Accepted Manuscript

This is a post-peer-review, pre-copyedit version of an article published in
Water, Air, and Soil Pollution by Springer.

The final authenticated version is available online at:
http://dx.doi.org/10.1007/s11270-018-3918-7

Olsen M, Moy F E, Mjelde M, Lydersen E. 2018. An In Situ Experimental Study of Effects on Submerged Vegetation After Activated Carbon Amendment of Legacy Contaminated Sediments.
Water, Air and Soil Pollution. 229 (8), 263.

Title:

An *in situ* experimental study of effects on submerged vegetation after activated carbon amendment of legacy contaminated sediments

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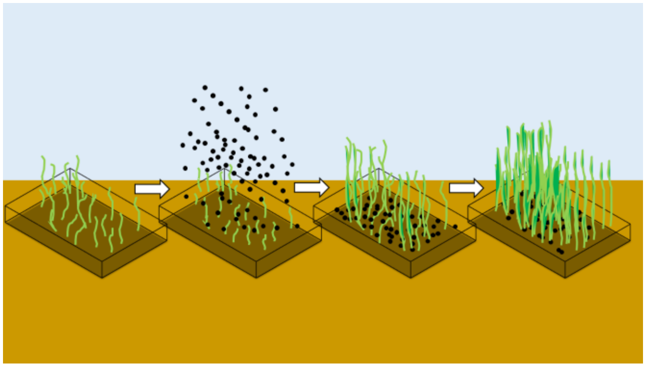
Abstract

Activated carbon (AC) amendment has been shown to reduce bioavailability of hydrophobic contaminants in the bioactive layer of sediment. Unwanted secondary effects of AC amendment could be particularly undesirable for ecologically important seagrass meadows, but so far only a few studies have been conducted on effects on submerged plants. The purpose of this study was to investigate effects on growth and cover of submerged macrophytes *in situ* after AC amendment. Test sites were established within a seagrass meadow in the severely contaminated Norwegian fjord Gunneklevfjorden. Here we show that AC amendment does not influence neither cover nor length of plants. Our study might indicate a positive effect on growth from AC in powdered form. Hence, our findings are in support of AC amendment as a low-impact sediment remediation technique within seagrass meadows. However, we

recommend further studies *in situ* on the effects of AC on submerged vegetation and biota. Factors influencing seasonal and annual variation in plant species composition, growth and cover should be taken into consideration.

Abstract art:



Introduction

Activated carbon (AC) amendment to contaminated sediments has been introduced as a low-impact approach for sediment remediation [1] and an alternative to removal or isolation of contaminated sediments. Several *in situ* and *ex situ* studies have reported on significant reduction in pore water concentration and bioavailability of hydrophobic contaminants in the bioactive layer of sediments after AC amendment[2-6]. However, recently there has been an awareness on the potential harmful secondary effects of AC amendment to benthic organisms and submerged vegetation[7, 8], though only a few studies have been conducted on secondary effects of AC amendment on submerged vegetation[7-9]. Laboratory studies have indicated reduced growth after amendment with AC [7]. However, in a long term study on recovery of benthic communities after amendment with different AC concentrations (0-10%), no significant effects were found in macrophyte densities between different AC treatments[9]. Lehmann, et al. [10] has evaluated the growth of terrestrial plants in soil amended with different types of manufactured black carbon, and found that biochar can greatly improve plant growth, while AC has shown somewhat diverging effects on growth of terrestrial plants [11, 12]. However, it is unclear whether observations in terrestrial systems can be translated to aquatic environments [7].

Secondary effects would be particularly undesirable for submerged meadows that already are experiencing a global decline [13, 14], as they are offering several important aquatic ecosystem services; providing foraging, shelter and breeding grounds to organisms [15-17], as well as functioning as carbon sinks [18]. Seagrass meadows are known to trap particles from the water column [19, 20], thus enhancing sediment deposition and reducing resuspension [21] and are therefore suspect to high concentrations of contaminants within polluted areas. Accordingly, submerged meadows may be important exposure sites for contaminants to inhabiting organisms, and recent studies have shown enhanced bioavailability of sediment Hg within vegetated areas[22-24], which may initiate a transfer of contaminants through food webs, with a potential to biomagnify at each trophic level. Thus, ecologically important submerged meadows within polluted areas potentially face the duality of being suspect to both remediation and conservation, which actualises the need to develop low-impact risk-reducing remediation strategies.

The purpose of the experiment was to investigate *in situ* whether amendment with powdered or granulated AC has effects on growth or cover of submerged macrophytes, prior to recommend it as a low-impact approach for remediation of contaminated sediments. To test the hypothesis of no variation between different treatments, test sites were established *in situ* within the submerged seagrass meadow found in the Norwegian brackish fjord Gunneklevfjorden (Figure 1).

Materials and Methods

Study site

The semi-enclosed brackish fjord Gunneklevfjord covers an area of approximately 0,7 km^2 and is connected to the river Skienselva to the north, and to the fjord Frierfjorden to the south (Figure 1). There are sills in both outlets, with the shallowest parts reaching only 2 meter depth. The main area in the southern part of the Gunneklevfjord is reaching 4-5 meter depth, while the northern part reaches down to 11 meter depth [25]. The salinity of surface waters in the Gunneklevfjord is typically in the range of 0.5-6 ‰. Periodically a halocline is found at 2-3 m depth and stagnant deep waters have been found with salinity in the range of 10 – 20 ‰ [25]. The fjord is hosting a large seagrass meadow in the south-eastern part of the fjord, covering approximately 70 000 m^2 and reaching from 0.5 to 2.5 meters depth. The seagrass meadow is classified as very important (of national value) due to its size and quality, according to the Norwegian Environment Agency [26]. In 2014 a survey identified 13 aquatic macrophytes in the Gunneklevfjord [27], with dominating species being the vascular plants *Elodea canadensis* and *Potamogeton crispus,* in addition to the charophyte *Chara virgata*. Brackish waters and varying salinity is a challenge to both marine and estuarine organisms, limiting the biological diversity of the fjord. Nevertheless, recent sampling of benthos and fish within the meadow has revealed high abundance of organisms and demonstrated the ecological importance of seagrass meadows[28]. Most of the species found in the fjord are freshwater species that have a tolerance for low and stable salinity. Since early 1900, the fjord has received substantial amounts of Hg and chlorinated compounds like dioxins/furans (PCDD/PCDF), octachlorstyren (OCS) and hexachlorbenzen (HCB) due to discharges from nearby industrial activities

[29]. Recent investigations have revealed sediment surface concentrations reaching 15.5 mg Tot-Hg kg^{-1} and 3.2 µg MeHg kg^{-1} [24].

Our *in situ* test sites for AC amendment were established within the seagrass meadow (Figure 1). Sediment was treated with thin layers (< 3 cm) of powdered or granulated activated carbon, approximately 2 kg AC/m^2 The limestone was included in the experiment as an alternative non-active capping material, which is traditionally placed on the sediments in much ticker layers than AC (>30 cm). In this experiment, limestone was added in a 3-5 cm layer, which is not as thick as a realistic treatment. Cover of plants was documented over a period of three months during the growing season in 2014 and then once in August 2015. Length of plants was measured once in 2014.

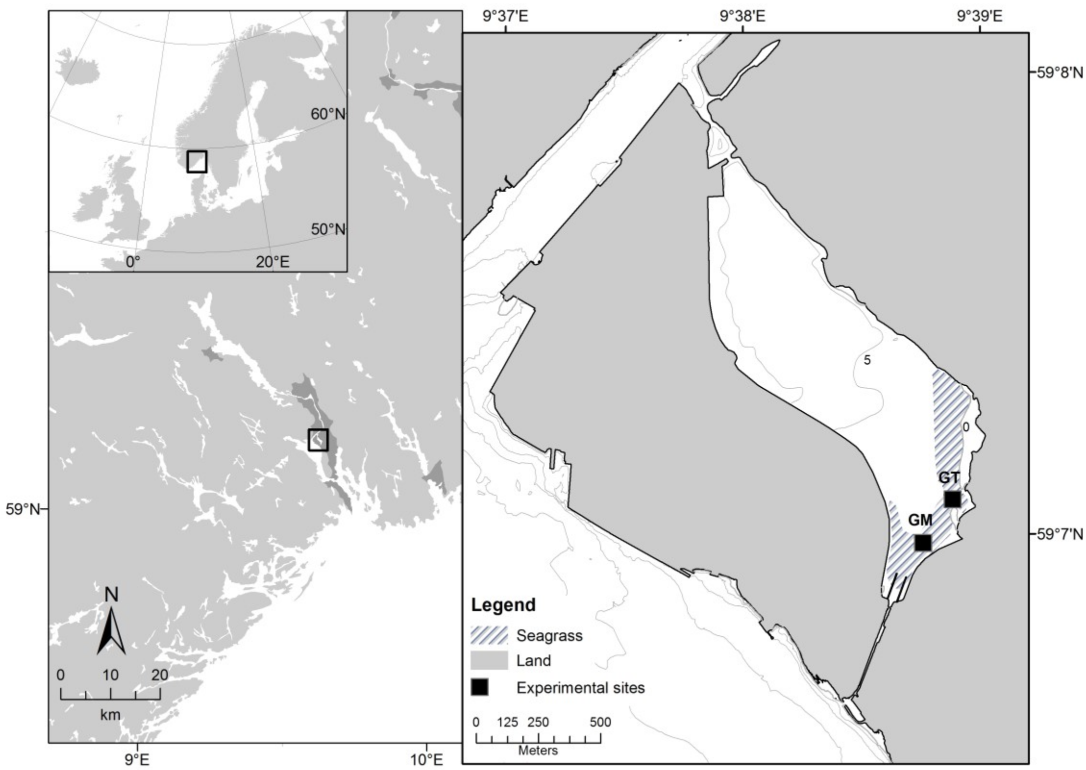


Figure 1. The study area Gunneklevfjord in southeastern Norway. The seagrass

area is shaded and the two experimental sites GM and GT is shown.

Placement of frames on seabed

Two *in situ* test sites (GT and GM) were established within the seagrass meadow with a distance of approximately 200 meters (Figure 1). The sites differed slightly in plant species composition at the initiation of the test. Site GT was dominated by *Chara virgata* while site GM was equally dominated by *Chara virgata* and *Potamogeton crispus*. In each site, 12 frames (80 x 120 cm) were placed on the seabed at 2 – 2.5 m depth, and with a distance of 5-10 meters between the frames, giving triplicate frames for each of three different treatments in addition to three untreated frames in both the test sites (controls). The frames were constructed by cutting off the bottom of bricklayer buckets, leaving a 10 cm high edge. To weigh down the frames heavy chains were attached to the outside of each frame. Each frame was marked with a rope and a buoy to the surface.

Capping of sediment within frames

The three treatments were distributed randomly to the frames within the two test sites GM and GT (Figure 2).

Site GM		
NON2	ACP3	LIM3
ACP1	ACG2	NON3
ACP2	ACG3	LIM2

Site GT		
LIM3	ACG1	ACP3
NON2	ACG3	LIM1
ACP2	ACP1	LIM2

NON1	LIM1	ACG1		ACG2	NON3	NON1

Figure 2. Placement of frames on the seabed within the two test sites GM and GT in the submerged meadow in Gunneklevfjord, and distribution of different treatments in triplicates (1-3). Treatment ACP=Powdered activated carbon, ACG=Granulated activated carbon, LIM=Limestone and NON= No treatment.

At each test site approximately 2 kg m^{-2} of powdered or granulated AC was added to three replicate frames each (named treatment ACP and ACG, respectively), without any pre-treatment. First, 1 kg m^{-2} of AC was added (8^{th} of July 2014), and the placement of the capping material within the frames was visually observed by the use of a subsea GoPro Hero3+ action camera after the capping material had settled, approximately one hour after application. Another 1 kg m^{-2} was added one week later. Limestone (Norstone, 0-8 mm; treatment LIM) was added in a 3-5 cm thick layer to three replicate frames at each site (8^{th} of July 2014). All capping materials were brought down to the seabed by the use of a pipe. A silt curtain was surrounding the pipe from the edge of the frame up to the water surface to limit loss of material outside the frames. Photos taken after capping revealed insignificant loss of capping materials outside the frames.

Monitoring cover

Documentation of cover of plants within the frames was done by photographing each frame from above with a waterproof GoPro Hero3 + Black edition camera. The camera body was attached to a rod and subsequently lowered into the water to about 30 cm over the seabed, consequently shooting one photo/2 second. Photography was completed on three occasions during the growing season in 2014 (time 1=6^{th} of

August 2014; time 2=27th of August 2014; time 3=29th of September 2014) and again one year later on one occasion in August 2015 (time 4=21th of August 2015). The first round of photography (time 1) was carried out 4 weeks after placement of capping material in the frames. At time 3 one frame of AC granulate (ACG) amendment in site GT and one untreated frame (NON) in site GM had been lost, giving a total of 22 frames photographed. At time 4 (August 2015) one more frame of AC granulate (ACG) and one of limestone (LIM) amendment had been lost from GT, giving 20 frames for both sites. The images were analysed by estimating the percentage cover of vegetation within each frame. The percentage cover was estimated manually using a 10x10 grid placed over the image. Percentage cover of plants in an identically sized area just outside each frame was similarly quantified as a non-treated reference for each frame. It was assumed that the area just outside each frame gave a better reference than the non-treated frames assigned as controls, given the natural patchiness of cover within the meadow. The ratio of the percentage cover outside (C_o) and within the frames (C_i) was used as a measure for the effect of treatment, expressed as the cover ratio (C_r).

$$C_r = C_i / C_o$$

The C_r calculated for the non-treated frames was used as a measure for effect of the frame itself.

<u>Measuring length of plants</u>

Plant material from inside the frames was collected three months after amendment using divers (at time 3). Divers cut plants from a square approximately 10x10cm within each frame and as close to the sediment surface as possible, for the measurement of plant length. Cut plants were put directly into plastic zipper bags under water. Immediately after sampling, the plants were brought ashore, and determined to species. For comparison of length of plants between treatments, only the most abundant species *Potamogeton crispus* in site GM was measured. All sampled plants were measured and the median plant length for each frame was used for comparison between treatments.

Statistical analysis

All statistical analyses were done using the computing program RStudio version 0.98.1056 running on R version 3.1.0 [30]. Correlation between percentage cover within and outside the frames was calculated using both parametric and non-parametric correlation coefficients and tests, as the data violated parametric assumptions being non-normally distributed. Differences in cover ratio (C_r) between treatments were tested using both parametric methods (ANOVA) and the non-parametric Kruskal-Wallis multiple comparison test. Differences in length of plants between treatments were tested using ANOVA and multiple regressions.

Results and Discussion

The central question of this study was whether amendment with powdered or granulated AC affects length or cover of macrophytes in a submerged meadow in the contaminated sediment site Gunneklevfjorden in Norway. The experiment revealed no significant effects of activated carbon whatsoever to the macrophytes, neither acute nor after one year. However, amendment with the non-active material limestone did reduce cover the first weeks after treatment. The results are presented and discussed below.

Effect of study design (frames) on percentage cover

To check for possible effects on percentage cover of plants from the frames themselves, the percentage cover observed outside and within the non-treated frames (treatment NON) were compared (Figure 3). There was no difference in cover ratio (C_r) between the two test sites for the untreated frames, hence data from both sites were merged when testing for effect of frames. Testing was done first for all sampling events merged (time 1, 2, 3 and 4), and then for the last sampling event in 2014 (time 3) separately.

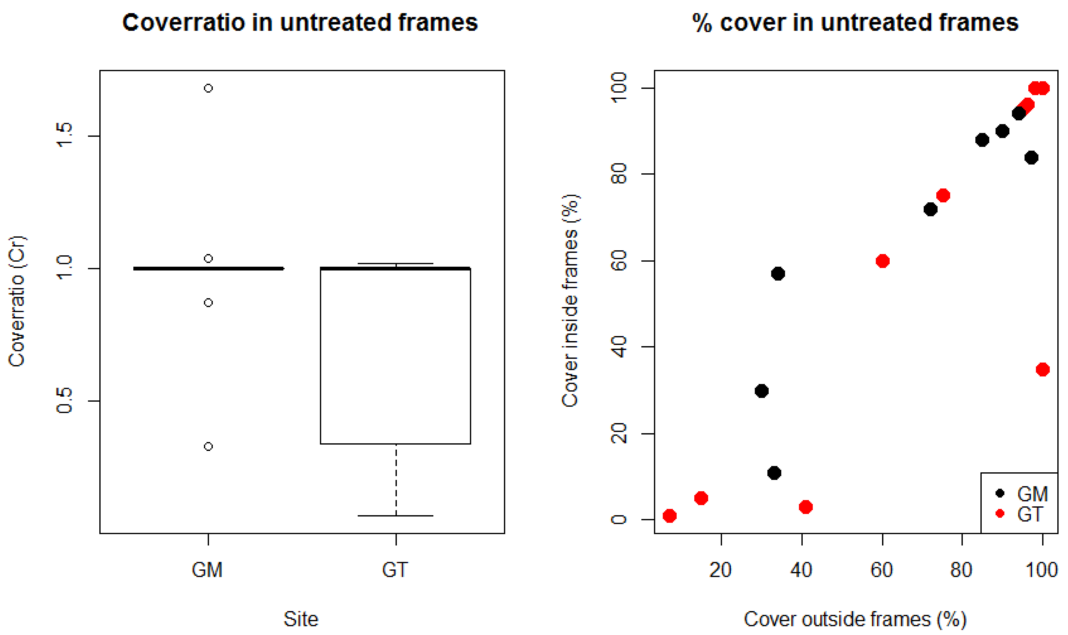


Figure 3. Difference in cover ratio (C_r) between the two sites GM and GT in the Gunneklevfjord (left) and comparison of the percentage cover observed outside and within the non-treated frames (treatment NON) for all sampling events merged (right).

Correlation of percentage cover outside and within NON-frames for all sampling events and both sites merged by Pearson's correlation coefficient and Spearman's rho was r=0.87 and r=0.85, respectively, with $p < 0.05$. Welch two sample t-test and Wilcoxon rank sum test were used for testing for difference in percentage cover between outside and within the frames. Neither of the tests showed significant difference between outside and within NON-frames.

Checking for correlation in percentage cover and for difference between inside and within frames for the last sampling event in 2014, did also give significant correlation and no significant difference (p>0,05). Based on the results for the untreated frames it was assumed that the placement of the frames on the seabed did not have any significant effect on the percentage cover of plants within the frames. Hence, effect of frames was not taken into consideration when testing for effect of treatments.

Effect on cover ratio (C_r)

Cover ratio (C_r) for each frame was calculated to look for effects of different treatments, and differences between treatments were tested using both parametric test (ANOVA and pairwise comparison using t-test) and non-parametric test (Kruskal-Wallis rank sum test and post hoc multiple comparison test after Kruskal-Wallis). There was a significant difference between the treatments (p<0,05) when all sampling events (time 1, 2, 3 and 4) were merged (Figure 4). The difference was caused by limestone (LIM), which was found to be significantly different from all other treatments, including the untreated frames (NON). No significant effects on C_r could be found for either powdered AC (ACP) or granulated AC (ACG).

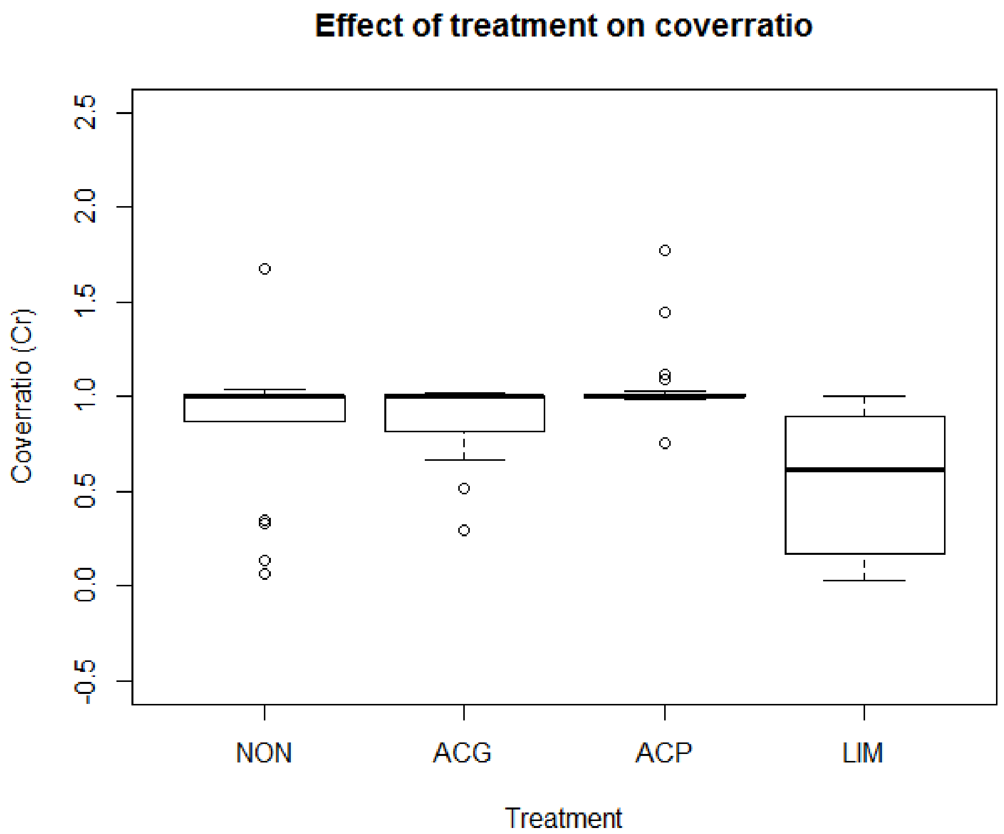


Figure 4. Comparison of cover ratio (C_r) for all treatments and all sampling events merged (time 1, 2, 3 and 4). Treatment ACP=Powdered activated carbon, ACG=Granulated activated carbon, LIM=Limestone and NON= No treatment.

The same tests were carried out separately for difference in C_r between treatments at each time of sampling (Figure 5). Significant variation in C_r between the treatments was found at all times of sampling during the first year (time 1, 2, 3), but not the second year (time 4). At time 1, 2 and 3 treatment LIM was found to be different from ACP ($p<0,05$), but none of the other treatments differed from each other in C_r.

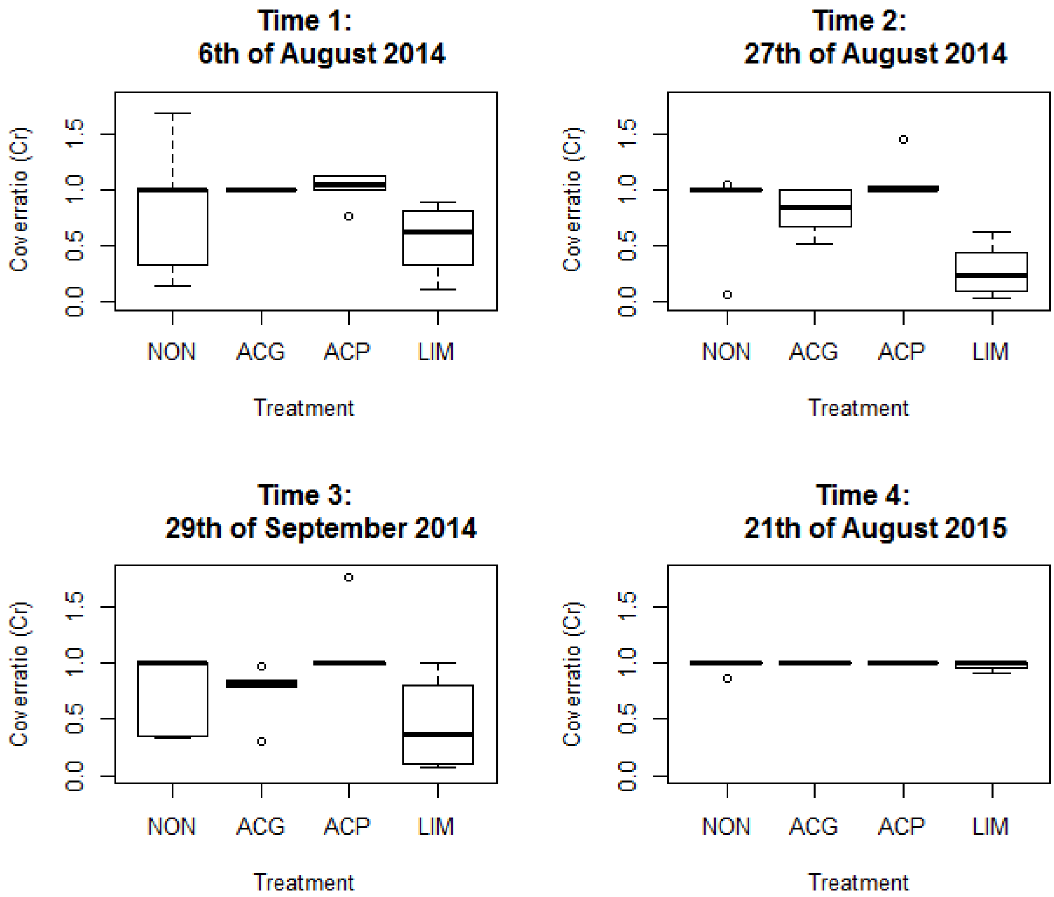


Figure 5. Comparison of cover ratio (C_r) between treatments at each time of sampling (time 1, 2, 3 and 4). Treatment ACP=Powdered activated carbon, ACG=Granulated activated carbon, LIM=Limestone and NON= No treatment.

Reduced cover of plants within frames amended with limestone the first year, may be caused by the mechanical disturbance of the plants by limestone. Limestone was added in a thicker layer (3-5 cm) and with larger grain size than AC. Also, limestone ($CaCO_3$) may have an influence on the water chemistry. Earlier studies have shown that addition of $CaCO_3$ have reduced or eliminated macrophyte biomass in hardwater lakes[31]. In addition, it is known that limestone ($CaCO_3$) may slowly dissolve and change the pH locally, subsequently reducing the CO_2 content of water. A local decrease in [CO_2] compared to [HCO3] may be one reason for the negative effect on cover. However, *Potamogeton crispus* can assimilate HCO_3 for growth, but it seems to prefer CO_2 as a carbon-source [32]. However, also AC may lower water pH with a potential for influencing water chemistry. Since water chemistry effects from addition of capping materials were not within the scope of this study, no measurements of [CO_2] or pH in water were carried out. The plant species in our study seem to senesces early in the season compared to similar species[31]. This may have an effect on the results.

During the study period, there was a marked change in the general cover of plants within the entire vegetation area. In August 2014 (time 3) the mean cover outside the frames was 88 %, while in August 2015 (time 4) the mean cover was 99 %. The species composition in the study sites also made a change from the first to the second year of study. In the first year the *Chara virgata* and *Potamogeton crispus* was the dominating species in the study area, while in 2015 *Potamogeton crispus* was barley seen. Our study reveals neither the cause of the general increase in cover of plants from 2014 to 2015, nor of the dominance of *Chara* over *Potamogeton crispus* observed in 2015. The change in cover and in species composition were observed not only within the frames but across the entire meadow. Therefore, we find it not likely that the changes were initiated by our treatments. The changes might rather be due to external factors such as light, nutrients or salinity, and to annual variation in competition between species. Salinity is recognised as the most important factor controlling species composition in brackish areas [33]. Occasional inflow of high salinity waters between sampling in August 2014 and September 2015 cannot be foreclosed.

Check of possible covariates influencing length of plants

To check whether site or number of different species within the frames had an influence on the length of plants, ANOVA was used to compare the median length of plants between the two sites GM and GT, and between groups of plants defined by numbers of species found when sampling (1, 2 or 3 species). Neither site nor number of species were found to give significant differences in length of plants, even though somewhat longer plants were found at site GT compared to GM (mean 30,5 cm and 26,3 cm, respectively) (Figure 6). Hence, site and number of species were not included as covariates when fitting models for length of plants.

Possible correlation between cover ratio and length

Correlation between percentage cover of plants and median length of plants within each of the non-treated frames (treatment NON) were found not to be significant ($p>0.05$ by Pearsons product-moment correlation). Also, a simple linear regression model fitted for length of plants showed that percentage cover was not a significant predictor. Hence, length of plants was not normalized to percentage cover before testing for effect of treatments.

No effects from treatments on length of plants

Variation in median length of plants between treatments was tested using ANOVA and pairwise comparison using t-test (Figure 6). Testing of differences in length was done within each site and for the sites merged. There were no significant differences in length of plants between the treatments.

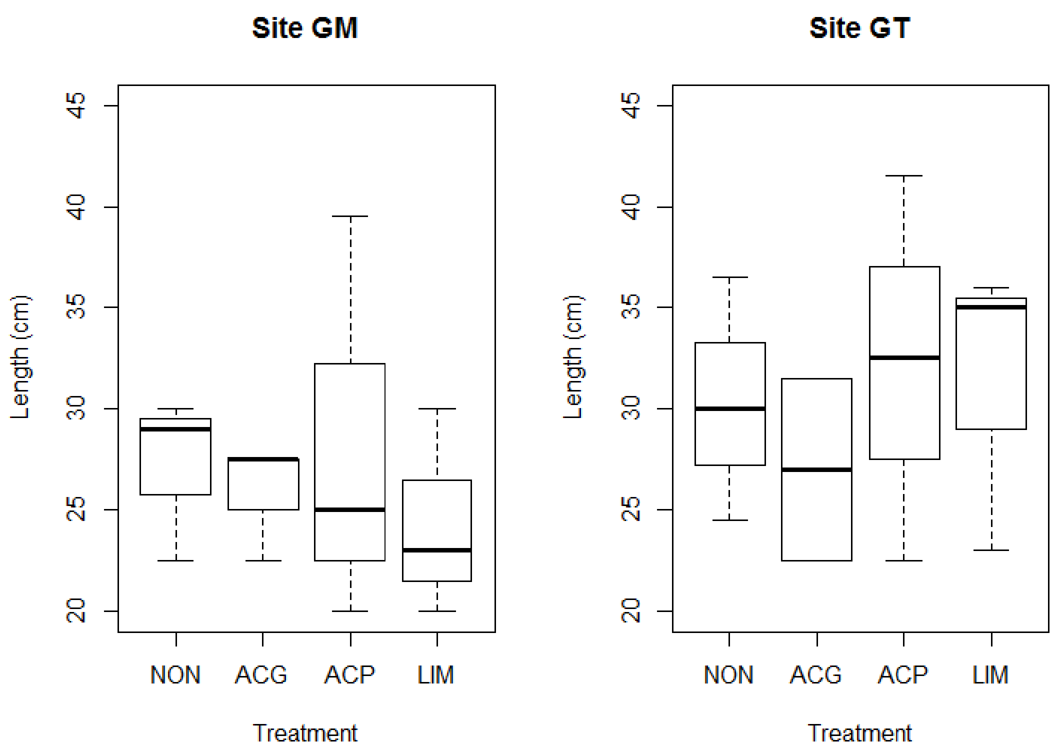


Figure 6. Comparison of median length of plants within frames of different treatments at two test sites in the Gunneklevfjord. Treatment ACP=Powdered activated carbon, ACG=Granulated activated carbon, LIM=Limestone and NON= No treatment.

Our results do not support earlier findings that AC in powdered form reduces plant growth [7, 12], and that AC in granulate form increases plant growth [12]. No significant effect was found after AC amendment on neither length nor cover of the plants within the study area in the Gunneklevfjord. The results are in support of AC amendment as a low-impact remediation method in areas of submerged vegetation. Still, since studies on secondary effects of AC amendment are few, knowledge is scarce and results are diverging, there is a need of more studies in-situ to understand the effects of activated carbon on submerged vegetation. Factors influencing seasonal and annual variation in plant species composition and cover should be taken into consideration when carrying out in-situ studies.

Acknowledgement

We thank Lise Ann Tveiten, Janne Kim Gitmark, Maia Røst Kile and Vetle B. Fredheim for assistance during sampling. This research was funded through a joint PhD by University college of southeastern Norway, University of Agder, Institute of Marine research and Norwegian Institute for Marine Research to Marianne Olsen.

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