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The impact of macrophyte removal on macroinvertebrates in the river Otra, Norway

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Master of Science in Animal biology

Preface

This thesis concludes my Master's Degree in Animal Biology at the Norwegian University of Life Sciences. This has been a weird year with the pandemic, and I am therefore extra grateful for having had this opportunity to conduct this study with the fieldwork and laboratory time that was necessary.

I would first like to thank my main supervisor Susanne Claudia Schneider and co-supervisor Kirstine Thiemer for supporting and guiding me all the way through. Thank you so much for your time and valuable input. I have very much appreciated and enjoyed being a small part of the MadMacs team. The fieldwork could not have been done without my supervisors and the help from Benoit Demars as well as my co-students, Astrid Torske and Emmanuel Bergan. Thank you for making the experience of the fieldwork enjoyable, exciting and informative.

Secondly, I would like to thank the Norwegian Institute for Water Research (NIVA) for allowing me to use their laboratory facilities and for providing the necessary equipment. A big thank you to Joanna Lynn Kemp for teaching me with great patience how to identify macroinvertebrates.

Financial support from NIVA and NMBU made it possible for me to conduct this thesis.

Thank you to Benjamin Misteli for giving me valuable input during the last part of my thesis.

Finally, thank you to my family and friends for being so supportive always and forever. I dedicate this thesis to my dad who was always my biggest supporter.

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Abstract

Aquatic macrophytes provide complex ecosystems in the freshwaters around the world. Sometimes, macrophytes can grow to very high biomasses and restrict recreational activities in which it starts being perceived as nuisance growth. Management often solves this by partially or fully removing the macrophytes. However, the full effect of macrophyte removal on the ecosystem is not fully known and often contradictive. One important feature of macrophytes is the high biodiversity of macroinvertebrates they can contain. Removing the macrophytes could potentially have negative impacts on the macroinvertebrate community inhabiting such an area. In this thesis a BACI design is used to investigate how macroinvertebrates are influenced by macrophyte removal in the oligotrophic river Otra, Norway. The river experience high *Juncus bulbosus* biomasses which prohibit human activities and is therefore periodically and partially removed with no knowledge of how it affects the macroinvertebrate community. Therefore, three sampling methods were used to randomly collect macroinvertebrates inhabiting the sediment, macrophytes and drift before, one week after and six weeks after macrophyte removal. Macroinvertebrate community composition had a shift after macrophyte removal. However, macrophyte removal did not affect macroinvertebrate density, diversity or taxa richness. The results of this study indicate that the management of macrophytes in the river Otra does not have a catastrophic influence on its macroinvertebrate community. In the future, how macrophyte removal affects the macroinvertebrate community in the long-term needs be investigated further. This will be important to preserve the biodiversity of the river.

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1. Introduction

1.1 Macrophytes as ecosystem engineers

Macrophytes are often the main primary producers in freshwater ecosystems (Mohamed, 2017; Su et al., 2019). They range from vascular plants to macroalgae that are fully or partly submerged in rivers, lakes and reservoirs and they can be seen with the naked eye (Rørslett & Johansen, 1996). Despite being primary producers, they provide habitats (Warfe & Barmuta, 2006; Mohamed, 2017), micro-environments (Warfe & Barmuta, 2006; Lusardi et al., 2018), good water quality (Verhofstad, 2017), high biodiversity (Kuiper et al., 2017), food for different organisms (Kaenel et al., 1998) and compete with cyanobacteria (Mohamed, 2017). Therefore, macrophytes are often used for monitoring ecosystem health (Rodrigues et al., 2019a). Macrophyte density and plant architecture have been related to macroinvertebrate abundance and richness, where higher macrophyte densities and complex plant architecture carry higher macroinvertebrate abundances and richness (Warfe & Barmuta, 2006; Scrine et al., 2017).

1.2 Macroinvertebrate importance

Macroinvertebrates are an important part of freshwater ecosystems. They can live their whole lifecycle under water or have their larval/nymphal stages in water and depart from the water in their adult life (Velle et al., 2017). Macroinvertebrates can also be semi-aquatic, where they spend time in both water and on land. Some examples of macroinvertebrates are aquatic or semi-aquatic insects, aquatic worms, snails, clams, amphipods and leeches. In rivers, they are part of the nutrient cycle, decomposition of decay and translocation of sediments in their ecosystem (Wallace & Webster, 1996; Zou et al., 2019). They are also important as they are a food source for several fish species (Wallace & Webster, 1996; Zou et al., 2019). Different macroinvertebrate species all have different tolerance levels for acidification, anthropogenic disturbances and natural disturbances (Schartau et al., 2008; Schneider & Petrin, 2017; Scrine et al., 2017). Therefore, macroinvertebrate diversity, density, richness and abundance are very well studied due to their ability to indicate the water quality of freshwater ecosystems (Buczyński et al., 2016; Rodrigues, et al., 2019b).

Macroinvertebrates inhabit different parts of freshwater ecosystems (Usseglio-Polatera et al., 2000; Beauger et al., 2006). Some live in or on the sediment, some live on macrophytes and others drift in the water column. Drifting invertebrates are important for dispersal and colonizing new areas to maintain their population or new suitable habitats (Baxter et al., 2017).

Drift is defined as either accidental or active movement to the open water where the macroinvertebrates are transported downstream (Brittain & Eikeland, 1988; Baxter et al., 2017). Drift is also something some aquatic insects go through as they go from an aquatic larval/nymphal/pupal stage into air-breathing adults with wings emerging from the water column (Brittain & Eikeland, 1988; Baxter et al., 2017). Macroinvertebrates who drift are the most vulnerable to predation as they do not have sheltering opportunities and therefore become available to aquatic predators as well as terrestrial predators such as birds, spiders and bats (Baxter et al., 2017). Drifting macroinvertebrates might start to drift from habitats in or on the sediment or from macrophytes. Depending on the sediment type (gravel, silt, sand, mud, organic material), it provides habitats with different foods and sheltering opportunities (Usseglio-Polatera et al., 2000). Whereas macrophytes provide an environment with lower velocity, hiding places from predators and food for macroinvertebrates (Thomas & Daldorph, 1991; Watson & Barmuta, 2011; Saulino & Trivinho-Strixino, 2018).

1.3 When macrophytes become a problem

Sometimes macrophytes can produce very high biomasses which will decrease river flow as well as opportunities for people to enjoy different recreations such as boating (Kuiper et al., 2017; Verhofstad, 2017). These mass developments of macrophytes are often dominated by one or two species of macrophytes and their growth could lead the ecosystem to become anoxic at times (Verhofstad, 2017). Mass developments of macrophytes are often caused by human disturbances such as hydropower development and eutrophication, and are generally seen in eutrophic rivers and lakes (Velle et. al, 2021). When such outgrowths happen, people often start to look at the massive macrophyte stands as a nuisance, and methods for their removal have been implemented to reduce the vegetation (Schneider et al., 2013; Verhofstad et al., 2017; Velle et al., 2021). However, dense macrophytes do not only cause problems as they also provide good water quality and higher faunal densities (Su et al., 2019; Velle et al., 2021).

In Northern Europe, the macrophyte *Juncus bulbosus* (L.) (Bulbous rush) can grow rapidly and extensively (Moe et al., 2012). *J. bulbosus* is a grass-like macrophyte that thrives in acidic environments (Brandrud & Roelofs, 1995; Rørslett & Johansen, 1996; Schneider et al., 2013). Massive Bulbous development has been observed to occur in limed sediments with a high concentration of CO_2 in the water layer (Lucassen et al., 1999; Brandrud, 2002). NH_4^+ (ammonium) and P (phosphorus) have also been related to enhancing *J. bulbosus* growth (Schneider et al., 2013; Schneider & Demars, 2020). In Norway, the mass development of *J.*

bulbosus is also found in oligotrophic waterbodies. One of these waterbodies is the river Otra, Norway (Rørslett & Johansen, 1996; Dalen, 2019), where *J. bulbosus* is especially a problem in certain parts of the river (Velle et al., 2019; Schneider & Demars, 2020). The macrophyte limits the recreational use of the river as it is very dense in some areas and stop water intake in hydropower plants (Dalen, 2019; Velle et al., 2019). Due to the mass developing macrophytes' many negative effects on the anthropogenic uses of the river, it is often decided to remove and reduce the macrophyte. There are many ways to remove and reduce macrophytes (Verhofstad, 2017). All of them disturb the ecosystem somewhat (Rørslett & Johansen, 1996). However, the question is how much the removal of macrophytes disturb the surrounding environment and its living organisms.

1.4 Removal of macrophytes

There are various methods to remove macrophytes; mechanical, chemical, biological and manual (Rørslett & Johansen, 1996; Aldridge, 2000). Chemical removal methods (i.e., herbicides) have been effective (Aldridge, 2000; Laughton et al., 2007); however, they are not legal in most countries anymore, and therefore rarely used. Biological removal, such as releasing grass carps (*Ctenopharyngodon idella*) or control agents can be a good method to keep macrophyte growth down (Rørslett & Johansen, 1996; Aldridge, 2000), but the grass carp should be native to the area if implemented for this purpose. Manual removal methods (by wading and/or diving) such as pulling out or cutting the vegetation manually have been used (Laughton et al., 2007; Bickel & Closs, 2009) as well as scythes (Rasmussen et al., 2021). These are often small scale and combined with other methods (Bickel & Closs, 2009). Mechanical methods are the most used as they provide good results of decreasing macrophyte biomass, and although this method is expensive, it is thought to be the most cost-effective (Bączyk et al., 2018). There are several ways mechanical macrophyte removal affects the ecosystem. It increases turbidity and disturbs the sediment mostly for a short amount of time (Rørslett & Johansen, 1996). The removed and loose macrophyte and sediment might not get fully collected and therefore flow downstream and affect the downstream ecosystem (Aldridge, 2000).

Mechanical methods that are most often used is dredging (Płaska et al., 2016) and mowing machines on boats or vehicles (Aldridge, 2000). Dredging is used to decrease macrophytes and remove sediment (Płaska et al., 2016; Zawal et al., 2016). Different weed boats and mowing machines are used for macrophyte removal which cuts the submerged vegetation (Aldridge,

2000). In the river Otra, Norway, several macrophyte removal methods have been tested to remove *J. bulbosus*, such as manipulation of water flow by hydropower plants (winter drawdowns and flushing flows), mowing and dredging (Johansen, 2002). These methods have shown to be effective at removing the macrophyte (Johansen, 2002). However, the removal is expensive and cause trouble downstream as the cut *J. bulbosus* biomass ends up in downstream areas and cause mass development there. *J. bulbosus* also seems to keep regrowing quickly (Vegge & Haraldstad, 2006). Therefore, removal practices are kept up when the vegetation grows back to its nuisance growth (Vegge & Haralstad, 2006). These practices are now mainly with mechanical mowing machines as they are effective in the short term although expensive (Johansen, 2002). Waterflow manipulation is difficult to conduct as it depends on the hydropower plants to possibly lose income and could lead to technical difficulties (Johansen, 2002).

1.5 The effects of macrophyte removal on macroinvertebrates

Macroinvertebrates are likely to be affected by macrophyte removal as habitats are temporarily disturbed until the regrowth of the plants. The impact of macrophyte removal on macroinvertebrates has been studied in several waterbodies around the world (Thomas & Daldorph, 1991; Kaenel et al., 1998; Aldridge, 2000; Miliša et al., 2006; Laughton et al., 2007; Bickel & Closs, 2009; Habib & Yousuf, 2014; Buczyński et al., 2016; Płaska et al., 2016; Zawal et al., 2016; Carey et al., 2017; Ward-Campbell et al., 2017; Lusardi et al., 2018). However, how macroinvertebrate communities respond to macrophyte removal are highly variable and there is still uncertainty on how macrophyte removal affects macroinvertebrates in different waterbodies. Previous studies have looked at several parameters describing the macroinvertebrate community, such as density, diversity (Shannon-Wiener index, Hurlbert index and Simpson's reciprocal diversity), taxa richness and assemblage with either a before-after (BA) design, control-impact (CI) design or a before-after-control-impact (BACI) design. Macroinvertebrate sampling also varied among the studies, where some sampling methods were taxa specific (Thomas & Daldorph, 1991; Aldridge, 2000; Laughton et al., 2007; Buczyński et al., 2016; Płaska et al., 2016; Zawal et al., 2016), others sampled within the macrophytes (Bickel & Closs, 2009; Habib & Yousuf, 2014; Carey et al., 2017) as well as in the sediment (Kaenel et al., 1998; Miliša et al., 2006; Ward-Campbell et al., 2017; Lusardi et al., 2018). Only Lusardi et al. (2018) have conducted a study on the impact of macrophyte removal on macroinvertebrates drifting. Today, no studies have evaluated the effects of macrophyte removal on macroinvertebrate communities in an oligotrophic Nordic river.

Therefore, in this study, I investigated the effects of macrophyte removal on macroinvertebrates in the oligotrophic river Otra, in Norway. Here a BACI design was used to investigate the impact of removing the macrophyte *J. bulbosus* on the macroinvertebrate density, diversity, richness and composition. The macrophyte removal method used in this study area was a mechanical mowing machine and harrow to decrease *J. bulbosus* biomass as much as possible. As macroinvertebrates inhabit different areas, macroinvertebrate samples were collected from the sediment and the macrophytes. Samples of drifting invertebrates were also collected as it was expected to represent dislodged or emigrating macroinvertebrates from the sediment and the macrophytes.

1.6 Research aims and hypothesis

This study aimed to determine 1) the immediate effects of mowing *J. bulbosus* on the macroinvertebrate density, diversity and composition and 2) how the macroinvertebrate density, diversity and composition was affected 6 weeks after the mowing of *J. bulbosus*.

I hypothesize that the cutting of *J. bulbosus* 1) will not influence taxa richness but affect macroinvertebrate density, diversity negatively and change the taxa composition associated with macrophytes, 2) will not influence taxa richness or macroinvertebrate density but affect diversity negatively and also change the taxa composition in/on the sediment, and 3) will positively affect density, diversity and taxa richness of drifting macroinvertebrates in both the surface drifters and sediment drifters. I also expected a change in the taxa composition of the drifting macroinvertebrates.

2. Methods

2.1 Study area

The river Otra is 245km long in Agder county, southern Norway. The riverhead is within Vinje municipality in Telemark county near the border of Agder county and runs through the municipalities; Bykle, Valle, Bygland, Evje, Hornnes, Iveland, Vennesla and has its outlet in the east harbour of Kristiansand into the North Sea. The southern part of the river from Vatnedalen in Bykle municipality consists of mainly gneiss and granite, with deciduous and coniferous woodlands while the northern part from Vatnedalen is dominated with metamorphic and sedimentary rock which buffers acid rain and has a vegetation of birch and moor (Dalen, 2019; Heggstad & Thorsnæs, 2020). It drains about 4000 km^2 of water from its surrounding

environment (Wright et al., 2017). The river is impacted by acid rain, hydropower regulation, agricultural runoff, urban pollution and increasingly by climate change (Dalen, 2019). In 1964, modifications of the hydropower development in the upper part of Otra started with several dams and tunnels to collect water from its many tributaries (Wright et al., 2017). The collection of water is stored in several deep basins (Wright et al., 2017). The study site for this thesis, the Rysstad basin (59°05'19.1"N 7°33'00.5"E; Fig. 1), is between two hydropower plants, upstream; Brokke hydropower plant and downstream; Hekni hydropower plant.

Rysstad basin is by the small village Rysstad in Valle municipality. The basin is approximately 4km downstream from Brokke hydropower plant and is the water intake of Hekni hydropower plant. Due to the hydropower plants, this reach is relatively slow running (Schneider & Demars, 2020). In this locality, brown trout (*Salmo trutta*) can be found. The water levels are depending on the output from Brokke and the intake of Hekni. Minimum waterflow during summer is 5m^3/s and in winter 2m^3/s (Otra Kraft DA, n.d.). At this study area, pH, conductivity and temperature were stable throughout the study time (Table 1). The riverbed in the research area consists mainly of fine sediment where there is no vegetation. Water depth had some variation between sites and time. The control site had an average water depth of 129cm±35.92 before macrophyte removal, 198.48cm±37.70 1 week after macrophyte removal, and 172.09cm±30.56 6 weeks after macrophyte removal. In the impact site, there was an average water depth of 142.34cm±42.38 before macrophyte removal, 194.28cm±41.86 1 week after macrophyte removal, and 178.56cm±38.97 6 weeks after macrophyte removal (unpublished data measured in June 2020). The control and impact site was approximately 500m x 60m each, respectively.

The study area is used for several recreational purposes such as boating, fishing and swimming, there is also a camping site and hotel and has high recreational value (Schneider & Demars, 2020). The macrophyte, *J. bulbosus*, covers large areas and creates almost a blanket in this area. It is therefore viewed as a nuisance as it makes it difficult to conduct recreational activities. Due to that, the vegetation is disked and removed often (about every 2-3 years) by Valle municipality and funded by KPS ("Krypsivprosjektet på Sørlandet"). Removal measures cost about 2 800 000 NOK annually (*Juncus bulbosus in the Otra river (Norway)*, 2020). Valle municipality and KPS agreed on cutting a set area in the timeframe of this study, meaning in the middle of June. The area that will be cleared of macrophytes is ≥ 1000 m^2, to enable fishing

from land for the local community and tourists. The mowing decreased *J. bulbosus* biomass but did not remove all the vegetation close to the sediment (Fig. 2).

Table 1. Environmental parameters at the study area (unpublished data measured in June 2020).

	Measurements	Average	S.D.	Median
Conductivity (µS)	48	8.1	0.5	7.9
pH	48	6.1	0.3	6.1
Temperature (°C)	48	9.2	2.1	9.5

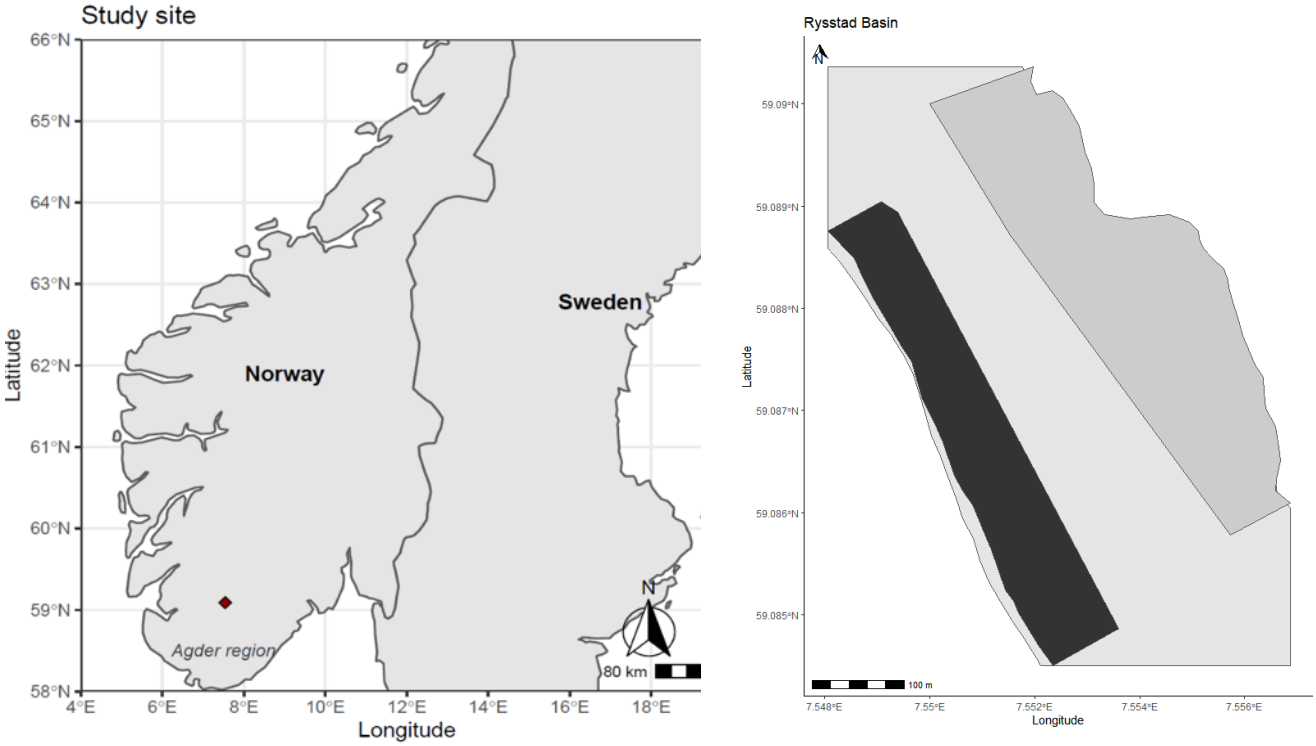

Figure 1. Map of study site and location of impact and control site in the Rysstad Basin. Dark grey = impact site, and light grey = control site. (Courtesy of K. Thiemer).

2.2 *Juncus bulbosus*

J. bulbosus is a grass-like macrophyte and is very polymorphic (Brandrud & Roelofs, 1995). The macrophyte species belongs to the family Juncaceae and is often to be found in

oligotrophic rivers and lakes in Europe and North America (Vellet et al., 2021). Normally, the buds of *J. bulbosus* are around 10-20cm long, although with the right environmental conditions it can grow to 3m long in very high biomasses which is when it is perceived as a nuisance (Velle et al., 2021). In northern Europe, there are several cases where it is reported to be growing in mass developments (Moe et al., 2012), and the river Otra is one of those rivers that experience these mass developments. *J. bulbosus* is part of the rivers natural vegetation and has probably occurred in moderate concentrations before river regulations were implemented (Schneider & Demars, 2020). The vegetation was first reported as a problem after hydropower plants were built (Schneider & Demars, 2020), and in the 1980s, Rørslett (1987) found that ~55% of the riverbed in the Rysstad basin was covered in *J. bulbosus.* Today *J. bulbosus* covers around ~80% (Velle et al., 2019).

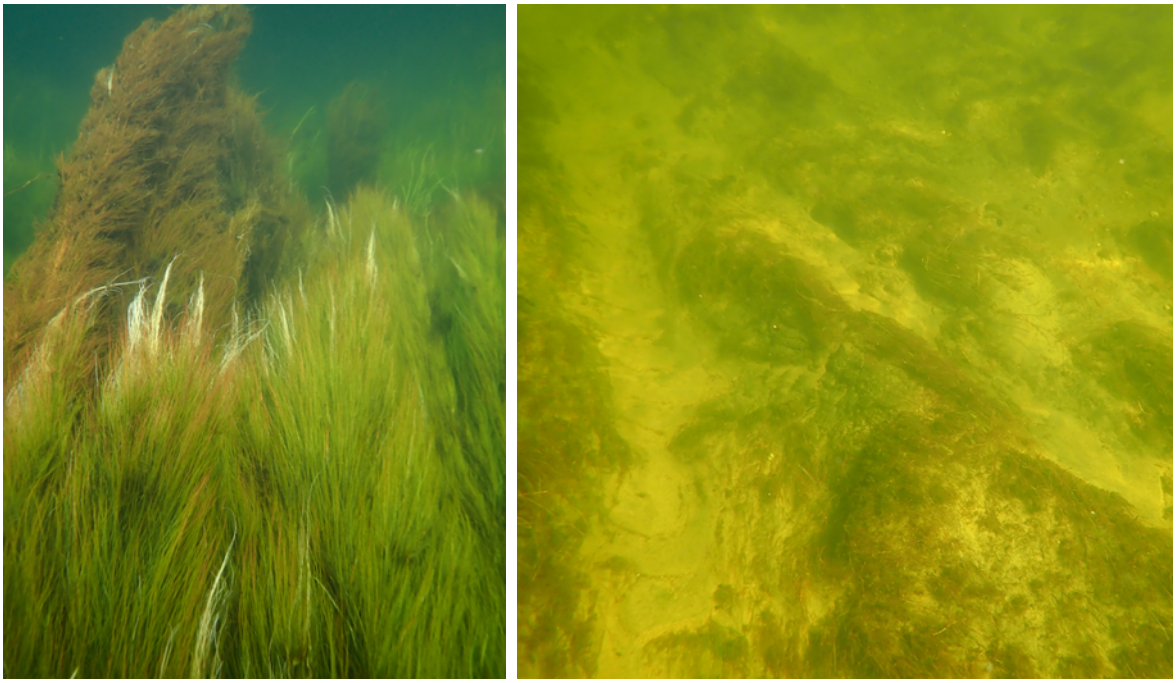

Figure 2. J. bulbosus in the river Otra, before (left) and after removal (right) (Courtesy of K. Thiemer).

2.3 Macroinvertebrate sampling

Different sampling methods for macroinvertebrates were used to get a complete representation of the macroinvertebrate communities that live on macrophytes, in/on sediment and drift downstream. All sampling followed a BACI design where samples were collected one week before macrophyte removal, right after macrophyte removal and six weeks after macrophyte removal. In the result section, the macroinvertebrates sampled the day after macrophyte

removal will be addressed as after- or 1 week after macrophyte removal. Samples were collected in June and August (for more details, see Table 2).

Table 2. Overview of sampling scheme of macroinvertebrates (the removal of the macrophytes took place from 15th – 23rd of June 2020).

Date	Method	Site	Comments
11.06.20	Sweep & Grab	Control & Impact	One week before the removal started.
12.06 - 13.06.20	Drift nets	Control & Impact	Put out between 22:00-23:40 in the evening and retrieved between 07:36-08:19 in the morning.
23.06.20	Sweep & Grab	Control	The last day of removal, because of the amount of field work that needed to be done on the 24th it was decided to take the control site invertebrates samples on this day instead of the day after since the control site should not be impacted by the mowing.
24.06.20	Sweep & Grab	Impact	The day after the removal was finished.
24.06 - 25.06.20	Drift nets	Control & Impact	Put out between 20:19-21:30 in the evening and retrieved between 07:44-09:12 the next morning.
05.08.20	Sweep & Grab	Control & Impact	6 weeks after removal.

2.3.1 Macroinvertebrates living on macrophytes

Macroinvertebrates associated with macrophytes were collected using sweep sampling. The sweep sampling was conducted from an anchored boat with a 250µm net with a contractable handle (Fig. 3). Five replicates were taken randomly in the control and impact site at each sampling site, respectively. The macroinvertebrates were collected by sweeping the net with an up-down motion pushing towards the macrophyte (*J. bulbosus*) in a stripe of approximately 30 cm width for approximately the length of the side on the boat (4.5m) for approximately 30 seconds. The bottom of the net was carefully dipped in the water afterwards (mesh size 250

µm) to get rid of organic matter before the sample was placed in a glass container (250mL). The net was then checked and washed down with alcohol to rinse off the remains into the glass jar. The glass containers were filled with 96% alcohol and labelled.

Macroinvertebrate density on the water plants was calculated using the following equation (1):

$$Macroinvertebrate\ density_{sweep} = \frac{n}{A} = \frac{n}{1.35m^2} \quad (1)$$

Where, n = number of individuals in the sample, and A = Length*Width = (4.5m * 0.30m) = 1.35m^2.

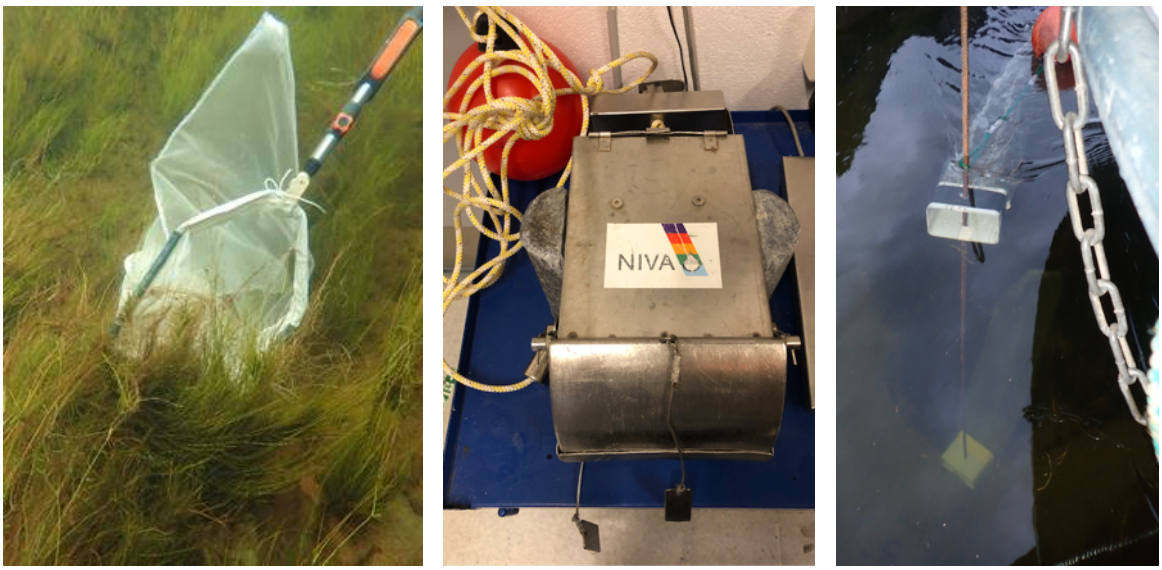

Figure 3. The equipment used for collecting macroinvertebrates. Left: Sweep net; Middle: Ekman grab; Right: drift nets in two heights.

2.3.2 Macroinvertebrates living in/on sediment

Macroinvertebrates in the sediment were collected using an Ekman Birge grab sampler (Fig. 3) due to mostly fine sediment. Five replicates were randomly taken each time and at each site. The grab samples were taken by placing the Ekman Birge grab with the opening on the sediment and shutting the opening fast. Then dragging the grabber rapidly but carefully up to the boat and placing the content of the grabber into a bucket. If a bigger stone was caught so that the "mouth" was not fully closed, the grab was placed onto another sediment patch to get a valid sample. The bucket samples were later rinsed in a 250µm net and subsampled depending on the sample size. When subsampling, the sample was homogenized and divided into two or four parts, depending on how big the sample was (Fig. 4). The subsample was then rinsed in

the net again before placing the sample in a glass jar (250mL) and filling it with 96% alcohol. The labelling was done in the same manner as with the sweep samples. The opening of the grab sampler was 15cm * 15cm, thus the sampled area was 0.0225m^2.

Macroinvertebrate density on the sediment was calculated using the following equation (2):

$$Macroinvertebrate\ density_{grab} = \frac{n}{A} = \frac{n}{0.0225m^2} \quad (2)$$

Where, n = number of individuals in the sample, and A = length*width = 0.15m*0.15m = 0.0225m^2.

Figure 4. Subsampling procedure (Left) and rinsing of grab samples in practise (Right).

2.3.3 Drifting macroinvertebrates

Macroinvertebrate drift was sampled using drift nets. Drift nets are nets designed for sampling macroinvertebrates in flowing waters in one place over a specific amount of time (Klemm et al., 2001). These samples collect macroinvertebrates actively or passively drifting in the water column, and they collect qualitative and quantitative data (Klemm et al., 2001). The drift nets were placed on a rebar, with one net for the bottom sample (250 µm) and one net for the surface sample (500µm) (Fig. 3). The 250 µm net was used to collect drifting macroinvertebrates close to the sediment, and it is less likely to be subject to clogging. Whereas the bigger 500 µm net was used to collect the surface drifters to avoid clogging. The net openings were 10 cm x 15 cm and had a hole drilled in them to get the rebar through and were fastened to the rebars using strips. Since the net opening is not that large it also helps in preventing clogging (Elliot, 1970). To fasten the drift nets in the river, the rebar was hammered down in the sediment until the

bottom net was close to the sediment surface. Altogether six rebars were constructed with 12 nets, where three rebars were randomly placed in the impact site and three randomly placed in the control site. This gave 12 samples, six samples from the bottom drifters and six of the surface drifters. Drift samples were collected one time before macrophyte removal and one time a few days after macrophyte removal, giving 24 samples for later sorting and identification. The drift nets were placed in the river in the evening and taken out in the morning. The samples were taken during the night-time due to predatory fish being present in the study area. Nocturnal drift increases with more predatory visual-feeding fish in the ecosystem (Baxter et al., 2017). Velocity was measured with an OTT MF pro (Water Flow meter) in front of each net opening in the evening and the morning. When the drift nets were taken out of the river, they were rinsed in the nets and put in separate glass jars with 96% alcohol. The samples were labelled in the same way as the sweep and grab samples.

Drift density was calculated using the following equation (3) (Baxter et al., 2017):

$$Macroinvertebrate\ density_{drift} = \frac{n * 100}{t * A * V} = \frac{100n}{t * 0.015m^2 * V} \quad (3)$$

N = number of individuals in the sample * 100, t = seconds left out in the river, A = opening of the area of drift net (length*width = 0.10m*0.15m = 0.015m^2) and V = flow velocity in m/s (Velocity plots can be found in Appendix N). Due to expecting a small number of drift density it will be expressed as drift density per 100m^3 of water.

2.4 Counting and Determination of macroinvertebrate samples

All samples were brought to a laboratory at NMBU/NIVA to determine the taxonomic composition and abundance of the collected macroinvertebrates. In the laboratory, the invertebrates were sorted out from the samples, counted and identified. For the sorting, the samples were rinsed from alcohol with a 60µm netting sheet and a sieve. The sample was further transferred from the netting sheet onto a tray to be sorted under a dissecting microscope (Fig. 5). The sorted invertebrates were placed in small glass containers with 96% alcohol. The grab samples were not subsampled when sorting the invertebrates. The sweep samples varied in size where those who filled over half the sample glass (150mL) from the study area were subsampled in the sorting tray. Small sweep samples were sorted completely, while the large samples were homogenised in the tray and divided into four equal parts. One fourth were first sorted and if time allowed another fourth was sorted. The sorting time for the subsamples was set to 5-6 hours per sample.

After the invertebrates had been sorted, they were investigated under a dissecting microscope for identification. The macroinvertebrates were identified to species level where possible. Ephemeroptera, Plecoptera and Trichoptera were identified to species where possible, however, some were too small and therefore left at genus, family or even order. Oligochaetes were left at subclass, Nematodes were left at phylum, Chironomids, Simuliidae, Ceratopogonidae and Sphaeriidae were left at family, while Chelifera and Hydra were left at genus and Hydrachnidae and Oribatei were left at subgroup and suborder, respectively. The following identification keys were used: Hubendick, 1949 (Lymnaeidae); Fitter & Manuel, 1986 (general macroinvertebrates); Lillehammer, 1988 (Plecoptera); Arnekleiv, 1995 (Ephemeroptera); Nilsson, A, 1996 (general macroinvertebrates); Raastad & Olsen, 1999 (general macroinvertebrates); Krogvold & Sand, 2008 (Ephemeroptera); Rinne & Larsen, 2017 (Trichoptera).

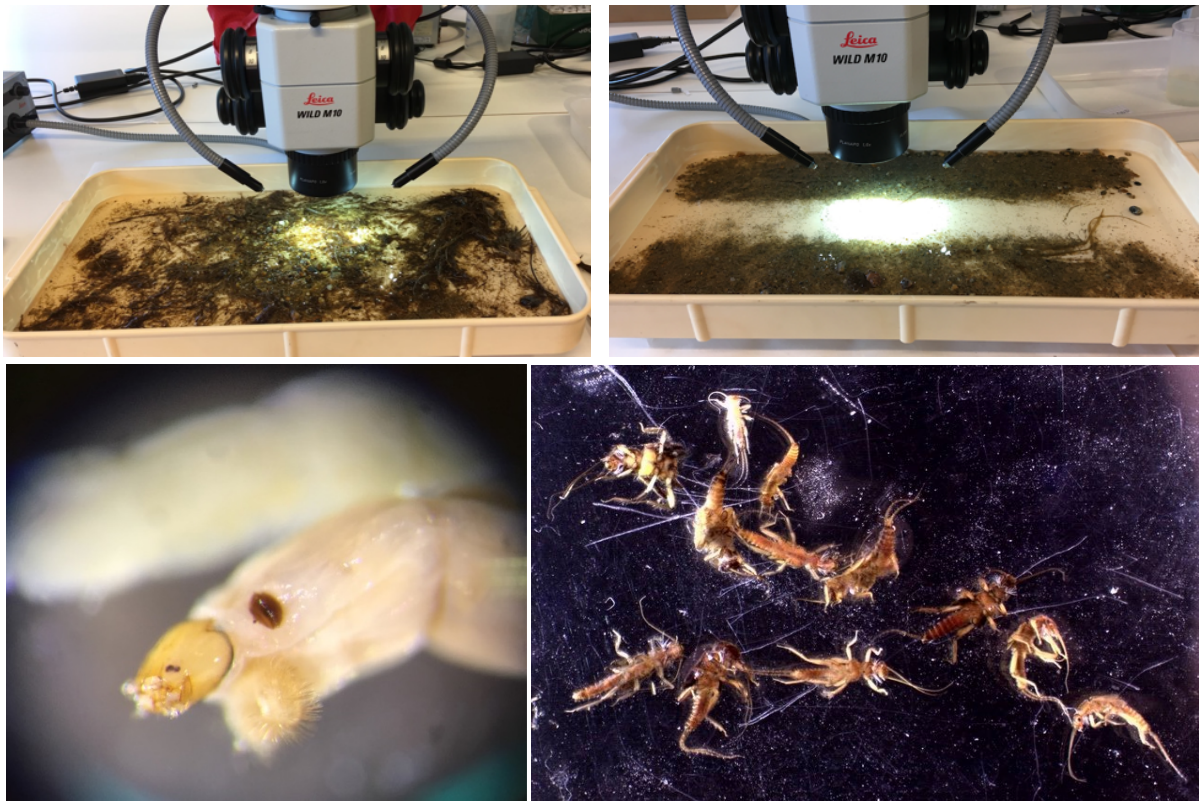

Figure 5. Sorting macroinvertebrates under a microscope (top), a chironomid (bottom left) and some Amphinemura species (bottom right) sorted from the samples.

2.5 Statistical analyses

The removal effect of *J. bulbosus* on the macroinvertebrate taxa were investigated by calculating and comparing different indices, including taxa richness, Shannon-Wiener index of diversity, Fisher alpha diversity index and Pielou's Evenness. These indices were calculated according to the following equations (4-6):

Taxa richness (S) = total number of taxa in a sample.

Fisher alpha index looks at the relationship between species richness and the number of individuals of each species. It is widely used in ecological community studies as it is not sensitive to a small amount of sample replicates. The expected number of species ($\hat{f}$) with n individuals is (Fisher et al., 1943):

$$S = a * \ln\left(1 + \frac{n}{a}\right) \quad (4)$$

Where S = number of taxa, n = number of individuals, a = Fisher's alpha

Shannon-Wiener Index ('H) takes into account the number of taxa as well as the number of individuals. A value of 0 would mean that the community only had one taxon, and higher values would mean communities containing many taxa with a few individuals (Shannon, 1948).

$$H' = -\sum\left(\frac{n_i}{N} \times ln\frac{n_i}{N}\right) (5)$$

Where n is the total number of one species and N is the total number of individuals of all species.

Pileou's Evenness (J') takes the Shannon-Wiener index (H') by the logarithm of the number of taxa (S) which compensates for the effect of species richness on the Shannon-Wiener index (Smith & Wilson, 1996):

$$J = \frac{H'}{H_{max}} = \frac{H'}{lnS} \quad (6)$$

For these indices, not all Ephemeroptera, Trichoptera and Plecoptera individuals could be identified to species level, thus only family levels were used. The individuals that could not be identified down to family level was left out of these indices as there was no certainty that they did not belong to a family that had already been identified. The data were tested for normality using the Shapiro-Wilk test and using Levene's test for homogeneity with the "rstatix" package

(Kassambara, 2020, version 0.7.0). The data was log-transformed where necessary. A two-way ANOVA test was used to test the BA, CI and BAxCI for differences in the indices mention above as well as macroinvertebrate density with the “rstatix” package.

A non-metric multidimensional scaling (NMDS) ordination was used to look for dissimilarity in macroinvertebrate assemblages on the BACI design using the metaMDS function from the “vegan” package (Oksanen et al., 2020, version 2.5-7). Species in Trichoptera were all put in one group, the same with species in Ephemeroptera and Plecoptera. This due to having a small amount of each species within those orders. Before using the ordination, a Shepard plot and stress plot was made to see that the ordination fit for use. The NMDS ordination was carried out on untransformed macroinvertebrate abundances in all sampling methods using the Bray Curtis similarity measure with set 100 iterations and two dimensions. To test if there are differences in macroinvertebrate assemblages in the BACI design an analysis of similarity (ANOSIM (R), vegan) was used, but only for the macroinvertebrates assemblage found within the macrophytes and in the sediment. ANOSIM was not used for the drifting invertebrates as the number of replicates per site and time was too small to give reliable results.

The statistics were performed in R software for Statistical computing version 3.6.2. (R Core Team, 2020). Figures were plotted using ggplot2 (Wickham & Chang, 2015).

3. Results

3.1 Taxon composition

In total, a number of 6102 macroinvertebrates were sampled in the sediment (see appendix A for complete taxa list). The most dominant of these was chironomid larvae with 3576 individuals and represented over half of the macroinvertebrate community (Fig. 6). Oligochaetes were the second most dominant group with 865 individuals, and the third most dominant group were nematodes with 644 individuals. Trichoptera was the group with the least individuals, 16 in total and of these were 8 *Lepidostoma hirtum*, 4 *Mystacides azurea* and 4 *Oxyethira* sp., respectively.

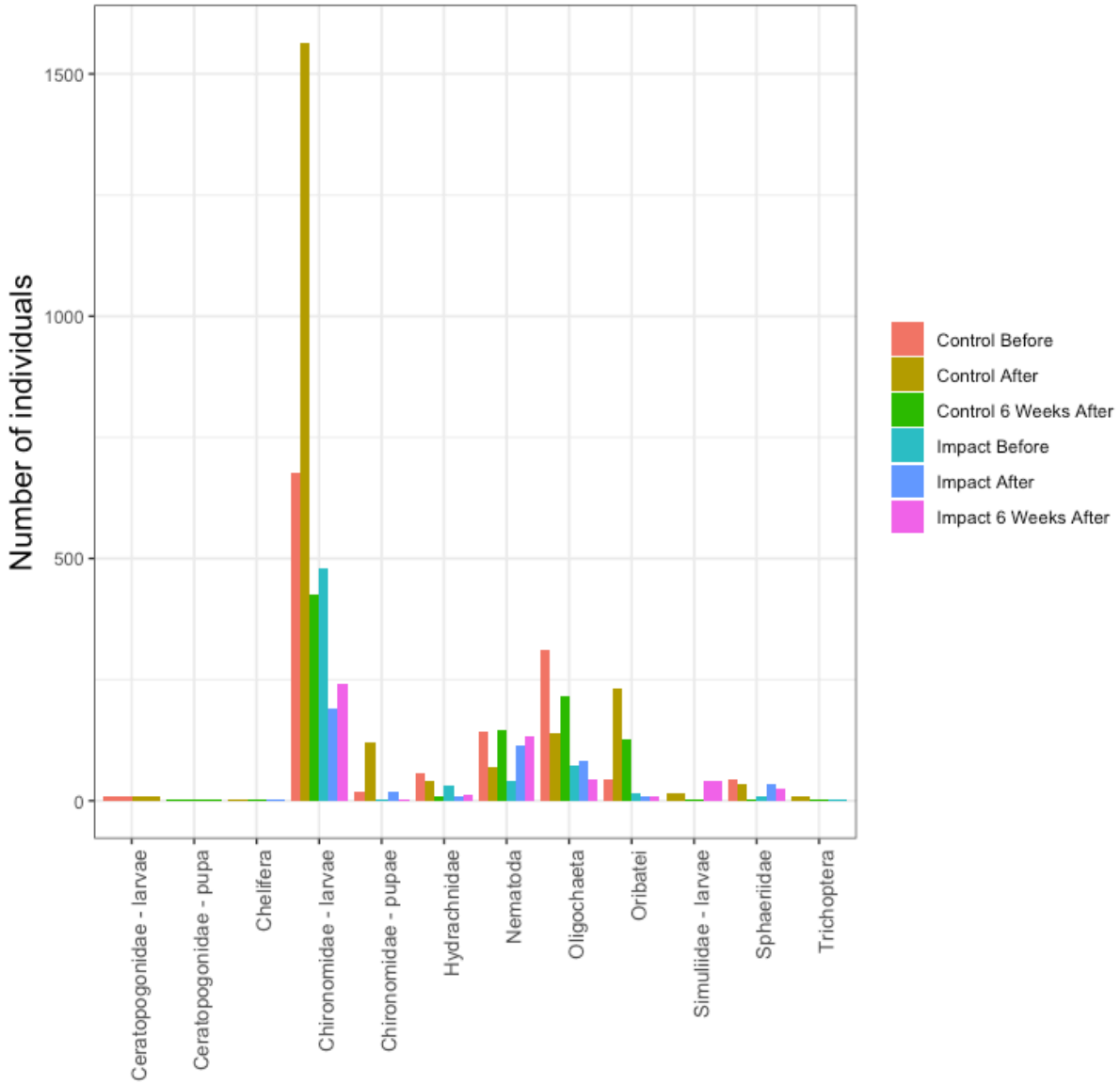

Figure 6. Total number of the taxa found in the sediment between site and time.

Within the macrophytes, a total of 13345 macroinvertebrates were collected. Chironomid larvae were the most dominant having a total of 10203 individuals (Fig. 7). A total of 122 individuals of Trichoptera were found, distributed among four families (Complete taxa list is available in Appendix B). A total of 53 individuals of Plecoptera were found between six families, and a total of 80 Ephemeroptera was collected between four families within the macrophytes. 289 individuals of Sphaeriidae were found, most of them in the impact site after macrophyte removal (64 individuals one week after removal and 110 individuals six weeks after removal).

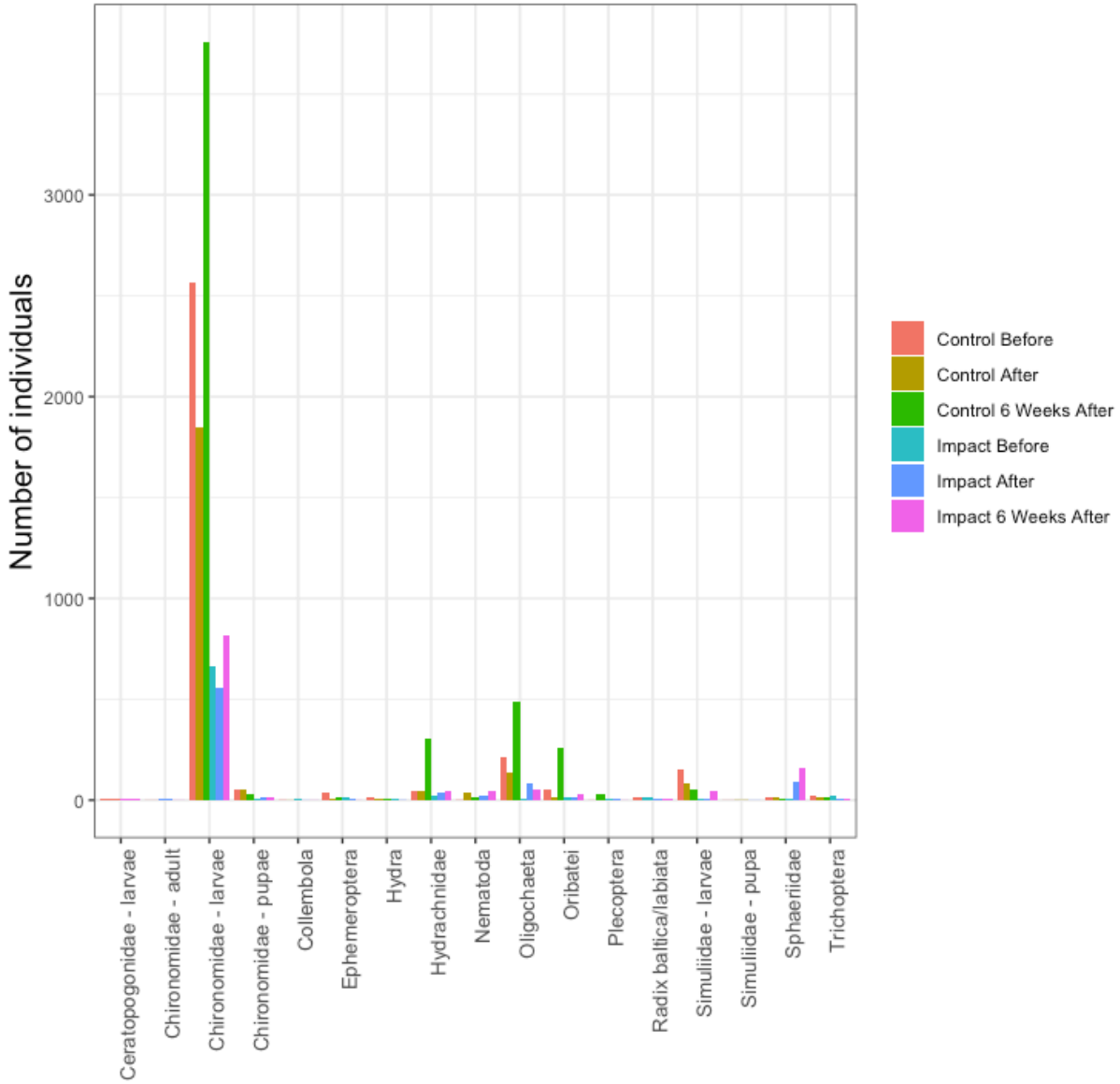

Figure 7. Total number of taxa found within the macrophytes between site and time.

A total of 557 surface drifting invertebrates were sampled. Chironomid larvae were the most abundant having a total of 212 individuals (Fig. 8). Chironomid pupae were the second most abundant, with a total of 128 individuals. 102 individuals of Plecoptera were found between five families, 90 individuals of these were *Amphinemura* sp. 13 individuals of Ephemeroptera were found between three families, and 6 individuals of Trichoptera were found between two families (Complete taxa list is available in Appendix C).

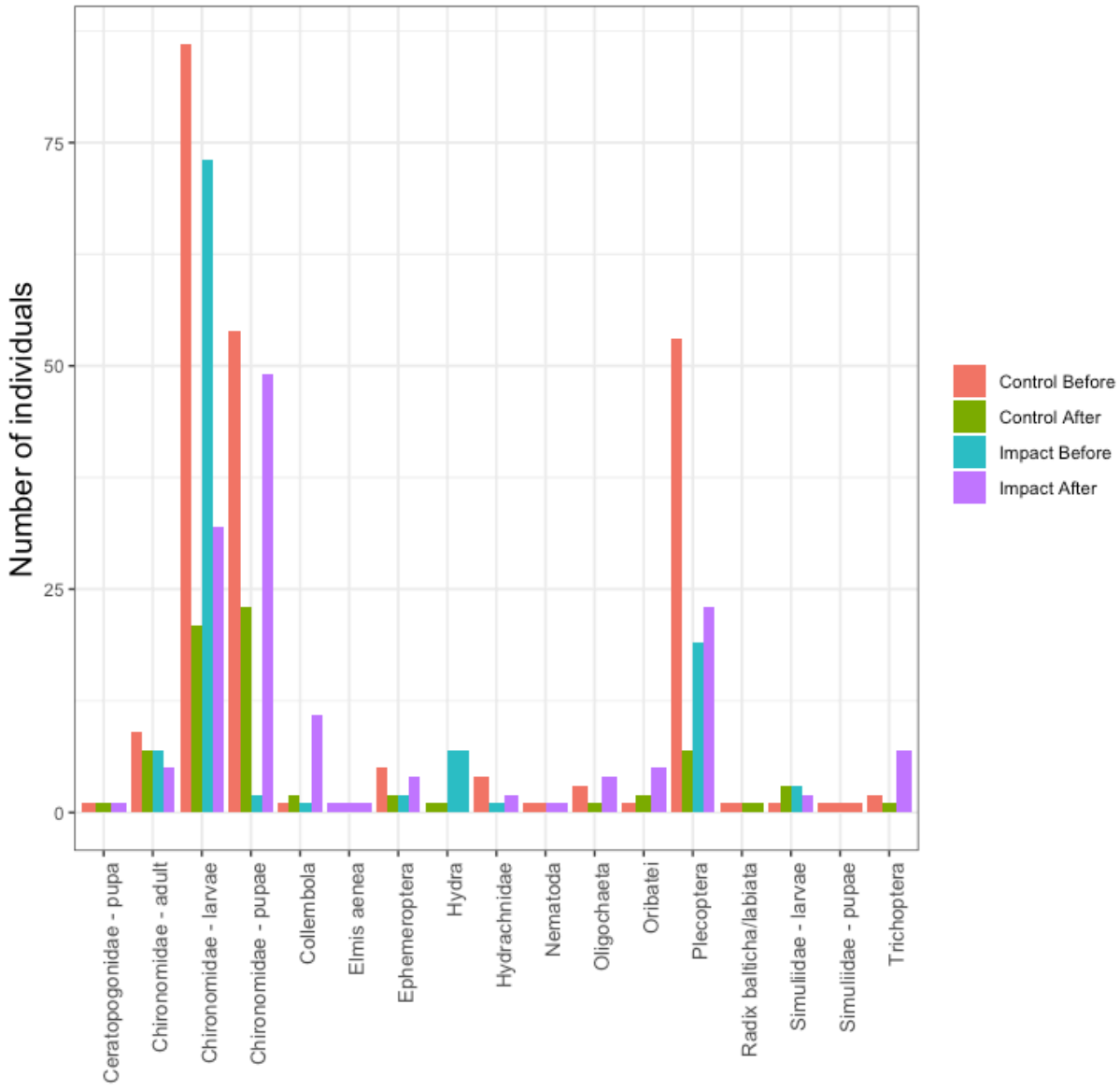

Figure 8. Total number of taxa found drifting in the surface between site and time.

The sediment drift invertebrates had a total of 1273 individuals. 964 of these were chironomid larvae and 70 chironomid pupae (Fig. 9). 24 individuals of Plecoptera were found, 22 of those belonging to the genus *Amphinemura* (Complete taxa list is available in Appendix C). 6 individuals were found of Ephemeroptera, all were in the family *Leptophlebidae,* and 6 individuals were found of Trichoptera between three families. 58 individuals of Sphaeriidae were found in the impact site after macrophyte removal.

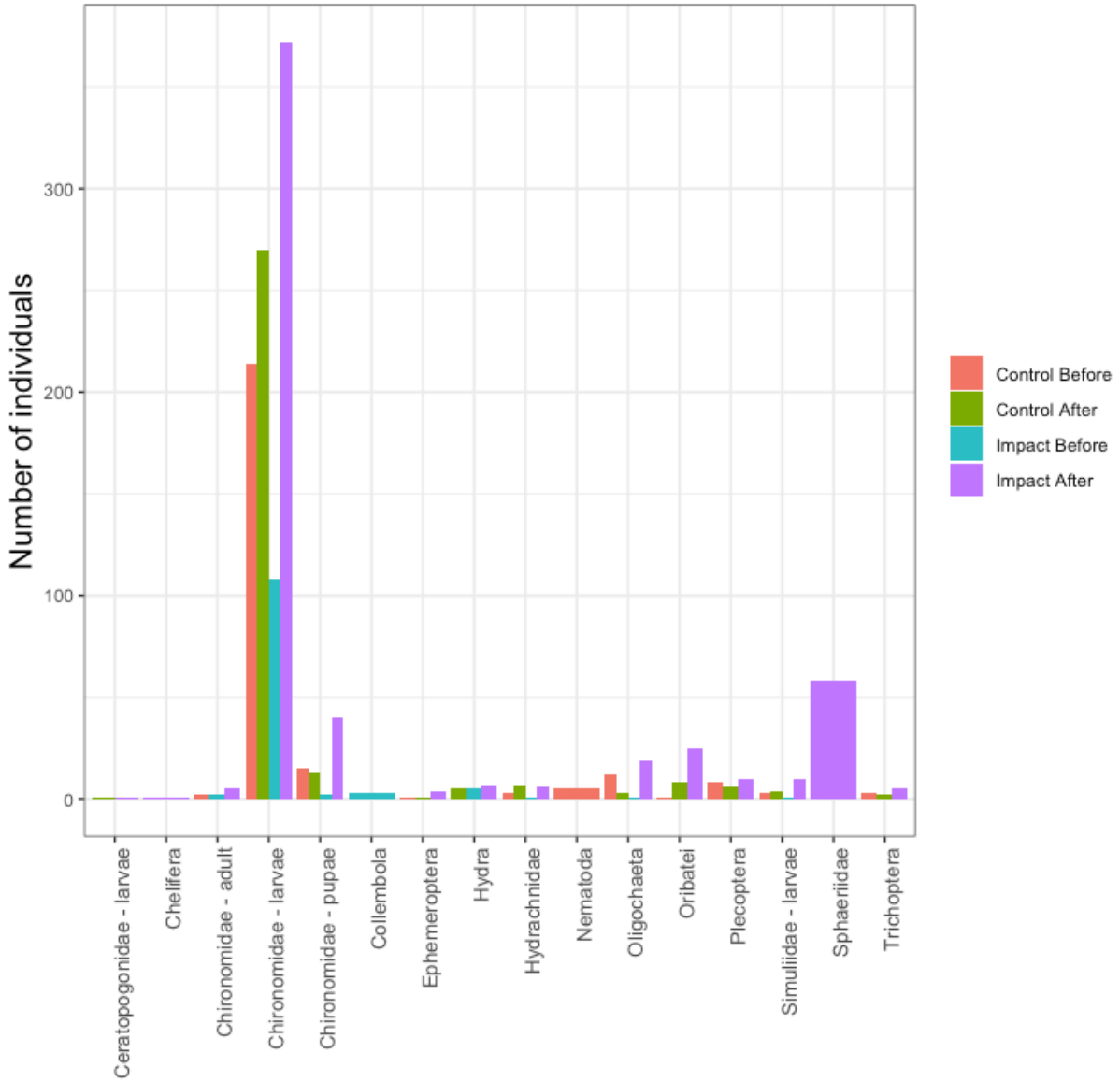

Figure 9. Total number of taxa found drifting near the sediment between site and time.

Significant effects of time and site on the macroinvertebrate community composition were found within the sediment (ANOSIM, $p = 0.0051$). However, the effect strength was weak, which entails considerable variation within groups rather than between groups ($R = 0.1662$). The NMDS plot for macroinvertebrates associated with the sediment shows large overlaps between groups and extensive variation within groups (Fig. 10 A). The groups highly overlap with the taxa found in the sediment. However, some taxa are more associated with some groups than others. For example, Sphaeriidae is more associated with the impact site in comparison to the control site (Fig. 10 B).

The macroinvertebrate community composition within the macrophytes was likewise significantly affected by time and site (ANOSIM, $p = 0.0052$). However, the effect strength was also weak here ($R = 0.2165$), indicating strong overlaps among groups (Fig. 10 C). The impact groups had less overlap between time than control groups (Fig. 10 C). Impact before macrophyte removal occurs furthest left along the NMDS1 axis with more associations to the taxa Plecoptera, Trichoptera and Ephemeroptera (Fig. 10 D). The impact site right after macrophyte removal occurs next to the impact site before removal along the NMDS1 axis. The impact site six weeks after removal occurs right above impact after along the NMDS2 axis.

The surface drifting macroinvertebrate samples show some similarities between the control groupings (Fig. 11 A). However, no overlap between impact before and impact after removal was found. Impact after was associated with Ephemeroptera and Trichoptera species more so than impact before (Fig. 11 B).

The sediment drifting macroinvertebrate assemblage did not have overlaps between groupings (Fig. 11 C). Dissimilarities between control before and after removal were seen along the NMDS2 axis, and impact before and after removal along the NMDS1 axis. Along the NMDS1 axis, the impact after macrophyte removal as well as both control groupings were more associated with Ephemeroptera, Trichoptera and Plecoptera compared to the impact site before macrophyte removal (Fig. 11 D).

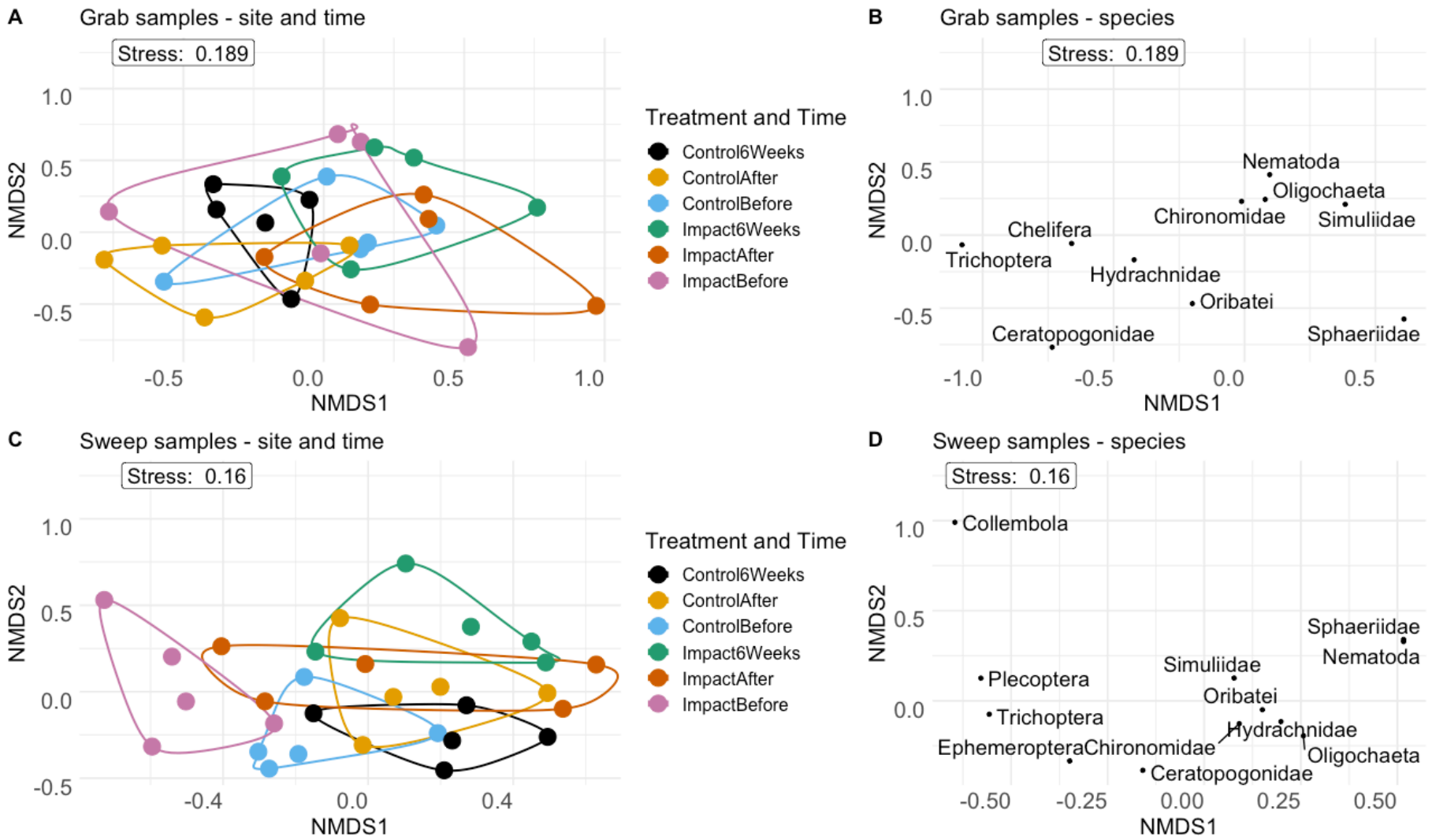

Figure 10. A - NMDS showing the dissimilarity of macroinvertebrate community composition between sites and times within the sediment. B – the same NMDS with the taxa composition within the sediment. C – NMDS showing the dissimilarity of macroinvertebrate community composition between the sites and treatment times within the macrophytes. D – the same NMDS with the taxa composition within the macrophytes.

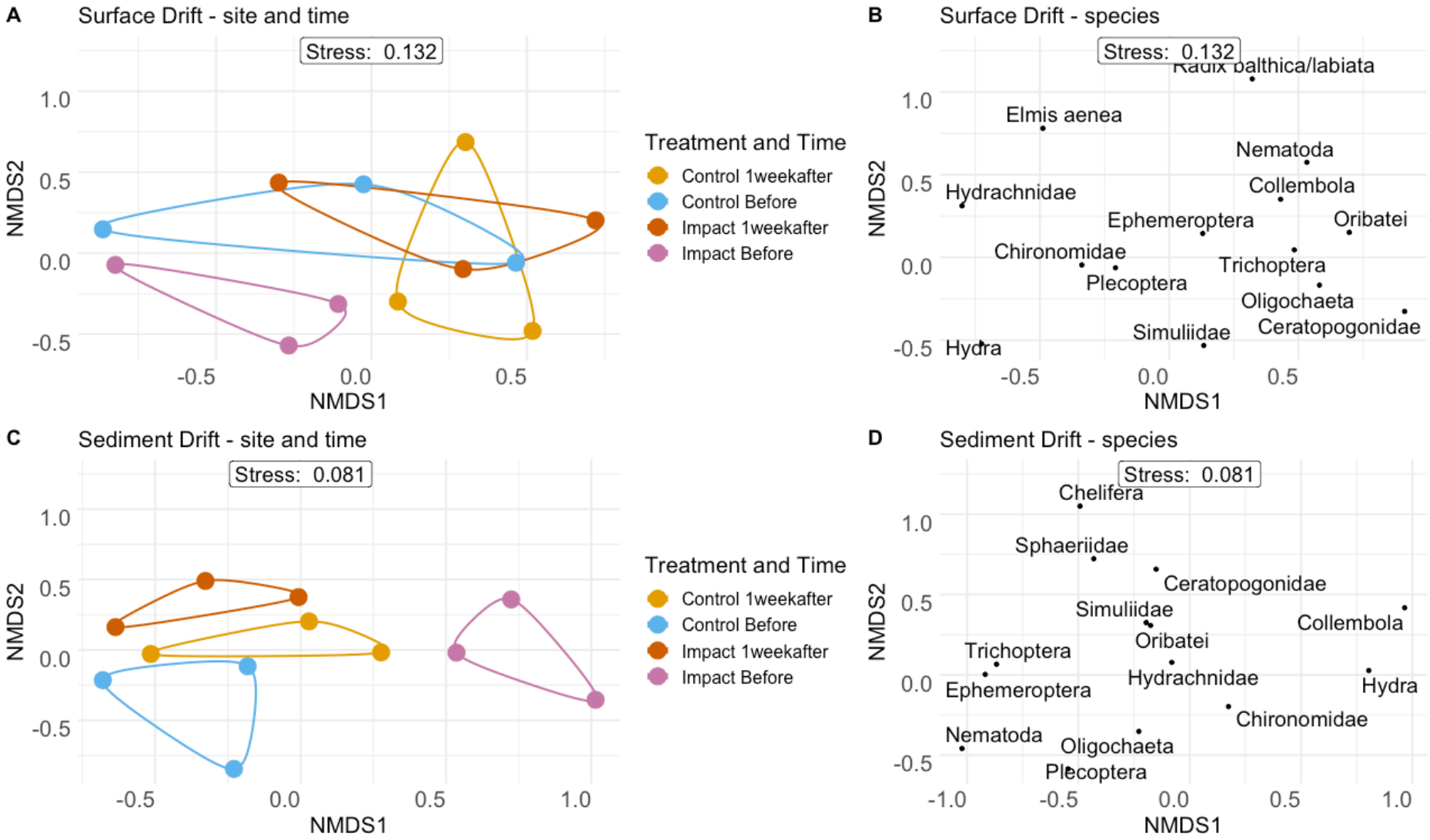

Figure 11. A - NMDS showing the dissimilarity of macroinvertebrate composition between the sites and treatment times within the surface drift. B – the same NMDS with the taxa composition within the surface drift. C – NMDS showing the dissimilarity of macroinvertebrate composition between the sites and treatment times within the sediment drift. D – the same NMDS with the taxa composition within the sediment drift.

3.3 Taxa richness

In general, the macroinvertebrate community found in the macrophytes represented a higher taxa richness than the macroinvertebrate communities found in the sediment and drift samples. Within the drifting macroinvertebrates, the surface drifters had a higher taxa richness compared to those drifting closer to the sediment.

A total of eight macroinvertebrate taxa was found within the sediment (impact: 6 taxa; control: 6 taxa) before macrophyte cutting, a total of 11 macroinvertebrate taxa (impact: 7 taxa; control: 8 taxa) one week after macrophyte cutting and a total of 10 taxa (impact: 7 taxa; control: 7 taxa) six weeks after macrophyte cutting (means and S.D. is available in Appendix D). Taxa richness was significantly higher in the control site compared to the impact site in the sediment (Two-Way ANOVA, $p = 0.007$; Full Two-Way ANOVA output is available in Appendix E). However, taxa richness did not differ between time ($p = 0.343$), and there was no significant interaction between site and time ($p = 0.574$; Fig.12 A).

Of the macroinvertebrates found in the macrophytes (Sweep samples), a total of 20 macroinvertebrate taxa were found before macrophyte cutting (impact: 13 taxa; control: 13 taxa). A total of 20 macroinvertebrate taxa were found one week after macrophyte cutting (impact: 12 taxa; control: 10 taxa) and a total of 20 macroinvertebrate taxa were found six weeks after cutting (impact: 12 taxa; control: 12 taxa). Taxa richness did not differ between sites (Two-Way ANOVA, $p = 0.801$; Fig.12 B), neither did it differ between time (Two-Way ANOVA, $p = 0.115$) or between site and time (Two-Way ANOVA, $p = 0.588$).

The surface drifting macroinvertebrates had a total of 17 taxa (impact: 6 taxa; control: 9 taxa) before cutting and a total of 19 taxa (impact: 11 taxa; control: 7 taxa) after cutting. Taxa richness was not significantly different between sites (Two-Way ANOVA, $p = 0.487$; Fig.12 C), nor between time (Two-Way ANOVA, $p = 0.226$) and no significant difference was found between site and time (Two-Way ANOVA, $p = 0.095$).

The sediment drifting macroinvertebrates had a total of 12 taxa (impact: 5 taxa; control: 9 taxa) before cutting and a total of 13 taxa (impact: 11 taxa; control: 7 taxa) after cutting. Taxa richness showed no significant difference between sites (Two-Way ANOVA, $p = 0.891$; Fig.12 D), time (Two-Way ANOVA, $p = 0.067$) or between site and time (Two-Way ANOVA, $p = 0.067$).

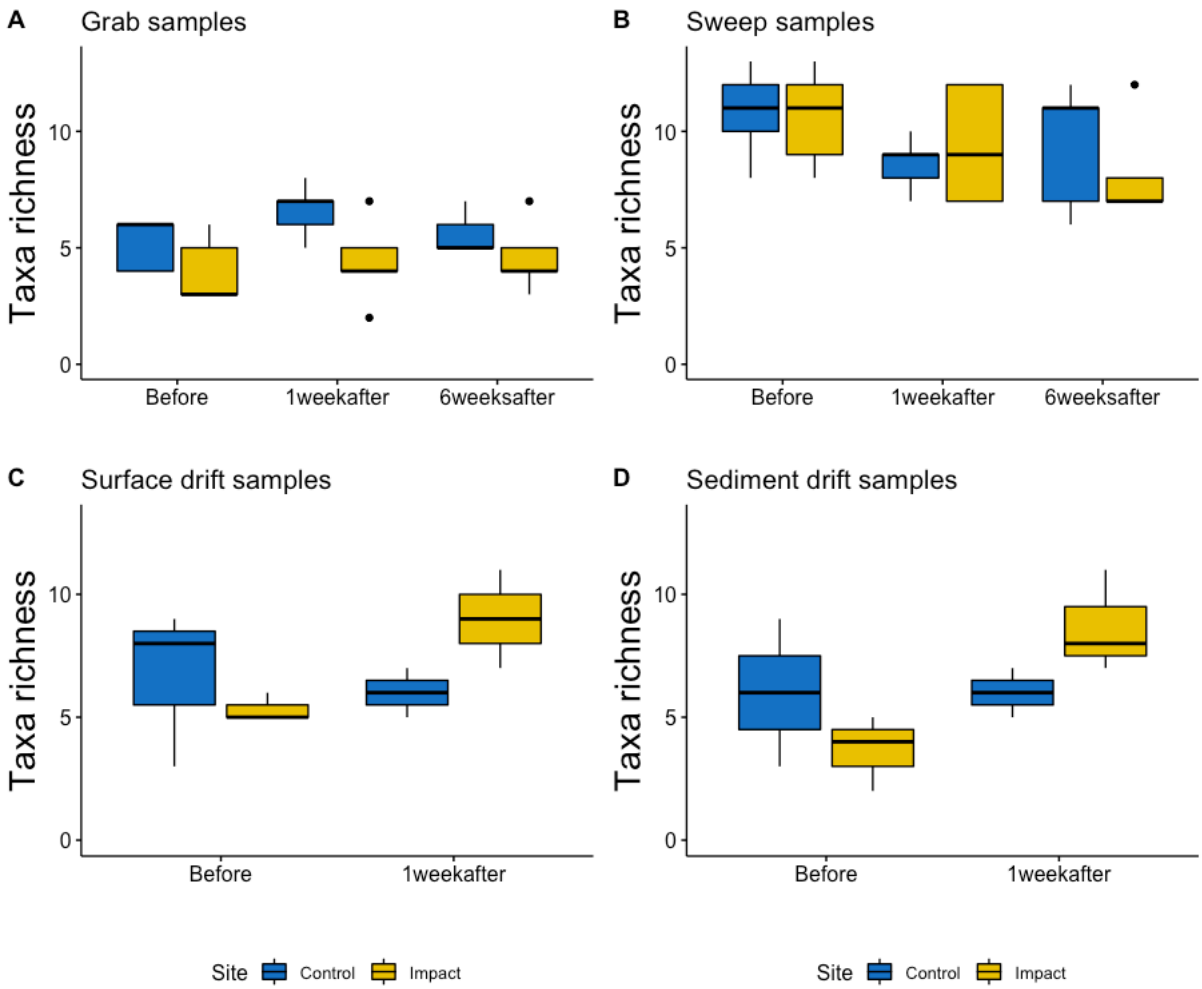

Figure 12. Taxa richness across treatment time between sites of macroinvertebrates living in/on the sediment (A), in the macrophytes (B), surface drifting macroinvertebrates (C) and sediment drifting macroinvertebrates (D). Horizontal bold lines represent the median, boxes the 25% and 75% quantiles and whiskers the minimum and maximum values.

3.4 Fisher alpha diversity

Macroinvertebrate alpha diversity within the sediment was lowest before removal ($\alpha_{control}$ = 0.992±0.241; α_{impact} = 0.943±0.45; Summary statistics can be found in Appendix F). The highest average diversity was one week after macrophyte removal in both control (α = 1.15±0.19) and impact site (α = 1.12±0.424). Alpha diversity decreased after 6 weeks of removal ($\alpha_{control}$ = 1.11±0.276; α_{impact} = 1.05±0.321). However, there was no significant difference in diversity between sites (Two-Way ANOVA, p = 0.718; Fig. 13 A), time (Two-Way ANOVA, p = 0.532) or between site and time (Two-Way ANOVA, p = 0.995; Full Two-Way ANOVA output is available in Appendix G).

The alpha diversity of macroinvertebrates in macrophytes was higher in the impact site than the control site (Two-Way ANOVA, p = 0.001; Fig. 13 B), and there was a significant difference between time (Two-Way ANOVA, p = 0.012). However, there was no significant difference between site and time (Two-Way ANOVA, p = 0.867). Alpha diversity of the macroinvertebrates found in the macrophytes had the highest average before removal ($\alpha_{control}$ = 2.03±0.356; α_{impact} = 2.72±0.407). It decreased right after removal ($\alpha_{control}$ = 1.54±0.111; α_{impact} = 2.40±0.795) and decreased further 6 weeks after removal ($\alpha_{control}$ = 1.48±0.445; α_{impact} = 1.9±0.198).

There was a significant difference between time (Two-Way ANOVA, p = 0.036; Fig. 13 C), but no significant difference between sites (Two-Way ANOVA, p = 0.578) or between site and time (Two-Way ANOVA, p = 0.398). Surface drifting macroinvertebrates had the highest average diversity right after removal ($\alpha_{control}$ = 2.61±0.654; α_{impact} = 3.28±0.874) and the lowest diversity average before macrophyte removal ($\alpha_{control}$ = 1.87±1.10; α_{impact} = 1.72±0.308).

Alpha diversity had no significant difference between sites (Two-Way ANOVA, p = 0.712), time (Two-Way ANOVA, p = 0.134) or between site and time (Two-Way ANOVA, p = 0.119) in the sediment drifting macroinvertebrates (Fig. 13 D). Alpha diversity of the sediment drifting macroinvertebrates in the control site was higher before (α = 1.55±0.481) macrophyte removal than after (α = 1.53±0.268) removal. In the impact site, diversity was lower before (α = 0.987±0.464) than after (α = 1.90±0.581) removal.

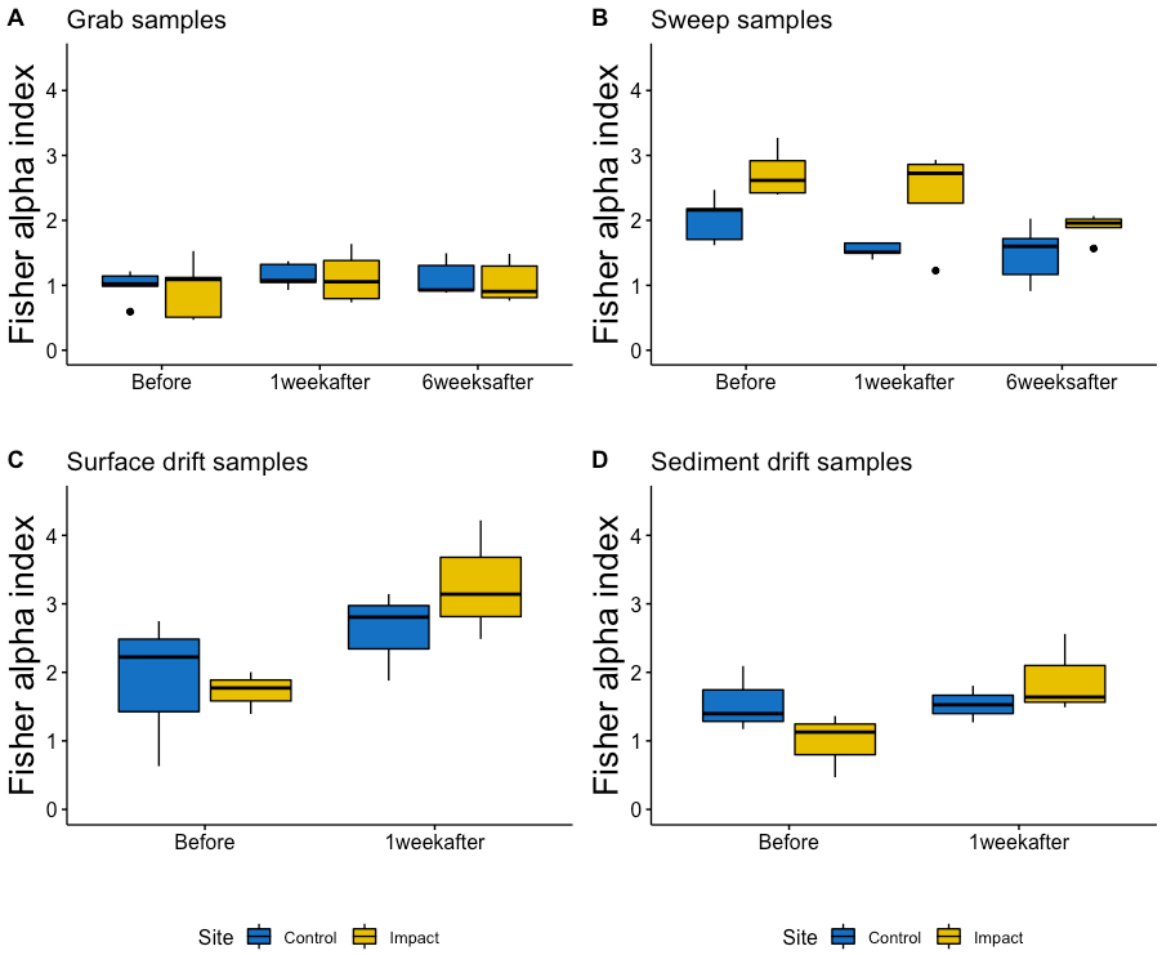

Figure 13. Fisher alpha diversity across time between sites of macroinvertebrates living in/on the sediment (A), in the macrophytes (B), surface drifting macroinvertebrates (C) and sediment drifting macroinvertebrates (D). Horizontal bold lines represent the median, boxes the 25% and 75% quantiles and whiskers the minimum and maximum values.

3.5 Shannon-Wiener diversity

Shannon-Wiener index (H') did not differ between site (Two-Way ANOVA, p = 0.690; Full Two-Way ANOVA output can be found in Appendix I), time (Two-Way ANOVA, p = 0.211) or between site and time (Two-Way ANOVA, p = 0.263) on the macroinvertebrates found in the sediment (Fig.14 A; Summary statistics is available in Appendix H).

Macroinvertebrate diversity in the macrophytes was higher in impact site (H'_{before} = 1±0.348; H'_{after} = 1.33±0.408; H'_{6weeks} = 1.10±0.186; Fig.14 B) than in control site (H'_{before} = 0.802±0.239; H'_{after} = 0.73±0.326; H'_{6weeks} = 0.884±0.227; Two-Way ANOVA, p = 0.005), respectively. A difference in diversity was not found between time (Two-Way ANOVA, p = 0.631) or between site and time (Two-Way ANOVA, p = 0.256).

The diversity of surface drifting macroinvertebrates showed no significant differences between site (Two-Way ANOVA, p = 0.368; Fig.14 C), time (Two-Way ANOVA, p = 0.149) or between site and time (Two-Way ANOVA, p = 0.454).

There was a significant difference between time (Two-Way ANOVA, p = 0.004) and between site and time (Two-Way ANOVA, p = 0.004) of the sediment drifting invertebrate diversity. Diversity of the sediment drifting invertebrates increased after the macrophyte removal in impact site (H'_{before} = 0.999±0.057; H'_{after} = 0.378±0.15; Fig. 14 D), while it was unchanged in the control site (H'_{before} = 0.562±0.158; H'_{after} = 0.562±0.146). There was no significant difference in diversity between sites (Two-Way ANOVA, p = 0.249).

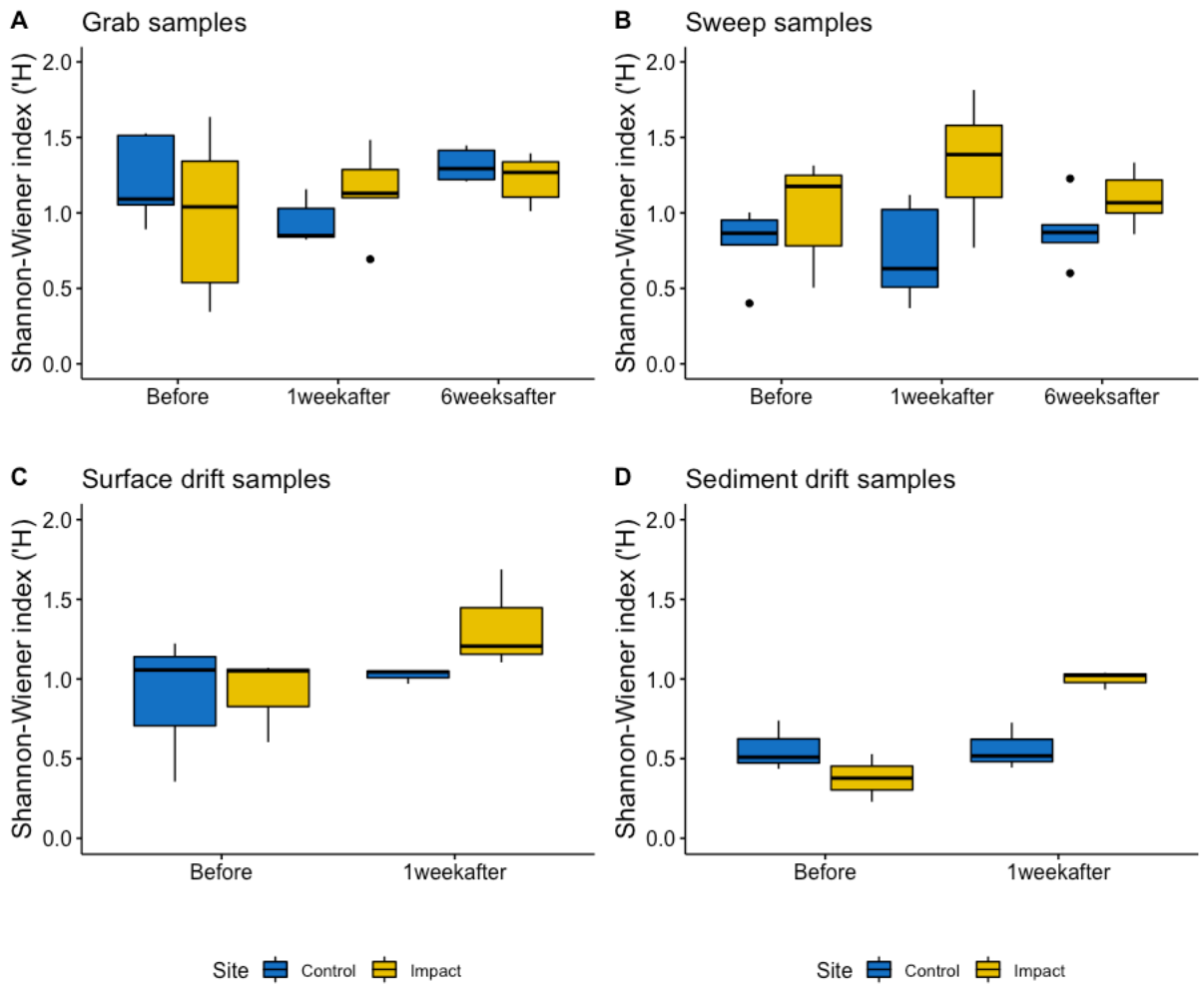

Figure 14. Shannon-Wiener diversity (H') across time between sites of macroinvertebrates living in/on the sediment (A), in the macrophytes (B), surface drifting macroinvertebrates (C) and sediment drifting macroinvertebrates (D). Horizontal bold lines represent the median, boxes the 25% and 75% quantiles and whiskers the minimum and maximum values.

3.6 Species evenness

There was a higher evenness within the macroinvertebrates found in the sediment in the impact site compared to the control site (Two-Way ANOVA, p = 0.047; Fig.15 A) and there was a significant difference between site and time (Two-Way ANOVA, p = 0.030). However, there was no significant difference between time (Two-Way ANOVA, p = 0.165; Full Two-Way ANOVA output can be found in Appendix K; Summary statistics is available in Appendix J).

Macroinvertebrate taxa evenness within the macrophytes was higher in impact site (J'_{before} = 0.438±0.174; J'_{after} = 0.621±0.232; J'_{6weeks} = 0.529±0.095; Fig.15 B) in comparison to control site (J'_{before} = 0.346±0.12; J'_{after} = 0.344±0.163; J'_{6weeks} = 0.399±0.07; Two-Way ANOVA, p =

0.006). Taxa evenness did not differ between time (Two-Way ANOVA, p = 0.388), or between site and time (Two-Way ANOVA, p = 0.377).

The surface drifting macroinvertebrate community had no significant difference in evenness between sites (Two-Way ANOVA, p = 0.397; Fig.15 C), no significant difference between time (Two-Way ANOVA, p = 0.189) and no significant difference between site and time (Two-Way ANOVA, p = 0.707).

The sediment drifting macroinvertebrate community had no difference in evenness between sites (Two-Way ANOVA, p = 0.322; Fig.15 D), time (Two-Way ANOVA, p = 0.268) or between site and time (Two-Way ANOVA, p = 0.138).

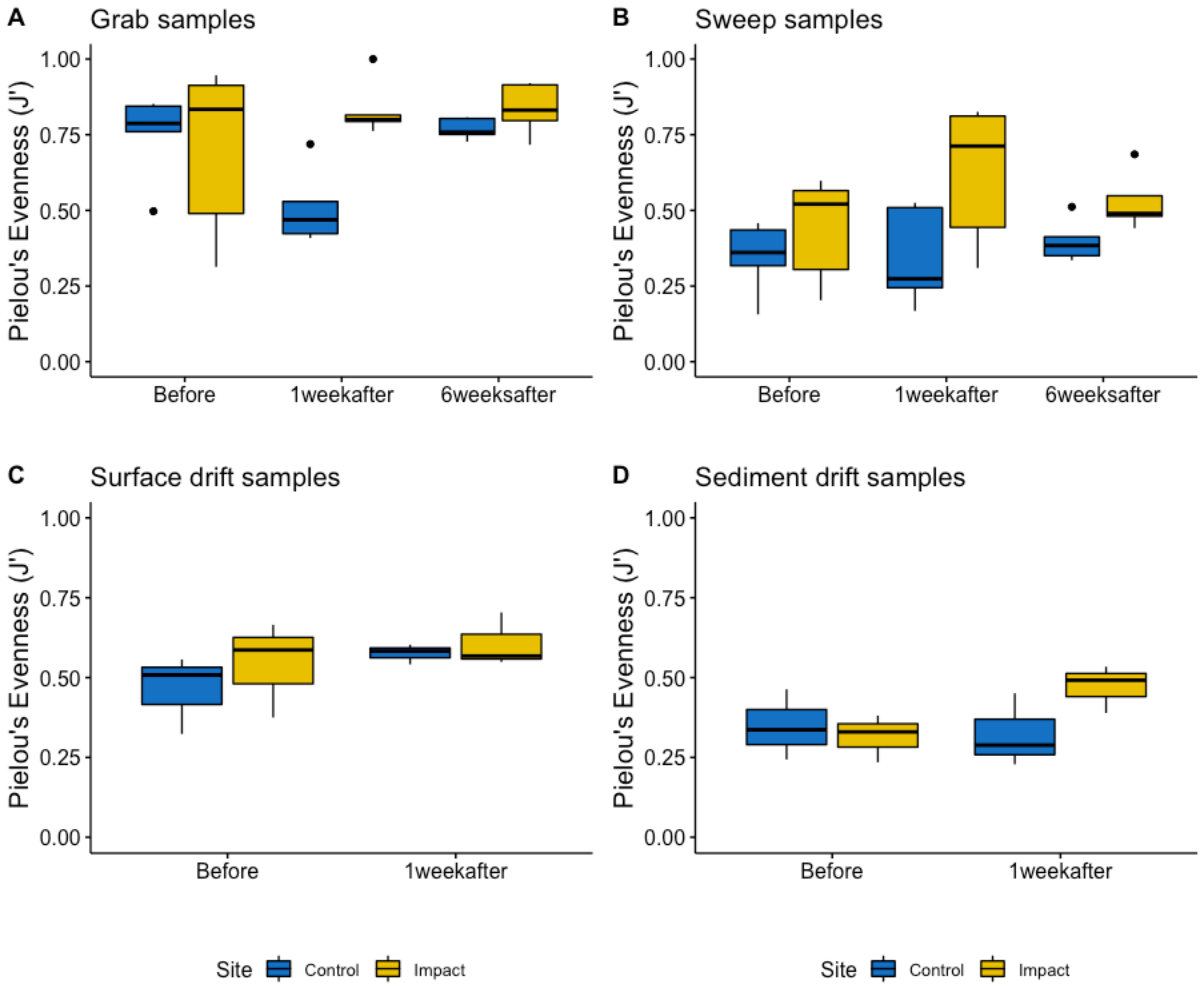

Figure 15. Pielou's evenness (J') across time between sites of macroinvertebrates living in/on the sediment (A), in the macrophytes (B), surface drifting macroinvertebrates (C) and sediment drifting macroinvertebrates (D). Horizontal bold lines represent the median, boxes the 25% and 75% quantiles and whiskers the minimum and maximum values.

3.7 Macroinvertebrate density

Macroinvertebrate density in the sediment (Fig. 16 A) was lower in the impact site ranging from 5831 individuals/m^2 to 4053 individuals/m^2 (Summary statistics can be found in Appendix L) compared to the control site ranging from 19876 individuals/m^2 to 8409 individuals/m^2 (Two-Way ANOVA, $p = 0.004$; Full Two-Way ANOVA output can be found in Appendix M) throughout time (Two-Way ANOVA, $p = 0.974$). There was no difference in density between site and time (Two-Way ANOVA, $p = 0.333$).

The macroinvertebrates found in the macrophytes (Fig. 16 B) also had higher densities in the control site ranging from 164 individuals/m^2 to 38.1 individuals/m^2 compared to the impact site ranging from 51.7 individuals/m^2 to 12.7 individuals/m^2 (Two-Way ANOVA, $p = 0.000691$). There was no difference in density between time (Two-Way ANOVA, $p = 0.069$) or between site and time (Two-Way ANOVA, $p = 0.216$).

Surface drifting macroinvertebrates (Fig. 16 C) had a significant decline in density after removal (Two-Way ANOVA, $p = 0.000546$). Where the control site went from a density of 51.3 individuals/100m^3 to 9.92 individuals/100m^3 and the impact site went from 66.2 individuals/100m^3 to 15 individuals/100m^3. However, no difference in density was found between site (Two-Way ANOVA, $p = 0.0384$) or between site and time (Two-Way ANOVA, $p = 0.508$).

Sediment drifting macroinvertebrate densities (Fig. 16 D) did not differ between sites (Two-Way ANOVA, $p = 0.908$), or time (Two-Way ANOVA, $p = 0.912$) and there was no significant difference in the interaction of site and time (Two-Way ANOVA, $p = 0.824$).

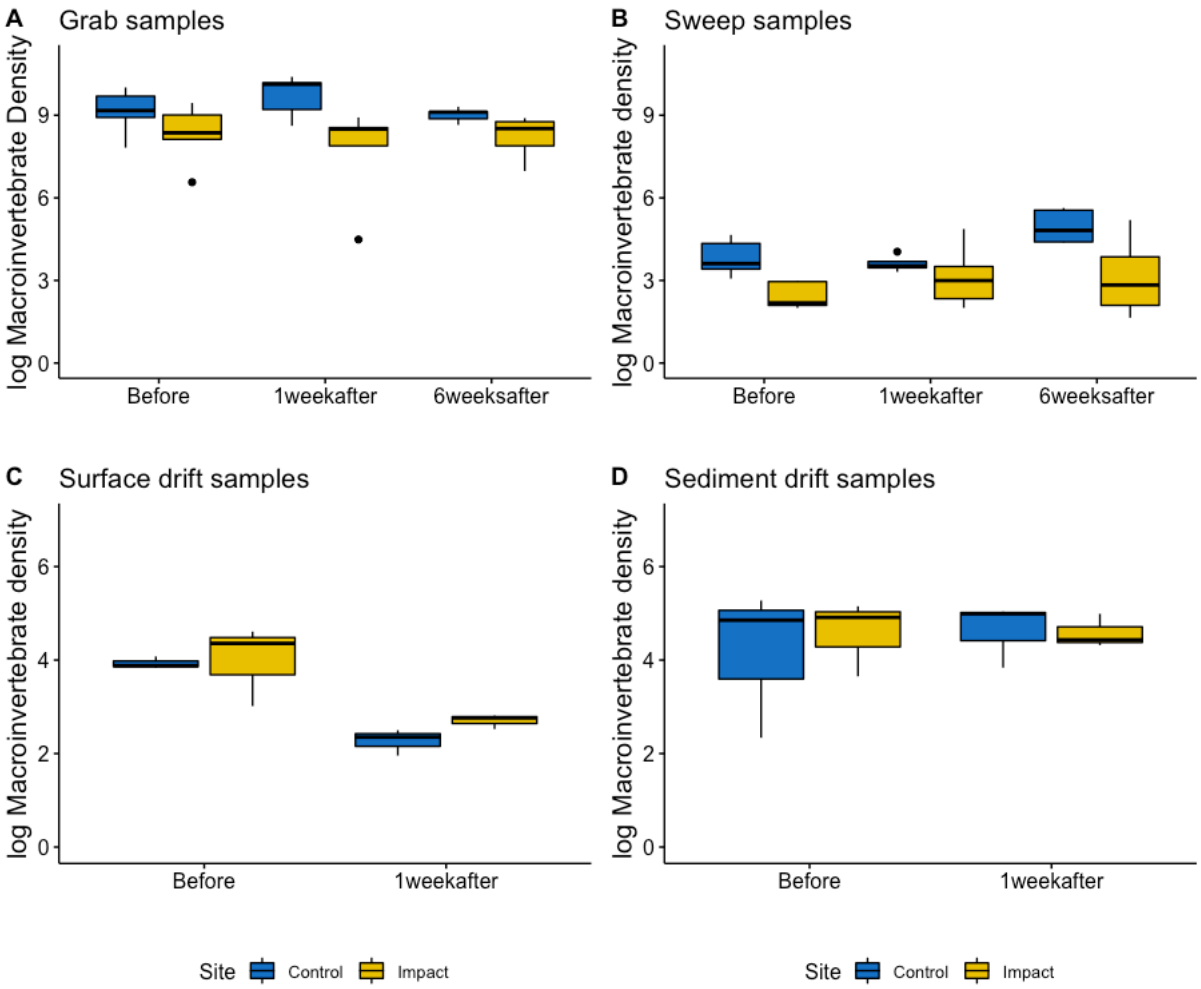

Figure 16. Macroinvertebrate density (log transformed) across time between sites of macroinvertebrates living in/on the sediment (A), in the macrophytes (B), surface drifting macroinvertebrates (C) and sediment drifting macroinvertebrates (D). Horizontal bold lines represent the median, boxes the 25% and 75% quantiles and whiskers the minimum and maximum values.

4. Discussion

In this study, the aim was to evaluate how the removal of *J. bulbosus* influence macroinvertebrate density, diversity and community composition in an oligotrophic river in Norway.

4.1 Effect of macrophyte removal on macroinvertebrate composition

In general, Chironomids were the most abundant taxa in the sediment (166,311 individuals/m^2), on the macrophytes (7,685 individuals/m^2), and as drift invertebrates (surface = 295 individuals/100m^3, sediment = 1134 individuals/100m^3). This is in concordance with previous studies from the river Otra (Velle et al., 2021). Velle et al. (2021) found chironomids to be dominant in both gravel and macrophyte areas. Although, they had a much smaller density in the gravel (5,897 individuals/m^2) and a larger density in the macrophytes (10,252 individuals/m^2), using a Surber sampler, compared to this study. In macrophyte-rich freshwaters, chironomids are commonly present in both the sediment and among macrophytes (Westlake et al., 1972), and are often dominant in most freshwaters (Ferrington, 2007) which includes oligotrophic waters (Kownacki et al., 2000). Within the sediment, there were also oligochaetes, nematodes, Hydrachnidae and Oribatei (Acari), Sphaeriidae, and some Simuliidae larvae. Velle et al. (2021) found Oligochaeta, Nematoda and Acari to be dominant after Chironomidae within the sediment in the river Otra. The macrophyte habitat held a higher taxa richness than that of any other habitat in this study. A reason for this might be the microhabitats *J. bulbosus* provide with refuge and feeding options (Kaenel et al., 1998; Warfe & Barmuta, 2006). The second most dominant taxon within the macrophytes was Oligochaeta, which Velle et al. (2021) also found. Nematoda took a less pronounced proportion in the taxa composition in this study than Velle et al. (2021). This could be explained by seasonal changes, as Velle et al. (2021) sampled macroinvertebrates in mid-September. Acari showed to be dominant in both studies within the macrophytes. The surface drifting macroinvertebrates' second most dominant taxon was Plecoptera which consisted mainly of the rheophilic genus *Amphinemura* (Miliša et al., 2006) and nocturnal drifters (Brittain & Eikeland, 1988). Other taxa known to drift during the night are Ephemeroptera and Simuliidae (Brittain & Eikeland, 1988), both were found in a small portion of the surface drifting taxa composition. In the sediment drifting macroinvertebrates, the second most dominant taxon was Sphaeriidae. Sphaeriidae prefers a habitat of fine sediment with low velocity (Kubíková et al., 2011), and they are slow dispersers (Kappes & Haase, 2012).

Removal of *J. bulbosus* was expected to influence the macroinvertebrate community of macroinvertebrates associated with macrophytes, sediment as well as drifting from upstream areas. The chironomids found in this study in and on the sediment decreased in the impact site after macrophyte removal. This was the case for chironomids in the macrophytes as well. However, there was a rise 6 weeks after removal in the number of chironomids found, which could mean they recovered quickly after the removal as Monahan & Caffrey (1996) experienced with their macroinvertebrates after macrophyte removal. A much higher number of chironomids were found in the control site compared to the impact site in the macrophytes. This might be due to differences in velocity between the two sites. The chironomid larvae found drifting near the surface decreased in both sites after macrophyte removal. While the chironomid larvae drifting near the sediment increased in both sites after removal. This might be due to higher velocities in the surface compared to the sediment, and as there were no macrophytes left near the surface to take refuge, the chironomids might drift lower in the water column for this purpose. The surface drifting chironomids consisted of a lot more chironomid pupae compared to the other habitats which could be explained by their active drift to the surface for adult emergence and their short pupal stage (Kranzfelder et al., 2015).

Simuliidae inhabits solid substrates to which they stay attached, and macrophytes are of great importance to them (Kaenel et al., 1998). Kaenel et al. (1998) found that macrophyte removal had a highly negative impact on Simuliidae abundance (Kaenel et al., 1998). In this study, Simuliidae larvae were found mostly 6 weeks after macrophyte removal in the impact site in both the macrophytes and the sediment, which might be explained by a higher velocity in this site after removal as the family requires swift flows as suspension feeders (Carey et al., 2017). More Simuliidae larvae were found in the control site in the macrophytes before macrophyte removal compared to the impact site which could be explained by the control site having higher velocity as more food for Simuliidae comes more frequently with higher velocities (Kaenel et al., 1998).

Sphaeriidae was more abundant in the impact site one week after macrophyte removal as well as 6 weeks after macrophyte removal in both the sediment and the macrophytes. The presence of Sphaeriidae in the macrophyte samples may imply that some sediment has been collected during the sampling. This was a challenge because there were only very tiny macrophyte patches close to the sediment left, after macrophyte removal. There was also Sphaeriidae found in the sediment drift samples, and only after macrophyte removal. This suggests that the family

was affected by macrophyte removal as an increase in velocity was seen after macrophyte removal in the impact site.

The taxa composition in both sediment and macrophyte showed higher dissimilarity within the groups than between the groups, although the composition in the impact site in the macrophytes had less overlap than the control site. This implies, with the ANOSIM test results considered, that there is an effect of macrophyte removal on the macroinvertebrate assemblage. The ordination showed that impact before removal is more associated with Ephemeroptera, Plecoptera, and Trichoptera species than after removal. Ephemeroptera species such as *Leptophlebia* sp. prefer macrophyte habitat and slow velocity which was found mostly in the control site and impact before removal (Buffagni et al., 2009; Buffagni et al., 2021). Ephemeroptera and Trichoptera are also the preferred food for brown trout (Schei & Jonsson, 1989). Therefore, the macroinvertebrates might have become easier prey as the macrophytes were removed. This might explain why there were only a few Ephemeroptera and Trichoptera found after macrophyte removal. As for the Plecoptera individuals, several of the individuals collected (*Amphinemura*, *Brachyptera risi*, *Leuctra,* and *Nemouridae*) are rheophilic and either prefer macrophyte as habitat or are versatile in their habitat preference (Schmedtje & Colling, 1996; Graf et al., 2009; Tachet et al., 2010; Graf et al., 2021). Nematoda and Sphaeriidae are more associated with the impact 6 weeks after the removal group. Nematodes and Sphaeriidae, both bottom dwellers (Kaenel et al., 1998; Kubíková et al., 2011), were more abundant after macrophyte removal as the macrophyte patches left were short. The composition of the macroinvertebrates within the sediment does not seem to be much affected by macrophyte removal, which contrasted with the expectations. The macroinvertebrate composition in the macrophytes shows differences in the impact site which suggests that macrophyte removal affects the macroinvertebrate composition found there. Previous studies on macrophyte removal effects on macroinvertebrates see clearer alterations of taxonomic composition (Miliša et al., 2006; Bickel & Closs, 2009; Habib & Yousuf, 2014; Carey et al., 2017; Lusardi et al., 2018). Miliša et al. (2006) found altered macroinvertebrate communities by macrophyte removal, with decreases in Chironomidae and other Diptera, Plecoptera and Oligochaeta. Bickel & Closs (2009) found altered macroinvertebrate community compositions after macrophyte removal, where fewer chironomids and more molluscs were found after removal. Habib & Yousuf (2014) found that macrophyte removal had a considerable negative effect on the phyla Arthropoda, Mollusca and Annelida. Carey et al. (2017) found altered

macroinvertebrate assemblages where especially Odonata was negatively affected by macrophyte removal.

The taxa composition of the surface drifting invertebrates had some overlap between groups but clear differences in the impact site were seen. The sediment drifting taxa composition of invertebrates showed much less variation within groups and larger variations between groups. The impact site after removal in the surface drift was more associated with Trichoptera and Ephemeroptera species than impact before. Lusardi et al. (2018) found that a Trichopteran species (*Brachycentrus*) was more prevalent to drift in the macrophyte removed area compared to where there were macrophytes. It seems that macrophyte removal has a bigger impact on some species more than others. For example, *Cyrnus* species and *Oxyethira* species (Trichoptera) was found only in the impact site after macrophyte removal in the surface drift, which prefer macrophytes as a habitat and occurs mainly in slow to medium running water (Tachet et al., 2010). These might have lost their preferred habitat or been dislodged from their habitat and accidentally drifted. The area of macrophyte removal tends to be small compared to the full area of high macrophyte biomass. Therefore, the dislodged macroinvertebrates can drift downstream into another rich macrophyte habitat.

4.2 Effect of macrophyte removal on macroinvertebrate diversity

Taxa richness in this study ranged from 5 to 13 taxonomic groups across the site and time. This was less variation than Ward-Campbell et al. (2017) found with their range from 2 to 19 taxonomic groups in a stream in Canada. Whereas Bickel & Closs (2009) found higher taxa richness varying between 26 to 29 taxonomic groups in their oligotrophic lake in New Zealand. Monahan & Caffrey (1996) found a taxa richness ranging from 8 to 23 taxonomic groups in 8 canal sites between times in Ireland. Taxa richness in the studies mentioned above was not significantly affected by macrophyte removal either. Between the sediment, macrophytes, surface and sediment drift, the highest amount of taxonomic groups were found in the macrophytes, next in the surface drift, sediment drift and the least amount of taxonomic groups were found in the sediment. Taxa richness was not affected by macrophyte removal in the sediment, macrophytes or the surface and sediment drift. This was expected of the taxa found in the sediment and the macrophytes, but not expected in the drift. Therefore, the hypothesis that the taxa richness of the surface and sediment drift would increase after macrophyte removal was rejected. This was based on the thought that as macrophytes were removed, taxa

that do not normally drift would be subject to accidental drift and therefore increase taxa richness. Of the drifting macroinvertebrates, there were finds of species like the Ephemeroptera *Leptophlebia vespertina* which prefers macrophytes as a habitat with standing- to slow-running water (Graf et al., 2008; Graf et al., 2021). *L. vespertina* was found drifting mostly in the impact site after macrophyte removal and could therefore have been subjected to more accidental drift by macrophyte removal. In concordance, *L. vespertina* individuals within the macrophytes were found in higher numbers before cutting than after in the impact site. Sphaeriidae was another taxon that most likely was subject to accidental drift. The taxon was found only in the sediment drift after macrophyte removal. This could imply that the higher velocity caused the higher drift. More drift sample replicates would have provided a more robust result to be sure if there was no impact of macrophyte removal.

Macroinvertebrate diversity (H') was overall highest in the sediment and the surface drift, next in the macrophytes, and lowest in the sediment drift. Fisher alpha diversity showed that the overall highest diversity was found in the surface drift, next in the macrophytes and sediment drift. The lowest alpha diversity was found in the sediment. Diversity (H') was generally lower compared to others studying the effects of macrophyte removal on macroinvertebrate diversity (H' = 1.59 – 1.3, Bickel & Closs, 2009; H' = 2.1 – 1.7, Habib & Yousuf, 2014; H' = 2.4 – 1.8, Lusardi et al., 2018). The diversity (H' and α) in the sediment did not change much between sites and times, and evenness was stable in all sites and times, except for the control site one week after the removal that showed to have less evenness than the other sites and times. An explanation for this could be taxa richness having a slight increase in the control site one week after removal, where diversity showed a slight decrease at that site and time. The macroinvertebrate diversity (H' and α) found in the macrophytes was higher in the impact site compared to the control site. Evenness was higher in the impact site compared to the control site as well. In the surface drift, macroinvertebrate diversity (H') showed no change between sites and times, and evenness was stable. Whereas the macroinvertebrate diversity (α) increased in both sites after macrophyte removal. This increase could be due to higher temperatures in the river. The macroinvertebrate diversity (H') found in the sediment drift had no change in the control site. However, there was an increase in the impact site after macrophyte removal, although not significant, and α diversity had no change in the sediment drift. There are signs of a positive influence of macrophyte removal due to the increasing H' in the sediment drift. However, it is difficult to say that there was an effect of removal on macroinvertebrate diversity in the sediment drift, as α diversity showed not to be influenced

by macrophyte removal. For better understanding, more samples should have been collected. Overall, macroinvertebrate diversity did not seem to be influenced much by macrophyte removal. Similar studies on the influence of macrophyte removal on macroinvertebrate diversity show a variety of results (Miliša et al., 2006; Bickel & Closs, 2009; Habib & Yousuf, 2014; Lusardi et al., 2018). The closest resembling the result of this study is Bickel & Closs (2009) which found no significant effect of macrophyte removal in an oligotrophic river. Others have found decreases in macroinvertebrate diversity after macrophyte removal (Miliša et al., 2006; Habib & Yousuf, 2014). In contrast, Lusardi et al. (2018) found an increase in macroinvertebrate diversity after macrophyte removal.

4.3 Effect of macrophyte removal on macroinvertebrate density

The sediment sampling provided higher macroinvertebrate densities (individuals/m^2) compared to the macrophyte sampling. The method of collection of macroinvertebrates in the sediment covered a smaller areal than the sweep net used to sample macroinvertebrates in the macrophytes. This resulted in higher densities in the sediment compared to the macrophytes. The drift densities (individuals/100m^3) in the sediment were higher compared to the surface drift. This was most likely due to the mesh size being larger in the surface net. Through time, macroinvertebrate density was stable in both sites in the sediment, in the macrophytes, and the sediment drift. However, the macroinvertebrate density in the surface drift decreased in both sites after macrophyte removal. This decrease might be explained by increasing water temperatures as they increased from ~6°C to ~10°C during the study (unpublished data measured in June 2020). The climate, pH, and conductivity had no difference between before and after removal times. The macroinvertebrate density in the sediment and the macrophytes was higher in the control site compared to the impact site. This could be due to smaller differences in pH and water temperature between sites. Or it could be due to the higher use for recreational activities in the impact (boating and fishing), and therefore be more prone to disturbances in this site compared to the control site. As the impact area is next to housing and camping sites whereas the control site is next to a mountain wall. The drifting macroinvertebrate density did not change between sites.

The macroinvertebrate density in the sediment did not change with the interaction between site and time, which was the original hypothesis. It was not expected that the macrophyte removal would disturb the sediment much, and if some macroinvertebrates would decrease due to

disturbance others might take refuge in the sediment as the macrophytes were removed. The macroinvertebrate density found in the macrophytes was also not influenced by macrophyte removal. Therefore, the hypothesis that macrophyte removal would affect macroinvertebrate density negatively was rejected. *J. bulbosus* is a complex macrophyte, and as it had such high biomass in the study area, there were expectations that there would be high amounts of invertebrates inhabiting it, as more complex structures of macrophytes are known to have higher densities of invertebrates (Warfe & Barmuta, 2006), and when the complex macrophyte was removed, expectations were that macroinvertebrate density would decrease. Which implies that the macroinvertebrates have compressed to a much smaller macrophyte habitat area than before cutting. Another reason for this result might be that sampling effort, although standardization was done as well as possible, could be at fault. There were such short and small patches of macrophytes left, in which the sweep net might have collected some sediment during sampling. This could be a reason why there is a higher amount of Sphaeriidae within the impact sweep samples after cutting. The surface and sediment drifting macroinvertebrate densities were not influenced by macrophyte removal. Therefore, the hypothesis that the drifting macroinvertebrate density would increase after macrophyte removal was rejected. This was a surprise as macrophytes provide more areas for distribution. Previous studies have found larger reductions (often more than 50%) in macroinvertebrate densities after macrophyte removal (Monahan & Caffrey, 1996; Kaenel et al., 1998; Habib & Yousuf, 2014; Lusardi et. al., 2018). Monahan & Caffrey (1996) found that the macroinvertebrate density rapidly recovered after macrophyte removal.

While there was only a small amount of the parameters that showed change on the macroinvertebrate community in this BACI design, there probably is some macroinvertebrates that are dislodged during macrophyte removal in the Rysstad basin. This could have been solved with more sampling during the fieldwork, as there are signs of this in the summary statistics. For example, the density S.D. shows above 50% of the mean densities in several replicates in all sampling methods, which would be reduced by more samples. However, it would have been difficult to find time to sort and identify extra samples during this thesis.

5. Conclusion

Investigating the consequences of macrophyte removal on macroinvertebrates in a Nordic oligotrophic river has not been done until now. In the present study, macrophyte removal surprisingly did not show many signs of influencing the macroinvertebrates in the river. The main consequences of macrophyte removal seem to be on macroinvertebrate composition. The differences probably lie in the changes within individual taxa numbers and not so much in major changes of taxa composition between site and time. Functional feeding groups should be further investigated as it is a good way to indicate disturbances (Park et al., 2008). Although the macroinvertebrate community in the river Otra did not seem to be much affected by macrophyte removal, Velle et al. (2021) found that high biomasses of *J. bulbosus* might enhance the ecosystem rather than negatively affect it. Removing the nuisance growth of *J. bulbosus* might still be necessary periodically to allow for recreational uses. Kaenel et al. (1998) found that macrophyte removal during the summer might have fewer consequences for macroinvertebrates than during spring. This might be a reason for the low response of macroinvertebrate parameters to macrophyte removal in the Otra river. The current macrophyte removal area in the Rysstad basin is large enough to be able to conduct recreational activities. However, the biomass of removed macrophyte compared to what is left in the area is small. Partial macrophyte removal might be preferred for the established ecosystem as it leaves possibilities for dispersal for the organisms in the nearby area. Greer et al. (2012) found that fish completely vanished from areas of complete macrophyte removal, whereas in partial removal areas the fish was still present. Earlier suggestions have been removing partial macrophyte stands where two-thirds of the macrophytes are left standing to maintain the established ecosystem (Dawson & Haslam, 1983). What is essential to be addressed is the long-term effect on the macroinvertebrate communities in freshwaters where macrophytes are periodically removed. As it is feared that periodically removed macrophytes, long-term, could potentially create a completely different macroinvertebrate community and therefore change the food-web which then alters the energy transfers in the ecosystem (Habib & Yousuf, 2014).

6. References

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7. Appendices

Appendix A: Macroinvertebrates found within the sediment

Time	Taxa	C1	C2	C3	C4	C5	I1	I2	I3	I4	I5	
Before	Oligochaeta		20	36	164	72	20		20	12	24	16
Before	Nematoda		4	40	72	24	4		8	4	20	8
Before	Hydrachnidae		36	4	8	8			8			24
Before	Oribatei		20	8		16		4	12			
Before	Sphaeriidae			20		20	4	4	4			
Before	Ceratopogonidae Larvae		8									
Before	Chironomidae Larvae		276	60	236	76	28	8	24	168	236	44
Before	Chironomidae Pupae				20						4	
Before	Lepidostoma hirtum											4
After	Oligochaeta		12	24	32	20	52	16	1	8	32	24
After	Nematoda		8	20	36		4	24			76	14
After	Hydrachnidae		4	4	4	12	16	4		4		
After	Oribatei		120	8	32	12	60	4		4		
After	Sphaeriidae		4		24		8	8	1	12	4	8
After	Ceratopogonidae Larvae		4	4								
After	Chelifera			4				4				
After	Chironomidae Larvae		384	152	444	72	512	52		28	52	58
After	Chironomidae Pupae		20	8	20	4	68	4		4	4	6
After	Simuliidae Larvae						16					
After	Lepidostoma hirtum		4									
After	Oxyethira					4						
6 weeks after	Oligochaeta		28	24	80	12	72	12	8		16	8
6 weeks after	Nematoda		36	16	40	24	30	20	16	48	44	4
6 weeks after	Hydrachnidae		4			4	2	8	4			
6 weeks after	Oribatei		8	24	20	64	12	8				
6 weeks after	Sphaeriidae			4				12		12		
6 weeks after	Ceratopogonidae Pupae			4								
6 weeks after	Chelifera		4									
6 weeks after	Chironomidae Larvae		48	84	104	100	90	92	28	32	76	12
6 weeks after	Chironomidae Pupae							4				
6 weeks after	Simuliidae Larvae			4				8	4	20	8	
6 weeks after	Mystacides azurea				4							

Appendix B: Macroinvertebrates found in the macrophytes

Time	Taxa	C1	C2	C3	C4	C5	I1	I2	I3	I4	I5	
Before	Oligochaeta		56	24	32	17	86			3	3	3
Before	Nematoda			1	1							
Before	Hydrachnidae		32	11	3		2	4	1	2	6	7
Before	Oribatei		34	3	9	2	6	1	6	1	4	3
Before	Sphaeriidae		12								3	
Before	Collembola		2						2		3	
Before	Ceratopogonidae larvae				2		3					
Before	Chironomidae larvae		1136	132	800	101	399	44	28	45	245	302
Before	Chironomidae pupae		12	4	18	2	19		3	1		3
Before	Chironomidae adult				1							
Before	Simuliidae larvae		140	3	4	1	1	1	2	3	2	1
Before	Simuliidae pupae			1								
Before	Baetis niger									1		1
Before	Centroptilum luteolum		10		2	1	1					
Before	Leptophlebiidae		2				2				4	
Before	Leptophlebia marginata						2			1		2
Before	Leptophlebia vespertina		8	3	5	1	1	1		2	1	
Before	Radix balthica/labiata				2	6	6	6			3	5
Before	Hydra		6	2	1	4					4	6
Before	Amphinemura											1
Before	Amphinemura borealis								2	2		
Before	Amphinemura sulcicollis										1	
Before	Brachyptera risi								1			
Before	Capnia											1
Before	Nemoura						1					
Before	Leuctra									1		
Before	Leuctra hippopus									1		
Before	Annitella obscurata									1		
Before	Apataniidae				2		1				3	
Before	Apatania stigmatella				1	1	1	6			1	
Before	Hydroptilidae									1		
Before	Hydroptila								1			
Before	Lepidostoma hirtum		4			3	6	2	2		5	1
Before	Oxyethira				2							
After	Oligochaeta		14	24	29	39	31	9	4	2	10	60
After	Nematoda		2	8		25	4	1			7	16
After	Hydrachnidae		26	4	3	6	7	2	3	4	7	24
After	Oribatei		7	3	3	4	1	3	2	2	1	4
After	Sphaeriidae		5	5		2		3	3	2	19	64
After	Collembola					1		1				
After	Chironomidae larvae		590	366	532	260	99	212	12	9	132	188
After	Chironomidae pupae		16	16	10	3	9	1	2	1	7	4
After	Chironomidae adult							3				
After	Simuliidae larvae		41	1	5	27	6	5	1	1	3	
After	Simuliidae pupae		1			1					1	
After	Centroptilum luteolum				1						1	
After	Leptophlebiidae		1					2				
After	Leptophlebia vespertina		1		1					1	1	
After	Siphlonurus alternatus		1									
After	Radix balthica/labiata											8
After	Hydra			2	2				2			
After	Amphinemura borealis							5				
After	Nemouridae							2				
After	Siphonoperla burmeisteri										1	
After	Leuctra							1				
After	Apatania stigmatella									1		
After	Hydroptilidae									1		
After	Lepidostomatidae				1			1				
After	Lepidostoma hirtum							5				
After	Leptoceridae						1					
After	Mystacides						1					
After	Oecetist estacea										1	
After	Oxyethira		1			11					1	
6 weeks after	Oligochaeta		148	66	98	104	70	2	13	3	3	34
6 weeks after	Nematoda			2		12		1	2		1	42
6 weeks after	Hydrachnidae		80	12	10	140	60	4	2	1		40
6 weeks after	Oribatei		88	26	2	116	30	4	11	3	1	12
6 weeks after	Sphaeriidae		4			4	2	12	35	3	1	110
6 weeks after	Collembola										1	
6 weeks after	Ceratopogonidae larvae											4
6 weeks after	Chironomidae larvae		1448	352	538	1144	276	43	184	39	51	496
6 weeks after	Chironomidae pupae		4	8		12	4		1	2	1	10
6 weeks after	Chironomidae adult									1		
6 weeks after	Simuliidae larvae		24	2	8	12	10	9	8	6	9	14
6 weeks after	Baetidae		8				1					
6 weeks after	Baetis vernus					1						1
6 weeks after	Ephemerella mucronata						1					
6 weeks after	Leptophlebiidae		4		2							
6 weeks after	Leptophlebia vespertina					1						
6 weeks after	Radix balthica/labiata								1			2
6 weeks after	Hydra			2			2					
6 weeks after	Plecoptera		12							1		
6 weeks after	Leuctra						1					
6 weeks after	Protonemura		4									
6 weeks after	Taeniopteryx nebulosa		12			1	2					
6 weeks after	Limnephilidae/Apataniidae					1						
6 weeks after	Apataniidae		4									
6 weeks after	Lepidostoma hirtum		12							1		1
6 weeks after	Mystacidesazurea											2

Appendix C: Macroinvertebrates found drifting

Net position	Time	Taxa	C1	C2	C3	I1	I2	I3	
Surface	Before	Oligochaeta				3			
Surface	Before	Nematoda		1					
Surface	Before	Hydrachnidae		2	1	1		1	
Surface	Before	Oribatei				1			
Surface	Before	Collembola		1					1
Surface	Before	Ceratopogonidae Pupae				1			
Surface	Before	Chironomidae Larvae		31	35	20	14	24	35
Surface	Before	Chironomidae Pupae		21	24	9			2
Surface	Before	Chironomidae Adult		1	7	1		2	5
Surface	Before	Simuliidae Larvae				1	1		2
Surface	Before	Simuliidae Pupae		1					
Surface	Before	Baetidae					1		
Surface	Before	Leptophlebidae		1					
Surface	Before	Leptophlebia marginata		1					
Surface	Before	Leptophlebia vespertina		2		1			
Surface	Before	Heptagenia fuscogrisea					1		
Surface	Before	Radix balthica/labiata		1					
Surface	Before	Hydra						5	2
Surface	Before	Amphinemura Larvae			3	1			
Surface	Before	Amphinemura borealis		10	2	24	10	3	1
Surface	Before	Amphinemura sulcicollis		4	1	5			1
Surface	Before	Brachyptera risi						1	
Surface	Before	Nemouridae				1	1		
Surface	Before	Nemoura						1	
Surface	Before	Nemoura cinerea		2					
Surface	Before	Taeniopteryx nebulosa						1	
Surface	Before	Lepidostoma hirtum				1			
Surface	Before	Mystacides azurea				1			
Surface	After	Oligochaeta		1			1		3
Surface	After	Nematoda							1
Surface	After	Hydrachnidae						2	
Surface	After	Oribatei			1	1	3		2
Surface	After	Elmis aenea						1	
Surface	After	Collembola				2			11
Surface	After	Ceratopogonidae Pupae			1				1
Surface	After	Chironomidae Larvae		9	9	3	12	6	14
Surface	After	Chironomidae Pupae		7	6	10	22	19	8
Surface	After	Chironomidae Adult		1	4	2	1	2	2
Surface	After	Simuliidae Larvae		1	2		2		
Surface	After	Baetis muticus							1
Surface	After	Leptophlebidae						1	
Surface	After	Leptophlebia marginata		1					
Surface	After	Leptophlebia vespertina				1	1	1	
Surface	After	Radix balthica/labiata				1			
Surface	After	Hydra			1				
Surface	After	Amphinemura Larvae					1		1
Surface	After	Amphinemura borealis		5	1	1	5	4	6
Surface	After	Amphinemura Adult						1	
Surface	After	Brachyptera risi					1		
Surface	After	Isoperla grammatica							1
Surface	After	Nemoura cinerea					1		
Surface	After	Siphonoperla burmeisteri					1		1
Surface	After	Apataniidae					1	1	
Surface	After	Apatania stigmatella							1
Surface	After	Lepidostoma hirtum			1			1	
Surface	After	Oxyethira							2
Surface	After	Cyrnus					1		

Net position	Time	Taxa	C1	C2	C3	I1	I2	I3	
Sediment	Before	Oligochaeta		1	3	8			1
Sediment	Before	Nematoda				5			
Sediment	Before	Hydrachnidae			2	1			1
Sediment	Before	Oribatei			1				
Sediment	Before	Collembola						2	1
Sediment	Before	Chironomidae Larvae		11	80	123	29	32	47
Sediment	Before	Chironomidae Pupae		1	11	3	1		1
Sediment	Before	Chironomidae Adult			1	1	1	1	
Sediment	Before	Simuliidae Larvae			2	1		1	
Sediment	Before	Leptophlebia vespertina				1			
Sediment	Before	Hydra					2	2	1
Sediment	Before	Amphinemura borealis		1	1	4			
Sediment	Before	Amphinemura sulcicollis				1			
Sediment	Before	Leuctra				1			
Sediment	Before	Apataniidae				3			
Sediment	After	Oligochaeta			2	1	12	4	3
Sediment	After	Hydrachnidae		6		1		4	2
Sediment	After	Oribatei		3	3	2	8	12	5
Sediment	After	Sphaeriidae					20	22	16
Sediment	After	Ceratopogonidae Larvae			1				1
Sediment	After	Chelifera							1
Sediment	After	Chironomidae Larvae		122	129	19	132	108	132
Sediment	After	Chironomidae Pupae		4	6	3	24	6	10
Sediment	After	Chironomidae Adult					2		3
Sediment	After	Simuliidae Larvae		2	2		4	2	4
Sediment	After	Leptophlebia vespertina		1			4		
Sediment	After	Hydra			4	1		4	3
Sediment	After	Plecoptera		1					
Sediment	After	Amphinemura Larvae					2		
Sediment	After	Amphinemura borealis		4	1		2		3
Sediment	After	Amphinemura sulcicollis					2		1
Sediment	After	Hydropsychidae							1
Sediment	After	Lepidostoma hirtum					2		
Sediment	After	Oxyethira					2		
Sediment	After	Wormaldia		1					
Sediment	After	Rhyacophilidae		1					

Appendix D: Taxa richness summary statistics. Max = total number of taxa, S.D. = standard deviation.

Taxa Richness summary statistics

Method	*Site*	*Time*	*Max*	*Mean*	*S.D.*
Sweep	Control	Before	13	10.8	1.92
		After	10	8.6	1.14
		6 weeks after	12	9.4	2.70
	Impact	Before	13	10.6	2.07

		After	12	9.4	2.51
		6 weeks after	12	8.2	2.17
Grab	Control	Before	6	5.2	1.10
		After	8	6.6	1.14
		6 weeks after	7	5.6	0.894
	Impact	Before	6	4	1.41
		After	7	4.4	1.82
		6 weeks after	7	4.6	1.52
Surface Drift	Control	Before	9	6.67	3.22
		After	7	6	1
	Impact	Before	6	5.33	0.577
		After	11	9	2
Sediment Drift	Control	Before	9	6	3
		After	7	6	1
	Impact	Before	5	3.67	1.53
		After	11	8.67	2.08

Appendix E: Two-Way ANOVA output of taxa richness.

		Taxa Richness		**Two-way ANOVA**		
Method	*Effect*	*DFn*	*DFd*	*F*	*p*	*ges*
Grab	Site	1	24	8.881	0.007*	0.270
	Time	2	24	1.119	0.343	0.085
	Site:Time	2	24	0.569	0.574	0.045
Sweep	Site	1	24	0.065	0.801	0.003

	Time	2	24	2.370	0.115	0.165
	Site:Time	2	24	0.543	0.588	0.043
Surface Drift	Site	1	8	0.532	0.487	0.062
	Time	1	8	1.723	0.226	0.177
	Site:Time	1	8	3.596	0.095	0.310
Sediment Drift	Site	1	8	0.02	0.891	0.002
	Time	1	8	4.50	0.067	0.360
	Site:Time	1	8	4.50	0.067	0.360

*p<0.05

Appendix F: Fisher alpha diversity summary statistics.

Fisher alpha index summary statistics

Method	*Site*	*Time*	*Mean*	*S.D.*
Sweep	Control	Before	2.03	0.356
		After	1.54	0.111
		6 weeks after	1.48	0.445
	Impact	Before*	2.72	0.407
		After*	2.40	0.795
		6 weeks after	1.9	0.198
Grab	Control	Before	0.992	0.241
		After	1.15	0.19
		6 weeks after	1.11	0.276
	Impact	Before	0.943	0.45
		After*	1.12	0.424
		6 weeks after	1.05	0.321

Surface Drift	Control	Before	1.87	1.10
		After	2.61	0.654
	Impact	Before	1.72	0.308
		After	3.28	0.874
Sediment Drift	Control	Before	1.55	0.481
		After	1.53	0.268
	Impact	Before	0.987	0.464
		After	1.90	0.581

*1 outlier removed

Appendix G: Two-Way ANOVA output of Fisher alpha diversity.

	Fisher alpha index			**Two-Way ANOVA**		
Method	*Effect*	*DFn*	*DFd*	*F*	*p*	*ges*
Grab	Site	1	24	0.134	0.718	0.006
	Time	2	24	0.649	0.532	0.053
	Site:Time	2	24	0.005	0.995	0.000429
Sweep	Site	1	24	13.362	0.001*	0.378
	Time	2	24	5.411	0.012*	0.330
	Site:Time	2	24	0.143	0.867	0.013
Surface Drift	Site	1	8	0.336	0.578	0.040
	Time	1	8	6.351	0.036*	0.443
	Site:Time	1	8	0.798	0.398	0.091
Sediment Drift	Site	1	8	0.146	0.712	0.018
	Time	1	8	2.781	0.134	0.258

	Site:Time	1	8	3.039	0.119	0.275

*p<0.05

Appendix H: Shannon-Wiener diversity summary statistics.

Shannon-Wiener index summary statistics

Method	*Site*	*Time*	*Mean*	*S.D.*
Sweep	Control	Before	0.802	0.239
		After	0.73	0.326
		6 weeks after	0.884	0.227
	Impact	Before	1.00	0.348
		After	1.33	0.408
		6 weeks after	1.10	0.186
Grab	Control	Before	1.22	0.288
		After	0.94	0.147
		6 weeks after	1.32	0.11
	Impact	Before	0.98	0.54
		After	1.14	0.292
		6 weeks after	1.22	0.161
Surface Drift	Control	Before	0.878	0.461
		After	1.02	0.045
	Impact	Before	0.908	0.264
		After	1.33	0.312
Sediment Drift	Control	Before	0.562	0.158

		After	0.562	0.146
	Impact	Before	0.378	0.15
		After	0.999	0.057

Appendix I: Two-Way ANOVA output of Shannon-Wiener diversity.

Shannon-Wiener index				Two-Way ANOVA		
Method	*Effect*	*DFn*	*DFd*	*F*	*p*	*ges*
Grab	Site	1	24	0.163	0.690	0.007
	Time	2	24	1.662	0.211	0.122
	Site:Time	2	24	1.414	0.263	0.105
Sweep	Site	1	24	9.576	0.005*	0.285
	Time	2	24	0.469	0.631	0.038
	Site:Time	2	24	1.444	0.256	0.107
Surface Drift	Site	1	8	0.911	0.368	0.102
	Time	1	8	2.548	0.149	0.242
	Site:Time	1	8	0.619	0.454	0.072
Sediment Drift	Site	1	8	2.659	0.142	0.249
	Time	1	8	16.044	0.004*	0.667
	Site:Time	1	8	15.968	0.004*	0.666

*$p<0.05$

Appendix J: Pielou's Evenness index summary statistics.

Pielou's Evenness summary statistics				
Method	*Site*	*Time*	*Mean*	*S.D.*

Sweep	Control	Before	0.346	0.12
		After	0.344	0.163
		6 weeks after	0.399	0.07
	Impact	Before	0.438	0.174
		After	0.621	0.232
		6 weeks after	0.529	0.095
Grab	Control	Before	0.748	0.146
		After	0.51	0.126
		6 weeks after	0.769	0.035
	Impact	Before	0.699	0.282
		After	0.834	0.095
		6 weeks after	0.836	0.085
Surface Drift	Control	Before	0.463	0.123
		After	0.576	0.031
	Impact	Before	0.542	0.15
		After	0.607	0.085
Sediment Drift	Control	Before	0.348	0.11
		After	0.323	0.115
	Impact	Before	0.315	0.074
		After	0.472	0.075

Appendix K: Two-Way ANOVA output of Pielou's Evenness index.

Pielou's Evenness				Two-Way ANOVA		
Method	*Effect*	*DFn*	*DFd*	*F*	*p*	*ges*
Grab	Site	1	24	4.364	0.047*	0.154
	Time	2	24	1.942	0.165	0.139
	Site:Time	2	24	4.090	0.030*	0.254
Sweep	Site	1	24	8.972	0.006*	0.272
	Time	2	24	0.986	0.388	0.076
	Site:Time	2	24	1.016	0.377	0.078
Surface Drift	Site	1	8	0.801	0.397	0.091
	Time	1	8	2.064	0.187	0.205
	Site:Time	1	8	0.152	0.707	0.019
Sediment Drift	Site	1	8	1.116	0.322	0.122
	Time	1	8	1.416	0.268	0.150
	Site:Time	1	8	2.713	0.138	0.253

*$p<0.05$

Appendix L: Macroinvertebrate density summary statistics.

Macroinvertebrate density summary statistics					
Method	*Site*	*Time*	*Mean*	*S.D.*	*Median*
Sweep (m^2)	Control	Before	54.2	35.5	37
		After	38.1	11.5	34.1
		6 weeks after	164	96.9	124
	Impact	Before	12.7	6.32	8.89
		After	40.3	51.4	20

		6 weeks after	51.7	74	17
Grab (m^2)	Control	Before	11591	7712	9600
		After	19876	11576	24889
		6 weeks after	8409	2056	9067
	Impact	Before	5831	4645	4267
		After	4053	2793	4889
		6 weeks after	4480	2587	4978
Surface Drift ($100m^3$)	Control	Before	51.3	6.76	48.4
		After	9.92	2.61	10.5
	Impact	Before	66.2	41.3	78
		After	15	2.23	15.8
Sediment Drift ($100m^3$)	Control	Before	111	93.6	128
		After	116	60.3	147
	Impact	Before	116	69.3	136
		After	102	39.2	84.2

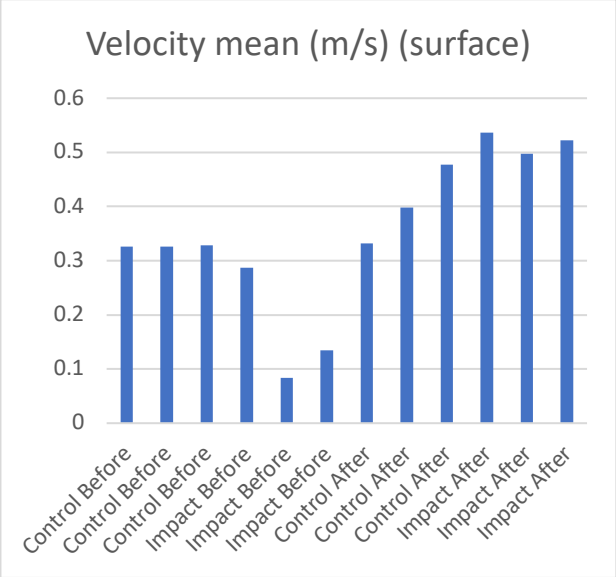
Appendix M: Two-Way ANOVA output of macroinvertebrate density.

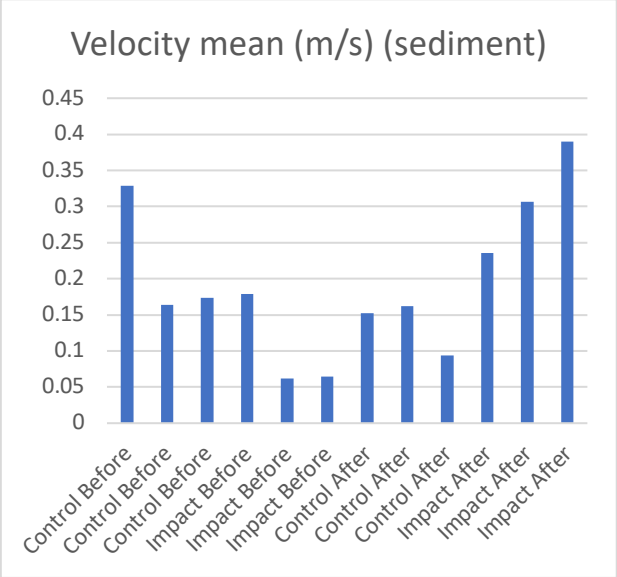
	Macroinvertebrate density			**Two-Way ANOVA**		
Method	*Effect*	*DFn*	*DFd*	*F*	*p*	*ges*
Grab	Site	1	24	10.331	0.004*	0.301
	Time	2	24	0.026	0.974	0.002
	Site:Time	2	24	1.152	0.333	0.088
Sweep	Site	1	24	15.152	0.000691*	0.387
	Time	2	24	3	0.069	0.200

	Site:Time	2	24	1.636	0.216	0.120
Surface Drift	Site	1	8	0.847	0.384	0.096
	Time	1	8	30.707	0.000546*	0.793
	Site:Time	1	8	0.480	0.508	0.057
Sediment Drift	Site	1	8	0.014	0.908	0.002
	Time	1	8	0.013	0.912	0.002
	Site:Time	1	8	0.053	0.824	0.007

*p<0.05

Appendix N: Mean velocity measured during drift sampling.





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