

**Effect of Artificial Light at Night and Invasion by *Pacifastacus leniusculus* on  
the Functional Diversity of Macroinvertebrates in Stream Ecosystems.**

Einfluss von künstlichem Licht in der Nacht und der Invasion von *Pacifastacus  
leniusculus* auf die funktionelle Diversität von Makrowirbellosen in  
Flussökosystemen.

**A**

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# Declaration

I hereby declare that this thesis work, titled “**Effect of Artificial Light at Night and Invasion by *Pacifastacus leniusculus* on the Functional Diversity of Macroinvertebrates in Stream Ecosystems**”, was completed by me as a part of the requirements for the **Master of Science** degree in **Environmental Science** at Rheinland-Pfälzische Technische Universität Kaiserslautern-Landau, Landau, Germany. This research was carried out under the guidance of Prof. Dr. Mirco Bundschuh and Dr. Alessandro Manfrin and has not been previously included in any other thesis or dissertation submitted for a degree or other qualifications. I confirm that this thesis work only makes use of the specified tools and does not rely on any online sources beyond those cited in the references.

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## Abstract

Human activities significantly impact freshwater ecosystems, introducing stressors like altered water flow, chemical pollution, invasive species, urbanisation, and artificial light at night (ALAN). These interactions can disrupt ecosystem diversity and function, posing unpredicted consequences and complicating management efforts. The growing use of ALAN in human settlements threatens the preservation of freshwater macroinvertebrate communities and can impact predator-prey interactions for both nocturnal and diurnal species. Understanding these interactions is crucial for effective management. Nocturnal species, such as signal crayfish (*Pacifastacus leniusculus*), reduce their activity under ALAN. Furthermore, ALAN can alter drift pattern of certain macroinvertebrates to avoid predation in illuminated conditions, while crayfish with UV-absorbing corneas might react differently to changes in ALAN spectral composition. This study investigates the individual and combined effects of ALAN and the invasive signal crayfish (*Pacifastacus leniusculus*) on the trait composition of benthic macroinvertebrate communities. We tested the following hypotheses in a 6-week Riparian Stream Mesocosm (RSM) experiment using a completely randomised design with 16 artificial stream channels: (a) ALAN attracts phototactic species and reduces drift, altering functional trait compositions and diversity; (b) crayfish presence impacts functional diversity and trait composition through predation, habitat changes, and alterations in drift pattern; (c) ALAN reduces signal crayfish feeding activity, influencing macroinvertebrate functional traits and diversity. Our results indicate that ALAN attracted phototactic macroinvertebrates, which led to trait composition changes both upstream and downstream. At ALAN sites, FRich increased significantly at the upstream and downstream positions, whereas FDisp and RaoQ entropy decreased significantly at the downstream positions compared to the controls during week 6. This implies that species were more functionally similar, and reducing trait dissimilarity among the species. Moreover, the presence of crayfish further affected the trait compositions, supporting their effect on macroinvertebrate drift dynamics. We observed high functional diversity (FD), such as functional evenness (FEve) and FRich, at the upstream and downstream positions, while FDisp and RaoQ entropy decreased at the downstream position of the crayfish sites during week 6 compared to the control. In addition, signal crayfish exposed to ALAN reduce crayfish activity, resulting in effects on macroinvertebrate trait composition. The combined stressors site had a higher FRich than the crayfish site and there was minimal change in FDisp and RaoQ entropy between the crayfish and combined stressors sites, indicating reduced crayfish activity due to the presence of ALAN. Our study provides strong evidence of the single and combined effect of ALAN and signal crayfish on the macroinvertebrate trait composition, as well as a significant increase in the abundance of scrapers (e.g., Chironomidae) at the downstream position in each treatment site.

**Keywords:** ALAN; signal crayfish; freshwater ecosystem; macroinvertebrate communities; functional diversity (FD); functional richness (FRich); functional dispersion (FDisp); functional evenness (FEve); Rao's quadratic entropy (RaoQ).

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## List of Abbreviations

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ALAN	Artificial Light at Night
RSM	Riparian Stream Mesocosm
HPS light	High Pressure Sodium light
LEDs	Light-emitting diodes
COB	Chip on Board
CWM	Community Weighted Mean
FD	Functional Diversity
FRich	Functional Richness
FDisp	Functional Dispersion
FEve	Functional Evenness
RaoQ	Rao's Quadratic Entropy
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Analysis of Multivariate Dispersions
ANOVA	Analysis of Variance
Tukey's HSD test	Tukey's Honestly Significant Difference (HSD) Test
BLMM	Bayesian Linear Mixed-effect Model
MCMC	Markov Chain Monte Carlo
NUTS	No-U-Turns Sampler
CI	Bayesian Credible Intervals
EMMEANS	Estimated Marginal Means

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## 1.0 Introduction

Human activities have substantial and widespread impacts on Earth's ecological systems (Vitousek et al. 1997), particularly with regard to aquatic ecosystems due to their preference for various human activities (Dudgeon et al. 2006, Ricciardi and Rasmussen 1998, Perkin et al. 2011). Various stressors such as changes in water flow (Poff et al. 1997), contamination from chemical substances (Likens et al. 1996), invasion by non-native species (Ricciardi and Rasmussen 1998), urban development (Morley and Karr 2002), artificial light (Perkin et al. 2011) all contribute to the deterioration of habitats and decline in biodiversity and in turn alter the functioning of aquatic ecosystems. Freshwater ecosystems often experience multiple concurrent stressors, and the interactions between these stressors can result in outcomes that deviate from the additive effects of each individual stressor (Schäfer et al. 2016). These interactions can manifest in various ways: additive (the combined effect equals the sum of individual effects), synergistic (the combined effect exceeds the sum of individual effects), or antagonistic (the combined effect is less than the sum of individual effects) (Piggott et al. 2015). For example, the study by Schäfer et al. (2016) identified weak positive correlations between toxicity and both nutrients and habitat degradation, implying possible synergistic interactions. In contrast, studies by Elbrecht et al. (2016) and Jackson et al. (2016) observed that the combined impacts of various stressors are frequently lower than the sum of their individual effects, indicating antagonistic interactions. However, the primary drivers of these stressors are human activities, especially land and water management practices, which have been identified as the primary sources contributing to the occurrence of multiple stressors in freshwater ecosystems (Davis et al. 2010). These stressors lead to changes in stream water flow, biogeochemical processes, and climate condition, potentially reducing biodiversity and causing shifts in ecosystem function (Wiederkehr et al. 2020, Burdon et al. 2020, Gál et al. 2019, Schindler and Smol 2006). In addition, the combined influence of these stressors can significantly affect the water quality and biological composition (Heathwaite 2010, Sabater et al. 2018). Agricultural activities, specifically, have been identified as significant contributors to water pollution, posing immediate and long-term risks to aquatic ecosystems (Cooper 1993). Among other human activities, urbanization has led to a yearly rise of 10% in the worldwide consumption of artificial lighting, making it crucial to identify the possible effects of Artificial Light at Night (ALAN) on freshwater macroinvertebrates and develop methodologies to mitigate these disturbances (Falchi and Bará 2013, Perkin et al. 2014a). However, most human settlements are set up near fresh water bodies, where more than 50% of the Earth's population lives within 3 km of a surface freshwater body (Kummu et al. 2011). For this reason, the growing utilization of artificial illumination poses a threat to the conservation of freshwater macroinvertebrate communities (Perkin et al. 2014a). ALAN leads to higher levels of light and longer exposure periods during the night (Manríquez et al. 2021), which can disrupt

the natural circadian rhythm of aquatic macroinvertebrates (Grubisic et al. 2019, Underwood et al. 2017). This disruption of biological rhythms is a key factor, as it has the potential to cause hormonal irregularities (Zeman et al. 2023), inhibit melatonin synthesis (Grubisic et al. 2019) and change in metabolism and behaviour (Rumanova et al. 2020). These disturbances can have extensive effects, impacting the fitness of organisms with specific physiological and behavioural characteristics and potentially leading to changes in communities and ultimately in ecosystems (Falcon et al. 2020, Russart and Nelson 2018). Despite the phototactic attraction to ALAN for freshwater invertebrates, there is still limited understanding of how ALAN affects macroinvertebrate communities and biological trait composition of these species in freshwater environments (Kühne et al. 2021). In addition, ALAN can interact with other stressors, such as ultraviolet radiation (Wrona et al. 2006), climate change (Häder et al. 2011), habitat fragmentation, pollution, (Pulgar et al. 2019) and biological invasion (Thomas et al. 2016) resulting in potential influence on freshwater ecosystems due to increasing urbanization. However, the combined effect of ALAN and other stressors on freshwater ecosystem have been poorly investigated.

Macroinvertebrate communities are aquatic insects that dwell at the bottom of stream bodies throughout their life cycle (Ren et al. 2015). Macroinvertebrates play a crucial role in the movement of organic material within stream ecosystems, serving as vital elements of freshwater environments (Hauer and Lamberti 2017). Moreover, they are pivotal in stream ecosystems by aiding in energy transfer and the reutilization of resources (Ren et al. 2015). Macroinvertebrate communities serve as biological indicators in streams because of their susceptibility to environmental shifts and their ability to indicate the overall well-being of the ecosystem (Deborde et al. 2016, Jenderedjian et al. 2007, Oliveira and Callisto 2010). They are utilized as indicators due to their stable community composition over time and their capacity to mirror the characteristics of various stream habitats (Rabeni et al. 2002). The presence, quantity and the functional diversity of benthic invertebrates can provide insights into environmental contamination (Ren et al. 2015). Considering all of these benefits, many researchers have utilized benthic invertebrate community assessments as a means to evaluate stream health or broader ecosystem conditions (Frithsen and Holland 1992, Oliveira and Callisto 2010, Simboursa et al. 2014, Callisto et al. 2004), and some research suggests that they may offer more sensible assessments of the effects of human activities compared to bacterial communities (Lear et al. 2009).

Effects of ALAN on macroinvertebrates can differ based on the species sensitivity to light. Most species like *Oligochaeta*, do not show attraction (negative phototactic behavior) to artificial light (Wodsedalek 1911, Moon 1940, Timm and Martin 2015). However, other species such as *Chironomidae*,

Tanypodinae, Baetis, Ephemerellidae, Tipulidae, Ceratopogonidae and Elmidae demonstrate a positive phototactic behavior towards artificial (Kühne et al. 2021, Ali et al. 1986, Kirkeby et al. 2013, Sanderson 1953). In addition, research has found that ALAN can suppress the drift behavior of species such as *Baetis* in the aquatic ecosystem (Holt and Waters 1967). Invertebrates usually consume food on the riverbed during daylight hours and then detach from it at night to relocate downstream in order to evade from predators (Allan 1987, Brittain and Eikeland 1988). A 50% decrease in drift among stream invertebrates was observed at lit reaches compared to the control site (dark) in natural forest streams (Perkin et al. 2014a). In addition, other studies have found different effects of ALAN on aquatic insects. For example, Henn et al. (2014) showed that it can disrupt the circadian rhythms of larval aquatic insects, reducing drift and possibly changing community structure. Perkin et al. (2014b) found a significant decrease in activity of adult aquatic insects in response to artificial light, which may implicate their dispersal and habitat colonization. However, according to Parkinson et al. (2020) and Kühne et al. (2021), ALAN might tend to aggregate the fluxes of emergent aquatic insects, possibly increasing their abundance. It was also observed that ALAN can increase the abundance of aquatic insects in riparian areas (Manfrin et al. 2017). In addition, Kühne et al. (2021) and Carannante et al. (2021) both pointed out the potential attraction of aquatic insects to artificial light, although Kühne et al. (2021) showed preference for longer wavelengths, and Carannante et al. (2021) demonstrated white LED lamp capture effect. In addition, a research study discovered that short-time exposure of ALAN reduced the abundance of shredder in ALAN site (Perkin et al. 2014a). Also, research has showed that ALAN intensities could alter the abundance of shredders (Franken et al. 2005). For example, Franken et al. (2005) found that an increase in the light intensity may lead to a rise in the growth of shredders, maybe due to the rise in algae in the leaf-biofilm. Since ALAN has the potential to alter the composition of the macroinvertebrate community and the functional feeding group, we expected that ALAN might also affect overall functioning in freshwater ecosystems. Limited research however has been conducted on the functional effect that ALAN can have on macroinvertebrate communities, such as alterations in ecological traits such as size, reproduction strategy, respiration method, locomotion, resistance form, voltinism.

Non-indigenous species, which are not naturally found in a specific ecosystem, pose a major threat to freshwater biodiversity (McCarthy et al. 2006). *Pacifastacus leniusculus*, a freshwater signal crayfish, originally from North America, is widely recognized as one of the most successful invaders in Europe (Kouba et al. 2015, Mathers et al. 2020a). The introduction of *P. leniusculus* species into new habitats can have diverse effects, such as altering the environment, engaging in competition with indigenous species, transmitting diseases, and alter predatory interactions increasing predatory pressure on key components of the food web such as macroinvertebrates (Crawford et al. 2006). The identification of



ecological consequences resulting from crayfish invasion is challenging, as it depends on a variety of factors including the size and condition of the crayfish's habitat, as well as the behaviors, size, and movement abilities of their prey (Crawford et al. 2006, Correia and Anastácio 2008, Stenroth and Nyström 2003). For example, Correia and Anastácio (2008) found that the existence of crayfish had a detrimental effect on the diversity of macroinvertebrates, with juvenile crayfish causing a more significant reduction in diversity compared to sub-adults and adults. A study by Ruokonen et al. (2014) observed that crayfish had a significant effect on macroinvertebrate communities in stony habitats compared to vegetated ones. Moreover, aquatic insects with limited mobility and crawling on the sediment are at risk of being preyed by the crayfish (Crawford et al. 2006), while highly mobile invertebrates (e.g. *Baetis* spp., *Gammarus*) are less affected by the predatory activity of the crayfish (Nyström et al. 1996, Nyström et al. 1999). Additionally, species that can burrow and live under mud or stones may be less preyed by the crayfish (Crawford et al. 2006, Mathers et al. 2020a). Other biological traits such as voltinism, life-cycle duration, feeding habits, and dietary preferences have been found to be affected by the presence of crayfish invasion (Mathers et al. 2020a). For instance, macroinvertebrate species that produce only one brood per year or take more than a year to complete their life cycle are detrimentally impacted by crayfish (Mathers et al. 2020a). Conversely, species that can complete their life cycle in less than one year might have the ability to endure predation by crayfish (Mathers et al. 2020b). According to Mathers et al. (2020a), the presence of *P. leniusculus* is associated with positive growth in aquatic invertebrates that consume microorganisms (e.g. shredders). This is because *P. leniusculus* can efficiently shred leaf litter into small fragments and fine particulate organic matter; such activities increase the availability of resources in the ecosystem, which may lead to a greater abundance of shredders (Doherty-Bone et al. 2018). Also, it was found that functional diversity indices such as functional richness (FRich), functional evenness (FEve), and Rao's quadratic entropy (RaoQ) showed a negative correlation with the invasion of signal crayfish (*P. leniusculus*) (Mathers et al. 2020a). This suggests that the presence of signal crayfish not only affects macroinvertebrates communities in term of composition, but also their functionality.

The presence of ALAN in freshwater environments has been noted to influence the development of predator-prey interactions, which could lead to changes in the traits of both predators and prey, ultimately affecting their dynamics (Kyba et al. 2011). ALAN has a strong effect on both nocturnal (which includes 60% of invertebrates) and diurnal species (Hölker et al. 2010). Signal crayfish (e.g. *P. leniusculus*) is known as nocturnal animal, but it has been found that their activity reduces when they are exposed to ALAN (Thomas et al. 2016). In addition, in presence of a stress factor such as the presence of the crayfish, macroinvertebrates might increase their active drift to avoid predatory pressure (Brittain and Eikeland 1988), while it has been found that ALAN in some taxa can inhibit

drifting behaviour to avoid exposure to predation in illuminated conditions (Perkin et al. 2014b). However, crayfish possess corneas that are capable of absorbing ultraviolet wavelengths, and this ability could influence how they react to shifts in the spectral composition of ALAN (Goldsmith and Fernandez 1968). A study findings concluded that ALAN has a significant impact on the behavior of *P. leniusculus*, leading to decreased activity and interactions among individuals, as well as an increased preference for shelters (Thomas et al. 2016). This altered behavior could have implications for the ecosystem, as ALAN can also impact the physiological and behavioral patterns of crayfish (Jackson and Moore 2019). For example, crayfish undergo physiological and behavioral shifts that can have a significant effect on aquatic environments (e.g., macroinvertebrate), affecting foraging behaviors and interactions (Belanger et al. 2017, Jackson and Moore 2019). On the other hand, ALAN can lead to an increase in the abundance of specific macroinvertebrates, such as amphipods and polychaetes, which are frequently targeted by predators like crayfish (Garratt et al. 2019). This shift in prey availability has the potential to impact interactions between predators and their prey (Garratt et al. 2019). Considering the documented impacts of ALAN on aquatic species, it is evident that both aquatic native and non-native species will react to ALAN (Thomas et al. 2016).

The main goal of this study was to understand single and combined effects of ALAN and invasive signal crayfish on functional diversity and traits composition of benthic macroinvertebrates. Here, we conducted a mesocosm experiment within 16 artificial stream channels. Mesocosms are downsized replicas of natural ecosystems that provide a practical approach for evaluating the impacts of stressors on populations, communities, and ecosystems (Coors et al. 2006). In addition, mesocosms enable for the investigation of the impact of stressors on various trophic and taxonomic levels within a context that is ecologically relevant (Boone and James 2005). However, in this study the following hypotheses were tested:

- a) ALAN is expected to attract a wider variety of phototaxis species (Kühne et al. 2021) and reduce drift patterns (Perkin et al. 2014a), leading to changes in macroinvertebrate functional trait compositions and functional diversity that are different from those in the control sites.
- b) The presence of crayfish is expected to alter functional diversity and trait composition (Mathers et al. 2020a) both for direct predation, habitat changes, and alterations in drift patterns (Flood et al. 2020).
- c) The presence of ALAN is expected to reduce signal crayfish feeding activity (Thomas et al. 2016), with resultant effects on macroinvertebrates functional trait composition and functional diversity that differ from stressors alone.

## 2.0 Methods

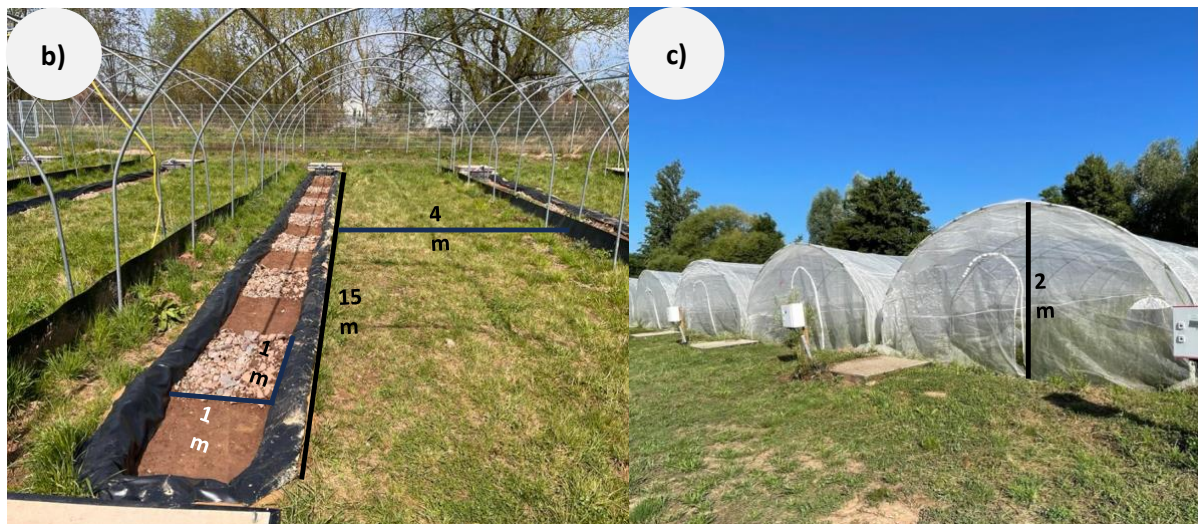
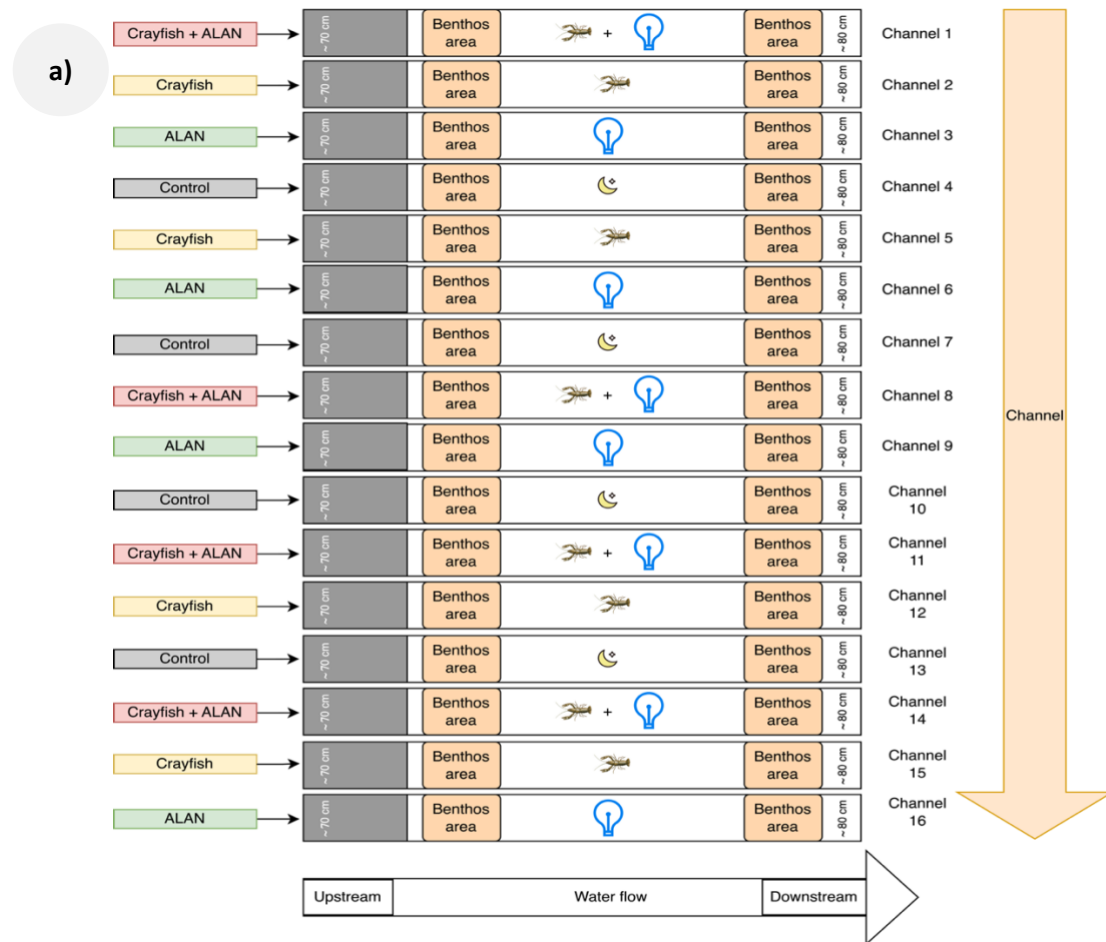
### 2.1 Experimental method:

#### 2.1.1 Study site and experimental setup

The study was performed at the Riparian Stream Mesocosm (RSM) facility, located nearby the Queich River in Rhineland-Palatinate, Germany, at coordinates 49°12'06.70" N, 8°08'33.80" E. The mesocosm facility consists of 16 separate units (Fig. 1a), each containing an artificial stream channel that is approximately 15 m long and 1 m wide (Fig. 1b). Additionally, it includes the adjacent riparian area measuring about 15 m by 4 m (Fig. 1b). To guarantee isolation from each other, each unit has a frame structure that is up to 2 m high and enclosed with mesh gauze with very small openings of just 1 mm (Fig. 1c). Water from the Queich River is directed independently into each stream, allowing for either recirculation or direct flow-through options. (Manfrin et al. 2023, Rovelli et al. 2024)

#### 2.1.2 Experimental design

The study was split into two main phase: the colonization phase and the subsequent treatment phase. During colonization, the channels initially underwent passive colonization. Artificial substrates were placed in the Queich River to be colonised, collected after 2 weeks as well as the organisms attached to them. Artificial substrates were then homogeneously spread into the RSM channels. The treatment phase began in the first week of May 2022 and continued for six weeks. Throughout this phase, four distinct treatments were applied across the 16 channels. Each treatment group was fully crossed by a two-factorial design with four RSM units being randomly assigned to each of the four treatments: signal crayfish, ALAN, the co-occurrence of signal crayfish and ALAN, and the control (i.e., neither signal crayfish nor ALAN). Throughout the experiment, the channels assigned to ALAN and crayfish + ALAN treatment were illuminated with LED light from 9:00 pm to 6:00 am (Fig. 1a). The lighting utilized was 24 V neutral white chip on board (COB) LED strips, 8 mm in thickness with a color temperature ranging from 4000 to 4500 K (24 V). These strips were powered by a dimmable 320 W transformer (HLG-320-24B). The strips were cut into 7 m pieces. Each pair of 7 m LED strips was supplied power by a LED-Trafo (Transformator), positioned centrally to cover a total distance of 14 m across each ALAN-exposed channel. The intensity of the light was adjusted to maintain approximately  $20 \pm 2$  lux at the center, with a slight gradient towards the edges, where the intensity dropped by about  $5 \pm 2$  lux.



**Figure 1. a)** The experimental design scheme consists of 16 replicated Riparian Stream Mesocosm (RSM) units (15 m x 6 m), arranged to study the effects of ALAN and the presence of the signal crayfish (*Pacifastacus leniusculus*) in benthic invertebrate communities. A two-way, fully factorial design was used across each treatment group, and four RSM units were assigned randomly to each of the four treatments: signal crayfish, ALAN, the interaction of signal crayfish and ALAN, and the control group (neither the presence of crayfish nor ALAN). Each channel has two benthos areas to collect the macroinvertebrate samples from both the upstream and downstream position. Icons indicate the presence of crayfish and ALAN in each channel: a crayfish icon indicates crayfish presence, a light bulb icon indicates ALAN exposure, and a moon icon indicates the absence of ALAN. **b)** An inside view of one RSM unit with the measurement of the artificial stream (15 m x 1 m) and the adjacent riparian areas (15 m x 4 m), was taken before the experiment began. **c)** An exterior view of the RSM units, including the height of the frame structure (2 m), was taken after the experiment began. Photo credits: SystemLink

### 2.1.3 Procedure for macroinvertebrate sampling

We collected a total of 64 macroinvertebrate samples both before (week (-1)) and after (week 6) the application of the treatments. Macroinvertebrate sampling followed the collection procedure described by (Surber 1970). First, the big stones were picked and scrubbed in the collecting net to displace pupae and other associated insects. Secondly, the substrate was vigorously scoured to a depth of 10 cm for two minutes, thus the current was carrying the sediment and organic debris that are native to the area's macroinvertebrate fauna into the net. Finally, the samples were placed immediately from the Surber net in plastic bags with labels and treated them with 96% ethanol. (Surber 1970)

### 2.1.4 Procedure for macroinvertebrate sorting and identification

Macroinvertebrate samples collected during the first and final weeks were identified down to the family, subfamily, or genus level. Each macroinvertebrate sample was stored in a plastic vial and preserved with 96% ethanol. The ethanol in each benthos sample was exchanged twice: first after 1-2 days and then again after a week. Each benthos sample was labelled with three identifiers: time point (week), specific channel name, and whether it was collected during the day or night. Macroinvertebrates were sorted from organic and inorganic detritus and separated into coarse taxonomical group for later identification. A stereomicroscope was utilised to count and identify each macroinvertebrate at the family level for Ephemeroptera, Trichoptera, Diptera, and Coleoptera. Gammarus spp. were identified at the genus level, whereas for the family Chironomidae, we distinguished Tanypodinae from other Chironomidae. For Oligochaeta, we did not do further identification and organisms were just counted.

## 2.2 Statistical analysis methods

### 2.2.1 Trait data generation from taxa list

To assign functional traits and calculate functional metrics (Functional Richness (FRich), Functional Evenness (FEve), Functional Dispersion (FDisp), Rao's quadratic entropy (RaoQ)), the "biomonitoR" package (R version 0.9.3) for R protocol explained in (Laini et al. 2022a) was followed. The Tachet database from the Freshwater Ecology website (<https://freshwaterecology.info/>) was used to assign traits to macroinvertebrates (Tachet et al. 2010). Fuzzy-coded data was utilized to assign 11 biological traits based on their 63 corresponding grouping features (Table 1). In the first step of statistical analysis, the "as\_biomonitor" function from the "biomonitoR" package for R (Laini et al. 2022a) was applied to the species abundance data (Fig. 2). This function compared our taxa list with the reference

macroinvertebrate taxonomic data table (Laini et al. 2022a). Taxa were grouped according to our dataset using the *"aggregate\_taxa"* function (Laini et al. 2022a). Subsequently, the *"assign\_traits"* function created a data frame that includes the names and taxonomic levels of the species in the taxonomic dataset, as well as the names and levels of related species in the trait dataset. It also shows the taxonomic distance between those species and their traits. The biological traits data were collected for each species in the taxonomic datasheet using the *"assign\_traits"* function from the *"biomonitorR"* package for R (Laini et al. 2022a). In the next step, *"average\_traits"* function was used to compute the average traits for each taxon, which is used in further statistical analysis. The analysis included different biological traits such as maximum potential size, life-cycle duration, voltinism, aquatic stages, reproduction strategy, dispersal strategy, resistance form, respiration method, locomotion and substrate relation, food consumed, and feeding group (Table 1). This fuzzy-coded trait dataset presents the macroinvertebrate affinities for each trait, ranging from zero (representing no affinity) to three or five (representing a significant affinity) (Chevene et al. 1994, Tachet et al. 2010). In addition, to achieve equal taxonomic weight between taxa, each biological grouping feature was normalized as proportional affinities so that the total of each trait equaled to 1 (White et al. 2017, Laini et al. 2023). (Fig. 2) For the multivariate and univariate analysis, the *"relevel"* function was used to specify the reference levels for the treatment, time point, and position factors. The reference levels were set as follows: "Control" for the treatment factor, "Upstream" for the stream position factor, and "Before Treatment" for the time point factor.

### 2.2.2 Functional diversity indices and CWM calculation

The *"biomonitorR"* package for R was utilized to compute various indices of functional diversity including functional dispersion (mean distance of each species from the centroid in the multidimensional trait space), RaoQ (pairwise differences among taxa), evenness (consistent pattern of species abundance distribution and differences in functional space), and richness (volume of the community's functional space) (Laliberté and Legendre 2010, Mathers et al. 2020a, Ricotta et al. 2014). These indices were derived from a data frame generated by the *"aggregate\_taxa"* function (Fig. 2). The computation involved using the mean trait value, selecting the *"taxa"* taxonomic level (i.e. a mixed taxonomic level) for trait data retrieval, and specifying "F" for fuzzy coded traits within the *"trait\_db"* function. The number of dimensions for the multidimensional spaces kept as low as possible (nbdim = 2), with the *"col\_blocks"* function defining a vector indicating the count of modalities per trait (Laini et al. 2022a). The default "Gower" method was employed to calculate functional distances and the "Cailliez" correction method was applied to adjust for any negative eigenvalues (Laini et al. 2022a). Functional indices values were standardized before the Bayesian mixed effect modelling (Laini et al.

2022b). In addition, community-weighted mean (CWM) of all trait values (weighted by species abundance) was computed from the data frame generated by “*aggregate\_taxa*” function. Two separate data frames were produced: one containing all functional indices (used for univariate analysis) and another for CWM (used in multivariate analysis) (Fig. 2).

**Table 1.** Functional trait names of macroinvertebrates with grouping features derived from the “biomonitoR” package.

Trait group	Trait modalities	Code	Trait group	Trait modalities	Code
Size	≤0.25 cm	SIZE_1	Life-cycle duration	≤1 year	LIFESPAN_1
	>0.25-0.5 cm	SIZE_2		>1 year	LIFESPAN_2
	>0.5-1 cm	SIZE_3	Voltinism	<1	VOLTINISM_1
	>1-2 cm	SIZE_4		1	VOLTINISM_2
	>2-4 cm	SIZE_5		>1	VOLTINISM_3
	>4-8 cm	SIZE_6	Aquatic stages	Egg	STAGES_1
	>8 cm	SIZE_7		Larva	STAGES_2
Reproduction strategy	Ovoviviparity	REPRODUCTION_1		Nymph	STAGES_3
	Isolated, free eggs	REPRODUCTION_2	Dispersal strategy	Adult	STAGES_4
	Isolated, cemented eggs	REPRODUCTION_3		Aquatic passive	DISPERSAL_1
	Clutches, cemented	REPRODUCTION_4		Aquatic active	DISPERSAL_2
	Clutches, free	REPRODUCTION_5		Aerial passive	DISPERSAL_3
	Clutches, in vegetation	REPRODUCTION_6		Aerial active	DISPERSAL_4
	Clutches, terrestrial	REPRODUCTION_7	Resistance form	Eggs/statoblasts	RESISTANCE_1
	Asexual	REPRODUCTION_8		Cocoons	RESISTANCE_2
Locomotion	Flier	LOCOMOTION_1		Housing against desiccation	RESISTANCE_3
	Surface swimmer	LOCOMOTION_2		Diapause/dormancy	RESISTANCE_4
	Full water swimmer	LOCOMOTION_3		None	RESISTANCE_5
	Crawler	LOCOMOTION_4	Respiration method	Tegument	RESPIRATION_1
	Burrower	LOCOMOTION_5		Gill	RESPIRATION_2
	Interstitial	LOCOMOTION_6		Plastron	RESPIRATION_3
	Temporarily attached	LOCOMOTION_7		Spiracle	RESPIRATION_4
	Permanently attached	LOCOMOTION_8		Hydrostatic vesicle	RESPIRATION_5
Food consumed	Microorganisms	FOOD_1	Feeding group	Absorber	FEEDING_1
	Detritus < 1 mm	FOOD_2		Deposit feeder	FEEDING_2
	Dead plant ≥1 mm	FOOD_3		Shredder	FEEDING_3
	Living microphytes	FOOD_4		Scraper	FEEDING_4
	Living macrophytes	FOOD_5		Filter-feeder	FEEDING_5
	Dead animal ≥1 mm	FOOD_6		Piercer	FEEDING_6
	Living microinvertebrates	FOOD_7		Predator	FEEDING_7
	Living macroinvertebrates	FOOD_8		Parasite	FEEDING_8
	Vertebrates	FOOD_9			

Note. Reprinted from “Macroinvertebrate Taxonomic and Functional Trait Compositions within Lotic Habitats Affected By River Restoration Practices”, by White et al., 2017, *Environmental management*, 60, p. 516 (<https://doi.org/10.1007/s00267-017-0889-1>).

### 2.2.3 Identifying the stressors effects on functional traits

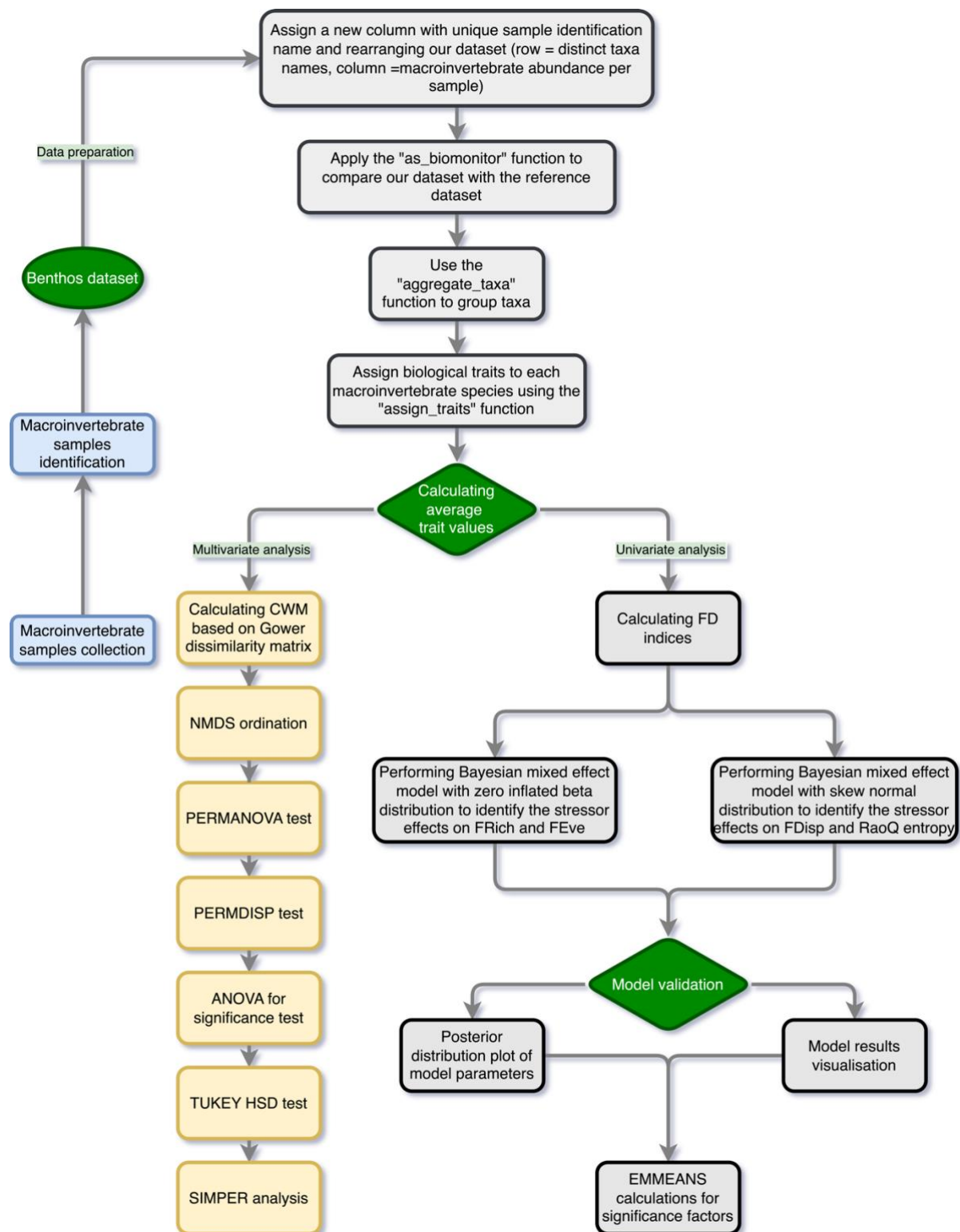
The multivariate difference in term of functional traits composition was visualized using “Non-metric Multidimensional Scaling” (NMDS) ordination across different treatments, time points and stream positions (White et al. 2017, Nichols et al. 2022). This multivariate ordination technique relied on a “Gower” dissimilarity matrix generated with the “*dist.ktab*” function from the “biomonitor” package for R. A “Permutational Multivariate Analysis of Variance” (PERMANOVA) was used to statistically assess the variations in functional dissimilarities (White et al. 2017), considering different treatments, time points (before and after treatment), and positions within the stream (upstream and downstream). The “*adonis2*” function from the “vegan” package (R version 2.6-4) (Dixon 2003) for R was used in this step. This statistical analysis considered the interactions among treatment, time point, and position factors. A “Permutational Analysis of Multivariate Dispersion” (PERMDISP) was conducted on the same dissimilarity matrix to assess the multivariate dispersion of macroinvertebrate traits, in relation to the significant factors and their interactions as identified by PERMANOVA results (Nichols et al. 2022). The analysis was performed using the “*betadisper*” function from the “vegan” package for R. The significance level of PERMDISP was evaluated through an “Analysis of Variance” (ANOVA). Pairwise Tukey’s HSD test was performed to determine the statistical significance of differences in mean values for each factor variable and their interaction terms (e.g., treatment, time point, position, and the combination of time point and position). This analytical approach involved using the “*permutest*” function from the “vegan” package for R. Mean trait dissimilarities were examined for variability across significant categorical factors using the “Similarity Percentage” (SIMPER), which was carried out with the “*simper*” function from the “vegan” package (White et al. 2017) for R.

### 2.2.4 Identifying the stressors effects on functional diversity indices

The influence of ALAN and signal crayfish on FD indices of macroinvertebrates was assessed by applying “Bayesian Linear Mixed-Effects Model” (BLMM), with variations observed across different treatments, time points (pre- and post-treatment effect), and stream positions. The “*brm*” function from the “brms” package for R (R version 2.20.4) (Bürkner 2017) was used for conducting each BLMM. Each BLMM considered interactions between the fixed factors, being Treatment, Time point, and position, while including the Channel name as a random factor. All community-level effect (e.g., Treatment, Time points, and Positions) priors were set simultaneously using the “*set\_prior*” function, and the parameters were determined based on the mean and standard deviation of each response variable (e.g., FD indices) (Bürkner 2017). The model parameter’s posterior distribution was computed through the use of Hamiltonian Markov Chain Monte Carlo no-U-turn (NUTS) sampling, which was executed in Stan using the “brms” package (Bürkner 2017, Bürkner 2018) for R. Each model was



estimated using three separate Markov Chain Monte Carlo (MCMC), consisting of 3000 iterations per chain (Bürkner 2017). The first 1500 iterations were used as warm-up periods to calibrate the sampler parameters (Bürkner 2017). The “NUTS” method was controlled via the “control” argument to reduce divergent transitions, which might bias the estimated posterior distribution (Bürkner 2017). To improve the accuracy and reliability of posterior samples, the “adapt\_delta” parameter was adjusted to 0.95 in order to decrease sampling, while limiting the “max\_treedepth” parameter to 15 for a more stable sampling process. For the FEve and FRich response variables, both values range from 0 to 1 and included zero values, “zero\_inflated\_beta” family was used with the “brms” package for R to account for the presence of zeros and variability in the data (Bürkner 2017). On the other hand, for RaoQ and FDisp variables, which also showed proportional distribution but exhibited left-skewness, models using “skew\_normal” family within the “brm” function. Model validation of each BLMM involved the use of diagnostic plots and posterior predictive checks, utilizing the “plot” and “pp-check” functions (Bürkner 2017). Subsequently, pairwise estimated marginal means (EMMEANS) were computed for each statistically significant variable and their interactions to evaluate the effects within the model framework by using “emmeans” function from the “emmeans” package for R (R version 1.8.9). BLMM not only compute an estimation for each parameter, but also compute its Bayesian 95% credible interval (CI) (Labusch et al. 2022). The significance of stressors or their interactions was considered significant if the 95% CI did not include zero (Labusch et al. 2022). Finally, the model outputs were visualized using the “ggplot2” package for R (R version 3.5.1) (Wickham 2016) and the “mcmc\_areas” function from the “brms” package for R. (Fig. 2) All statistical analysis were performed using R version 09.0+463 (RStudio Team 2023). Additionally, ChatGPT was used to paraphrase and develop statistical analysis methods (OpenAI 2024).



**Figure 2.** The conceptual flow diagram represents the process and analysis of the benthos dataset to identify the effects of ALAN, signal crayfish, and their interaction on the functional traits and diversity of macroinvertebrate communities at different treatments, time points, and stream positions within this study. First, macroinvertebrate samples are collected and identified. Next, the data is prepared by giving each sample a unique ID, comparing it to reference datasets, grouping taxa, assigning biological traits, and finding the average trait value. The multivariate analysis consists of CWM, NMDS ordination, PERMANOVA, PERMDISP, ANOVA, Tukey HSD, and SIMPER analysis. The univariate analysis consists of FD indices. To determine the stressors impact, we use Bayesian mixed effect models with zero-inflated beta distributions of FRich, FEve, and skew normal distributions of FDisp, and RaoQ to determine the impact of the stressors. We validate the model by creating posterior distribution plots, visualising the results, and then performing EMMEANS calculations.

### 3.0 Results

#### 3.1 Effect on macroinvertebrate trait composition

The PERMANOVA result revealed significant impacts of time point (differences in treatment period), stream position factor, and their interaction (e.g., Time point x Position) on trait composition within the multidimensional trait space among the studied treatment sites (Table 2). However, the analysis did not show any significant effects of the treatment factor or its interactions with position, time point, or with both factor (e.g., Treatment x Time Point, Treatment x Position, Treatment x Time Point x Position) on the macroinvertebrate trait composition.

**Table 2.** Three-factor permutational analysis of variance (PERMANOVA) results for macroinvertebrate trait composition

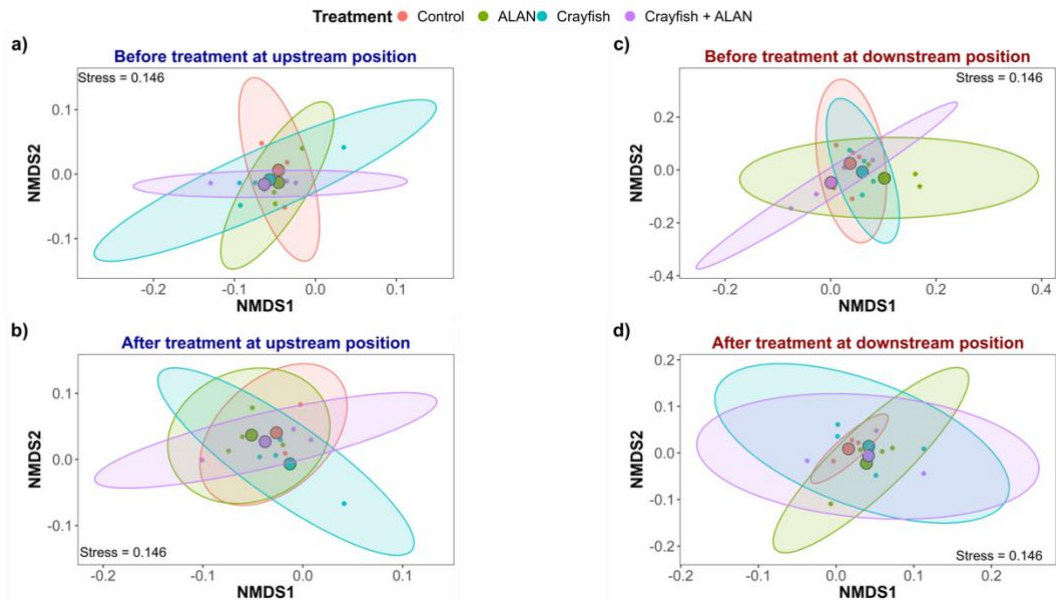
Predictor Variable	DF	SS	R <sup>2</sup> value	Pseudo-F statistic	P-value
Treatment	3	0.023	0.036	1.04	0.391
<b>Time point</b>	<b>1</b>	<b>0.027</b>	<b>0.042</b>	<b>3.61</b>	<b>0.012*</b>
<b>Position</b>	<b>1</b>	<b>0.158</b>	<b>0.245</b>	<b>21.01</b>	<b>0.001**</b>
Treatment x Time point	3	0.013	0.020	0.57	0.882
Treatment x Position	3	0.027	0.043	1.22	0.304
<b>Time point x Position</b>	<b>1</b>	<b>0.020</b>	<b>0.032</b>	<b>2.76</b>	<b>0.047*</b>
Treatment x Time point x Position	3	0.011	0.018	0.52	0.935
Residual	48	0.362	0.561		
Total	63	0.644	1.000		

Notes: PERMANOVA was performed with 999 permutations and calculation based on a Gower dissimilarity matrix. Significant p-values are denoted in boldface font. Significance levels: 0 '\*\*\*'; 0.001 '\*\*'; 0.01 '\*'; 0.05 ' '.

The variable time point had a significant effect on the trait composition of macroinvertebrates; it explained 4.2% of the observed variation, which means that trait composition changed over time (Table 2). The composition of traits also varied across different stream positions, accounting for 24.5% of the observed variability (Table 2). Also, the interaction between time points and position factors (e.g., Time point x Position) was significant and explained 3.2% of the total variation. This showed that temporal effects on trait variabilities were different for positions upstream and downstream.

The NMDS ordination supported the findings from the PERMANOVA analysis. Prior to treatment application (Week (-1)), the NMDS ordination showed a similarity in trait composition between the control and treatment groups at the upstream positions. This suggests a homogeneous trait composition without clear distinction prior to the treatment (Fig. 3a). Furthermore, before applying any treatments (Week (-1)), we observed a difference in trait composition among the treatment groups at the downstream position, suggesting possible minor inherent differences in trait distributions (Fig.

3c). In contrast, NMDS ordination showed changes in the functional trait composition following *P. leniusculus* introduction, exposure to ALAN, and their combined effects (e.g., signal crayfish exposed to ALAN) during the post-treatment assessment (Week 6). These changes were evident when compared to control conditions at both upstream and downstream positions (Fig. 3b and 3d, Fig. S1a).



**Figure 3.** Macroinvertebrate trait composition is displayed through non-metric multidimensional scaling (NMDS). The ordination used to illustrate the impact of ALAN, the presence of *P. leniusculus species*, and their interaction before and after treatment at different stream positions: **a)** comparing trait composition among various treatment groups prior to treatment at the upstream positions; **b)** illustrating trait composition comparisons between each treatment group and the control after treatment at the upstream position; **c)** displaying trait composition differences between each treatment and the control group before treatment at the downstream position; **d)** presenting changes in trait compositions between each treatment group and the control site after treatment at the downstream position. Each ellipse represents a 95% confidence area for each treatment level: Control = red, ALAN = green, crayfish = light blue, and crayfish + ALAN = purple.

Multivariate group dispersion of macroinvertebrate trait composition was homogeneous across all treatment groups. This means that the average distances to the median of macroinvertebrate trait dissimilarities were not significantly different between the treatment groups (Table 3, Fig. S1c). However, significant variations in trait dispersion were observed within stream positions (e.g., upstream, and downstream), and two different time points (e.g., before and after-treatment) (Table 3, Fig. S1d & e). Furthermore, the interaction between time points and stream position was found to be statistically significant (Table 3, Fig. S1b), suggesting that the heterogeneity of trait dispersion between upstream and downstream positions changed during the study period.

Tukey's HSD test was conducted on the PERMDISP model for each significant factor or their interactions (e.g., time point, position, and time point x position) that were identified from ANOVA. Tukey's HSD test revealed a significant decrease in the dispersion of macroinvertebrate overall traits within the

multivariate space following the end of the treatment period (week 6) compared to the trait dispersion measured before the start (week(-1)) of the experiment (Table 4, Fig. S1e). Moreover, the downstream

**Table 3.** Table for analysis of variance (ANOVA) to assess the significance of PERMDISP results. Evaluating the variation in macroinvertebrate trait compositions across various grouping factors.

Predictors / Residuals	DF	Sum Sq	Mean Sq	F value	P-value
Treatment	3	0.005	0.001	1.52	0.218
Residuals	60	0.070	0.001		
<b>Time point</b>	<b>1</b>	<b>0.009</b>	<b>0.009</b>	<b>7.76</b>	<b>0.007**</b>
Residuals	62	0.076	0.001		
<b>Position</b>	<b>1</b>	<b>0.007</b>	<b>0.007</b>	<b>5.84</b>	<b>0.018*</b>
Residuals	62	0.079	0.001		
<b>Time point x Position</b>	<b>3</b>	<b>0.015</b>	<b>0.005</b>	<b>4.29</b>	<b>0.008**</b>
Residuals	60	0.070	0.001		
Treatment x Time Point x Position	15	0.022	0.001	0.93	0.536
Residuals	48	0.075	0.001		

Notes: Each ANOVA was performed on the output from the PERMDISP test, where response variable was a Gower dissimilarities distance matrix of trait composition. Significance p-values were denoted in boldface font. Significance levels: 0 '\*\*\*', 0.001 '\*\*', 0.01 '\*', 0.05 '.'

position exhibited a significant increase in overall trait dispersion compared to the upstream position (Table 4, Fig. S1d). Tukey's HSD test also revealed a significant interaction between time point and position only with regards to the variation in trait dispersion between upstream and downstream positions prior to the initiation of treatment (e.g., Week (-1)\_Downstream vs Week (-1)\_Upstream). However, other comparisons, such as Week6\_Downstream vs. Week6\_Upstream, Week6\_Upstream vs. Week (-1) Upstream, and Week6\_Downstream vs. Week (-1) Downstream, did not show any statistical significant differences (Table 4, Fig. S1b).

**Table 4.** Comparison of average trait distribution distances in multivariate space by Tukey's HSD test, across various significant factors and their interactions within groups.

Group Comparison	Mean Difference	95 % Confidence Interval		p-value
		Lower Bound	Upper Bound	
<b>Week 6-Week (-1)</b>	<b>-0.024</b>	<b>-0.041</b>	<b>-0.006</b>	<b>0.007**</b>
<b>Downstream-Upstream</b>	<b>0.021</b>	<b>0.003</b>	<b>0.039</b>	<b>0.018*</b>
<b>Week (-1)_Downstream-Week (-1)_Upstream</b>	<b>0.035</b>	<b>0.003</b>	<b>0.067</b>	<b>0.025*</b>
Week6_Downstream-Week6_Upstream	0.010	-0.021	0.043	0.805
Week6_Upstream-Week(-1)_Upstream	-0.004	-0.036	0.027	0.985
Week6_Downstream-Week(-1)_Downstream	-0.028	-0.060	0.003	0.097

Notes: Tukey's HSD test was conducted to identify significant mean differences for the time point factor, position factor, and their interaction term. Asterisks next to p-values indicate a significant difference between the two groups at a level of  $p < 0.05$ . Significance levels: 0 '\*\*\*', 0.001 '\*\*', 0.01 '\*', 0.05 '.'

Similarity Percentages Analysis (SIMPER) was performed to identify the traits that significantly contributed to differences in composition at the downstream position compared to the upstream positions (including treatment and time point factors), as well as differences in trait composition after treatment compared to before treatment (including treatment and position factors) (Table 5, Table 6). Macroinvertebrate species associated with filter feeding and parasitism, consuming microorganisms, detritus, and living macroinvertebrates and typically reproducing less than once a year, were found to be more abundant in downstream positions compared to the upstream positions (Table 5). In contrast, shredders and scrapers that feed on decomposed plant material along with taxa capable of active aquatic dispersal showed higher abundance at upstream positions (Table 5) compared to the downstream positions.

**Table 5.** Results of the Simper analysis. The table compares the average dissimilarity of trait group assemblages, which accounted for up to 70% of the dissimilarity between upstream and downstream positions. Traits are listed based on the cumulative percentage of the average stream position dissimilarity.

Trait	Grouping feature	Average contribution	Std. dev	Upstream	Downstream	Cum. (%)	p-value
Aquatic active	Dispersal	0.00126	0.00097	<b>0.254</b>	0.235	9.6	<b>0.001</b>
Aerial passive	Dispersal	0.00183	0.00119	0.151	<b>0.189</b>	14.13	<b>0.001</b>
Shredder	Feeding group	0.00246	0.00193	<b>0.237</b>	0.200	28.17	<b>0.001</b>
Scraper	Feeding group	0.00198	0.00132	<b>0.312</b>	0.294	31.41	<b>0.002</b>
Filter feeder	Feeding group	0.00108	0.00068	0.048	<b>0.070</b>	34.39	<b>0.001</b>
Parasite	Feeding group	0.0009	0.00051	0.029	<b>0.049</b>	43.18	<b>0.001</b>
Microorganism	Food	0.00017	0.00010	0.007	<b>0.010</b>	45.99	<b>0.001</b>
Detritus <1 mm	Food	0.00135	0.00103	0.165	<b>0.191</b>	48.54	<b>0.001</b>
Dead plant ≥1 mm	Food	0.00205	0.00123	<b>0.165</b>	0.121	51.00	<b>0.001</b>
Dead animal ≥1 mm	Food	0.00076	0.00052	<b>0.064</b>	0.048	58.16	<b>0.001</b>
Living macroinvertebrates	Food	0.00139	0.00091	0.143	<b>0.167</b>	60.38	<b>0.001</b>
≤1 year	Life cycle	0.00306	0.00230	0.802	<b>0.839</b>	66.5	<b>0.002</b>
>1 year	Life cycle	0.00306	0.00230	<b>0.197</b>	0.160	68.47	<b>0.002</b>

Additionally, after the treatment period ended (week6), there was an increase in the abundance of macroinvertebrate species characterized by passive dispersal mechanisms, including scraper and piercer feeding groups which mainly feed on dead animal matter (Table 6). Conversely, deposit feeders, which primarily consume microorganisms and living macrophytes, were more abundant before the start (week-1) of the experiment (Table 6).

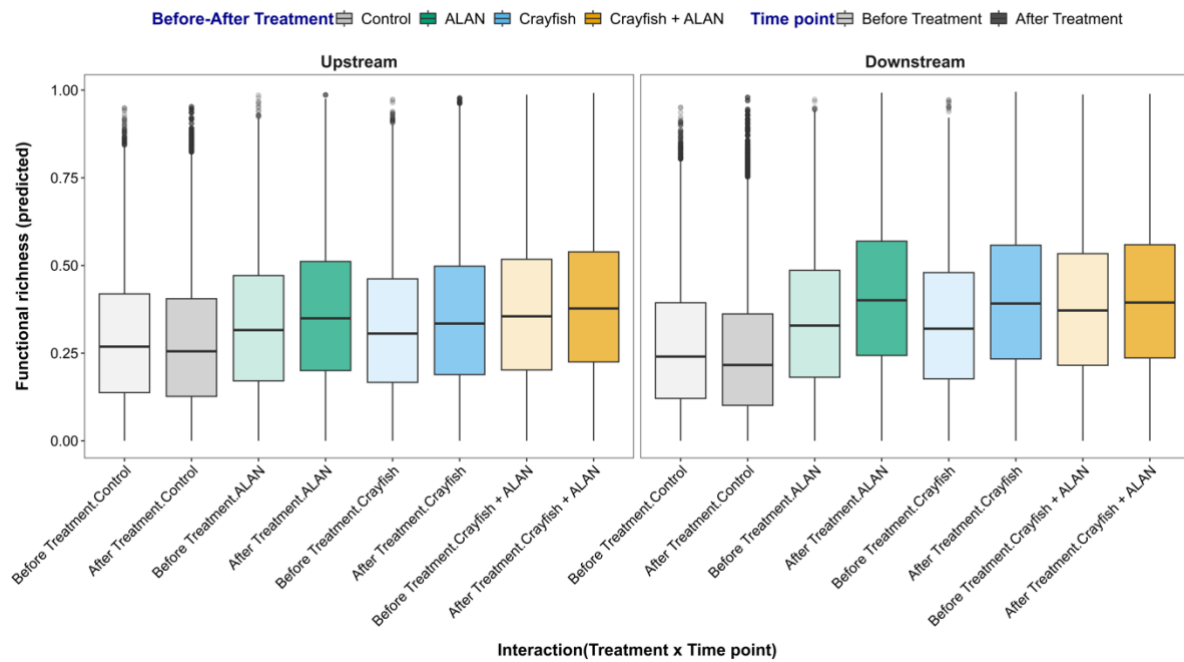
**Table 6.** Results of the Simper analysis. The table compares the average dissimilarity of trait group assemblages, which accounted for up to 70% of the dissimilarity between before and after treatment. Traits are listed based on the cumulative percentage of the average two different treatment periods dissimilarity.

Trait	Grouping feature	Average contribution	Std. Dev.	Before Treatment	After Treatment	Cum. (%)	p-value
Aquatic passive	Dispersal	$1.8 \times 10^{-3}$	$1.31 \times 10^{-3}$	0.375	<b>0.4019</b>	4.3	<b>0.001</b>
Aerial active	Dispersal	$2.43 \times 10^{-3}$	$1.84 \times 10^{-3}$	<b>0.208</b>	0.1838	15.5	<b>0.011</b>
Deposit feeder	Feeding group	$1.14 \times 10^{-3}$	$7.95 \times 10^{-4}$	<b>0.138</b>	0.1233	22.41	<b>0.007</b>
Scraper	Feeding group	$1.91 \times 10^{-3}$	$1.48 \times 10^{-3}$	0.292	<b>0.3148</b>	28.81	<b>0.032</b>
Piercer	Feeding group	$1.54 \times 10^{-5}$	$6.35 \times 10^{-5}$	0	<b>0.0003</b>	34.91	<b>0.001</b>
Microorganism	Food	$1.44 \times 10^{-4}$	$1.01 \times 10^{-4}$	<b>0.009</b>	0.0084	43.67	<b>0.046</b>
Living macrophytes	Food	$4.07 \times 10^{-4}$	$3.93 \times 10^{-4}$	<b>0.076</b>	0.0708	54.13	<b>0.003</b>
Dead animal $\geq 1$ mm	Food	$6.80 \times 10^{-4}$	$5.08 \times 10^{-4}$	0.051	<b>0.0616</b>	56.63	<b>0.001</b>

### 3.2 Effects of ALAN, crayfish and their interaction on functional indices

#### 3.2.1 Effect on functional richness (FRich)

The Bayesian model indicates that the overall interaction effect of ALAN and *P. leniusculus* (effects included both time points and stream positions) had a significant positive influence on FRich (estimates = 0.39, 95% CI [0.06 to 0.70]) (Fig. 4, Table S1), compared to the control.



**Figure 4.** The Bayesian model predicts the effects of different treatments and time points on functional richness (FRich) at upstream and downstream positions. Four colors represent the different treatments: grey for the control, green for ALAN, blue for crayfish, and orange for the crayfish and ALAN interaction. The boxplots' color transparency reflects the time point factor: lighter tones indicate the before-treatment effects (Week (-1)), while darker tones indicate the after-treatment effects (Week-6).

Estimated marginal means (EMMEANS) calculated from the Bayesian model were used to quantify the individual impacts of each treatment on FRich compared to the control site at both upstream and downstream positions across the two different treatment periods (week(-1) & week-6). At week 6, the RSM channels that were exposed to ALAN had a significant increase in FRich compared to the controls (Table 7, Fig. 4) at both upstream (estimate = -0.441, 95% CI [-0.869 to -0.023]) and downstream positions (estimate = -0.840, 95% CI [-1.412 to -0.308]).

**Table 7.** Emmeans of Bayesian CI comparing among different treatment sites at both upstream and downstream positions over two different time periods to quantify the effects of different stressors and their interaction on FRich.

Site comparisons	Before treatment at upstream position			After treatment at upstream position		
	Estimate	2.5%	95%	Estimate	2.5%	95%
Control – ALAN	-0.243	-0.548	0.071	<b>-0.441</b>	<b>-0.869</b>	<b>-0.023</b>
Control – Crayfish	-0.220	-0.538	0.116	-0.400	-0.825	0.018
<b>Control – (Crayfish + ALAN)</b>	<b>-0.387</b>	<b>-0.718</b>	<b>-0.078</b>	<b>-0.522</b>	<b>-0.932</b>	<b>-0.071</b>
Crayfish – (Crayfish + ALAN)	-0.163	-0.582	0.288	-0.121	-0.669	0.474
Site comparisons	Before treatment at downstream position			After treatment at downstream position		
	Estimate	2.5%	95%	Estimate	2.5%	95%
Control – ALAN	-0.405	-0.813	0.012	<b>-0.840</b>	<b>-1.412</b>	<b>-0.308</b>
Control – Crayfish	-0.390	-0.825	0.021	<b>-0.806</b>	<b>-1.330</b>	<b>-0.240</b>
<b>Control – (Crayfish + ALAN)</b>	<b>-0.559</b>	<b>-0.987</b>	<b>-0.117</b>	<b>-0.776</b>	<b>-1.330</b>	<b>-0.235</b>
Crayfish – (Crayfish + ALAN)	-0.166	-0.718	0.426	0.028	-0.667	0.756

Notes: The estimates or mean, lower 95% credible intervals (2.5%), upper 95% credible intervals (95%) of posterior coefficient estimate comparisons among different treatment groups are presented. Effect was considered as significant if a 95% credible intervals for a parameter estimate does not include zero. Significant CI's presented here in boldface font.

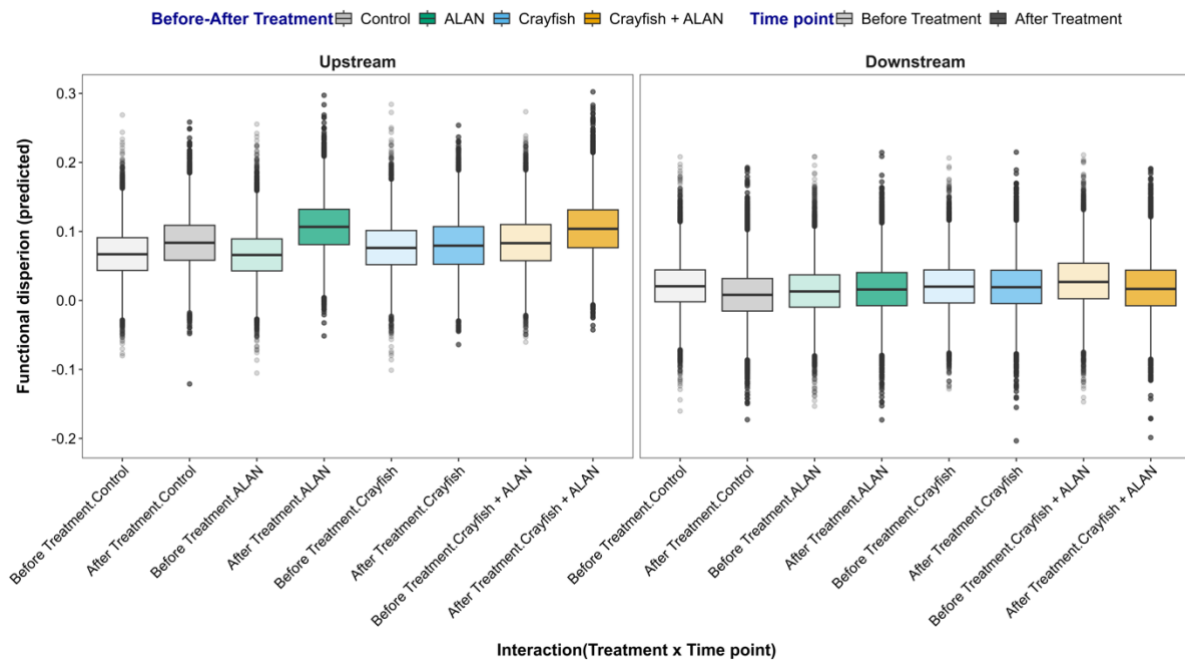
Additionally, at week 6, FRich was higher in the signal crayfish site compared to the control site (Table 7, Fig. 4) at the downstream positions (estimate = -0.806, 95% CI [-1.330 to -0.240]).

Moreover, at Week 6, the combined stressor site (e.g., signal crayfish exposed to ALAN) had a significantly higher FRich value compared to the controls (Table 7, Fig. 4) at both upstream (estimate = -0.522, 95% CI [-0.932 to -0.071]) and downstream (estimate = -0.776, 95% CI [-1.330 to -0.235]) positions.



## 3.2.2 Effect on functional dispersion (FDisp)

The Bayesian model suggests a significant overall decrease in FDisp at the downstream positions compared to the upstream positions, with an estimate of -0.05 [95% CI: -0.08 to -0.02]) across all the treatments and time points (Table S2, Fig. 5).



**Figure 5.** The Bayesian model predicts the effects of different treatments and time points on functional dispersion (FDisp) at upstream and downstream positions. Four colors represent the different treatments: grey for the control, green for ALAN, blue for crayfish, and orange for the crayfish and ALAN interaction. The boxplots' color transparency reflects the time point factor: lighter tones indicate the before-treatment effects (Week (-1)), while darker tones indicate the after-treatment effects (Week-6).

EMMEANS calculated from the Bayesian model were used to quantify changes in FDisp at the downstream positions compared to the upstream positions across all the treatments and time points (week(-1) and week-6). Pairwise EMMEANS indicated that at Week 6, there was a significant decrease in FDisp at the downstream positions across all the treatment sites compared to the upstream positions. This is supported by the 95% credible intervals which did not encompass zero (Table 8, Fig. 5).

Furthermore, a similar significant reduction in FDisp was observed at the downstream positions before the start of the experiment across all treatments. This is also supported by the 95% credible intervals which did not include zero (Table 8, Fig. 5).

**Table 8.** Emmeans of Bayesian CI, comparing between upstream and downstream position for different treatment sites over two different time periods to quantify the changes on FDisp.

Group Comparison	Control – Before treatment			Control – After treatment		
	Estimate	2.5 %	95%	Estimate	2.5%	95%
Upstream – Downstream	<b>0.046</b>	<b>0.015</b>	<b>0.077</b>	<b>0.075</b>	<b>0.034</b>	<b>0.119</b>
	ALAN – Before treatment			ALAN – After treatment		
Upstream – Downstream	<b>0.052</b>	<b>0.015</b>	<b>0.087</b>	<b>0.089</b>	<b>0.043</b>	<b>0.137</b>
	Crayfish – Before treatment			Crayfish – After treatment		
Upstream – Downstream	<b>0.056</b>	<b>0.015</b>	<b>0.093</b>	<b>0.060</b>	<b>0.012</b>	<b>0.109</b>
	Crayfish + ALAN – Before treatment			Crayfish + ALAN – After treatment		
Upstream – Downstream	<b>0.055</b>	<b>0.017</b>	<b>0.094</b>	<b>0.086</b>	<b>0.041</b>	<b>0.130</b>

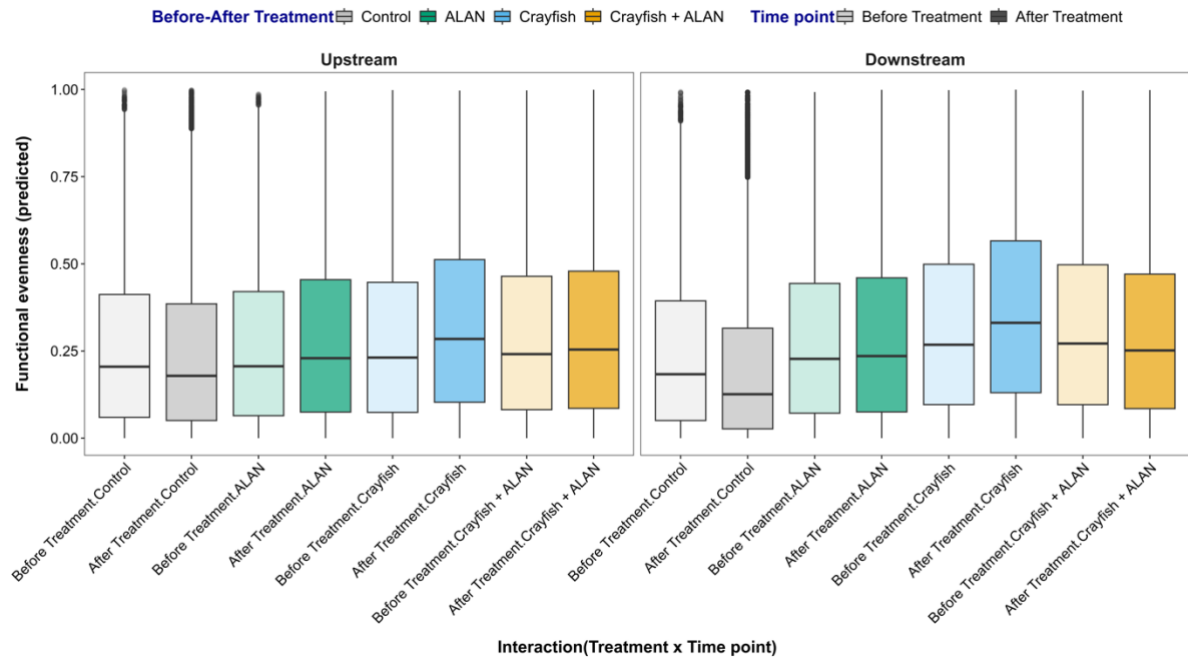
Notes: The estimates or mean, lower 95% credible intervals (2.5%), upper 95% credible intervals (95%) of posterior coefficient estimate comparisons between upstream and downstream are presented. Effect was considered as significant if a 95% credible intervals for a parameter estimate does not include zero. Significant credible intervals presented here in boldface font.

### 3.2.3 Effect on functional evenness (FEve)

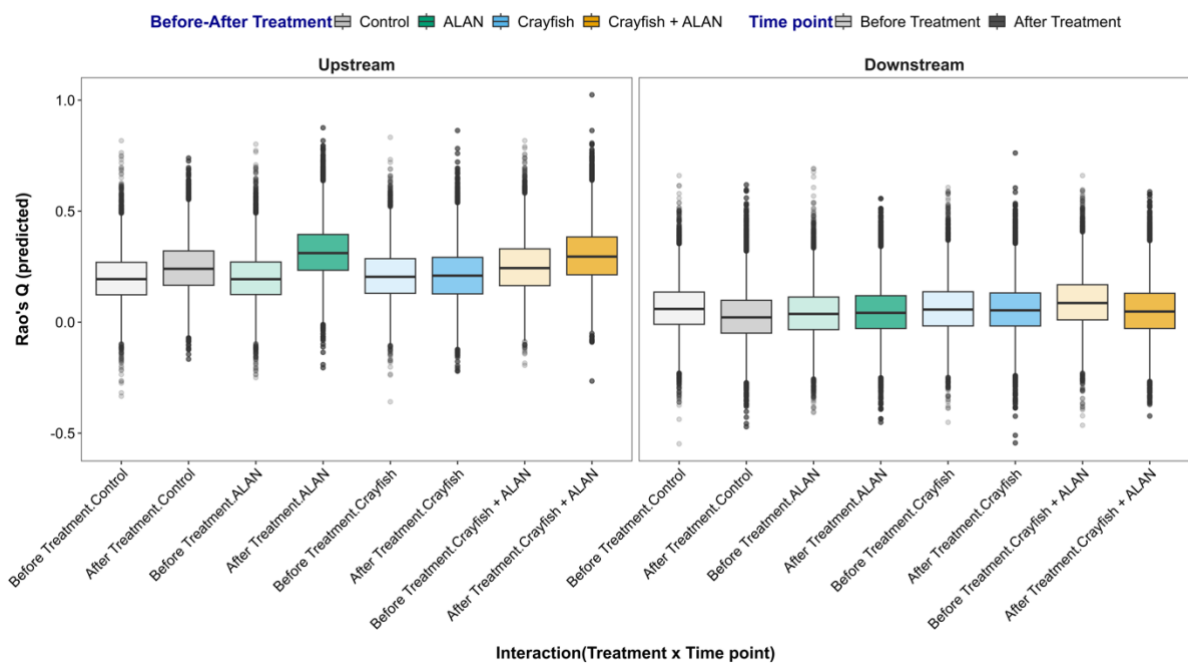
The Bayesian model suggests that there was no significant effect of the treatments or their interaction with time point (e.g., week (-1), week-6) and position factor (e.g., upstream, downstream) on FEve (Table S3, Fig. 6).

### 3.2.4 Effect on Rao's quadratic entropy (RaoQ)

The Bayesian model indicates a significant decrease in overall RaoQ entropy value at the downstream positions compared to the upstream positions, with an estimated effect size of -0.13 (95% CI: -0.23 to -0.04) including all treatments and time points (week(-1), week-6) (Table S4, Fig. 7). Additionally, pairwise EMMEANS calculated from Bayesian credible intervals indicated that at week 6, RaoQ entropy value showed a significant decrease (95% CI excluded zero) in the downstream positions compared to the upstream positions across all treatment sites (Table 9, Fig. 7). Moreover, the same significant reduction trend in RaoQ entropy value (95% CI excluded zero) was observed at the downstream positions compared to the upstream positions before the start of the experiment (week(-1)) (Table 9, Fig. 7).



**Figure 6.** The Bayesian model predicts the effects of different treatments and time points on functional evenness (FEve) at upstream and downstream positions. Four colors represent the different treatments: grey for the control, green for ALAN, blue for crayfish, and orange for the crayfish and ALAN interaction. The boxplots' color transparency reflects the time point factor: lighter tones indicate the before-treatment effects (Week (-1)), while darker tones indicate the after-treatment effects (Week-6).



**Figure 7.** The Bayesian model predicts the effects of different treatments and time points on Rao's quadratic entropy (RaoQ) at upstream and downstream positions. Four colors represent the different treatments: grey for the control, green for ALAN, blue for crayfish, and orange for the crayfish and ALAN interaction. The boxplots' color transparency reflects the time point factor: lighter tones indicate the before-treatment effects (Week (-1)), while darker tones indicate the after-treatment effects (Week-6).

**Table 9.** Emmeans of Bayesian CI, comparing between upstream and downstream positions for different treatment sites over two different time periods to quantify the changes on RaoQ entropy.

Group Comparison	Control – Before treatment			Control – After treatment		
	Estimate	2.5 %	95%	Estimate	2.5%	95%
Upstream – Downstream	<b>0.133</b>	<b>0.039</b>	<b>0.227</b>	<b>0.218</b>	<b>0.094</b>	<b>0.349</b>
	ALAN – Before treatment			ALAN – After treatment		
Upstream – Downstream	<b>0.156</b>	<b>0.047</b>	<b>0.263</b>	<b>0.268</b>	<b>0.126</b>	<b>0.416</b>
	Crayfish – Before treatment			Crayfish – After treatment		
Upstream – Downstream	<b>0.150</b>	<b>0.031</b>	<b>0.263</b>	<b>0.156</b>	<b>0.010</b>	<b>0.298</b>
	Crayfish + ALAN – Before treatment			Crayfish + ALAN – After treatment		
Upstream – Downstream	<b>0.157</b>	<b>0.043</b>	<b>0.282</b>	<b>0.250</b>	<b>0.109</b>	<b>0.387</b>

Notes: The estimates or mean, lower 95% credible intervals (2.5%), upper 95% credible intervals (95%) of posterior coefficient estimate comparisons between upstream and downstream are presented. Effect was considered as significant if a 95% credible intervals for a parameter estimate does not include zero. Significant credible intervals presented here in boldface font.

## 4.0 Discussion

Stressors caused by human activities are widespread in freshwater environments, with climate change (Schindler and Smol 2006), water stress (Sabater et al. 2018), and a variety of compound stressors (Schäfer et al. 2016) all exerting substantial influence. The understanding of interaction between multiple stressors is complex due to several influencing factors. Interactions between multiple stress factors involve direct impacts on the ecosystem, indirect effects, and interactions among the stressors themselves (Glibert et al. 2022). For example, elevated temperatures have the potential to increase the occurrence of detrimental algal blooms, subsequently causing hypoxia, which impacts different species in diverse ways (Glibert et al. 2022). This suggests that a single stressor has the potential to change the effects of another stressor through indirect mechanisms, thereby adding complexity to the understanding of their interactions (Glibert et al. 2022). However, determining the ecological impacts of interactions between multiple stressors is crucial in freshwater ecosystems due to their potential to lead to unexpected consequences, which can complicate efforts to manage the effects of these stressors (Lange et al. 2018, Birk 2019, Kath et al. 2018). In this study, we performed a mesocosm experiment aimed at assessing the single and combined effects of ALAN and invasive crayfish *P. leniusculus* on the functional trait composition of macroinvertebrate communities.

### 4.1 Responses of macroinvertebrate traits to ALAN

At week 6, a clear shift in the macroinvertebrate trait composition was observed in the ALAN sites compared to the control treatments at both upstream and downstream positions (Fig. 3b, 3d, Fig. S1a). It is possible that these changes happened because our sampling site had more phototaxis species (e.g., Chironomidae, *Gammarus*, Tanypodinae, and *Baetis*) than the negative phototaxis species (e.g., Oligochaeta). These results support some aspects of our first hypotheses, which speculated that ALAN could alter the functional trait composition of macroinvertebrates compared to the control sites. This is due to the attraction of macroinvertebrates to ALAN, and the presence of more phototaxis species (e.g., Chironomidae) in the studied samples. In addition, the phototactic behavior of macroinvertebrates changes depending on the wavelength of light (Kühne et al. 2021). In the present study, natural LEDs with a 450 nm wavelength was used. In contrast, a study by Kühne et al. (2021) observed that aquatic insects are significantly drawn to longer wavelengths of ALAN, such as green (517 nm) and yellow (588 nm) LEDs, than the shorter wavelengths of LEDs. These discrepancies may be attributed from physiological distinctions in how different insect orders perceive light (Kühne et al. 2021). Furthermore, during Week 6, the significant differences in macroinvertebrate functional traits did not observe between the ALAN site and the control sites at both upstream and downstream

positions. But the abundance of scrapers, which feed on detritus and are capable of passive aquatic dispersal (e.g., Chironomidae), was observed to be lower in the ALAN site compared to the control site at the upstream position during week 6 (Fig. S5, Table 5, Table 6). However, at the downstream position, the abundance of scrapers were increased at the ALAN sites compared to the control sites during the same week. These results did not support another aspect of our first hypotheses, which suggested that the presence of ALAN reduced macroinvertebrate drift. Water inflow from the nearby Queich River, which potentially carries chemical signals from predators into our experimental stream environment, could be the cause of this unexpected increase in scraper abundance at the downstream position of the ALAN site (McIntosh et al. 1999). On the other hand, there were more shredders (which eat dead plants, like *Gammarus*) in the upstream position at the ALAN sites during week 6 than in the downstream position (Fig. S5, Table 5). Shredders, such as *Gammarus* species, prefer upstream positions as a result of environmental influences such as variations in water flow, precipitation, temperature, and water levels (Hultin 1971). These elements induce positive rheotaxis, leading *Gammarus* to move against the current through an aquatic active dispersal strategy (Hultin 1971). This behaviour offsets downstream movement and aids in sustaining their presence in favourable environments, representing an adaptive response to environmental circumstances (Hultin 1971, Hughes 1970).

At the end of the experiment (week 6), FRich was significantly higher at the ALAN sites compared to the control sites at both upstream and downstream positions (Table 7). However, in the ALAN site, there were no significant changes in other FD indices, such as FEve, FDisp, and RaoQ entropy, compared to the control sites at both upstream and downstream positions at the same time (week 6). These findings support other aspects of our first hypotheses. Specifically, ALAN is expected to change the functional trait composition of macroinvertebrates, leading to different functional diversity (measured by FRich and FEve) at the ALAN site compared to the control site, which alligns with our first hypotheses. However, Species richness affects functional richness by increasing the overall FD of an ecosystem (Dalerum et al. 2012). For example, Dalerum et al. (2012) conducted a simulation showing that an increase in the number of species leads to a proportional increase in both FD and functional redundancy (FR), as long as similar functional components overlap across all pairs of species. This indicates that a larger number of species brings about a broader range of functional traits, ultimately enriching the ecosystem's functionality (Dalerum et al. 2012). Another study also found a strong correlation between FRich and species richness, as well as between FEve and diversity with species evenness and diversity (Heino 2008). From this perspective, it can be said that the ALAN site exhibited greater macroinvertebrate abundance and diversity in comparison to the control site. Our findings support previous studies, suggesting that the existance of High Pressure Sodium (HPS) or LED lighting changes the taxonomical composition and abundance of macroinvertebrates (Garratt et al.

2019, Juvigny-Khenafou et al. 2024). In addition, in the present study, we observed a significant increase in FRich after 6 weeks at both upstream and downstream positions, without any significant effect on FEve. Halpern and Floeter (2008) found that with the rise in species diversity within reef fish communities, new species have a tendency to integrate into already rich functional groups instead of creating new ones. This results in increased FRich but reduced FEve, as there is an uneven distribution of species across functional groups, resulting in increased redundancy within specific groups (Halpern and Floeter 2008). Another potential explanation for these outcomes is that different species can coexist together and support ecosystem functions by inhabiting distinct ecological niches, thus raising FRich without impacting the evenness of species distribution (Goswami et al. 2017). However, we observed a significant decrease in FDisp at the downstream position of the ALAN sites compared to the upstream positions during week 6 (Fig. 5, Table 8), indicating an increase in functional similarity among species in the downstream positions (Valdivia et al. 2017). In contrast, during week 6, RaoQ entropy significantly decreased at the downstream position in the ALAN site compared to the upstream position (Fig. 7, Table 9). This means that species were less different from each other on the basis of average trait value (Botta-Dukát 2005). The possible reason behind this consequence is that most of the macroinvertebrates in the downstream positions were scrapers (e.g., Chironomidae, *Baetis*) during week 6, while at the upstream position, the abundance of feeding groups like scrapers (e.g., Chironomidae, *Baetis*) and shredders (e.g., *Gammarus*) was more evenly distributed during week 6 (Fig. S5).

#### 4.2 Responses of macroinvertebrate traits to *P. leniusculus* species invasion

Our study did not find any statistically significant effects of *P. leniusculus* invasion on the composition of macroinvertebrate traits, similar to the observed effects of ALAN on macroinvertebrate traits. Nonetheless, there was a substantial change in the composition of macroinvertebrate traits in week 6 as a result of *P. leniusculus* presence (see Fig. 3b & 3d, Fig. S1a) compared to the control sites at upstream and downstream positions. This also provides evidence supporting aspects of our second hypotheses, indicating that the presence of crayfish would alter macroinvertebrate trait composition. PERMDISP results showed a wider of macroinvertebrate trait dispersions at the downstream positions within the crayfish site and reduced trait variability at the upstream position compared to the control site during week 6 (Fig. S1a). These results support our hypotheses about the impact of *P. leniusculus* on macroinvertebrate drift dynamics. Because macroinvertebrates have the potential to shift from upstream to downstream positions. These movements can occur as a result of their natural activities, including food-seeking and foraging, or as a deliberate response to perceived threats from predators in their surroundings (Brittain and Eikeland, 1988). Another potential explanations for the higher

abundance of macroinvertebrate traits in the downstream positions may include a preference of signal crayfish for upstream positions for their better survival and reproductive circumstances (Rosewarne et al. 2013), ultimately leading to decreased predation risk for macroinvertebrates at the downstream position. For example, Rosewarne et al. (2013) conducted a study where signal crayfish were seen moving upstream, although their progress was hindered by a weir when compared to a control stretch without any barriers. This would indicate that, despite physical barriers encountered, signal crayfish continue in their attempts to move upstream for better environmental conditions or less competition. This result supports other aspects of our second hypotheses, that the presence of signal crayfish changes the macroinvertebrate drift pattern because of habitat changes caused by the crayfish. Additionally, the abundance of scrapers, which feed on detritus and are able to passive aquatic dispersal, was higher in the signal crayfish sites during week 6 at the upstream position compared to the controls, as represented by Chironomidae (see Fig. S5, Table 5, Table 6). The experimental design may be the reason why the macroinvertebrate like Chironomidae is not showing response to the introduction of signal crayfish. Indeed, macroinvertebrates and crayfish are native to the Queich, indicating that invertebrates are already used to the presence of crayfish. Whereas the abundance of scrapers (e.g., Chironomidae) and shredders (e.g., *Gammarus*) which feed on decomposed plant material, were less abundant in the crayfish site at the downstream position compared with the control sites (see Fig. S5, Table 5, Table 6). Abundance of shredder and scrapers can be reduced either by direct predation of crayfish or due to the alteration of the habitat (indirect effect) of the shredder or scrapers (Dunoyer et al. 2014, Usio and Townsend 2004). Crayfish alter the physical habitats of shredders and scrapers, affecting the survival and functionality of these organisms that rely on structures such as leaf litter and fine sediment composition. By disturbing the sediment and reducing available leaf litter, crayfish can indirectly reduce the quality and suitability of the habitat for these organisms. (Usio and Townsend 2004, Dunoyer et al. 2014)

The other aspect of our second hypotheses, which suggested that the presence of crayfish would alter functional diversity in the macroinvertebrate community, was supported. This study observed that having *P. leniusculus* in the crayfish sites for 6 weeks could cause the FRich to increase significantly at the downstream position compared to the control sites (Fig. 4, Table 7). In addition, during week 6, the FEve positively increased at both the upstream and downstream positions compared to the control sites, but not significantly (Fig. 6, Table S3). Previous studies have supported our finding that the existence of crayfish can result in either an increase in macroinvertebrate abundance leading to a greater variety in trait distribution or no impact on invaded areas (Usio and Townsend 2004, Keller and Ruman 1998, Grandjean et al. 2011, Crawford et al. 2006, Albertson and Daniels 2016). However, the influence of crayfish invasion on macroinvertebrate communities is shaped by various factors such as



selective predation, the movement patterns of different taxa, handling time for prey, environmental conditions in their habitat, and crayfish density (Mathers et al. 2016, Usio and Townsend 2004). As a part of selective predation, crayfish initially consume medium and large-sized Tanypodinae, which in turn reduces the predatory impact on other macroinvertebrates (Usio and Townsend 2004). This could be one reason why our experimental site with crayfish contained a higher FD (measured by FRich and FEve) compared to the control sites during week 6 (Fig. 4, Fig. 6, Table 7). Chironomidae is commonly targeted by crayfish due to their scarce locomotion ability (Whitledge and Rabeni 1997, Usio and Townsend 2004). But a study conducted by Keller and Ruman (1998) reported that after a 17 day exposure to crayfish, there were no significant changes in the abundance of Diptera and Trichoptera, while there was a decrease in the abundance of Ephemeroptera. One potential reason for these outcomes could be that crayfish encountered challenges in preying on small invertebrates, especially insects at a particular stage of development (Keller and Ruman 1998). The relatively short duration of our study, which spanned only six weeks, could be another potential explanation for the wider variability of macroinvertebrate traits observed in the crayfish site compared to the control sites at downstream position during week 6 (Fig. S1c). It is feasible that an extended exposure of macroinvertebrates to crayfish might result in a more pronounced or statistically significant influence on the variability of macroinvertebrate traits. For example, different studies have found that the long-term presence of invasive crayfish can lead to shifts in macroinvertebrate community composition, decreased functional diversity, and modifications to habitat structure (Mathers et al. 2016, Mathers et al. 2020a). Another aspect to be considered in future research to identify the impact of crayfish invasion on macroinvertebrates is the potential influence of increased crayfish population density. A study found that declining patterns of macroinvertebrate abundance and diversity are associated with increasing crayfish density (Galib et al. 2022). It is possible that higher densities of crayfish might trigger a response; but the densities observed in our study were comparable to the natural densities found in the Queich River. However, another factor that may influence our findings is the presence of stone beds within our experimental stream. A previous study found that crayfish can use large gravels or stones as shelter, which may affect their behavior and engineering effects (Albertson and Daniels 2016). Furthermore, our macroinvertebrates samples were contained highly mobile species like *Baetis* spp., *Gammarus*, and Ephemeroptera, which can avoid being preyed upon by crayfish. This capacity of evasion might lead to a shift in community composition favoring these more mobile taxa at the cost of less mobile species, such as those belonging to the Trichoptera species (Mathers et al. 2016, Nyström et al. 1996, Nyström et al. 1999). However, during week 6, we observed a significant decrease in FD indices such as FDisp and RaoQ entropy at the downstream position in the crayfish sites compared to the upstream position (Table 8, Table 9). The possible explanation for this is that scrapers (e.g.,

Chironomidae) were more abundant at the downstream position of the crayfish site, while the abundance of scrapers (e.g., Chironomidae, Baetis), shredders (e.g., Gammarus), deposit feeders (e.g., Oligochaeta), and predators (e.g., Tanypodinae) was more evenly distributed at the upstream position compared to the downstream position (Fig. S5).

#### 4.3 Responses of macroinvertebrate traits to ALAN and *P. leniusculus* interactions

The present study did not find any statistically significant interacting effect between ALAN and *P. leniusculus* on macroinvertebrate trait composition compared to the crayfish or ALAN site during week 6. However, at week 6 with both stressors combined, there was a slight shift in trait composition at the upstream and downstream position compared to the crayfish site (see Fig. 3b, 3d; Fig. S1a). This finding support some aspects of our third hypotheses, which speculated that ALAN reduces feeding activities of crayfish, with resultant effects on macroinvertebrate trait composition. Also, the PERMDISP results showed that there was a higher variance in macroinvertebrate trait dispersions at the downstream position for the combined stressors sites in week 6 compared to the upstream position (Fig. S1a). This pattern may be due to active behavioral drift by macroinvertebrates as they try to avoid predation pressure (Brittain and Eikeland 1988). In addition, feeding group such as scraper (e.g., Chironomidae), slightly increased in the combined stressors sites at the downstream position during week 6, compared to the crayfish sites (see Fig. S5, Table 5, Table 6). Usio and Townsend (2002) found that predatory Tanypodinae was reduced by crayfish and indirectly increased collector or gatherer Chironomidae, which may indicate a community shift in Chironomidae.

Additionally, measures of functional diversity such as FRich showed higher values at the combined stressor sites (e.g., crayfish exposed to ALAN) compared to the crayfish and control sites during week 6 at upstream position (Fig. 4, Table 7). In addition, FRich was significantly increased in the combined stressor sites at upstream and downstream position during week 6 compared to the control sites (Fig. 4, Table 7). This suggests no negative impact on macroinvertebrate trait variability at upstream and downstream position during week 6 compared to the control sites. These results support another aspect of our third hypotheses, suggesting that the presence of ALAN should affect signal crayfish feeding activity, which will be different from the stressor alone. A possible explanation for the observed results may be attributed to the nocturnal behavior of *P. leniusculus* species, which tends to show higher activity during nighttime (Thomas et al. 2016). It was found that exposure to HPS lighting at a intensity level of  $12 \pm 5$  lux for short periods significantly reduces the activity and interactions within this species (Thomas et al. 2016). As a result, this decrease in activity could lead to decreased predation pressure at the combined stressor site compared to the crayfish site (Thomas et al. 2016, Fischer et al. 2020). However, FEve was lower at the combined stressor sites at upstream and downstream position

compared to the crayfish site in week 6 (Fig. 6). A reduction in macroinvertebrate functional evenness at the crayfish sites occurred because crayfish selectively preyed on specific groups of macroinvertebrates, for example, the Culicidae, Chironomus, Tanytarsini, and Orthocladinae (Correia and Anastácio 2008). This finally lowers the functional trait diversity in the community, making its functional balance out of order; in this way, functional evenness may be lower (Correia and Anastácio 2008). Moreover, crayfish modify abiotic conditions like increasing the level of sediment (indirect effect), which in turn impacts the functional trait composition of macroinvertebrate communities by rendering the habitat not suitable for some of the taxa (Mathers et al. 2020a). In contrast, other FD indices including FDisp and RaoQ entropy, demonstrated minimal changes in the combined stressor sites compared to the crayfish site at the upstream and downstream position in week 6 (Fig. 5, Fig. 7). However, during week 6, we observed a significant decrease in FD indices such as FDisp and RaoQ entropy at the downstream position in the combined stressors sites compared to the upstream position (Table 8, Table 9). The possible explanation for this is that scrapers (e.g., Chironomidae) were more abundant at the downstream position of the combined stressors sites (crayfish + ALAN), while the abundance of scrapers (e.g., Chironomidae, *Baetis*), shredders (e.g., *Gammarus*), deposit feeders (e.g., Oligochaeta), and predators (e.g., Tanytarsini) was more evenly distributed at the upstream position (Fig. S5).

#### 4.4 Last remarks and conclusions

During week 6, the present study observed that the ALAN, crayfish, and combined stressor sites (e.g., signal crayfish exposed to ALAN) exhibited more macroinvertebrate functional traits and functional diversity (measured by FRich and FEve) compared to the control sites at both upstream and downstream positions. The combined stressor site had more functional traits and higher FD (e.g., FRich) compared to the crayfish site, indicating that ALAN reduces the activity of signal crayfish and has a positive influence on the macroinvertebrate community. Furthermore, the abundance of scraper was higher in the downstream position compared to the upstream position during week 6 across all treatments, indicating macroinvertebrate drifts to reduce predation pressure. However, the effect of ALAN and signal crayfish and their interaction on macroinvertebrate trait composition was not investigated in the linked aquatic-terrestrial ecosystems in the present study. It was found that ALAN affects aquatic-terrestrial interactions by changing the community structure and behaviour of macroinvertebrates that influence energy and nutrient flux across these ecosystems (Sullivan et al. 2019). For example, riparian predator and detritivore dominance under ALAN was found to drive changes in food web characteristics such as food chain length and the contribution of aquatically derived energy (Sullivan et al. 2019). In addition, the present study did not consider crayfish size as an

effect, and the impact of signal crayfish and its interaction with ALAN were evaluated only over a six-week period. But it was found that Crayfish like juvenile *Procambarus clarkii*, significantly decrease the diversity of aquatic macroinvertebrates (Correia and Anastácio 2008), which may also influence the energy and nutrient flow from aquatic to terrestrial ecosystems based on the role of macroinvertebrates in nutrient cycles and as a food source for terrestrial predators. For example, macroinvertebrates such as grazer, shredder, gatherer, filterer, and predator contribute to decomposition and material translocation (Wallace and Webster 1996). These activities help in nutrient cycling within the stream ecosystem (Wallace and Webster 1996), which can impact adjacent terrestrial ecosystems. Moreover, while we did observe a positive influence on the macroinvertebrate trait composition in the ALAN, crayfish, and combined stressor sites at upstream and downstream positions compared to the controls during week 6, the data from the long-term monitoring sites of English rivers found that the presence of *P. leniusculus* significantly reduced the majority of macroinvertebrate abundance (Mathers et al. 2016). Future research should focus on the long-term impacts (e.g., 1–2 years) of *P. leniusculus*, considering crayfish size in interaction with ALAN in linked aquatic-terrestrial ecosystems. In addition, the present study used LED lights with 450 nm intensities as the ALAN source. But, future research should investigate the effects of different wavelengths of ALAN, such as green and yellow LEDs with higher wavelengths (e.g., wavelength > 450 nm), on *P. leniusculus* and their interacting effect on phototaxis aquatic insects. Because it has been found that phototaxis aquatic insects are more attracted to green and yellow LEDs with higher intensities, for instance, 517-588 nm, than short-wavelength LEDs, for example, blue LEDs with a wavelength of 455 nm (Kühne et al. 2021). We expected that different wavelengths of ALAN would alter the predation impact of *P. leniusculus* on macroinvertebrates. However, the effects of ALAN, signal crayfish, and their interaction on macroinvertebrate functional traits observed in this study may not prevail when the chronic effects of these stressors are evaluated in isolation and combined for a long-term period. Furthermore, by comparing species diversity with functional diversity, we can more effectively identify the individual and combined effects of multiple stressors.

## 5.0 References

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## 6.0 Appendix

### 6.1 Supplementary tables

**Table S 1.** Results from the Bayesian model summary, calculating the effects of different stressors and their interaction on FRich over different treatment periods (week-1 & week 6) at both upstream and downstream positions.

Predictors	Estimates	Est. Error	2.5%	95%	Bulk_ESS	Rhat
Intercept	-0.85	0.18	-1.21	-0.52	5982	1
ALAN	0.24	0.16	-0.06	0.56	5160	1
Crayfish	0.22	0.17	-0.10	0.56	5405	1
<b>Crayfish + ALAN</b>	<b>0.39</b>	<b>0.17</b>	<b>0.06</b>	<b>0.70</b>	<b>6457</b>	<b>1</b>
After treatment	-0.05	0.16	-0.36	0.26	6649	1
Downstream	-0.11	0.15	-0.39	0.20	5415	1
ALAN x After	0.20	0.17	-0.14	0.53	7260	1
Crayfish x After	0.18	0.17	-0.16	0.52	6576	1
(Crayfish + ALAN) x After	0.14	0.17	-0.20	0.48	6383	1
ALAN x Down.	0.17	0.17	-0.15	0.49	7197	1
Crayfish x Down	0.17	0.17	-0.16	0.49	6398	1
(Crayfish + ALAN) x Down	0.17	0.17	-0.17	0.50	6725	1
After x Down.	-0.09	0.16	-0.41	0.23	6560	1
ALAN x After x Down.	0.24	0.17	-0.12	0.57	6578	1
Crayfish x After x Down.	0.24	0.18	-0.10	0.58	8325	1
(Crayfish + ALAN) x After x Down.	0.08	0.18	-0.25	0.43	6935	1

Notes: The estimates or mean, estimated error (Est. Error), lower 95% credible intervals (2.5%), upper 95% credible intervals (95%) of posterior coefficient estimates are presented, as well as effective sample size (Bulk\_ESS) and the R-hat measure of parameter convergence. Effect was considered as significant if a 95% credible intervals for a parameter estimate does not include zero. Significant credible intervals presented here in boldface font.

**Table S 2.** Results from the Bayesian model summary, assessing the effects of various stressors and their interactions on FDisp across different treatment periods at both upstream and downstream positions.

Predictors	Mean	Est. Error	2.5%	95%	Bulk_ESS	Rhat
Intercept	0.06	0.01	0.04	0.09	2614	1
ALAN	0	0.02	-0.03	0.04	2505	1
Crayfish	0.01	0.02	-0.02	0.05	2029	1
Crayfish + ALAN	0.02	0.02	-0.02	0.06	2133	1
After treatment	0.02	0.02	-0.02	0.05	1955	1
<b>Downstream</b>	<b>-0.05</b>	<b>0.02</b>	<b>-0.08</b>	<b>-0.02</b>	<b>2293</b>	<b>1</b>
ALAN x After	0.02	0.02	-0.02	0.07	2852	1
Crayfish x After	-0.01	0.02	-0.05	0.03	2665	1
(Crayfish + ALAN) x After	0	0.02	-0.04	0.05	2366	1
ALAN x Down.	-0.01	0.02	-0.05	0.03	2803	1
Crayfish x Down	-0.01	0.02	-0.05	0.03	2846	1
(Crayfish + ALAN) x Down	-0.01	0.02	-0.05	0.03	3012	1
After x Down.	-0.03	0.02	-0.07	0.01	2232	1
ALAN x After x Down.	-0.01	0.03	-0.06	0.04	3113	1
Crayfish x After x Down.	0.03	0.03	-0.03	0.08	3038	1
(Crayfish + ALAN) x After x Down.	0	0.03	-0.05	0.05	2566	1

Notes: The estimates or mean, estimated error (Est. Error), lower 95% credible intervals (2.5%), upper 95% credible intervals (95%) of posterior coefficient estimates are presented, as well as effective sample size (Bulk\_ESS) and the R-hat measure of parameter convergence. Effect was considered as significant if a 95% credible intervals (CI) for a parameter estimate does not include zero. Significant credible intervals presented here in boldface font.

**Table S 3.** Results from the Bayesian model, assessing the effects of different stressors and their interaction on FEve over different treatment periods at both upstream and downstream positions.

Predictors	Mean	Est. Error	2.5%	95%	Bulk_ESS	Rhat
Intercept	-1.06	0.23	-1.52	-0.63	5708	1
ALAN	0.18	0.19	-0.18	0.55	6654	1
Crayfish	0.24	0.19	-0.13	0.61	7007	1
Crayfish + ALAN	0.26	0.18	-0.10	0.63	6121	1
After treatment	-0.08	0.17	-0.41	0.26	6793	1
Downstream	-0.07	0.17	-0.41	0.28	6623	1
ALAN x After	0.15	0.19	-0.21	0.53	7664	1
Crayfish x After	0.26	0.19	-0.13	0.63	7547	1
(Crayfish + ALAN) x After	0.11	0.19	-0.26	0.49	6245	1
ALAN x Down.	0.14	0.19	-0.24	0.52	6796	1
Crayfish x Down	0.21	0.20	-0.18	0.61	7886	1
(Crayfish + ALAN) x Down	0.17	0.19	-0.20	0.54	7358	1
After x Down.	-0.19	0.18	-0.54	0.16	7237	1
ALAN x After x Down.	0.16	0.20	-0.24	0.55	8079	1
Crayfish x After x Down.	0.22	0.20	-0.17	0.60	8293	1
(Crayfish + ALAN) x After x Down.	0.09	0.19	-0.30	0.47	7160	1

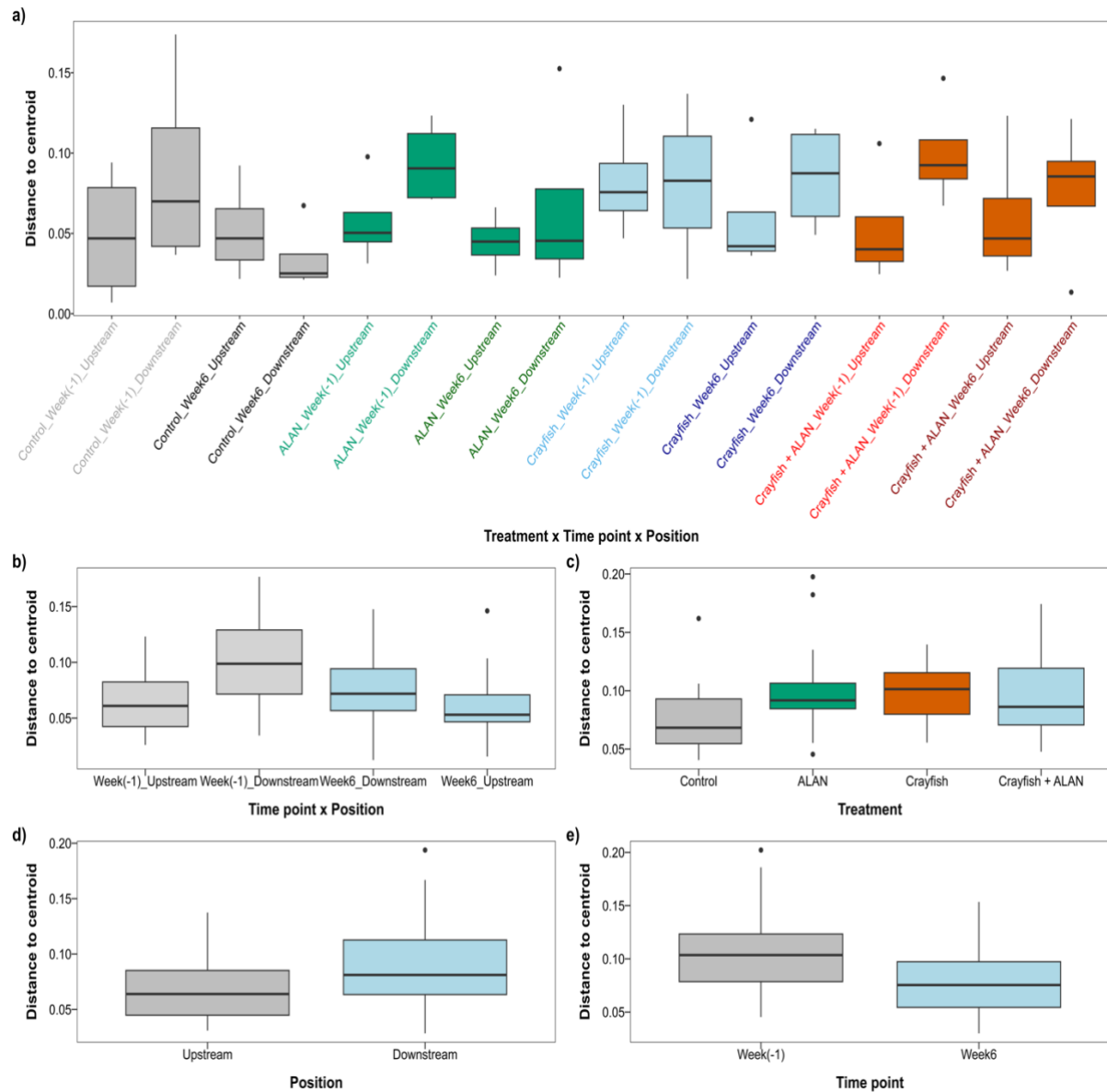
Notes: The estimates or mean, estimated error (Est. Error), lower 95% credible intervals (2.5%), upper 95% credible intervals (95%) of posterior coefficient estimates are presented, as well as effective sample size (Bulk\_ESS) and the R-hat measure of parameter convergence. Effect was considered as significant if a 95% credible intervals for a parameter estimate does not include zero.

**Table S 4.** Results from the Bayesian model summary, calculating the effects of different stressors and their interaction on RaoQ over different treatment periods at both upstream and downstream positions.

Predictors	Mean	Est. Error	2.5%	95%	Bulk_ESS	Rhat
Intercept	0.19	0.04	0.10	0.27	2119	1
ALAN	0.02	0.06	-0.10	0.13	2313	1
Crayfish	0.03	0.06	-0.09	0.15	2048	1
Crayfish + ALAN	0.07	0.06	-0.05	0.19	1754	1
After treatment	0.05	0.05	-0.05	0.15	1589	1
<b>Downstream</b>	<b>-0.13</b>	<b>0.05</b>	<b>-0.23</b>	<b>-0.04</b>	<b>2118</b>	<b>1</b>
ALAN x After	0.07	0.07	-0.06	0.21	2697	1
Crayfish x After	-0.04	0.07	-0.17	0.09	2963	1
(Crayfish + ALAN) x After	0.01	0.07	-0.13	0.13	2342	1
ALAN x Down.	-0.02	0.07	-0.15	0.10	2682	1
Crayfish x Down	-0.02	0.07	-0.14	0.11	2600	1
(Crayfish + ALAN) x Down	-0.02	0.06	-0.15	0.10	2489	1
After x Down.	-0.09	0.06	-0.21	0.04	1772	1
ALAN x After x Down.	-0.03	0.08	-0.19	0.14	2748	1
Crayfish x After x Down.	0.08	0.08	-0.09	0.24	3079	1
(Crayfish + ALAN) x After x Down.	-0.01	0.08	-0.16	0.16	2458	1

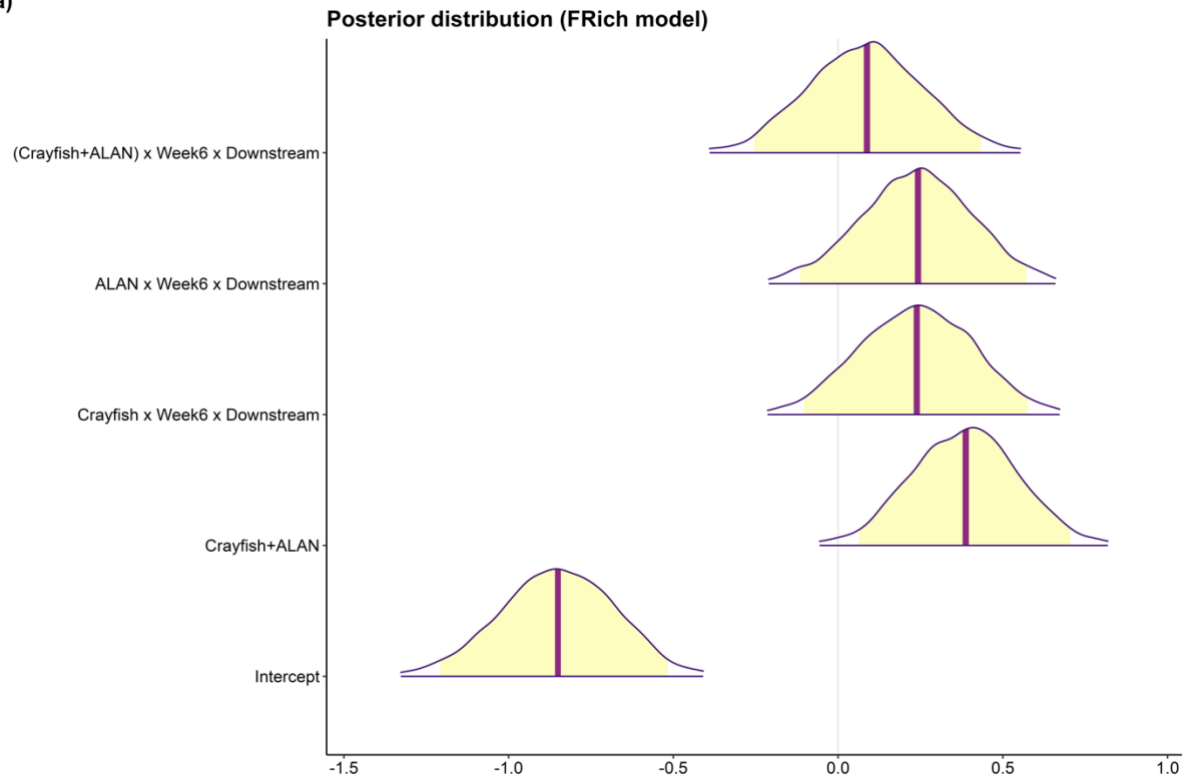
Notes: The estimates or mean, estimated error (Est. Error), lower 95% credible intervals (2.5%), upper 95% credible intervals (95%) of posterior coefficient estimates are presented, as well as effective sample size (Bulk\_ESS) and the R-hat measure of parameter convergence. Effect was considered as significant if a 95% credible intervals for a parameter estimate does not include zero. Significant credible intervals presented here in boldface font.

## 6.2 Supplementary figures

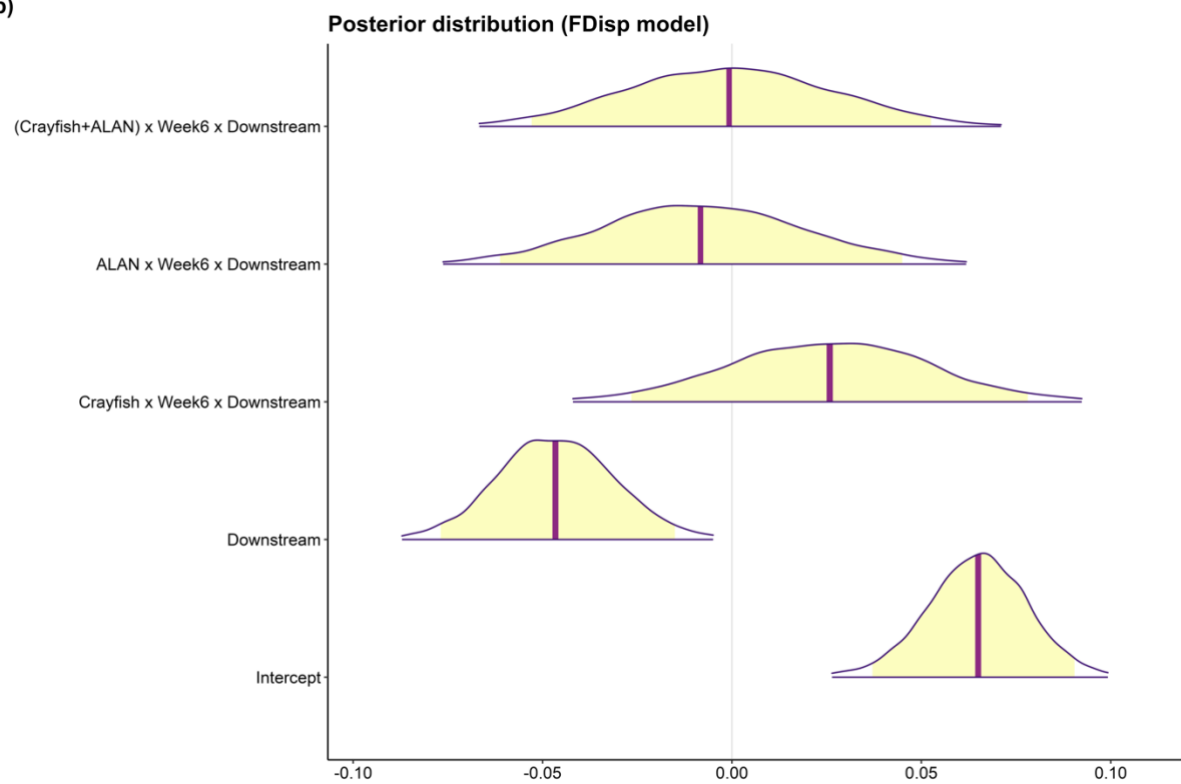


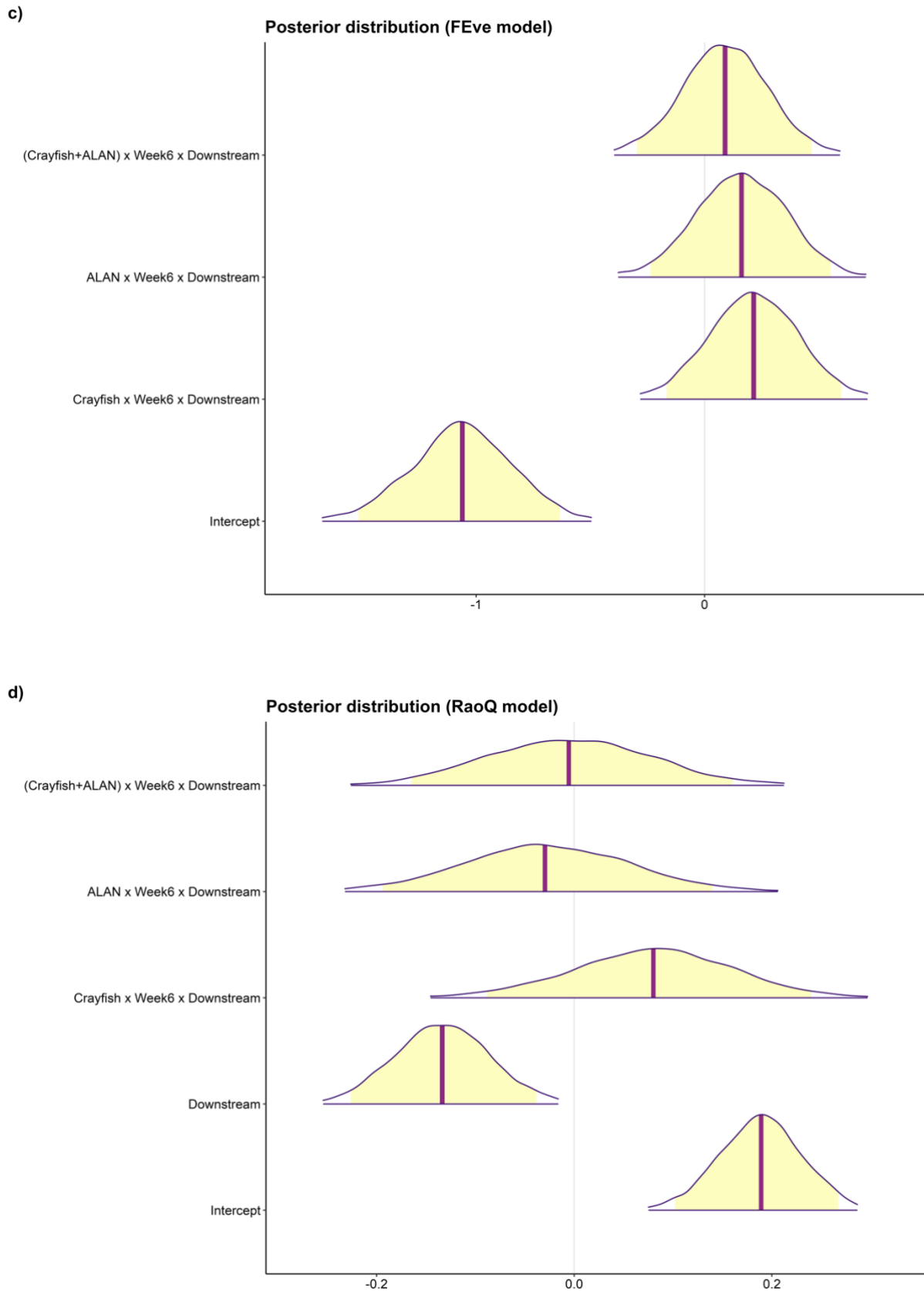
**Figure S 1.** PERMDISP results visualization. Comparing mean centroid of Gower's dissimilarity matrix for macroinvertebrate trait measured as multivariate dispersion across treatment, time point, position, and their interaction. Mean group centroid comparison of macroinvertebrate trait for each predicted factor variable: **a)** interaction including treatment; time point, and position; **b)** interaction between time point and position; **c)** Treatment; **d)** Stream position; **e)** Before-after treatment effect (time point)

a)

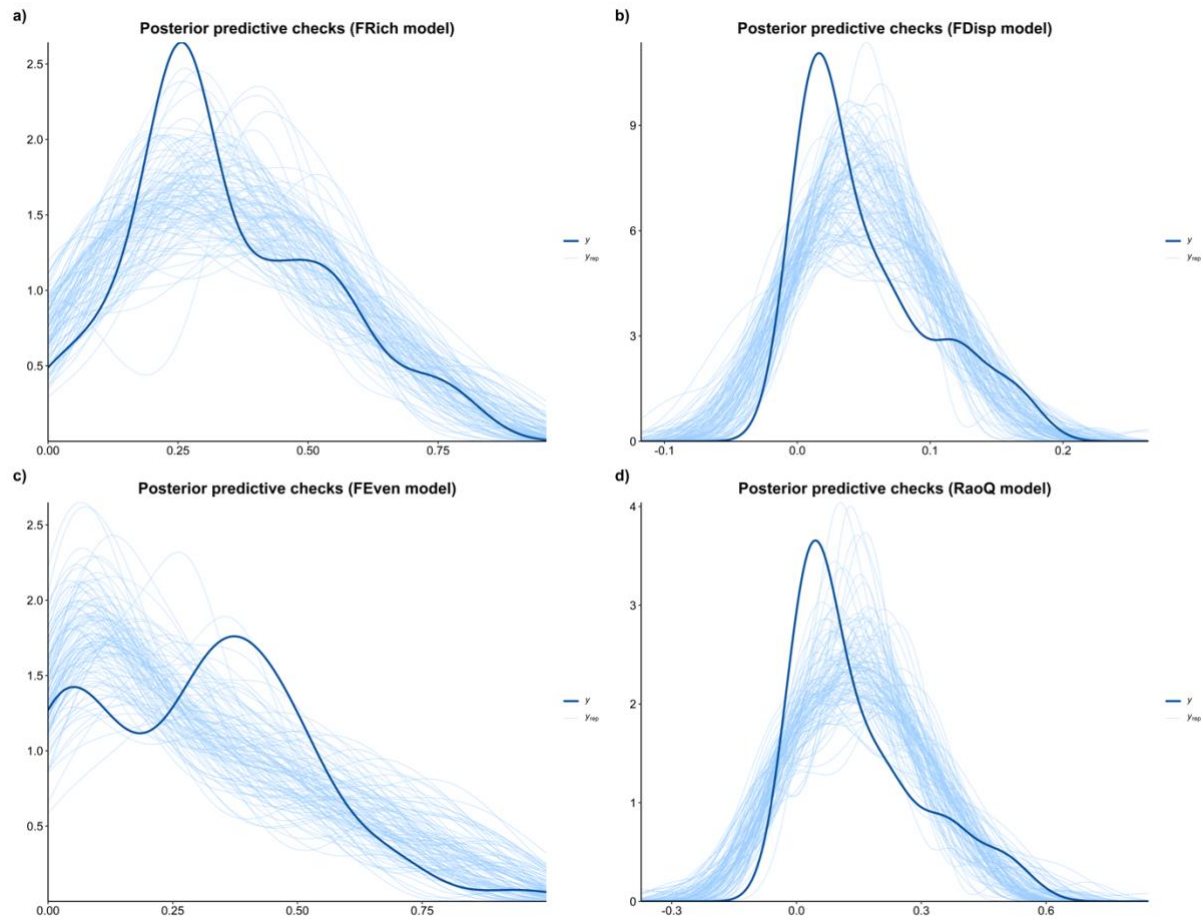


b)





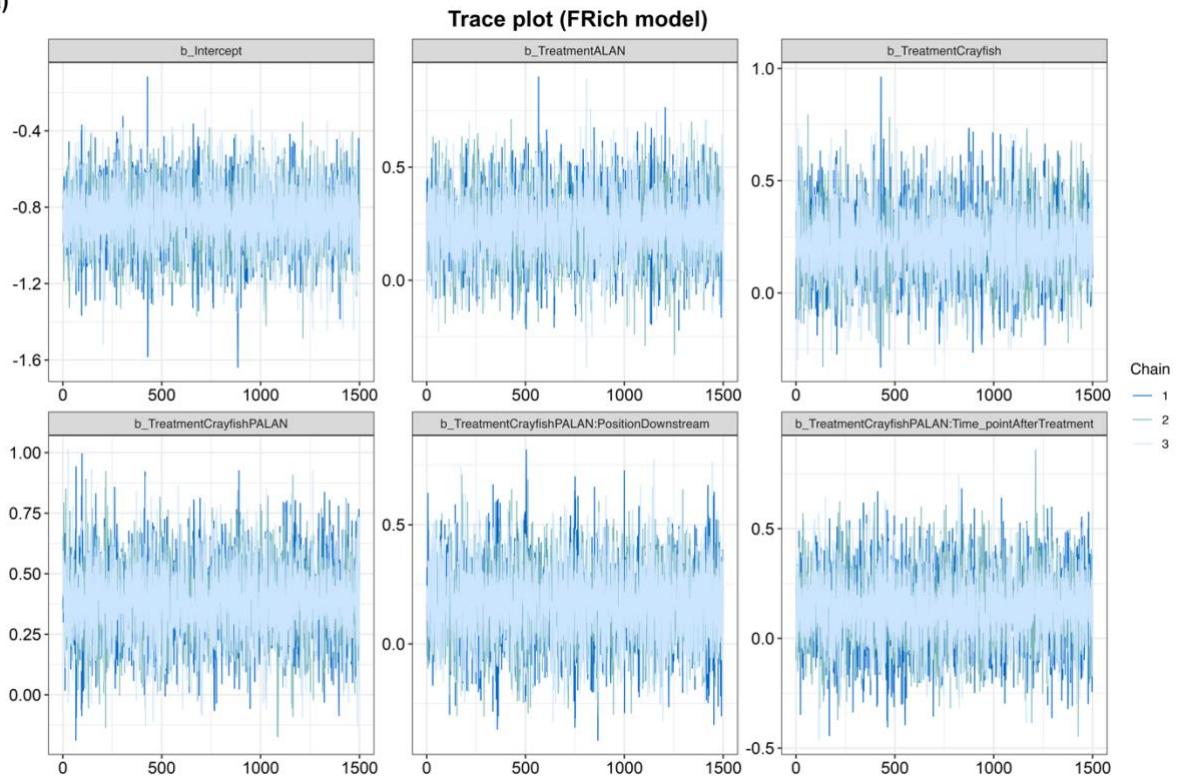
**Figure S 2.** Markov chain Monte Carlo (MCMC) draws from the posterior distribution of model parameters for all FD indices with medians and 95% credible intervals: **a)** posterior distribution for Frich model; **b)** posterior distribution for FDisp model; **c)** posterior distribution for FEve model; **d)** Posterior distribution for RaoQ entropy model. Fill shows 95% credible intervals, solid vertical lines represent medians.



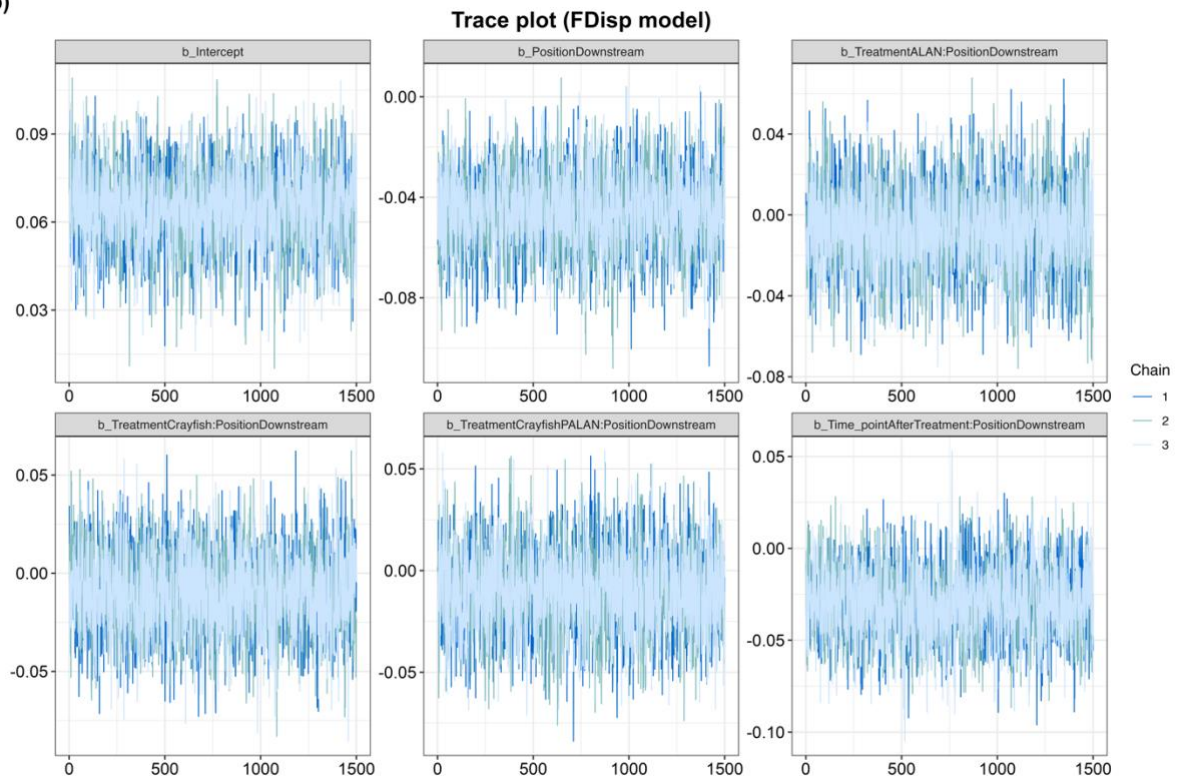
**Figure S 3.** Graphical visualization of posterior predictive check comparing the observed distribution of four FD indices (solid dark blue line or  $y$ ) to 100 simulated datasets from the posterior predictive distribution (light blue line or  $y_{rep}$ ): **a)** posterior predictive check of FRich model; **b)** posterior predictive check of FDisp model; **c)** posterior predictive check of FEve model; and **d)** posterior predictive check of RaoQ entropy model.

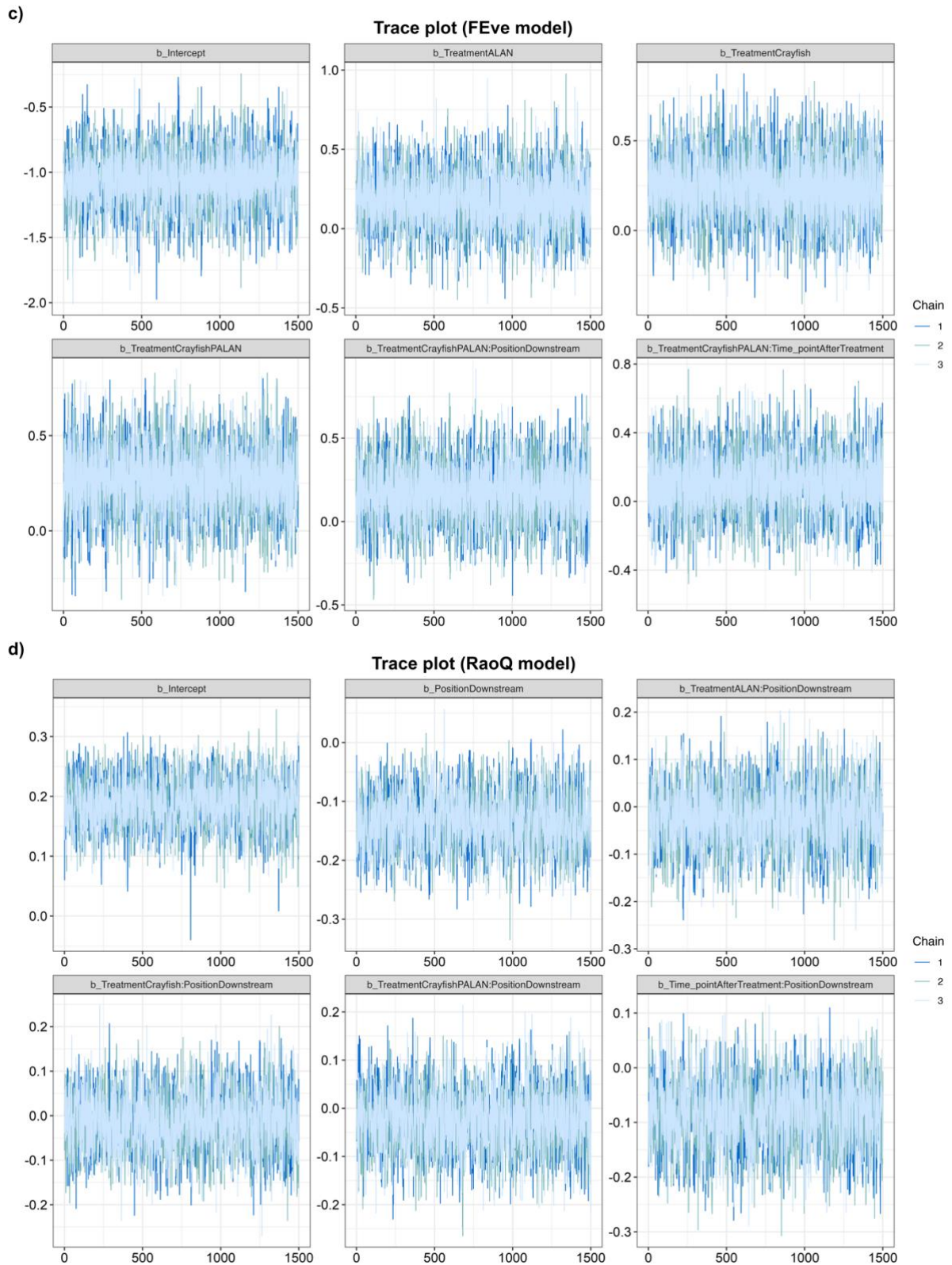


a)

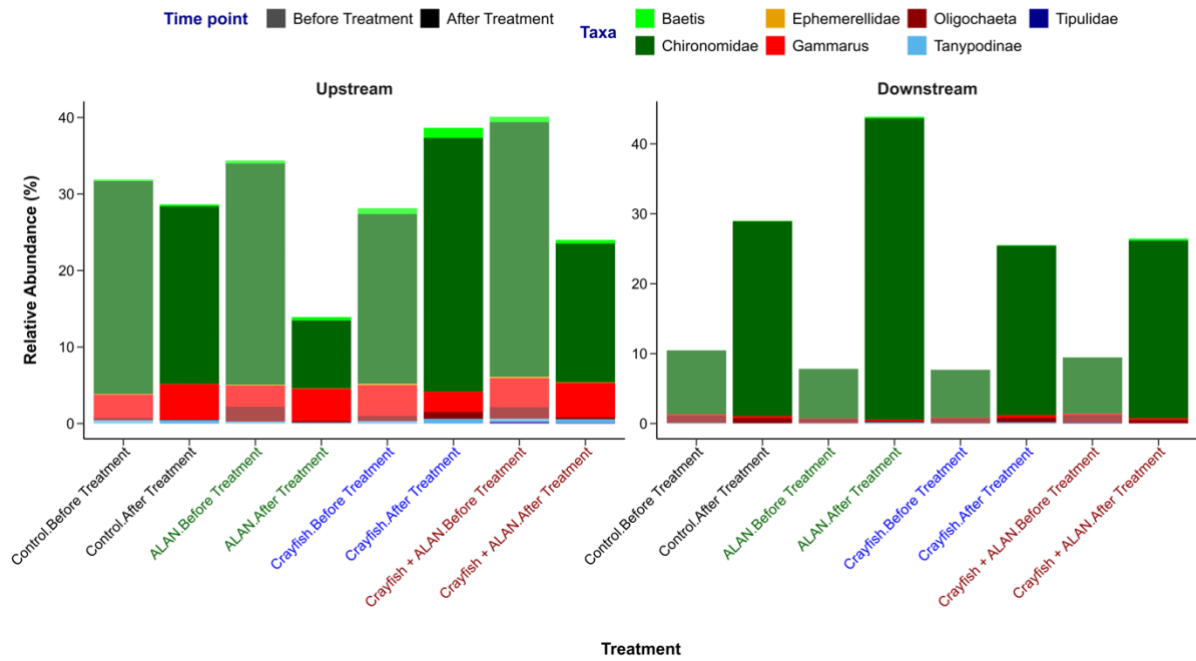


b)





**Figure S 4.** Post-warmup trace plot for the parameters of the Bayesian linear mixed-effect models estimated in the FRich (a), FDisp (b), FEve (c), and RaoQ (d) model across different treatments over two different time periods at both upstream and downstream positions. The three chains for each model seem to exhibit high similarity with some minor random fluctuations, suggesting robust convergence. The horizontal axis of the posterior parameter trace plot represents iterations during initialization of the MCMC procedures while the vertical axis depicts corresponding parameter values. It is important to highlight that only 1500 post-warmup iterations per chain are utilized in each FD's index model to optimize visualization while ensuring convergence and reliable parameter estimation.



**Figure S 5.** Relative abundance (%) bar chart of the seven most abundant species (each with an total abundance greater than 70, across the whole study site) across different treatments at two different time points at upstream and downstream positions. The x-axis text colors represents different treatments: black for Control, green for ALAN, blue for Crayfish, and red for the Crayfish and ALAN interaction. The transparency of the stacked bar reflects the time point factors, with lighter tones indicating the before-treatment effects (Week -1) and darker tones indicating the after-treatment effects (Week 6).