Chemosphere 359 (2024) 142284

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Enhancing nitrogen removal through macrophyte harvest and installation of woodchips-based floating beds in surface-flow constructed wetlands

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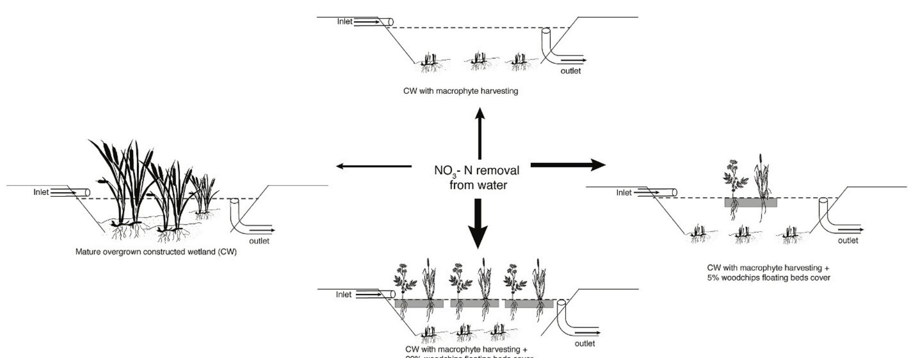
HIGHLIGHTS

- Macrophyte harvesting may improve N removal capacity of surface-flow constructed wetlands.
- Woodchips-based floating beds support plant growth and plant N assimilation.
- *Glyceria maxima* and *Filipendula ulmaria* grow well on woodchips-based floating beds.
- Plant roots and woodchips enhance floating bed denitrification potential.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: T Cutright

Keywords:
Floating wetlands
Aquatic plants
Wetland biofilm
Nitrogen uptake
Wetland restoration

ABSTRACT

Wetland management maintains nitrogen (N) removal capacity in mature and overgrown constructed wetlands (CWs). We evaluated whether CW management by macrophyte harvesting, and subsequent installation of woodchips-based floating beds (WFBs) planted with *Glyceria maxima* and *Filipendula ulmaria* improved N removal. In sixteen heavily overgrown experimental CWs, we applied four treatments: i) only macrophyte harvesting, ii) 5% of the harvested-CW surface covered with WFBs, iii) 20% WFBs cover, and iv) a control treatment (heavily overgrown). N removal was determined in all wetlands at nine occasions. Plant biomass accrual, N assimilation, and denitrification genes *nirS*, *nirK*, *nosZI* and *nosZII* on plant roots and woodchips from WFBs were estimated. Macrophyte harvesting improved N removal of heavily overgrown CWs, whereas subsequent WFB installation only sometimes improved N removal. Mean N removal efficiencies (± standard deviation) overall were 41 ± 15 %, 45 ± 20 %, 46 ± 16 % and 27 ± 8.3 % for treatments i to iv, respectively. Relative biomass production, root length and root surface area for *G. maxima* (mean ± standard deviation: 234 ± 114 %, 40 ± 6.5 cm, 6308 ± 1059 cm^2g^{-1}, respectively) were higher than those for *F. ulmaria* (63 ± 86 %, 28 ± 12 cm, 3131 ± 535 cm^2g^{-1}, respectively) whereas biomass N assimilation was higher for *F. ulmaria* (1.8 ± 0.9 gNm^{-2} of WFB) than for *G. maxima* (1.3 ± 0.5 gNm^{-2} of WFB). Denitrification gene abundance was higher on plant roots

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https://doi.org/10.1016/j.chemosphere.2024.142284
Received 4 March 2024; Received in revised form 30 April 2024; Accepted 6 May 2024
Available online 6 May 2024

than on woodchips while *G. maxima* hosted higher root denitrification gene abundance than *F. ulmaria.* We conclude that macrophyte harvesting improves N removal in heavily overgrown CWs. WFBs installation has the potential to support plant growth and denitrification in surface-flow constructed wetlands. Further studies need to evaluate the long-term effects of macrophyte harvesting and WFB installation on N removal in CWs.

1. Introduction

Constructed wetlands (CWs) are a cost-efficient nature-based solution for water nitrogen (N) removal (Vymazal, 2011; reviewed by Wang et al., 2018). The two main N removal pathways in CWs are N assimilation by plants and the conversion of dissolved nitrate (NO_3^-) to nitrogen gas (N_2) gas through denitrification (Choudhury et al., 2022). For both pathways, aquatic plants play a major role. Their root systems assimilate N and together with submerged stems, leaves, and litter enhance microbial denitrification by providing attachment surfaces as well as supplying labile dissolved organic carbon (DOC) for microbial growth and activity (Wu et al., 2022; Allen et al., 2023; Martínez-Espinosa et al., 2021). Denitrification is often a greater contributor to N removal than plant N uptake in CWs (Choudhury, 2018; Hallin et al., 2015). Since microorganisms performing denitrification are substratum associated, abundant attachment surfaces for microbial growth are imperative to maximize N removal (Soana et al., 2023).

N removal in CWs usually increases with wetland age and cover of wetland vegetation until wetlands become mature (Nilsson et al., 2020; Gu et al., 2023). However, in heavily overgrown wetlands, the hydraulic efficiency and N removal performance may be reduced (Sabokrouhiyeh et al., 2020; Bodin et al., 2012). Although the aim of wetland creation is the long-term nutrient removal, it is unclear how these systems perform over time spans of more than one decade (Nan et al., 2023; Craft, 1996). Most wetland N removal studies are based on short-term observations from mesocosms, newly constructed wetlands, as well as on specific wetland successional phases such as initial-, vegetation colonization- and mature vegetation phases (Kadlec and Wallace, 2009; Land et al., 2016; Vymazal, 2018; Nilsson et al., 2020). Long-term observations of N removal in wetlands past maturation are lacking (Nan et al., 2023). Therefore, we need to examine different management practices to improve CW performance past maturation.

Matured CWs generally perform well with respect to N removal (Koskiaho and Puustinen, 2019). However, both organic and inorganic solids may accumulate over time until CWs become silted up and heavily overgrown, thus reducing water residence time in wetlands and, in consequence, N removal (Wang et al., 2021). In CWs, high net primary production, a eutrophic environment, reduced water flow, and anaerobic conditions in sediments may result in organic matter accumulation (Wang et al., 2021; Fraser and Keddy, 2005; Kayranli et al., 2010). This organic material, along with vegetation overgrowth, may channelize the water flow, thus reducing water surface contact with wetland biofilm as well as water residence time. Overgrowth and channelization may further reduce nutrient removal efficiency in unmanaged CWs (Koskiaho et al., 2003; Smith et al., 2019). Therefore, management of wetland vegetation may improve N removal efficiency in overgrown CWs.

In heavily overgrown CWs, harvesting of macrophytes and root mat removal should reduce channelization and clogging (Wang et al., 2021) and enhance an even water distribution across the wetland area, thus increasing the contact between water and wetland surfaces, and water residence time. Since macrophyte harvesting removes surfaces for biofilm attachment, floating bed (FB) installation in wetlands may compensate for this initial loss. FBs planted with emergent macrophytes develop extensive root systems stretching into the water column and provide vast attachment surfaces for bacteria (Shen et al., 2021). Especially in newly harvested CWs, this should promote N removal through biomass N assimilation and by enhancing denitrification (Schwammberger et al., 2019; Zhang et al., 2024; Choudhury et al., 2019a). Thus, installing planted FBs after macrophyte harvest should further enhance N removal capacity of heavily overgrown CWs.

Our study aimed to evaluate these two wetland management options. The main objectives were: i) to investigate the effects of wetland management through macrophyte harvesting and installation of woodchips-based floating beds (WFBs) on N removal in overgrown CWs, and ii) to investigate the potential of WFBs for macrophyte growth, for plant N assimilation, and as denitrification gene hotspot.

2. Materials and methods

2.1. Study site

The field experiment was conducted in 2020 at Halmstad University's experimental wetland area, in southern Sweden (56°43′45″N, 12°43′33″E). The experimental wetlands are constructed free-water surface wetlands with a heavy clay bottom, mimicking constructed semi-natural wetlands in an agricultural landscape and are fed with N-rich (7 mg L^{-1} total-N) ground water. The facility consists of 18 CWs, with a water surface area of 8.8 × 3 m (L × W) and a water depth of approximately 0.8 m. Each CW contains an inlet and outlet pipe, with inlet pipes equipped with faucets to enable flow rate manipulations (for more information, see Bodin et al., 2012). Seventeen years after their construction, the CWs were heavily overgrown with macrophytes and partly filled with a dense root mat.

2.2. Experimental treatments and construction of woodchips-based floating beds

In May 2020, twelve heavily overgrown CWs were emptied and cleared from dense emergent vegetation. The root mat (up to ca. 50 cm thick) was removed when thicker than ca. 20 cm, using a hand saw. All plant litter from harvesting was cleaned out before applying our four experimental treatments with four replicates each to 16 CWs. Treatments consisted of: i) *harvested*: harvesting all macrophytes from heavily overgrown CWs, ii) *+5% WFB*: harvesting and covering 5% CW surface area with WFBs, iii) *+20% WFB*: harvesting and covering 20% CW surface area with WFBs, and iv) *control*: heavily overgrown CWs remained unmanaged. After the initial harvest, a secondary macrophyte harvest was performed at the start of August in all but the *control* treatments. Emergent macrophytes were cut (ca. 5 cm) above the bottom without disturbing the sediment. At the end of the experiment, wetland area covered with newly grown macrophytes was estimated visually by dividing the CWs into 10 similar squares. Macrophyte species were categorized as emergent, floating-leaved, submerged, free-floating, moss or filamentous green algae (Table S1 in Supplementary material). A one-way ANOVA was performed to test for difference in emergent macrophyte cover between different CW management treatments (*control*, *harvested*, *+5% WFB* and *+20% WFB*) at the end of the experiment. Emergent macrophytes were the dominant plant type and were present in all CWs (Table S1, Supplementary material). At the end of the experiment, there was still significantly higher macrophyte cover (%) in *control* than in *harvested* treatments (one-way ANOVA; $F_{3,12} = 49.32$, $p < 0.001$). There were no differences in macrophyte cover within the harvested treatments (*harvested*, *+5% WFB* and *+20% WFB*; Tukey's pairwise comparison, $p > 0.05$).

WFBs were constructed with woodchips, jute sheets and jute rope. Freshly cut birch (*Betula* spp.) woodchips were purchased from a local supplier. Jute sheets, bands and ropes were used to sew jute sacks to a size of 57 × 50 × 8 cm (L × W × H). These were made into FBs by filling them with 0.018 m^3 (ca. 6 kg) fresh woodchips (for WFB construction and pictures, see Fig. 1). Before the start of the experiment, the WFBs were submerged in water for three to four days to soak the woodchips and decrease tannin concentrations. In the *+5% WFB* treatment 4 WFBs and, in the *+20% WFB* treatment 16 WFBs were installed.

To investigate macrophyte growth potential and N assimilation on WFBs, a perennial grass *Glyceria maxima* (Hartm.) Holmb. (reed sweet grass) and a perennial herb *Filipendula ulmaria* (L.) Maxim. (meadowsweet) were collected nearby. *F. ulmaria* is not strictly a macrophyte but grows on WFBs (Choudhury et al., 2019a). WFBs were planted with a mixture of *G. maxima and F. ulmaria* (n = 4, per species) prior to the experiment (Fig. 1). The initial planting biomass on WFBs was 8.9 ± 2.8g and 20.0 ± 7.2g (dry mass per WFB: mean ± standard deviation) for *G. maxima* and *F. ulmaria*, respectively. At the beginning of the experiment, we collected samples of each species, recorded the fresh weight and then oven dried the samples at 60 °C for 7 days before measuring the dry weight. The fresh weight-dry weight relationship was then used to determine the initial dry weight of each species on the WFBs. In the *+5% WFB* treatment, 2 of 4 WFBs, and in the *+20% WFB* treatment, 8 of 16 WFBs were planted with macrophytes.

WFB macrophytes were harvested at the end of the experiment. Macrophytes were collected separately for each species' root and shoot biomass, and oven dried at 60 °C to determine dry mass. Total (shoots and roots) relative biomass production (%) per species and WFB was calculated according to Eq. (1), where *W1* is the initial total dry mass and *W2* the end total dry mass.

$$Relative\ biomass\ production = \frac{(W2 - W1)}{W1} \times 100 \quad \text{Eq. 1}$$

We also measured three plant morphologies: maximum root length (cm), specific root surface area per dry mass ($cm^2\ g^{-1}$) and shoot number increase during the experiment for *G. maxima*. Maximum root length was measured in the field while specific root surface area was measured at laboratory through image analysis (for details, see Appendix 1, Supplementary material).

2.3. Nitrogen removal in CWs

Water samples were collected from inlet distribution tanks and outlet of each CW twice a month during June, July and August and three times in September, resulting in 9 sampling occasions. At all sampling occasions, outlet water temperature was measured. Water samples were taken to the laboratory and frozen until further analyses for total-N and NO_3–N concentrations using a flow injection analyser (FIAstar 5000 Analyzer; FOSS, Hoganas, Sweden) in accordance with the standards AN 5202 (total-N) and AN 5201 (NO_3–N). Similar to earlier studies (Weisner and Thiere, 2010; Nilsson et al., 2020), virtually all N (99.5 %) in the in- and outflow water to and from the wetlands was dissolved NO_3^-. We therefore used water NO_3–N concentrations for calculation of N removal efficiency, N removal rate and k_a throughout. At each sampling occasion, flow rates were measured for each CW by recording outlet outflow, using a measuring cup and a stopwatch. If needed, flow rate (Q) was adjusted manually at the inlet faucet to maintain the target flow of 2.4 L min^{-1}. Areal hydraulic loading rate (HRL) was thus set to 0.13 m d^{-1} for all wetlands.

Wetland N removal was expressed as N removal efficiency (%; Eq. (2)), N removal rate (g $m^{-2}\ d^{-1}$; Eq. (3)), and first-order area-based removal rate coefficient with modified Arrhenius temperature adjustment k_a (m d^{-1}; Eq. (4); Kadlec, 2005).

$$N\ removal\ efficiency = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \quad \text{Eq. 2}$$

$$N\ removal\ rate = \frac{Q \times (C_{in} - C_{out})}{A} \quad \text{Eq. 3}$$

Fig. 1. Construction and pictures of a woodchips-based floating bed (WFB), A) jute sheet and freshly cut birch woodchips, B) sack made of woodchips and jute sheet, C) *Glyceria maxima* and *Filipendula ulmaria* planted on a WFB, D) plant root growth below a WFB at the end of the experiment.

$$k_a = \frac{N \times Q \times \left(\left(\frac{C_{in}}{C_{out}} \right)^{\frac{1}{N}} - 1 \right)}{A \times \theta^{(T-20)}} \quad \text{Eq. 4}$$

Where C_{in} is the N concentration (g m^{-3}) in inlet water, C_{out} is the N concentration (g m^{-3}) in outlet water, Q is the flow rate (m^3 d^{-1}), A is the wetland surface area (m^2), N is the number of continuously stirred tank reactors (CSTRs), θ is the temperature coefficient and T is the water temperature (°C). We used $N = 2$ according to a previous tracer study performed in these wetlands (Bodin et al., 2012) and a θ value of 1.088 according to Kadlec (2005).

For plant N content, dried root and shoot samples were ground to a homogenous powder and weighed into tin capsules. An ISO 9001:2008 certified Flash EA 1112 series NC analyser equipped with Thermo-Scientific MAS 200R auto-sampler analysed plant N content.

2.4. Bacterial denitrification genes on WFBs

We quantified ***nirS***, ***nirK***, ***nosZI*** and ***nosZII*** denitrification gene abundances on WFB plant roots and woodchips as a proxy for biofilm denitrification potential (Hallin et al., 2015). Woodchips were collected from randomly selected planted (n = 4) and unplanted WFBs (n = 4). Four individual plants of *G. maxima* and *F. ulmaria* each were randomly collected from WFBs. For each plant, representative subsamples were collected from the whole root system. Roots were then gently washed with wetland water to get rid of particles, gently dried with paper towels to get rid of excess water and cut into small pieces. Plant root and woodchip samples were stored at −80 °C until extraction using a DNeasy® PowerWater® Kit (QIAGEN). Extracted DNA was stored at −80 °C until later determination of its quantity and quality by spectrophotometry (Infinite M200; Tecan AG). Real-time quantitative polymerase chain reaction (qPCR) assays were performed using a RotorGene® Q equipment (QIAGEN; see Appendix 2 and Table S2 of Supplementary material for details).

2.5. Statistical analyses

A mixed model ANOVA was performed to test for treatment effects on i) N removal efficiency, ii) N removal rate, and iii) temperature adjusted first-order area-based removal rate coefficient k_a, at different time points (i.e. sampling occasions). Treatments and time were fitted as fixed factors and individual CW as random factor. Tukey's HSD was used for post-hoc pairwise comparison of N removal between treatments at each time point. Data was square root transformed for N removal efficiency, and $\log_{10}$ transformed for N removal rate and k_a, to achieve normal distribution.

To identify the potential effect of WFB installation, i.e., the effect of the *+5% WFB* and the *+20% WFB* treatments compared to the *harvested* treatment on N removal efficiency, removal rate and k_a value, we calculated effect sizes as Cohen's d (Eq. (5); Cohen, 1988).

$$d = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{(n_1 - 1)\, SD_1{}^2 + (n_2 - 1)\, SD_2{}^2}{n_1 + n_2 - 2}}} \quad \text{Eq. 5}$$

where, X_1 is the WFB treatment mean and X_2 is the *harvested* treatment mean; n_1 is the number of replicates for WFB treatments and n_2 for *harvested* treatment; SD_1 is the standard deviation of WFB treatments and SD_2 of *harvested* treatment. The effect size indicates direction and strength of a treatment effect (WFB treatments) on a variable by comparing the treatment effect to a control (here the *harvested* treatment). An effect size of $d = 0.2$ indicates a small, 0.5 a medium and 0.8 a large effect (Cohen, 1988; Lakens, 2013).

Independent samples t-tests were used to investigate difference between *G. maxima* and *F. ulmaria* in relative biomass production, plant biomass N assimilation, maximum root length, specific root surface area and denitrification gene abundance, as well as to test for differences in denitrification gene abundances between woodchips and plant roots.

3. Results

3.1. N removal in CWs

Treatment, time, and their interactions affected N removal efficiency and N removal rate, whereas only time and its interaction with treatment affected the N removal coefficient k_a (Table 1). On average, N removal efficiency and N removal rate were higher in all harvested treatments (*harvested*, *+5% WFB* and *+20% WFB*) than in the control treatment. However, there was no difference in average k_a between harvested and control treatments. Within the harvested treatments there was no general difference between the *harvested*, *+5% WFB* and *+20% WFB* treatments. Irrespective of management treatment, N removal efficiencies in CWs were higher at the end of June and beginning of July than in September (Fig. 2a). N removal rates followed a similar temporal pattern with lower values at the end of September (16th and 22nd) than during the high summer (from June 25th to August 10th, Fig. 2b). The temperature adjusted N removal coefficient k_a was also lower in the end of September (16th and 22nd) than during the high summer (Fig. 2c).

In the *control* treatment, N removal was relatively stable throughout the experiment. Thus, the differences in N removal between harvested treatments and the *control* treatment coincided with the extremely high N removal in harvested treatments during June through August (Fig. 2a–c). In September, no differences between treatments were discernible. Significant treatments effects on all three N removal parameters were found on July 13th (Fig. 2a–c; one-way ANOVA, $p < 0.05$).

N removal efficiency also differed between the treatments on the 8th of July and on the 10th and 17th of August (Fig. 2a; one-way ANOVA, $p < 0.05$, for pairwise comparisons see Table S2 in Supplementary material). CWs with macrophyte harvesting (*harvested*, *+5% WFB* and *+20% WFB* treatments) showed higher N removal efficiency than *control* treatments on the 8th and 13th of July and on the 10th of August (Fig. 2a; Tukey's pairwise comparison, $p < 0.05$). On the 17th of August, only the *+20% WFB* treatment exhibited higher N removal efficiency than the *control* treatment (Fig. 2a; Tukey's pairwise comparison, $p < 0.05$) whereas the difference between *harvested* and *control* treatment was near significant ($p = 0.05$).

N removal rate differed between treatments on the 9th of June, the 25th of June, the 13th of July, and the 10th of August (Fig. 2b; one-way ANOVA, $p < 0.05$, for pairwise comparisons see Table S3 in Supplementary material). Only the *+20% WFB* showed higher removal rates than the *control* treatment on the 9th of June, while on the 25th of June and on the 13th of July, all harvested treatments (*harvested*, *+5% WFB*, and *+20% WFB*) exhibited higher N removal rates than the *control* treatment (Fig. 2b; Tukey's pairwise comparison, $p < 0.05$). On the 10th of August, the N removal rate was higher in the *+5% WFB* and the *+20% WFB* treatment than in the *control* treatment, whereas the *harvested* treatment did not differ from any of the other treatments (Fig. 2b; Tukey's pairwise comparison, $p < 0.05$).

The removal coefficient k_a differed between the treatments on the 25th of June and the 13th of July (Fig. 2c; one-way ANOVA, $p < 0.05$). On both dates, treatments *+5% WFB* and *+20% WFB* showed marginally insignificantly higher k_a values than *control* treatments (Fig. 2c; for pairwise comparisons see Table S4 in Supplementary material).

The effect sizes (Cohen's d) of two WFB treatments on N removal parameters differed during the experiment. When comparing the *+5% WFB* to the *harvested* treatment, effect sizes for the whole experiment indicate weak effects for N removal efficiency ($d = 0.22$) and N removal rate ($d = 0.20$) but a near medium effect for N removal coefficient k_a ($d = 0.41$). A comparison of the *+20% WFB* to the *harvested* treatment gave a medium effect on N removal coefficient k_a ($d = 0.51$) and a near medium effect on N removal efficiency ($d = 0.35$) and N removal rate (d

Table 1
Mixed model ANOVA investigating the effect of experimental treatment (between subjects), time (within subjects), and their interaction on N removal efficiency (%), N removal rate (g m^{-2} d^{-1}) and temperature adjusted first-order area-based removal rate coefficient k_a (m d^{-1}).

Source of variation		N removal efficiency (%)[b]			N removal rate (g m^{-2} d^{-1})[c]			k_a (m d^{-1})[c]		
	df_{num}[a]	df_{den}[a]	F	p	df_{den}[a]	F	p	df_{den}[a]	F	p
Treatment	3	12	8.01	0.003	12	4.49	0.025	12	3.09	0.068
Sampling date	8	96	44.03	<0.001	96	35.09	<0.001	96	32.32	<0.001
Sampling date × treatment	24	96	1.90	0.015	96	1.75	0.030	96	1.77	0.027

[a] Numerator *df* (*df* num) were identical across all analyses, whereas the effective denominator *df* (*df* den) differed.
[b] Data was square root transformed.
[c] Data was log transformed.

= 0.35).

3.2. Plant biomass production and N assimilation

We observed that both *G. maxima* and *F. ulmaria* planted on WFBs survived the whole experiment. Total macrophyte (combined shoot and root) biomass on WFBs at the end of the experiment ranged from 48 to 180 g m^{-2} for *G. maxima* and from 29 to 251 g m^{-2} for *F. ulmaria*. Relative biomass production of *G. maxima* was significantly higher than for *F. ulmaria* (Table 2). Initially, each WFB was planted with 4 individual shoots of *G. maxima*, which multiplied into 11.4 ± 4.2 (mean ± SD) shoots per WFB by the end of the experiment. N assimilation into total plant biomass on WFBs was significantly higher for *F. ulmaria* than for *G. maxima* during the experiment (Table 2). Further, *G. maxima* and *F. ulmaria* growth on WFBs differed; maximum root lengths and specific root surface area were both significantly greater for *G. maxima* than for *F. ulmaria* (Table 2).

3.3. Denitrification gene abundance on plant roots and woodchips

Denitrification gene abundance was greater on plant roots than on woodchips, due to the greater abundances of *nirS* and *nosZI* genes on roots than on woodchips (Fig. 3; independent samples t-tests, $p < 0.01$). Denitrification gene abundance also differed between plant species, with significantly higher abundance of *nirS*, *nirK*, *nosZI* and *nosZII* on *G. maxima* than on *F. ulmaria* roots (Table 2).

4. Discussion

Our study highlighted that (i) management of overgrown CWs through harvesting of macrophytes and removal of root mats can enhance N removal in CWs and that (ii) macrophytes grow well on WFBs, develop extensive root systems, and host more denitrification genes on their roots than can be found on woodchips inside WFBs.

4.1. Enhanced N removal through macrophyte harvest

In our study, N removal efficiency and N removal rate increased about 1.5 times after macrophyte harvest in 17-year-old heavily overgrown CWs compared to previous long-term N removal measurements in the same CWs (Nilsson, 2018). Previous studies have also shown that harvesting increased N removal in CWs (Lv et al., 2024; Zhang et al., 2017). The effects of harvesting on N removal were more evident during the first two to three months in our experiment, which coincided with high summer (end of June to the beginning of August) when N removal is normally highest in temperate climates (Bastviken et al., 2009; Nilsson et al., 2020). Yang et al. (2016) found that summer harvesting of *Phragmites australis* led to higher total-N removal than in unharvest CW microcosms. Vymazal (2020) reported daily N removal of 0.46 g N m^{-2} – 1.04 g N m^{-2} in CWs managed by *P. australis* harvesting. This is identical to the daily N removal found in our harvested treatments (0.46 g N m^{-2} – 0.54 g N m^{-2}) while unharvested CWs had much lower daily N removal (0.30 g N m^{-2}). The overall enhanced N removal in the harvested CWs may be attributed to increased contact between N-rich water and wetland bottom surfaces. Moreover, the high N removal during the first months of the experiment could be result of harvesting of aboveground plant biomass and removal of plant litter that stimulates the release of root exudates in the form of DOC (Wang et al., 2023). This might affect microbial community composition and enhance denitrification activity, and thus increase N removal in CWs (Allen et al., 2023; Zheng et al., 2018). Therefore, macrophyte harvesting can have a seasonal boosting effect on N removal in CWs that might contribute to very high N removal in harvested CWs during summer as shown in our study. However, this boosting effect diminished at the end of growing season, as we could not find any difference in N removal between the treatments in September. Since we conducted our experiment during only one growing season, caution should be taken when generalizing our results without further long-term *in situ* studies to confirm our findings. There is no standard guideline about the time of the year or how often macrophytes should be harvested in CWs (Vymazal, 2020). Therefore, the frequency and timing of macrophyte harvesting in CWs should also be studied further. For example, *Typha* spp. showed significant reduction in vegetation cover after two annual cuttings (Wilcox et al., 2018) while Toet et al. (2005) reported that harvesting *P. australis* in autumn over two years did not affect the vitality of the stand. Harvesting of *P. australis* and *Phalaris arundinacea* two times a year usually showed higher N removal compared to a single harvest (Okada and Vymazal, 2023 and references therein). Additionally, the timing of macrophyte harvesting might have other environmental effects. For example, Kasak et al. (2020) recommended harvesting wetland macrophytes at the end of the growing season in September to minimize methane (CH_4) emissions from harvested CWs, which notably increased when macrophytes were harvested during summer.

4.2. N removal parameters and WFB addition

We did not observe differences in N removal parameters between the three harvested management options (*harvested*, *+5% WFB* and *+20% WFB*). This means that the WFB installation in our study did not have the desired positive effect on N removal parameters after harvesting, although woodchips-based bioreactors have been shown to increase N removal from water in horizontal and vertical flow CWs treating agricultural drainage (Audet et al., 2021). However, the installation of WFBs at 20% cover did increase N removal parameters at some sampling occasions during high summer compared to the *control* treatment when harvesting alone showed no effect. This indicates that WFBs have the potential to increase N removal, but possibly have to be installed at higher cover density to show clear measurable main effects. In our study, only 50% of the WFBs were planted with macrophytes. This might be another reason why we observed no significant effect of planted WFBs on N removal. In a FB experiment planted with *Carex appressa*, 8% FB coverage showed 25% total-N removal efficiency (Schwammberger et al., 2020) while 40% coverage of FBs planted with *Iris pseudacorus* resulted in total-N removal efficiency of 65% (Gao et al., 2019). Further, the medium effect size of the treatment with 20% WFB cover on N removal parameters in our study indicates that by increasing the surface

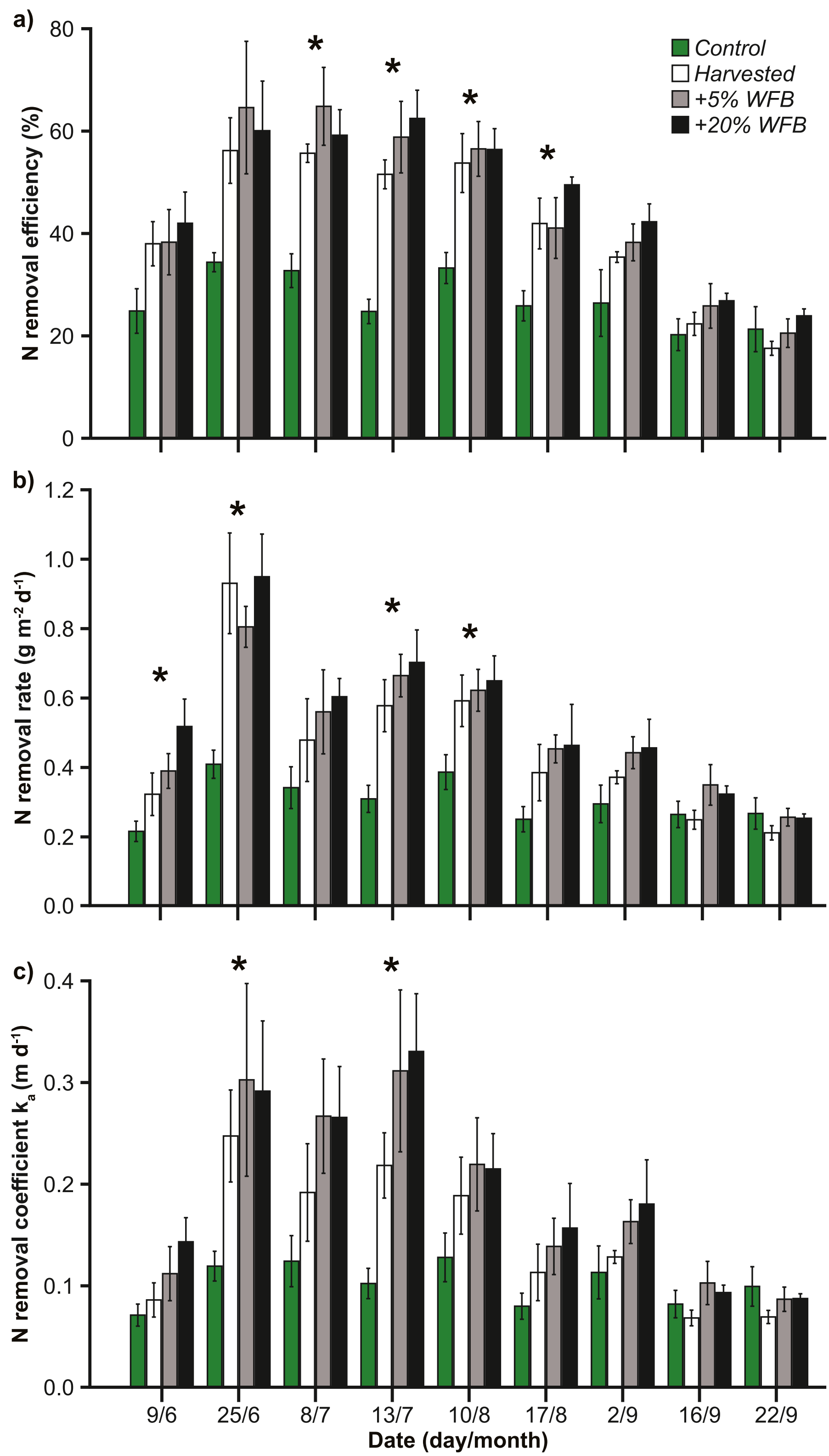

Fig. 2. Nitrogen (N) removal efficiency (a), N removal rate (b), and temperature adjusted N removal coefficient k_a (c) in constructed wetlands (CWs) under four wetland management treatments during nine water sampling occasions (*control*, overgrown CWs that remained unmanaged; *harvested*, harvesting all macrophytes from overgrown CWs; *+5% WFB*, harvesting and covering 5% CW surface area with WFBs; *+20% WFB*, harvesting and covering 20% CW surface area with WFBs). Columns represent average removal in four replicate wetlands, and error bars represent standard error. Asterisks mark significant differences (one-way ANOVA, $p < 0.05$) among treatments.

Table 2
Independent samples *t*-test investigating the difference in relative biomass production, biomass N assimilation, maximum root length, specific root surface area (SRSA), and denitrification gene abundances on roots between *G. maxima* and *F. ulmaria* planted on woodchips-based floating beds. Gene abundances are presented as gene copy numbers per gram dry weight of root (copies g-DW^{-1}). Significant effects are denoted as <0.05 or <0.01.

Variables	Mean (SD)		t	*df*	p
	G. maxima	*F. ulmaria*			
Relative biomass production (%)	233.51 (113.84)	62.73 (86.27)	6.09	50	<0.01
Biomass N assimilation (g N m^{-2})[a]	1.32(0.46)	1.79(0.86)	−2.09	50	<0.05
Maximum root length (cm)	40(6.49)	28(11.72)	4.03	38	<0.01
SRSA (cm^2 g^{-1})	6307.68 (1059)	3131(535)	4.63	4	<0.05
nirK (copies g- dry weight $root^{-1}$)[a]	2.94×10^9	3.62×10^8	6.10	5	<0.05
nirS (copies g- dry weight $root^{-1}$)[a]	2.53×10^9	2.22×10^8	3.94	5	<0.01
nosZI (copies g- dry weight $root^{-1}$)[a]	1.63×10^9	8.74×10^7	6.65	5	<0.01
nosZII (copies g- dry weight $root^{-1}$)[a]	9.25×10^8	3.27×10^7	3.59	5	<0.05

[a] Data was log transformed to meet the assumptions of parametric tests before statistical analyses.

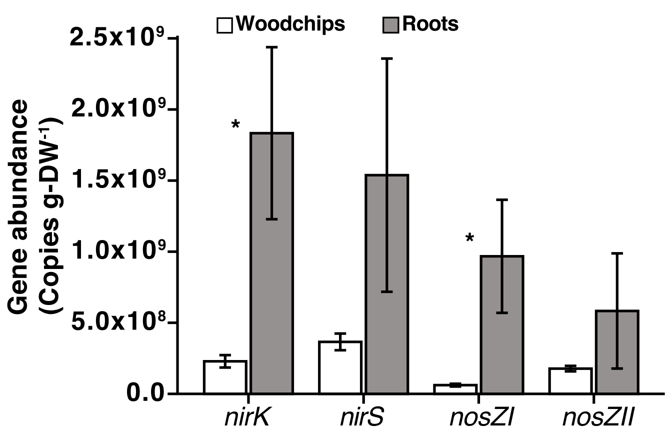

Fig. 3. Denitrification gene abundances of *nirK*, *nirS*, *nosZI* and *nosZII* on plant roots and woodchips in floating beds at the end of the experiment. Asterisks mark significant differences (independent samples t-tests, $p < 0.05$) between woodchips and plant roots, and error bars represent standard error.

area of WFBs, it is possible to enhance N removal in CWs. It was found that 5–45% coverage of floating bed treatment is needed to achieve satisfactory removal of pollutants in lakes, ponds or reservoirs (reviewed by Shen et al., 2021). However, caution should be applied when covering extended wetland surfaces with WFBs, as woodchips bioreactors treating tile drainage water showed increased chemical oxygen demand (COD) and total organic carbon (TOC) in outlet compared to inlet water (Povilaitis et al., 2020). Moreover, WFBs might release dissolve organic matter when first immersed in water (von Ahnen et al., 2023), which might lead to a short-term reduction in water column light penetration due to browning, temporarily hindering submerged aquatic plant growth (Choudhury et al., 2019b). Besides nutrient removal, deploying WFBs in newly harvested or newly constructed CWs may speed up initial establishment of emergent aquatic plants, and thus improve N removal in the early successional stages, as has been shown for initial planting of newly constructed CWs (Nilsson et al., 2020).

4.3. Plant growth and denitrification on WFBs

Our *in situ* experiment showed that growth of macrophytes on WFBs is feasible. However, biomass production appears reduced compared to previous studies. The total root and shoots biomass on WFBs after 3.5 months of growth was lower for both *G. maxima* and *F. ulmaria* than the above ground biomass of both plants in other FB studies. *F. ulmaria* grown on FBs for three growing seasons in northern Sweden showed above ground biomass production of 249–968 g m^{-2} dry weight (Choudhury et al., 2019a). *G. maxima* grown on FBs for around two years to treat nitrified meat processing effluent in New Zealand showed a biomass production of 7.2 kg m^{-2} (van Oostrom, 1995). The discrepancy between previous studies and our results might be due to the shorter duration of our experimental macrophyte growth on WFBs. We expect that these species will produce higher biomass on WFBs after being adapted to their new floating environment. This is supported by a long-term study, where a mixture of six macrophyte species grown on floating beds showed a much higher total fresh biomass production of 147 kg m^{-2} of floating bed over 4 years of growth (White, 2021). The higher relative biomass production of *G. maxima* highlights that this species may grow better than *F. ulmaria* on WFBs. This was also evident from increase of individual shoot number of *G. maxima* at the end of the experiment. Rhizomes with terminal buds of *G. maxima* can grow into aerial shoots during the whole year in presence of favourable environmental conditions (Sundblad, 1990). Therefore, plant growth strategy is an important aspect to consider while selecting species for planting on WFBs. If plant growth on WFBs continues for several growth seasons, plants on WFBs might also overgrow. However, this should not negatively affect CW N removal capacity since plant growth is mostly confined to the floating beds and thus does not create channelization.

In contrast to plant biomass production, we observed higher N assimilation into plant biomass for *F. ulmaria* than for *G. maxima*. This is due to *F. ulmaria*'s higher N concentration per dry mass in shoot (1.6% ± 0.46%) and roots (1.6% ± 0.25%) than in *G. maxima*'s roots (1.1% ± 0.04%) and shoots (1.4% ± 0.33%). In a previous study, N concentration in *F. ulmaria* aboveground biomass planted on FBs ranged from 1.5% to 2.2% N in dry biomass (Choudhury et al., 2019a). In line with our study, the difference in N content in plant biomass was also observed between *I. pseudacorus*, *Carex* spp., *P. australis*, *T. latifolia* and *Lythrum salicaria* grown on floating beds (reviewed by Alberto et al., 2021). The N content of *G. maxima*'s aboveground biomass in our study (1.4% of dry mass) is the same as reported in *G. maxima* in southern Sweden (Sundblad and Robertson, 1988). Besides biomass growth and N assimilation, we found difference between *G. maxima* and *F. ulmaria* with respect to root characteristics. The longer root length and larger root surface area of *G. maxima* indicate that this macrophyte has higher potential to increase denitrification in the water column (down to 40 cm in depth) by providing surface area and DOC (root exudates) for bacterial growth and activity. Maximum root length for *Carex appressa* was measured as 219 cm after one year of growth on FBs (Schwammberger et al., 2020). Therefore, it is expected that root length under floating beds will increase with time and that root of different species might grow differently. The roots of *G. maxima* also supported higher abundances of denitrification genes *nirS*, *nirK*, *nosZI* and *nosZII* in our experiment. Moreover, abundances of *nirK*, *nirS* and *nosZI* genes on the roots of *G. maxima* in our experiment were much higher than the abundances of same genes (6.20×10^8, 2.90×10^8 and 4.02×10^8 for *nirK*, *nirS*, *nosZ* genes, respectively) on the roots of *Canna indica* in FBs applied in China (Zhang et al., 2018). Besides on plant roots, we also observed denitrification genes on woodchips. Woodchips have high specific surface area and release DOC, thus providing a suitable habitat for the growth and activity of denitrifying bacteria (Audet et al., 2021), which in turn enhances the denitrification potential of WFBs.

5. Conclusions

Our field experiment showed that management of CWs through macrophyte harvesting and deployment of WFBs can enhance overall N removal in heavily overgrown CWs. We also showed that WFBs have potential for N removal through plant biomass N assimilation, and that roots and woodchips in and under WFBs host denitrification genes, thus indicating denitrification potential. In this study we used only two plant species. A larger number of species should be screened for their suitability for growth and N assimilation on FBs. Additionally, the succession of plant species on WFBs should be investigated in long-term experiments. Because WFBs are artificially made, they provide an opportunity to manipulate species diversity to test for possible synergistic effects between species. Therefore, future studies should consider macrophyte species diversity and ecosystem process of N cycling in WFBs. In addition, it is important to further investigate how often macrophyte harvesting should be performed in CWs for maintaining optimal N removal. Long-term N removal monitoring in CWs, after the initial boost effect of macrophyte harvest, is needed to make such a decision.

CRediT authorship contribution statement

Maidul I. Choudhury: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Josefin E. Nilsson:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Samuel Hylander:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Marc Hauber:** Writing – review & editing, Investigation. **Per Magnus Ehde:** Writing – review & editing, Validation, Resources, Investigation. **Stefan E.B. Weisner:** Writing – review & editing, Validation, Resources, Investigation. **Antonia Liess:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was funded by Stiftelsen Oscar och Lili Lamms Minne (grant no. FO2019-0012) to MC and SH and a grant for eutrophication prevention (övergödningsårgärder anslag 1:11) from Vattenmyndigheten Västerhavet to AL. AL, JN, SW and PME were supported by the Swedish Environmental Protection Agency [grant number 19/114]. We thank Matyas Baan for field and laboratory assistance, Kuno Kasak and Mikk Espenberg for supporting qPCR analysis, and Halmstad University and Linnaeus University for support and use of facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.142284.

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