favourable conditions, reproducing before the environment deteriorates, while long-cycle species could be negatively affected by sustained particle deposition, hindering their development and survival. A significant association was also identified for the trait large body size with suspended solids ($p = 0.011$), which is consistent with studies suggesting that larger organisms are more susceptible to high turbidity (Peeters et al., 2004).

On the other hand, traits such as gill respiration ($R^2 = 0.30$, $p = 0.072$) and long life cycle ($R^2 = 0.26$, $p = 0.132$) showed trends that, although not significant, could be ecologically relevant if a larger sample size or multi-temporal data were available. Traits such as skin respiration, shredder feeding, number of life cycles per year (one and more than one) showed no clear associations.

Overall, these results partially support hypothesis 3 although significant associations were identified for one key trait, most models were not globally significant. It is important to note that, although the analysis focused on key physicochemical water parameters, other factors not considered may have influenced the expression and distribution of functional traits. Among these are the coverage and structure of aquatic and riparian vegetation, which probably played a decisive role in habitat structuring. Macrophytes not only act as filters for nutrients and sediments, but also provide shelter, support for periphyton, and essential microhabitats for different functional groups (Sand-Jensen, 1998; Giller et al., 2004). Variables such as the intensity of surrounding agricultural use (type and amount of fertiliser, frequency of tillage), local hydromorphological conditions (e.g. micro-variations in flow) and the distance between remediated and unremediated sections may also have had an influence. Added to this are larger-scale factors such as landscape fragmentation and climate change. All these elements can act as additional ecological filters, modulating the functional composition of communities without necessarily causing detectable changes in the water quality variables analysed.

5.4 Limitations

This study presented several methodological limitations that should be taken into account when interpreting the results. First, the sampling design included only one event per site, which prevents capturing the temporal variability characteristic of aquatic ecosystems. This lack of temporal replication can reduce the ability to detect real and generalizable effects (Bonada et al., 2005; Bêche and Resh, 2007). However, this design can also be understood as a substitution of time for space, a common methodological strategy in ecology when prioritizing sampling a larger number of sites rather than performing repeated samples over time (Pickett, 1989; Blois et al., 2013). This approach allows capturing greater spatial heterogeneity, which can be valuable in exploratory studies, although it limits the ability to assess seasonal or interannual dynamics in the biological community.

Another methodological limitation of the study is the use of a pairwise comparison approach between remediated and unremediated reaches, rather than a before-after-control-impact (BACI) design. This choice makes it difficult to directly attribute observed effects to remediation interventions, as there is no pre-intervention baseline. Furthermore, because remediated sites are located downstream of unremediated sites within the same ditch, spatial dependencies exist between pairs that may limit the independence of observations. Nevertheless, this type of design is common in applied studies where historical or pre-intervention data are nonexistent or very limited. In many cases, restoration initiatives prioritize practical implementation over rigorous ecological monitoring, making the adoption of robust BACI approaches difficult (Downes et al., 2008; Palmer et al., 2005; Underwood., 1992). Therefore, although the approach used has limitations, it reflects the real-life conditions under which many remediations are carried out in agricultural landscapes.
Furthermore, several CWM estimates were based on taxa represented by few individuals, which may distort the mean value and reflect occasional dominance rather than consistent community patterns (Martini et al., 2020). Furthermore, natural heterogeneity among agricultural ditches and high variability in environmental parameters (especially CH_4 and Total P) make it difficult to detect systematic patterns with a single sampling point (Groffman et al., 2006; Hering et al., 2010).

Additionally, organisms were identified at different taxonomic levels (family, genus, or species), depending on the information available for their identification and the level of functional detail required for each group. This variability can make it difficult to compare taxa and influence functional analyses, as traits assigned to broader levels are less specific. Although this practice is common in functional studies, it is recognised that more uniform identification could improve the ecological accuracy of the results (Schmidt-Kloiber & Hering, 2015).

Finally, although appropriate statistical models were applied and collinearity was assessed using variance inflation factors (VIF < 2), the small sample size (n = 38 sites) limits the statistical power of the analyses (Gotelli et al., 2013).

Despite these limitations, the results offer a valuable exploratory overview of the relationship between water quality and functional traits in agricultural ditches and can serve as a basis for future more extensive studies with temporal replication and greater statistical power.

6. Conclusions

This study examined the effect of agricultural ditch remediation on aquatic macroinvertebrates and their relationship with water quality through a joint analysis of functional traits, traditional metrics, and physicochemical parameters. The results showed no statistically significant differences in water quality between remediated and unremediated reaches. While lower maximum values and less dispersion were observed for some contaminants such as methane, total phosphorus, and suspended solids in the remediated reaches, high variability between sites and the time-based nature of the sampling limited the possibility of detecting consistent patterns.

Classical taxonomic indicators, such as richness, abundance, and EPT metrics, also did not reflect significant differences between conditions. However, some remediated reaches had higher average values, which could indicate subtle ecological improvements, although these should be interpreted with caution. Functional analysis revealed statistically significant and ecologically relevant associations between certain traits and water parameters.

Organisms with medium body size showed differentiated responses to total phosphorus (positive) and suspended solids (negative), and similar responses were observed in short life cycle and long life cycle traits to turbidity, although the full models were not always significant. These associations could reflect a greater competitive capacity of medium-sized organisms in moderately eutrophic conditions, while the presence of suspended solids could limit their feeding or locomotor activity by obstructing sensory or respiratory mechanisms.

The ASPT index, used as an indicator of ecological status, showed mixed results: in five sites, the remediated reach had a higher status, while in four others it was lower. Most pairs presented equivalent status, suggesting that the effects of remediation may be slight, local, or dependent on other factors, such as landscape connectivity, land-use type, or time since intervention.

Finally, the use of a functional approach proved useful for detecting ecological signals even under conditions of low sampling effort. However, the lack of statistical significance in some models highlights the need for future studies with larger sample sizes, temporal replication, and better habitat characterization to validate the observed trends and better understand the mechanisms linking remediation to ecological responses.

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Popular science summary

In agricultural landscapes, it is common to find small ditches used to drain excess water from fields. Although they often go unnoticed, these ditches also carry nutrients, sediments, and pollutants to nearby rivers and lakes, which can affect water quality and aquatic life.
Traditionally, these ditches are built straight and deep, causing water to flow quickly and leaving little room for wildlife. However, in recent years, a new design called a ‘two-stage ditch’ has begun to be implemented. This type of ditch incorporates side platforms that allow water to spread during heavy rains, filter sediments and nutrients, and create more diverse habitats for aquatic organisms.

In this study, the evaluation focused on whether rehabilitated ditches contribute to improvements in water quality and ecological conditions for small aquatic organisms such as aquatic insects, snails and crustaceans. To achive this, 19 sites in southern and central Sweden were analysed, comparing sections of traditional ditches with other nearby sections that had been rehabilitated.

The results showed that, although there were no clear improvements in nutrient or pollutant levels, differences were found in the types of organisms present. Some species associated with more diverse habitats and aquatic vegetation were more common in the remediated ditches. In addition, by analysing certain “functional traits” characteristics such as body size or breathing patterns patterns were identified that could reflect adaptations to the new environmental conditions.
These findings suggest that, although the benefits are not always reflected in water chemistry, ecological analysis based on the functions of organisms can provide important information about ecosystem health. Therefore, two-stage ditches represent a promising option for improving biodiversity and ecological quality in agricultural areas and should be considered in environmental restoration strategies.

Appendix 1

Appendix 1. Functional traits and modalities

Functional traits	Modalities	Abrevation
Maximal potential size	≤ 0.25 cm	Size_0.25
	> 0.25-.5 cm	Size_0.5
	> 0.5-1 cm	Size_1
	> 1-2 cm	Size_2
	> 2-4 cm	Size_4
	> 4-8 cm	Size_8
	> 8 cm	Size_8plus
Life cycle duration	≤ 1 year	Life_Cycle_short
	> 1 year	Life_Cycle_long
Potential number of cycles per year	< 1	Cycle_Num_lt1
	1	Cycle_Num_1
	> 1	Cycle_Num_gt1
Dispersal	aquatic passive	Disp_aq_pass
	aquatic active	Disp_aq_act
	aerial passive	Disp_air_pass
	aerial active	Disp_air_act
Respiration	tegument	Resp_teg
	gill	Resp_gill
	plastron	Resp_plastron
	spiracle	Resp_spiracle
	hydrostatic vesicle	Resp_vesicle
Feeding habits	absorber	Feed_absorb
	deposit feeder	Feed_deposit
	shredder	Feed_shred
	scraper	Feed_scrape
	filter-feeder	Feed_filter
	piercer	Feed_pierce
	predator	Feed_pred
	parasite	Feed_parasite
Substrate (preferendum)	flags/boulders/cobbles/pebbles	Substr_rock
	gravel	Substr_gravel
	sand	Substr_sand
	silt	Substr_silt
	macrophytes	Substr_macros
	microphytes	Substr_micros
	twigs/roots	Substr_roots
	organic detritus/litter	Substr_detritus
	mud	Substr_mud
Current velocity (preferendum)	null	Flow_null
	slow	Flow_slow
	medium	Flow_med
	fast	Flow_fast
Trophic status (preferendum)	oligotrophic	Troph_oligo
	mesotrophic	Troph_meso
	eutrophic	Troph_eu

Appendix 2

Appendix 2. Variance Inflation Factors (VIF) calculated for each environmental predictor in the multiple linear regression models per functional trait. All VIF values were below 2, indicating low multicollinearity among predictors.

Functional trait	Environmental variable	VIF
Medium body size	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04
Long life cycle	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04
Short life cycle	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04
Large body size	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04
Shredder feeding	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04
Skin Respiration	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04
Gill respiration	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28

Functional trait	Environmental variable	VIF
	Q	1.04
One life cycle per year	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04
More than one life cycle per year	CH_4	1.07
	Total P	1.37
	SS	1.45
	DO	1.12
	Conductivity	1.28
	Q	1.04

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