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Aquatic worms (Tubificidae) facilitate productivity of macrophyte *Azolla filiculoides* in a wastewater biocascade system

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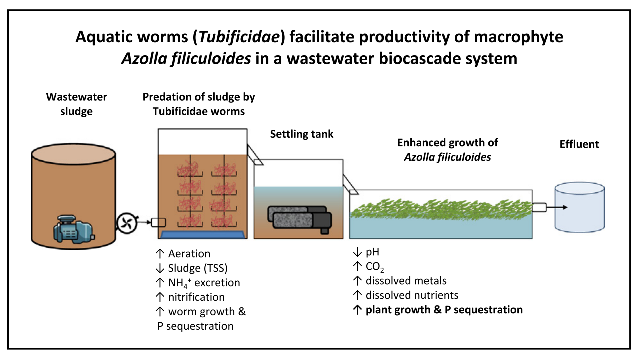
HIGHLIGHTS

- A biocascade was developed incorporating ecological processes in wastewater treatment.
- *Tubificidae* predation resulted in 45% sludge reduction.
- In total the biocascade sequestered 133 mmol P m^{-3} d^{-1} via biomass production.
- *Tubificidae* facilitated increased productivity of *A. filiculoides* on wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to high waste sludge disposal at wastewater treatment plants and increasing phosphorus scarcity, there is a need to combine waste removal and nutrient recovery. One way to achieve this is by incorporating ecological processes into wastewater treatment. Positive species interactions, such as facilitation, are critical to increase productivity of biomass and nutrient recovery. In this study we showed the potential of using ecological principles including interspecific facilitation processes of aquatic plants (*Azolla filiculoides*) and worms (Oligochaeta, Tubificidae) in waste recovery and biomass production. This was investigated by developing a biocascade with monocultures of plants and aquatic worms that was fed on activated sludge. Tubificidae had an average relative growth rate of 0.02 g g^{-1} DW d^{-1} whereby sludge predation resulted in 45% sludge reduction. When Tubificidae were present in the biocascade, *A. filiculoides* biomass production significantly increased to a relative growth rate of 0.15 g g^{-1} DW d^{-1}. The activity of Tubificidae mostly affected total suspended solids, chemical oxygen demand and ammonium concentration in the first compartment of the biocascade. Additionally, nitrification rates increased and the water acidified, leading to increased carbon dioxide concentrations and dissolved phosphorus-binding metals (zinc, iron, aluminium and manganese) that stimulated *A. filiculoides* growth. The high sludge reduction (45%) and phosphorus sequestration (133 mmol m^{-3} d^{-1}) show a strong potential of the biocascade for combined sludge waste reduction and phosphorus recovery from wastewater.

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Nomenclature

TSS [g L^{-1}] Total suspended solids
RGR [g g^{-1} DW d^{-1}]
Relative growth rate
DW [g] Dry weight
WW [g] Wet weight
t [d] Time
$f_{dw/ww}$ [−] Dry weight to wet weight conversion factor
TP [mmol L^{-1}] Total phosphorus
TN [mmol L^{-1}] Total nitrogen
COD [g L^{-1}] chemical oxygen demand
TIC [μmol L^{-1}] Total inorganic carbon.
HCO_3 [μmol L^{-1}] Bicarbonate
CO_2 [μmol L^{-1}] Carbon dioxide
NH_4^+ [μmol L^{-1}] Ammonium
NO_3^- [μmol L^{-1}] Nitrate
PO_4^{3-} [μmol L^{-1}] Phosphate
Al [μmol L^{-1}] Aluminium
Ca [μmol L^{-1}] Calcium
Fe [μmol L^{-1}] Iron
K [μmol L^{-1}] Potassium
Mg [μmol L^{-1}] Magnesium
Mn [μmol L^{-1}] Manganese
Mo [μmol L^{-1}] Molybdenum
Na [μmol L^{-1}] Sodium
N [μmol L^{-1}] Nitrogen
P [μmol L^{-1}] Phosphorus
S [μmol L^{-1}] Sulphur
Zn [μmol L^{-1}] Zinc

1. Introduction

Wastewater treatment plants (WWTPs) are a point source of pollution and produce large volumes of sludge with a limited application potential that needs to be disposed against high cost. Annually, approximately 10 million ton of activated sludge is produced in Europe (Gendebien et al., 2010). Waste sludge is often incinerated and sludge disposal is estimated to account for 50% of the total operation cost of the WWTP (Davis and Hall, 1997; Tchobanoglous et al., 1991). Additionally, effluent discharges from municipal WWTPs are causing eutrophication in freshwaters via nutrient input (Ahearn et al., 2005). At the same time, an increasing global food production requires more extensive fertilization of agricultural land with nitrogen (N) and phosphorus (P). Production of N via the Haber-Bosch process is a costly process that requires extensive use of non-renewable resources (> 1% of the global energy consumption) and P is non-renewable as it can only be obtained from a limited number of P mines (Chowdhury et al., 2017; Ullmann et al., 1985). Although there are several methods to recover P from sludge (e.g. direct use of sewage sludge, precipitation in form of struvite, recovery from ashes through thermochemical treatment), they often have limited application potential (Cieślik and Konieczka, 2017) and there is not one solution to combat the scarcity of P in the future. Therefore, there is an urge to implement the new concept of circular economy in wastewater management, in order to reuse sewage sludge as a valuable resource of matter and energy (Kacprzak et al., 2017).

An example of a system with such potential is a constructed wetland, which acts as a buffer zone between the WWTP and the natural environment (Brix, 1994). Here, the soil, microbes, aquatic macrophytes and animals together remove N and P while growing biomass. N is removed via coupled nitrification and denitrification and P is partly removed via adsorption and precipitation reactions (Reddy and DeLaune, 2008). Macrophytes play a minor role in the uptake of N and P, and are estimated to take up 5 – 10% of the N load and 5% of the P load in municipal wastewater, respectively (McJannet et al., 1995; Thable, 1984), but they do play an important indirect role in nutrient removal processes, especially floating-leaved species such as *Azolla* spp. (Stottmeister et al., 2003; Tang et al., 2017). Their roots, for example, provide surface area for attached microbial growth and release oxygen that stimulate nitrification and aerobic degradation (Brix, 1997). In contrast, aquatic animals are rarely studied or considered in the construction of wetlands, even though they have the potential to fulfil similar valuable functions such as nutrient uptake and remediation of heavy metals and microbial contaminants (Gifford et al., 2007). For example, for bivalve molluscs it has been shown that they can reduce nitrogen and phosphorus levels (van der Schatte Olivier et al., 2020) and some species, e.g. *Crassostrea virginica*, have even been identified as hyperaccumulator for heavy metals accumulating >2000 mg kg^{-1} copper (Gifford et al., 2004). Although this potentially creates opportunities to purify water and produce high-grade materials through biomass production, their biomass is often not harvested. Examples of high-grade materials include simple applications of aquatic worm and macrophyte biomass, as fish feed or green fertilizer (Mount et al., 2006; Wagner, 1997), but also the use of valuable specific biomass components, such as amino acids, fatty acids or enzymes while biomass could also be processed into oleo chemicals or biodiesel for instance (Brouwer et al., 2016; Elissen et al., 2010).

In order to implement circular economy in wastewater management, ecological theory could be used and applied in WWTP engineering (Graham and Smith, 2004). Currently, WWTPs largely use biological purification with aerobic sludge flocs or granules based on natural principles already. Depending on the substrate composition of the sewage water entering the WWTP, such as organic carbon loading, the microbial population and community composition changes over time (Li et al., 2008). At low substrate loadings, a higher species diversity is observed compared to high substrate loadings, which leads to dominant and fast-growing microbial species. This is similar to self-purification processes in natural ecosystems (Benoit, 1971), where few organisms act when pollution occurs and the biodiversity of the system decreases. Aquatic organisms can make polluted conditions more favourable, thereby promoting other species to grow. In this way, the ecosystem can stabilize again after it has been polluted if no critical pollution limits are exceeded and the growth of other species is facilitated.

Such positive interaction between species, where at least one of the species benefits and harm is caused to neither, is called interspecific facilitation (Bruno et al., 2003). Examples of interspecific facilitation can be found in applications such as restoration ecology and agricultural systems (Bertness and Callaway, 1994; Renzi et al., 2019). In restoration ecology, an example of this are mussels that act as biofilters (Giangrande et al., 2005). When mussels are abundant the water is filtered and thereby environmental conditions change, like a reduction in turbidity. Indeed, studies have shown that freshwater bivalves, e.g. *Dreissena polymorpha*, facilitate plant growth by reducing turbidity (Gagnon et al., 2020; Gao et al., 2017). Next to this, interspecific facilitation processes are used in intercropping systems with multiple species (Li et al., 2014; Zhang and Li, 2003), which enables organisms to exploit a greater portion of available resources and increase productivity of species (Bruno et al., 2003; Cardinale et al., 2002). Hence, by applying facilitative interactions between species of different functional groups in WWTP engineering, this could aid in the simultaneous increase in both biomass production and nutrient recovery.

Therefore, the aim of this study is to explore aquatic animal-plant interspecific facilitation processes in a WWTP system in order to mimic self-purification of ecosystems and increase productivity of valuable biomass on wastewater. Specifically, we tested whether (i) the aquatic worm of the family of Tubificidae was able to reduce sludge, grow on wastewater sludge and sequester nutrients (ii) the macrophyte *A. filiculoides* was able to grow and sequester nutrients from wastewater and (iii) facilitation processes by Tubificidae could increase the productivity of *A. filiculoides*. This was investigated by using a biocascade which is defined as a series of connected compartments through which wastewater flows and monocultures of different species, including aquatic animals, plants and associated microorganisms, are grown allowing for optimal biomass exploitation. In this experiment, wastewater flows first though a compartment containing Tubificidae subsequently entering the connected compartment with *A. filiculoides* monoculture. While research already established evidence that sludge reduction by aquatic worms is possible (Hendrickx et al., 2009) and the potential of some macrophytes species to grow and recover nutrients (Stottmeister et al., 2003), the use of interspecific facilitation processes to increase productivity in such a WWTP system has not been investigated yet. We expected complementary growth of the species through consumption of particles and nutrient regeneration (Vanni, 2002) by Tubificidae and uptake of dissolved nutrients through *A. filiculoides*, and in this way increasing *A. filiculoides* productivity by interspecific facilitation mechanisms.

2. Material and methods

2.1. Aquatic species

We selected the aquatic oligochaete specimens of the family of Tubificidae and the aquatic macrophyte *A. filiculoides* for the cascade, as they are known to grow well on domestic wastewater (e.g. Costa et al., 1999; de Valk et al., 2017). Tubificidae can be found in sediment of freshwaters with a low oxygen concentration and a high organic carbon loading. A. filiculoides often grows on the surface of polluted waters with high ammonium (NH_4^+), nitrate (NO_3^-) and especially high phosphate (PO_4^{3-}) concentrations (De Lyon and Roelofs, 1986). Tubificidae were bought at a local wholesale (Aquadip B.V, The Netherlands) and kept in the laboratory at 18 °C in aerated aquaria containing wastewater for at least 1 month to adapt. Note that the wastewater sludge used for the adaptation period was not the same as in the experiment, but had a similar TSS concentration (3.5 g L^{-1}). Each aquarium received a starting worm density of 20 g wet weight (WW) L^{-1} of carefully dry-blotted Tubificidae, since a pilot experiment showed the highest survival for this density (Fig. S1). *A. filiculoides* was obtained from cultures of the Radboud University. Before the start of the experiment, *A. filiculoides* was acclimatized to the experimental conditions by growing them on effluent wastewater for one week with a PO_4^{3-} concentration of 0.60 mmol L^{-1} and a dissolved inorganic nitrogen concentration (NH_4^+ and NO_3^-) of 0.76 mmol L^{-1}. Subsequently, the plants were washed with demineralised water and 37.5 g WW of carefully dry-blotted *A. filiculoides* was introduced per aquarium. This weight corresponds to a cover of approximately 75% of the water surface of each aquarium (Fig. 1) and a density of 0.75 kg m^{-2} in order to prevent overcrowding effects (>2 kg WW m^{-2}; Van Hove, 1989).

2.2. Experimental design

To evaluate the growth and purification potential of Tubificidae and *A. filiculoides* in wastewater, we used a three-compartment cascade with four replicates and three treatments (Fig. 1). Each compartment step consisted of 12 aquaria with a volume of approximately 2.5 L. Wastewater from the inlet container first flowed into compartment 1 containing 12 aerated aquaria (Fig. 1), with a size of 16 × 10 × 21 cm (l × w × h) and covered by a lid. Of these 12 aquaria, 4 were stocked with Tubificidae worms and 8 without worms, serving as control. Tubificidae were grown on carrier material in racks (artificial substrate) in order to improve their growth and increase their harvest potential (Fig. 1). Via the overflow pipe of compartment 1 at 16 cm height, water flowed into compartment 2 (Fig. 1), which consisted of 12 aquaria with a size of 17.5 × 13 × 15 cm (l × w × h) and also covered by a lid. The overflow was situated at 11 cm height. Compartment 2 functioned as a settling tank in order to remove excess sludge particles leaving compartment 1, by using two aquarium filter pumps with a filter capacity of 200 L h^{-1} (Aqua-Flow 100, Superfish). Compartment 3 consisted of 12 aquaria of 50 × 10 × 10 cm (l × w × h) with an overflow outlet at 5 cm height of which 8 were stocked with *A. filiculoides* and 4 served as control. The first two compartments and the control aquaria of the third compartment were kept in the dark by covering them with black foil in order to prevent growth of photoautotrophs. Water from the third compartment flowed into the outlet, which consisted of 12 closed buckets to prevent evaporation.

This design resulted in three treatments: 1) a ‘worms-plants’ treatment with aquaria in compartment 1 containing Tubificidae and aquaria in compartment 3 containing *A. filiculoides*; 2) a ‘control-plants’ treatment where compartment 1 did not contain Tubificidae (as a control), and aquaria in compartment 3 contained *A. filiculoides*; and 3) a double control treatment without Tubificidae and *A. filiculoides* (Fig. 1). The experiment was performed in a water bath with a temperature of 15 °C in a greenhouse with additional lamps (400 W high-pressure sodium lamps, Hortilux-Schréder, Monster, The Netherlands) maintaining a light:dark cycle of 16 h:8 h. Lights turned on when the natural light intensity fell below 250 W m^{-2} (300 μmol m^{-2} s^{-1} PAR; Quantum sensor, Skye Instruments LTD, Wales, England). Every two weeks municipal wastewater was collected from the aeration tank at WWTP Nijmegen and contained activated sludge (2.2 ± 0.6 (±SD) g TSS L^{-1}). This wastewater was subsequently stored at 4 °C and hereafter supplied to our three-compartment cascade via 30 L containers. The activated sludge in the 30 L container was resuspended with a submersible pump (Fig. 1) and was kept at 15 °C before supplying to our cascade. The container was refreshed every other day. Wastewater was pumped into the cascade using peristaltic pumps (Masterflex L/S; Cole-Palmer, Chicago, USA) with a tube size of 4.8 mm (Masterflex L/S tubing size 15; Cole-Palmer, Chicago, USA) and a flow rate of 1.04 ± 0.04 (SD) L h^{-1}. The cascade was batch fed and the pumps ran 15 min per hour for 3 h in a row (daily from 12:30 h to 15:30 h). In this way, the theoretical hydraulic retention time (HRT) was approximately 3 days, and excess sludge particles were allowed to be filtered out of the system by the filter pumps in compartment 2. Demineralised water was added to each aquarium daily to compensate for the evapotranspiration losses during the experiment. The outlet buckets were emptied weekly to determine the volume that ran through the three-compartment cascade. The total duration of the experiment was 4 weeks, with an acclimation period of 13 days for the Tubificidae beforehand (Fig. 2).

2.3. Biomass growth and nutrient sequestration

Samples of the Tubificidae populations were taken only at the start (day −13) and the end (day 28) of the experiment, while *A. filiculoides* was harvested once or twice a week to prevent overcrowding (Fig. 2). At the start, 75–100 g WW *A. filiculoides* ($n = 4$) and 75 g WW Tubificidae (n = 4) from the culture were harvested to determine the initial element content. These samples (at $t = 0$) were used as the control in the analyses. All harvested worms and plants were washed with demineralised water to remove residue from its surface. Wet weight was measured and subsequently worms and plants were dried in an oven to a constant weight at 70 °C for at least 24 h. To determine total element concentrations, 500 mg of the resulting dry worm and plant samples were grounded, digested and analysed for Aluminium (Al), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P) and sulphur (S) content, using ICP-OES analyses (following the same procedure as in Temmink et al., 2018).

The relative growth rate (RGR, g g^{-1} DW d^{-1}) for *A. filiculoides* and Tubificidae during the experiment was calculated according to formula 1:

$$RGR = \frac{\ln(DW_2) - \ln\left(WW_1 * f_{dw/ww}\right)}{t_2 - t_1} \quad (1)$$

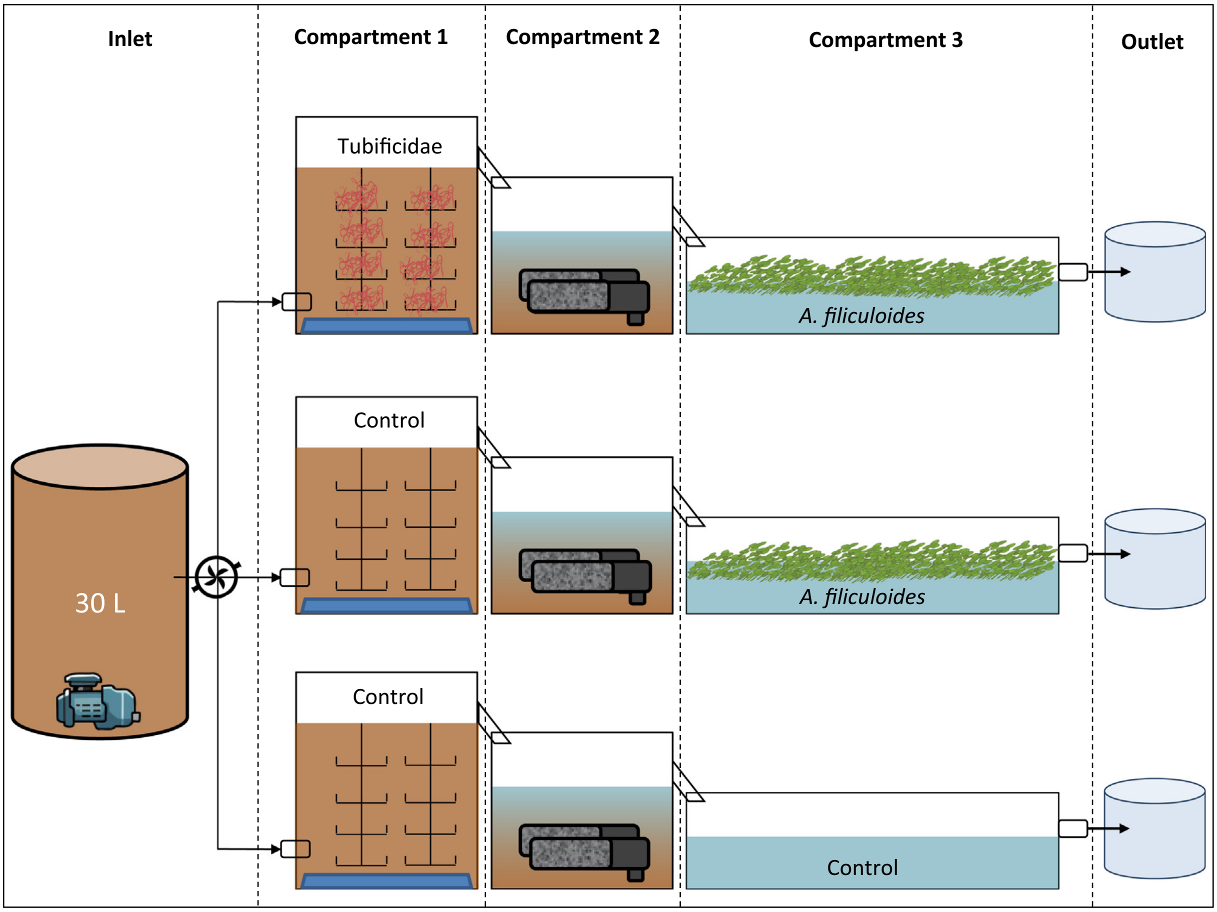

Fig. 1. Experimental setup of the three-compartment cascade with Tubificidae and *A. filiculoides*.

Where DW_2 is the dry weight (g) of time 2, WW_1 is the wet weight at time 1 and $f_{dw/ww}$ is the dry weight to wet weight ratio of the species. For *A. filiculoides*, the RGR was calculated each week. For Tubificidae, DW_2 was the dry weight at the end of the experiment (day 28) and WW_1 is the initial wet weight (g) of Tubificidae at day −13.

Additionally, the specific growth rate (μ_T, d^{-1}) of Tubificidae was calculated according to formula 2:

$$\mu_T = \frac{DW_2 - \left(WW_1 * f_{dw/ww}\right)}{\left(WW_1 * f_{dw/ww}\right) \times (t_2 - t_1)} \quad (2)$$

2.4. Physical-chemical water properties

Weekly water temperature, dissolved oxygen (DO) concentration and pH were measured in the morning, using a multiparameter portable meter (HQ40d, Hach, Loveland, USA) for temperature and DO and for pH a handheld meter (Multi 340i meter, Wissenschaftlich-Technische Werkstätten GmbH) connected to a pH probe (Orion 9156BNWP; Thermo Fisher Scientific). Filtered and unfiltered water samples were taken from each new inlet bucket, and once a week from aquaria in compartment 1, compartment 3 and outlet buckets. Water samples were filtered through a Whatman® puradisc filter with a pore size of 0.45 μm and concentrations of PO_4^{3-}, NH_4^+ and NO_3^- were colorimetrically measured (as in Geurts et al., 2008) with an Auto Analyzer 3 system (Bran & Luebbe, Norderstedt, Germany). Filtered water samples were further analysed for aluminium (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), Molybdenum (Mo), phosphorus (P), sulphur (S), and zinc (Zn), using inductive coupled plasma spectrometry (ICP-OES iCAP 6000, Thermo Fisher Scientific, Waltham, USA). Unfiltered water samples were analysed for total phosphorus (TP), total nitrogen (TN) and chemical oxygen demand (COD) using Dr. Lange® test kits. Total suspended solids (TSS) were measured according to Standard Methods (APHA, 1998). Additionally, total inorganic carbon concentrations (TIC) were measured in unfiltered water samples with an infrared gas analyzer (IRGA, ABB Advance Optima, Zürich, Switzerland), where after carbon dioxide and bicarbonate concentrations were calculated (as in van Bergen et al., 2019). An overview of the analyses, instruments and protocols used can be found in table S1.

2.5. Statistical analyses

2.5.1. Univariate analyses

Differences in worm biomass between day −13 and the end of the experiment (day 28) were tested by a *t*-test. For plant biomass we used a linear mixed model to determine differences over time between plants grown downstream of the worm or control aquaria. We used a *t*-test or Mann–Whitney *U* test (when data was not normally distributed) to test differences of physical-chemical parameters between the control and worm treatment in the first compartment at sampling day 0 and 28 in order to see how the system had changed. Differences between treatments in compartment 3 at sampling day 0 and 28 were tested with one-way ANOVA, followed by a post hoc Tukey test. Levene's test was used to test homogeneity of variances and for the cases that variances were not homogenous, we used a Kruskall–Wallis test followed by a pairwise Mann–Whitney U test with Bonferroni adjusted *p*-values. All univariate statistical analyses were performed with SPSS 23.0. All average values are shown with their SD (± 1SD).

2.5.2. Multivariate analyses

The effect of Tubificidae in compartment 1 and *A. filiculoides* in compartment 3 on physical-chemical water properties over time were analysed by the principal response curves analysis (PRC). The PRC analysis is a multivariate technique based on the redundancy analysis ordination technique (Van den Brink et al., 1999). For this, we ln(x + 1) transformed our physical-chemical water properties data. The PRC diagram shows on its horizontal axis time and on its vertical axis the regression coefficient (c_{dt}) of the first Principle Component of the treatment effects deviating from the control. Parameter weight (B_k), located at the right side of the diagram, indicates the weight of physical-chemical water properties measured and can be interpreted as the affinity of each physical-chemical parameters with the response graph shown in the diagram (Van den Brink et al., 1999). The effect of the different treatments on the physical-chemical parameters was statistically tested by using Monte Carlo permutation test. The multivariate statistical analyses were performed using the CANOCO for Windows® software package, version 5 (ter Braak and Šmilauer, 2012).

3. Results

The wastewater sludge from the aeration tank of the WWTP in Nijmegen used to feed the cascade slightly varied in physical-chemical composition over time (Table S2). Total suspended solids (TSS) was on average 2.02 g L^{-1} (±1.2), with a mean chemical oxygen demand (COD) of 3.3 (±0.9) g L^{-1} and a pH between 6.5 and 7.2. The mean TP concentration was 3 (±0.4) mmol L^{-1} and TN concentration was 10.3 (±2.6) mmol L^{-1}. The average phosphate concentration in wastewater was 260 (±199) μmol L^{-1}. Most dissolved inorganic nitrogen (DIN) in the wastewater was ammonium (319 ± 232 μmol L^{-1}), and a smaller fraction was nitrate (87 ± 167 μmol L^{-1}).

3.1. Effects of worms (compartment 1)

3.1.1. Growth and sequestration

Worm biomass significantly increased from 8.6 (±0.02) to 18.8 (±2.5) gram dry weight (paired t-test, $t(3) = -8.0$, $P = 0.004$), which resulted in a specific growth rate of 0.03 ± 0.007 d^{-1} and a RGR of 0.02 ± 0.003 g g^{-1} DW d^{-1}. We observed that almost all worms had settled on the carrier material at the end of the experiment. Additionally, we found that most of the measured elements accumulated in worm biomass over time (Fig. S2A). The highest accumulation was found for iron and manganese with more than 200% on average (Fig. S2A). Although phosphorus did not strongly accumulate, it was the element with the highest concentration in worm biomass with a mean content of 478 μmol g^{-1} DW.

3.1.2. Physical-chemical water properties

Aquatic worms strongly affected the physical-chemical properties of the wastewater (Table 1, S3 and Fig. S3, S4). The PRC diagram of physical-chemical water properties in compartment 1 shows that the worm treatment deviates from the control during the entire experiment (Fig. 5A). Of the total variance, 55% was assigned to the different treatments and the PRC diagram displayed a significant ($P = 0.002$) amount of this treatment variance (91%). Sampling time explained 26% of the total variance, and difference in replicates accounted to 19% of all variance. TSS and COD received the highest positive weights in the first PRC diagram, hence indicating a decrease in the worm treatment compared to the control. TSS reduction by worms was only 22% at day −11 but after the pumps were turned on TSS reduction increased and reached at day −7 a reduction rated similar to the rest of the experimental period (Fig. 3). From day 0 on, the average reduction of sludge by worms was 45%, and TSS concentration was significantly lower in worm aquaria compared to control aquaria (Fig. 3, Table 1). Additionally, COD in the worm aquaria was significantly reduced, with on average 1.2 g L^{-1} less COD than in the control treatment (Table 1, Fig. S3).

While TP concentration was significantly lower in the worm treatment, all dissolved nutrients and elements increased in the aquaria containing Tubificidae (Table 1, S3), caused by the degradation of particulate organic matter by the Tubificidae worms. The most negative weight of the first PRC axis in compartment 1 is NH_4^+, thus the largest difference caused by the treatment is the increased NH_4^+ concentration. Additionally, manganese, iron, aluminium and zinc received high negative weight (Fig. 5), indicating an increase in concentration caused by the Tubificidae worms.

3.2. Combination of worms and plants (compartment 3)

3.2.1. Plant growth and nutrient sequestration

The RGR of *A. filiculoides* did not differ between treatments in the first week and was approximately 0.1 g g^{-1} DW d^{-1}. After the first week the RGR of *A. filiculoides* increased and harvest frequency had to be increased to twice a week in order to prevent overcrowding. The difference in RGR between the treatments increased over time (Fig. 4A) and at the end of the experiment the RGR of downstream of the worm treatment increased to 0.15 ± 0.01 g g^{-1} DW d^{-1}, while *A. filiculoides* downstream of the control compartment only increased to 0.12 ± 0.01 g g^{-1} DW d^{-1}. The RGR calculation of *A. filiculoides* downstream of the control compartment was based on the biomass harvested that combined both *A. filiculoides* and floating algae, as it was not possible to separate this well. The cumulative biomass in the plant compartment behind the worm compartment was significantly higher over time (Fig. 4, Table S5, $p < 0.001$, $F = 29.2$). Although we found an increase in cumulative biomass of *A. filiculoides* grown downstream the control aquaria too, this was mainly caused by floating algae (Fig. 4).

Through time, the ratio *A. filiculoides*:floating algae decreased from approximately 0.95 at the start to 0.38 at the end of the experiment (Fig. 4B). Simultaneously with the biomass decrease of *A. filiculoides* downstream of the control treatment, most plants turned yellow and red (Fig. 4C2). In general, we found a decrease in element concentration

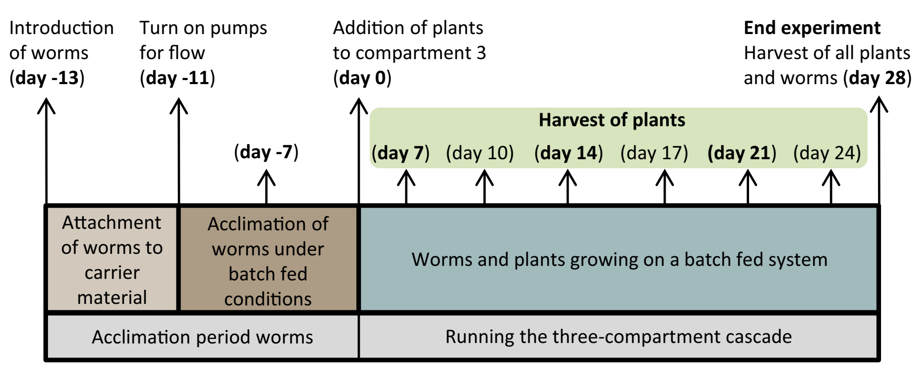

Fig. 2. Schematical overview of the experimental design including the harvest days of worms and plants. In bold are the days that physical-chemcial water properties have been measured.

Table 1
Physical-chemical water properties of the compartments 1 and 3, at day 0 and the last day (day 28) of the experiment. The physical-chemical water properties of day 0 have been measured just before the addition of plants to compartment 3 on that day. Values are shown as mean ± SD and different characters indicate significant ($P < 0.05$) differences between treatments.

	Compartment 1				Compartment 3					
	Day 0		Day 28		Day 0			Day 28		
	Control	Worms	Control	Worms	Control	Control + plants	Worms + plants	Control	Control + plants	Worms + plants
Water quality										
pH	$7.2^{a} \pm 0.2$	$6.2^{b} \pm 0.1$	6.3 ± 0.3	6.4 ± 0.1	$7.3^{a} \pm 0.2$	$7.4^{a} \pm 0.1$	$6.9^{b} \pm 0.1$	$6.4^{a} \pm 0.2$	$8.3^{b} \pm 0.3$	$4.0^{c} \pm 0.1$
O_2 (mg L^{-1})	9.3 ± 0.2	9.0 ± 0.2	9.5 ± 0.2	9.1 ± 0.4	7.8 ± 0.7	7.6 ± 0.4	6.9 ± 0.7	$8.1^{a} \pm 0.4$	$14.3^{b} \pm 0.3$	$8.6^{a} \pm 0.4$
Water temp (°C)	17.4 ± 0.3	18.0 ± 0.5	16.9 ± 0.2	17.0 ± 0.2	17.1 ± 0.2	17.4 ± 0.3	17.5 ± 0.2	17.4 ± 0.8	17.8 ± 0.7	18.1 ± 0.7
COD (mg L^{-1})	$4436^{a} \pm 306$	$2600^{b} \pm 165$	$2730^{a} \pm 206$	$1495^{b} \pm 78$	$29.6^{a} \pm 1.3$	$27.9^{a} \pm 3.0$	$91^{b} \pm 5.0$	$28.2^{a} \pm 3.6$	$32.5^{a} \pm 2.25$	$114.8^{b} \pm 13.0$
TSS (g L^{-1})	4.0 ± 0.5^{a}	2.3 ± 0.1^{b}	2.8 ± 0.2^{a}	1.4 ± 0.03^{b}						
TIC (μM)	140 ± 57.3	114 ± 33.1	$113^{a} \pm 41.9$	$170^{b} \pm 22.3$	$356^{a} \pm 186$	$448^{a} \pm 29$	$73.8^{b} \pm 13.4$	$96.1^{a} \pm 43.7$	$26.4^{b} \pm 29.2$	$33.1^{b} \pm 22.8$
HCO_3 (μM)	102 ± 54.2	52.9 ± 18.4	54.2 ± 33.0	83.0 ± 10.5	$297^{a} \pm 175$	$413^{a} \pm 22$	$7.6^{b} \pm 2.7$	$53.6^{a} \pm 32.5$	$25.7^{ab} \pm 28.4$	$0.1^{b} \pm 0.1$
CO_2 (μM)	$37.4^{a} \pm 13.1$	$61.1^{b} \pm 16.0$	58.6 ± 20.2	86.8 ± 19.5	$58.9^{a} \pm 15.4$	$34.4^{b} \pm 12.4$	$66.2^{a} \pm 15.3$	$42.5^{a} \pm 12.7$	$0.3^{b} \pm 0.3$	$33.0^{a} \pm 22.7$
Nutrients										
NH_4 (μmol L^{-1})	$9.9^{a} \pm 10.5$	$1615^{b} \pm 156$	$41^{a} \pm 18$	$1412^{b} \pm 417$	$1.6^{a} \pm 3.1$	$3.1^{a} \pm 3.0$	$810^{b} \pm 93.7$	0^{a}	$3.0^{a} \pm 2.8$	$327^{b} \pm 306$
NO_3 (μmol L^{-1})	$1402^{a} \pm 229$	$3265^{b} \pm 238$	$1897^{a} \pm 226$	$3534^{b} \pm 340$	$1135^{a} \pm 203$	$1008^{a} \pm 24$	$2826^{b} \pm 407$	$1504^{a} \pm 68$	$954^{b} \pm 115$	$2811^{c} \pm 314$
PO_4 (μmol L^{-1})	$110^{a} \pm 64.5$	$463^{b} \pm 21.5$	$424^{a} \pm 36.3$	$551^{b} \pm 15.3$	$79^{a} \pm 33.4$	$77^{a} \pm 14.1$	$453^{b} \pm 43.0$	$423^{a} \pm 17.4$	$251^{b} \pm 97.8$	$530^{c} \pm 6.2$
Total N (mmol L^{-1})	$14.1^{a} \pm 3.2$	$9.8^{b} \pm 0.9$	10.1 ± 2.3	9.8 ± 0.8	$1.4^{a} \pm 0.3$	$1.2^{a} \pm 0.04$	$3.8^{b} \pm 0.2$	$1.7^{a} \pm 0.1$	$1.2^{b} \pm 0.1$	$3.8^{c} \pm 0.7$
Total P (mmol L^{-1})	$3.6^{a} \pm 0.2$	$2.7^{b} \pm 0.2$	$3.2^{a} \pm 0.4$	$2.8^{b} \pm 0.1$	$0.1^{a} \pm 0.04$	$0.1^{a} \pm 0.01$	$0.5^{b} \pm 0.02$	$0.5^{a} \pm 0.03$	$0.2^{b} \pm 0.1$	$0.7^{c} \pm 0.2$
Elements										
Aluminium (μmol L^{-1})	$2.0^{a} \pm 0.3$	$4.5^{b} \pm 0.3$	$0.3^{a} \pm 0.2$	$2.7^{b} \pm 0.8$	$0.9^{a} \pm 0.6$	$0.5^{a} \pm 0.1$	$1.8^{b} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$4.4^{b} \pm 0.6$
Iron (μmol L^{-1})	$1.8^{a} \pm 0.1$	$6.6^{b} \pm 0.6$	$2.2^{a} \pm 0.5$	$8.9^{b} \pm 1.3$	$1.6^{a} \pm 0.3$	$1.3^{a} \pm 0.2$	$5.8^{b} \pm 0.7$	$2.0^{a} \pm 0.1$	$0.7^{b} \pm 0.1$	$8.2^{c} \pm 1.8$
Manganese (μmol L^{-1})	$0.2^{a} \pm 0.1$	$1.2^{b} \pm 0.1$	$0.2^{a} \pm 0.2$	$1.5^{b} \pm 0.3$	$0.1^{a} \pm 0.1$	$0.03^{a} \pm 0.01$	$2.4^{b} \pm 0.2$	$0.3^{a} \pm 0.2$	$0.01^{a} \pm 0.01$	$3.0^{b} \pm 0.8$
Zinc (μmol L^{-1})	$2.6^{a} \pm 0.8$	$5.7^{b} \pm 0.2$	$3.4^{a} \pm 1.1$	$5.8^{b} \pm 1.2$	$0.5^{a} \pm 0.1$	$0.4^{a} \pm 0.04$	$1.6^{b} \pm 0.2$	$0.9^{a} \pm 0.1$	$0.7^{a} \pm 0.1$	$2.1^{b} \pm 0.5$
Calcium (μmol L^{-1})	789 ± 67.9	802 ± 15	$1044^{a} \pm 66$	$1163^{b} \pm 45$	$767^{a} \pm 14$	$756^{a} \pm 10$	$874^{b} \pm 16$	$835^{a} \pm 26$	$568^{b} \pm 100$	$1040^{c} \pm 12$
Phosphorus (μmol L^{-1})	$129^{a} \pm 72$	$602^{b} \pm 53$	$537^{a} \pm 67$	$1167^{b} \pm 12$	$95.0^{a} \pm 32$	$87.4^{a} \pm 11$	$478^{b} \pm 16$	$506^{a} \pm 29$	$290^{b} \pm 107$	$819^{c} \pm 33$
Potassium (μmol L^{-1})	$512^{a} \pm 32$	$721^{b} \pm 14$	$828^{a} \pm 50$	$1021^{b} \pm 41$	$451^{a} \pm 23$	$435^{a} \pm 9$	$566^{b} \pm 10$	$652^{a} \pm 22$	$479^{b} \pm 24$	$371^{c} \pm 78$
Magnesium (μmol L^{-1})	$322^{a} \pm 44$	$560^{b} \pm 16$	$585^{a} \pm 40$	$889^{b} \pm 17$	$285^{a} \pm 17$	$270^{a} \pm 7$	$455^{b} \pm 13$	$490^{a} \pm 16$	$452^{a} \pm 37$	$699^{b} \pm 12$
Molybdenum (μmol L^{-1})	$0.10^{a} \pm 0.004$	$0.13^{b} \pm 0.01$	$0.07^{a} \pm 0.01$	$0.09^{b} \pm 0.004$	0.09 ± 0.01	0.09 ± 0.002	0.08 ± 0.01	$0.06^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.03^{b} \pm 0.01$
Sulphur (μmol L^{-1})	$456^{a} \pm 15.5$	$506^{b} \pm 11$	$691^{a} \pm 41$	$778^{b} \pm 21$	$382^{a} \pm 4$	$382^{a} \pm 5$	$413^{b} \pm 8$	$498^{ab} \pm 21$	$473^{a} \pm 9$	$511^{b} \pm 20$

in the biomass of *A. filiculoides* situated after the worms in the cascade (except phosphorus and sodium), while the concentration in the biomass of *A. filiculoides*, including floating algae, in the cascade without worms showed an increase at the end of the experiment compared to the start (Fig. S2B). The concentration of phosphorus inside *A. filiculoides* was in the range of 0.3–0.6 mmol g^{-1} DW.

3.2.2. Physical-chemical water properties

The effects of Tubificidae were also observed in the physical-chemical water properties of compartment 3 (Table 1, S4 and Fig. S3, S4). The PRC analysis showed that sampling date explained 12% of the total variance, displayed on the horizontal axis (Fig. 5B). Differences in replicates accounted to 13% of all variance, while the largest part, 75% of the total variance, could be attributed to treatment (Monte Carlo *p*-value = 0.002). Of this variance, 84% is explained by the first axis, shown on the vertical axis (Fig. 5B). HCO_3 and pH received the highest parameter weights, being indicative of prominent decreases in the worms-plants treatment (Table 1, Fig. 5B, S3, S4). Nutrient and element concentrations received a negative parameter weight of the PRC axis (Fig. 5B), denoting an increase in concentration in the worms-plants treatment. NH_4^+ and the metals aluminium, iron and manganese show lowest parameter weights, as similar to compartment 1 (Fig. 5A, Table 1). Though, different from compartment 1 is the significant increase of N and P compared to the control. However, total nutrient concentrations were still lower in worm treatment compared to control in compartment 2 when measured before sludge removal (compartment 2; worms: total P of 0.87 mmol L^{-1}, total N of 2.0 mmol L^{-1}, and control: total P of 1.1 mmol L^{-1}, total N of 3.0 mmol L^{-1}). Hence, the observed increase is a result of the larger fraction of the nutrients present in dissolved form in worm aquaria, which in turn were not removed as sludge in compartment 2 and remained in the system.

Lastly, in the control-plants treatment, we observed that *A. filiculoides* significantly decreased NO_3^-, PO_4^{3-} and the macronutrients potassium, calcium and iron in the water column (Table 1, S4). For PO_4^{3-} we found on average a 25% decrease in the water and for NO_3^- an even higher decrease of 40% (Table S4), consequently resulting of a decrease in total N and P concentration in the water column (Table 1).

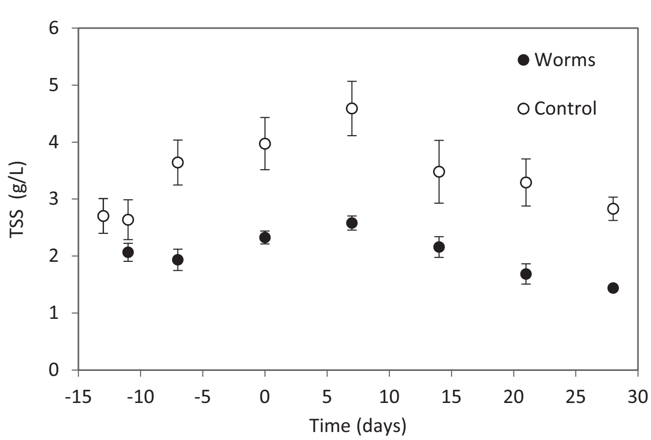

Fig. 3. Total suspended solids concentration (TSS; g L^{-1}) ± SD in the worm aquaria ($n = 4$; filled circles) and the control aquaria ($n = 8$; open circles) during the experiment.

4. Discussion

4.1. Main findings

This study demonstrated the interspecific facilitation processes of aquatic plants and animals in a biocascade system fed on wastewater by showing that sludge predation by Tubificidae worms can facilitate the biomass production of the aquatic macrophyte *A. filiculoides*, confirming our hypothesis. To our knowledge this is the first study that demonstrates increased biomass production on wastewater by

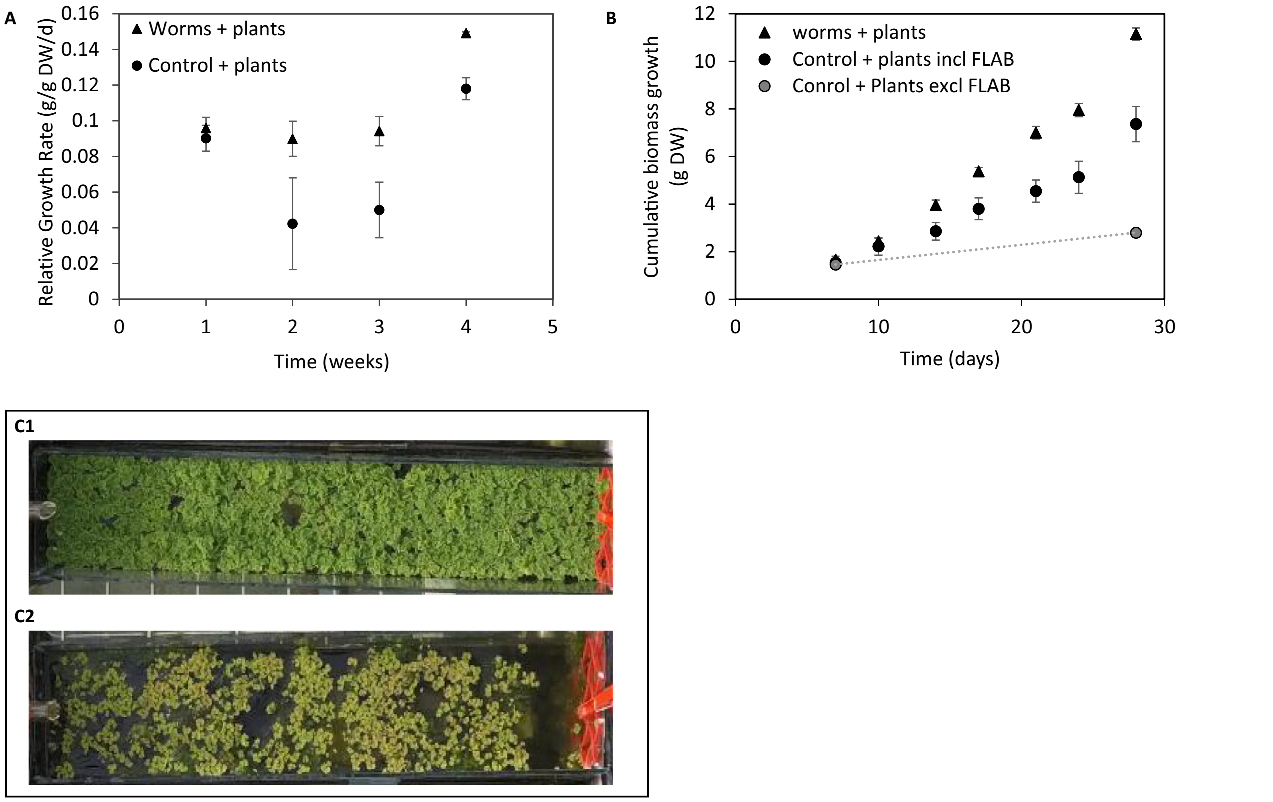

Fig. 4. Relative growth rate (g g^{-1} d^{-1}; mean ± SD) over time (A) and cumulative biomass growth (g DW; mean ± SD) over time (B) for the *A. filiculoides* plants grown downstream worms or control aquaria; and photos of *A. filiculoides* in the worm cascade (C1) and the control cascade (C2) at the end of the experiment. FLAB stands for Floating Algae Beds.

interspecific facilitating mechanisms, while organisms were grown in monoculture. Tubificidae reduced sludge TSS by approximately 45%, while more than doubling in biomass. Tubificidae grew well on carrying material, which optimized biomass production and creates potential for harvesting biomass in the future. The activity of Tubificidae mostly affected TSS, COD, NH_4^+ and metals, but also NO_3^- and PO_4^{3-} concentration in the first compartment of the biocascade. This increase in dissolved nutrients and metals was caused by the degradation of particulate organic matter (POM) by worms. Subsequently, water from the Tubificidae compartment flowed into the *A. filiculoides* compartment, where it affected the water composition causing a lower HCO_3^- concentration and pH while the concentration of zinc, iron, aluminium and manganese increased compared to the control system without Tubificidae (Fig. 5). These changes stimulated *A. filiculoides* growth, which was significantly higher in the presence of Tubificidae and prevented algae growth (Fig. 4).

4.2. Effects of worms

Tubificidae reduced sludge and were successfully grown on wastewater sludge. By introducing higher organisms, aquatic Tubificidae worms, that are able to consume bacterial biomass in sludge this resulted in an extended food chain in the activated sludge system (Ratsak et al., 1993) and biomass conversion. An average Tubificidae growth of 99.5 g DW m^{-3} d^{-1} and a maximum sludge consumption rate of 164.5 g DW m^{-3} d^{-1} in the biocascade were observed. The Tubificidae specific growth rate was 0.03 d^{-1} on average and in the range reported by other studies on aquatic worms *Lumbriculus variegatus* (family Lumbriculidae) and *Aulophorus furcatus* (subfamily Naididae; 0.01–0.11 d^{-1} specific growth rate) (Buys et al., 2008; Cai et al., 2017; Tamis et al., 2011). The sludge consumption (a 45% reduction) in our study was also in the range of that reported in other studies (16–75%, Buys et al., 2008; Hendrickx et al., 2009; Lou et al., 2011; Tamis et al., 2011; Zhang et al., 2020; Zhu et al., 2016), while the sludge to biomass conversion factor was higher (approximately 1.65 g sludge TSS g^{-1} worm TSS), than reported in other studies (e.g. 0.5–1 g TSS g-1 TSS worms; Tamis et al., 2011). Hence, by preying on bacterial biomass in sludge, energy transfers in the food chain resulting in a decrease of sludge due to the dissipation of energy (Wang et al., 2017) and incorporation into worm biomass. In addition, it has been suggested that sludge predation by worms could convert it into a better biodegradable form and increase overall biodegradability (de Valk et al., 2017; Tamis et al., 2011).

4.3. Effects of plants

A. filiculoides was able to grow on wastewater and had a maximum RGR of 0.15 g g^{-1} DW d^{-1} when Tubificidae were present in the biocascade. We used the same *A. filiculoides* strain as Temmink et al. (2018) and found a slightly higher RGR than the maximum RGR identified in their study (0.1 g g^{-1} DW d^{-1}), where *A. filiculoides* was grown on a N-free nutrient solution, similar to the quality of natural surface waters. This RGR might be inherent to this *A. filiculoides* strain as higher RGRs are reported for different strains (Temmink et al., 2018). The RGR in our experiment was in the range reported by other studies (Peters et al., 1980; van Kempen et al., 2016; Wagner, 1997), indicating that the species can successfully grow on wastewater. In addition, the P-sequestration per gram biomass in our study (0.3–0.6 mmol g^{-1} DW) was higher than the maximum sequestration reported in other studies that grew *A. filiculoides* on a nutrient solution similar to natural surface waters (0.16 and 0.35; Temmink et al., 2018; van Kempen et al., 2016). This indicates the potential of growing *A. filiculoides* on wastewater for phosphorus recovery. The element concentration in *A. filiculoides* was lower in the treatment with Tubificidae, which is a result of higher growth rates due to a dilution effect. Nevertheless, total element sequestration by *A. filiculoides* was higher in the presence of Tubificidae

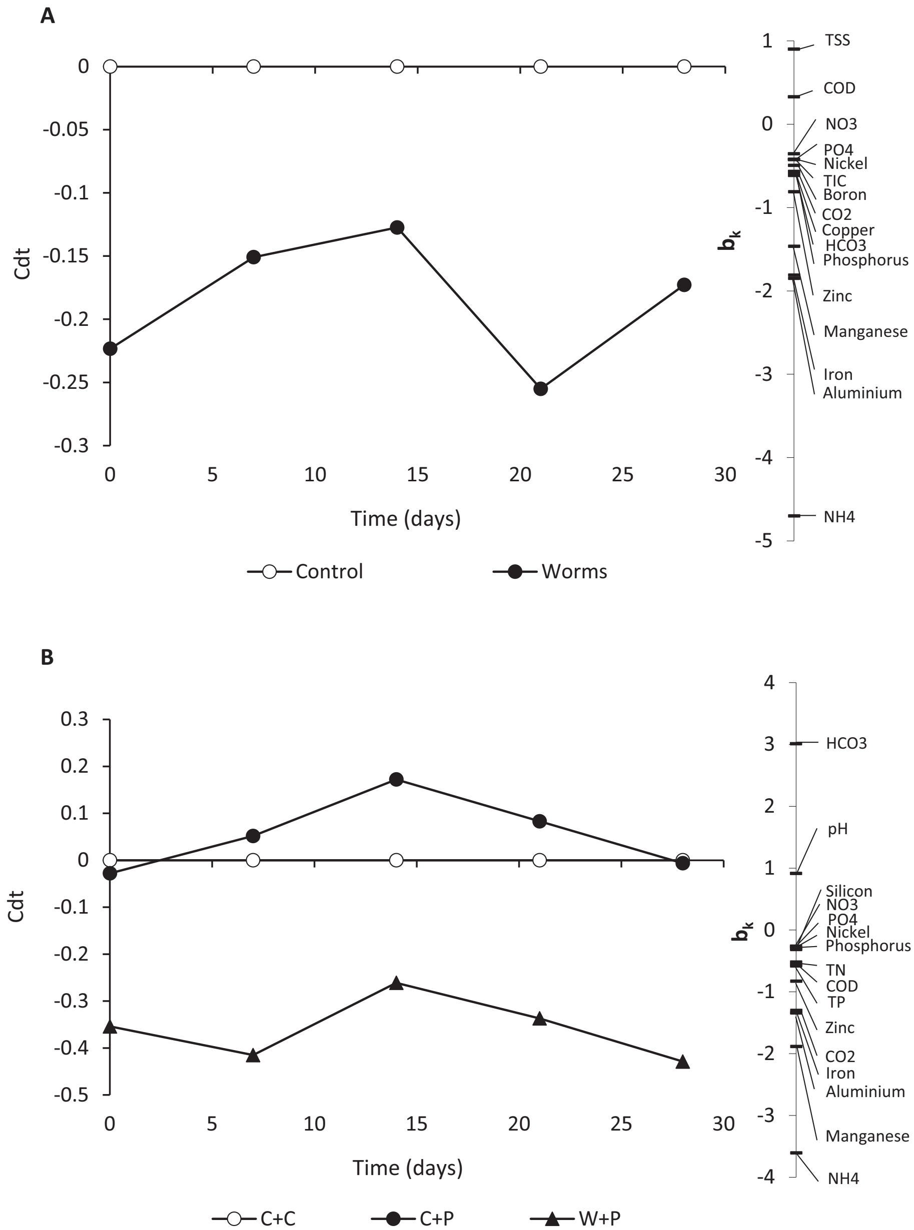

Fig. 5. PCR of compartment 1 (A) and compartment 3 (B), where C + C is the control without worms and plants; C + P is the treatment without worms in the first compartment, but with plants in the third compartment; and W + P is the treatment with worms and plants. The lines represent the course of the regression coefficient (C_{dt}) of the first Principle Component of the treatment levels in time. A) 55% of the total variance could be attributed to treatment, of which 91% is explained by the first axis, shown on the horizontal axis. B) 75% of the total variance could be attributed to treatment, of which 84% is explained by the first axis, shown on the vertical axis. The weight (bk) can be interpreted as the affinity of the environmental variables with the Principal Response Curves.

in the biocascade due to the increased biomass production. Tubificidae and *A. filiculoides* together sequestered 133 mmol P m^{-3} d^{-1} in the biocascade via biomass production. This shows the potential of the biocascade for P recovery from wastewater and thereby reuse (Shilton et al., 2012).

4.4. Facilitation mechanism

The increased growth of *A. filiculoides* in the biocascade with Tubificidae could not be explained by the increased availability of macronutrients, as the wastewater used in our study had high P concentrations in all treatments (> 90 μM, Table S2). When *A. filiculoides* grew in the absence of Tubificidae, plant growth was lower and pH values were high. The availability of phosphorus bound metals (iron, manganese, aluminium and zinc) was influenced by pH (Fig. S5). High pH values caused those metals to precipitate and thereby becoming less available to *A. filiculoides* (Fig. S5). It is known that *A. filiculoides* turns yellow when it has no access to iron (Wagner, 1997). Temmink et al. (2018) showed that phosphorus-induced iron chlorosis can occur in *A. filiculoides* when P concentrations are high (> 50 μM). Hence, it is likely that the concentration of phosphorus bound metals in the oxygenated water decreased due to precipitation reactions causing *A. filiculoides* to turn yellow/red with lower growth rates (Fig. 6). A pH above 8 in the *A. filiculoides* compartment in the absence of Tubificidae (Table 1) also might have inhibited the uptake of P by *A. filiculcoides*, as in the presence of Tubificidae the Fe content in the plants decreased while the P content increased with low pH values (Fig. S2B; da Silva Cerozi and Fitzsimmons, 2016). In contrast to the treatment without Tubificidae, no metal limitation occurred when Tubificidae were present in the biocascade as the growth rate increased.

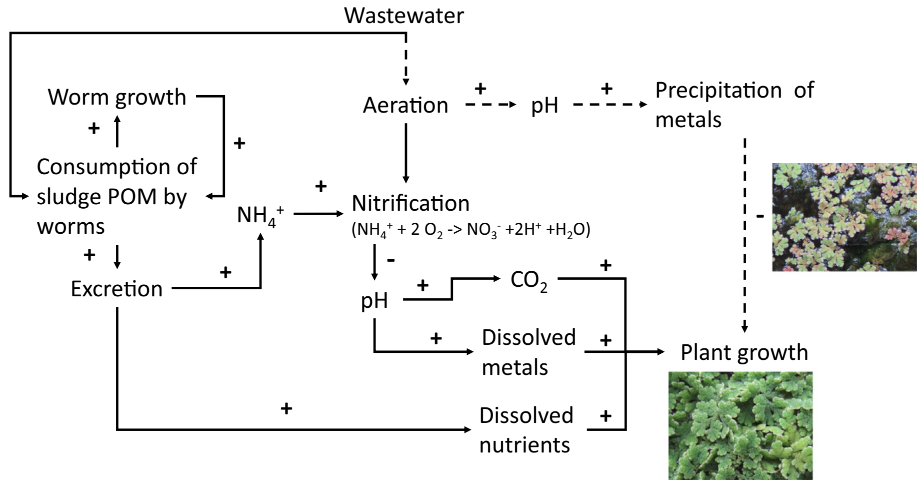

Fig. 6. Schematic overview of hypothesized mechanisms involved in tubificid worm growth facilitating plant growth. Positive arrows show an increasing effect, while negative arrows show a decreasing effect on a parameter. The dashed arrows indicate the mechanisms causing *A. filiculoides* to turn yellow/red with lower growth rates, while bold arrows indicate the facilitation of increased *A. filiculoides* growth. POM stands for particulate organic matter.

In the treatment with Tubificidae the water in the system showed increased NH_4^+ and NO_3^- (due to nitrification of NH_4^+) concentrations, a lower oxygen concentration and a lower pH (4 ± 0.1) with significantly higher concentrations of metals (Fe, Mn, Al and Zn) compared to the other treatments. The lowering of the pH and increased CO_2 may additionally have stimulated *Azolla* growth (Brouwer et al., 2018; van Kempen et al., 2016). Although reaeration of the Tubificidae compartment likely stimulated precipitation of phosphorus bound metals, this effect was reversed by acidification caused by nitrification of NH_4^+ via the reaction: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$. Mineralization of particulate organic matter by Tubificidae caused a high excretion of NH_4^+, which together with the high oxygen supply, stimulated nitrification rates by microorganisms. The protons released during the nitrification process, caused a decrease in pH (and a relative increase in CO_2), which increased the concentration of dissolved metals in the water as shown in the PRC (Fig. 5) and facilitated growth of *A. filiculoides* (Fig. 6). Microorganisms could have also contributed to the mineralization of organic matter and increased CO_2 production.

Similar to the macronutrients, the concentration of P bound metals inside the plants were lower when *A. filiculoides* grew in the presence of Tubificidae due to a dilution effect (Fig. S2). Also the uptake of nutrients by the floating algae that grew well in the absence of Tubificidae might have influenced the concentrations of (micro)nutrients inside the plants.

4.5. Facilitation processes in other studies

Observing facilitation between Tubificidae worms and *A. filiculoides* is in line with a previous study demonstrating the promoting effect of *Tubifex tubifex* (Tubificidae) on the productivity of the macrophytes species *Elodea canadensis* and *Myriophyllum spicatum* (Mermillod-Blondin and Lemoine, 2010). However, the proposed mechanism responsible for the enhanced macrophyte growth by worms differed from the mechanism observed in this study. While Mermillod-Blondin and Lemoine (2010) postulated that the enhanced macrophyte growth was a result of a reduction of anoxic conditions in sediments, our hypothesized mechanism involves facilitation by degradation of organic material by worms causing changes in physical-chemical water properties and elevation of micronutrient stress.

4.6. Optimisation of the biocascade system

With our biocascade of Tubificidae worms and *A. filiculoides*, we showed that interspecific facilitation processes can be used to increase the productivity of these species in a wastewater fed system. However, extending our biocascade with other positive plant-plant or animal-plant interactions is needed to increase further biomass production and nutrient removal. For this we could learn from other disciplines such as restoration ecology, where ecosystem engineers are being used to facilitate growth of other species (Halpern et al., 2007) or agricultural systems, in which overyielding is achieved by positive interactions between plant species. An example of extending the biocascade could be by adding freshwater bivalves, like *Dreissena polymorpha*, in combination with submerged macrophytes, since it has been shown that freshwater bivalves facilitate submerged plant growth by reducing turbidity (Gagnon et al., 2020; Gao et al., 2017). Next to that, a study on restoration of peat-forming wetland communities showed that complementarity between macrophyte species was the dominant mechanism causing overyielding for biomass production (van Zuidam et al., 2019). Complementarity can either be caused by interspecific resource partitioning among functional plant groups, or through interspecific facilitation processes (Hooper et al., 2005), but the authors stated interspecific facilitation as being the more likely explanation (van Zuidam et al., 2019). Thus, using plant–plant facilitation or plant-animal facilitation between species of different functional groups, i.e. species that process different resources and have a different ecosystem function, in wastewater engineering shows great potential for further increasing biomass production and nutrient recovery.

5. Conclusions

This work showed the potential of applying the ecological principle of interspecific facilitation in engineered systems for sludge reduction, phosphorus recovery and biomass production. The Tubificidae worms grew well on carrier material and facilitated *A. filiculoides* growth in this simple biocascade. Although complex, the processes in this cascade (Fig. 6) exemplify of how to make use of facilitating processes in order to achieve higher productivity similar to agricultural practices using wastewater. As nutrients are mobilised by the activity of the Tubificidae, an adapted design could enable the production of more *A. filiculoides* biomass resulting in a higher phosphorus recovery. Furthermore, the biocascade system could be expanded with other species such as submerged macrophytes and/or mussels, which act as biofilters. We postulate that optimizing interspecific interactions will strongly improve the success of biocascade-use in wastewater management practices.

CRediT authorship contribution statement

AS, LL and PV initiated the project. All authors contributed to the conceptual framework. LS and TB conducted the experiments and data analyses. LS and TB wrote the manuscript, and all authors contributed to improved versions of the manuscript.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147538.

References

Ahearn, D.S., Sheibley, R.W., Dahlgren, R.A., Anderson, M., Johnson, J., Tate, K.W., 2005. Land use and land cover influence on water quality in the last free-flowing river draining the western Sierra Nevada, California. J. Hydrol. 313 (3), 234–247.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Benoit, R., 1971. In: Ciacco, L. (Ed.), Water and Water Pollution Handbook. Marcel Dekker, Inc, New York.

van Bergen, T.J.H.M., Temmink, R.J.M., van Tweel-Groot, L., Bakker, W.J., Rehlmeyer, K., Koks, A.H.W., Waajen, A.C., Roelofs, J.G.M., Grootjans, A.P., van der Heide, T., Lamers, L.P.M., 2020. Self-facilitation and negative species interactions could drive microscale vegetation mosaic in a floating fen. J. Veg. Sci. 31 (2), 343–354.

Bertness, M.D., Callaway, R., 1994. Positive interactions in communities. Trends Ecol. Evol. 9 (5), 191–193.

ter Braak, C.J., Šmilauer, P., 2012. Canoco Reference Manual and User's Guide: Software for Ordination, Version 5.0, Microcomputer Power, Ithaca, NY, USA.

Brix, H., 1994. Use of constructed wetlands in water pollution control: historical development, present status, and future perspectives. Water Sci. Technol. 30 (8), 209–223.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol. 35 (5), 11–17.

Brouwer, P., van der Werf, A., Schluepmann, H., Reichart, G.-J., Nierop, K.G., 2016. Lipid yield and composition of Azolla filiculoides and the implications for biodiesel production. BioEnergy Res. 9 (1), 369–377.

Brouwer, P., Schluepmann, H., Nierop, K.G., Elderson, J., Bijl, P.K., van der Meer, I., de Visser, W., Reichart, G.J., Smeekens, S., van der Werf, A., 2018. Growing Azolla to produce sustainable protein feed: the effect of differing species and CO2 concentrations on biomass productivity and chemical composition. J. Sci. Food Agric. 98 (12), 4759–4768.

Bruno, J.F., Stachowicz, J.J., Bertness, M.D., 2003. Inclusion of facilitation into ecological theory. Trends Ecol. Evol. 18 (3), 119–125.

Buys, B.R., Klapwijk, A., Elissen, H., Rulkens, W., 2008. Development of a test method to assess the sludge reduction potential of aquatic organisms in activated sludge. Bioresour. Technol. 99 (17), 8360–8366.

Cai, L., Gao, D., Wang, K., Liu, H.-t. and Wan, X.-m., 2017. Sludge reduction using aquatic worms under different aeration regimes. Environ. Technol. 38 (6), 737–743.

Cardinale, B.J., Palmer, M.A., Collins, S.L., 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415 (6870), 426–429.

Chowdhury, R.B., Moore, G.A., Weatherley, A.J., Arora, M., 2017. Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. J. Clean. Prod. 140, 945–963.

Cieślik, B., Konieczka, P., 2017. A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of "no solid waste generation" and analytical methods. J. Clean. Prod. 142, 1728–1740.

Costa, M.L., Santos, M.C., Carrapiço, F., 1999. Biology, Ecology and Management of Aquatic Plants. Springer, pp. 323–327.

Davis, R., Hall, J., 1997. Production, treatment and disposal of wastewater sludge in Europe from a UK perspective. Eur. Water Pollut. Control 2 (7), 9–17.

De Lyon, M., Roelofs, J.G.M., 1986. Waterplanten in relatie tot waterkwaliteit en bodemgesteldheid, Katholieke Universiteit (Laboratorium voor Aquatische Oecologie).

Elissen, H., Mulder, W., Hendrickx, T., Elbersen, H., Beelen, B., Temmink, H., Buisman, C., 2010. Aquatic worms grown on biosolids: biomass composition and potential applications. Bioresour. Technol. 101 (2), 804–811.

Gagnon, K., Rinde, E., Bengil, E.G., Carugati, L., Christianen, M.J., Danovaro, R., Gambi, C., Govers, L.L., Kipson, S., Meysick, L., 2020. Facilitating foundation species: the potential for plant–bivalve interactions to improve habitat restoration success. J. Appl. Ecol. 57 (6), 1161–1179.

Gao, H., Qian, X., Wu, H., Li, H., Pan, H., Han, C., 2017. Combined effects of submerged macrophytes and aquatic animals on the restoration of a eutrophic water body—a case study of Gonghu Bay, Lake Taihu. Ecol. Eng. 102, 15–23.

Gendebien, A., Davis, B., Hobson, J., 2010. Environmental, Economic and Social Impacts of the Use of Sewage Sludge on Land. Project Interim Reports, Final Report Part III.

Geurts, J.J., Smolders, A.J., Verhoeven, J.T., Roelofs, J.G., Lamers, L.P., 2008. Sediment Fe: PO_4 ratio as a diagnostic and prognostic tool for the restoration of macrophyte biodiversity in fen waters. Freshw. Biol. 53 (10), 2101–2116.

Giangrande, A., Cavallo, A., Licciano, M., Mola, E., Pierri, C., Trianni, L., 2005. Utilization of the filter feeder polychaete Sabella. Aquac. Int. 13 (1–2), 129–136.

Gifford, S., Dunstan, R., O'Connor, W., Roberts, T., Toia, R., 2004. Pearl aquaculture—profitable environmental remediation? Sci. Total Environ. 319 (1–3), 27–37.

Gifford, S., Dunstan, R.H., O'Connor, W., Koller, C.E., MacFarlane, G.R., 2007. Aquatic zooremediation: deploying animals to remediate contaminated aquatic environments. Trends Biotechnol. 25 (2), 60–65.

Graham, D.W., Smith, V.H., 2004. Designed ecosystem services: application of ecological principles in wastewater treatment engineering. Front. Ecol. Environ. 2 (4), 199–206.

Halpern, B.S., Silliman, B.R., Olden, J.D., Bruno, J.P., Bertness, M.D., 2007. Incorporating positive interactions in aquatic restoration and conservation. Front. Ecol. Environ. 5 (3), 153–160.

Hendrickx, T., Temmink, H., Elissen, H., Buisman, C., 2009. Aquatic worms eating waste sludge in a continuous system. Bioresour. Technol. 100 (20), 4642–4648.

Hooper, D.U., Chapin Iii, F., Ewel, J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D., Loreau, M., Naeem, S., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. 75 (1), 3–35.

Kacprzak, M., Neczaj, E., Fijałkowski, K., Grobelak, A., Grosser, A., Worwag, M., Rorat, A., Brattebo, H., Almås, Å., Singh, B.R.J.E.r., 2017. Sewage Sludge Disposal Strategies for Sustainable Development. vol. 156 pp. 39–46.

van Kempen, M.M., Smolders, A.J., Bögemann, G.M., Lamers, L.P., Roelofs, J.G., 2016. Interacting effects of atmospheric CO 2 enrichment and solar radiation on growth of the aquatic fern Azolla filiculoides. Freshw. Biol. 61 (5), 596–606.

Li, A.-j., Yang, S.-f., Li, X.-y., Gu, J.-d.J.W.R., 2008. Microbial Population Dynamics During Aerobic Sludge Granulation at Different Organic Loading Rates. 42(13) pp. 3552–3560.

Li, L., Tilman, D., Lambers, H., Zhang, F.S., 2014. Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. New Phytol. 203 (1), 63–69.

Lou, J., Sun, P., Guo, M., Wu, G., Song, Y., 2011. Simultaneous sludge reduction and nutrient removal (SSRNR) with interaction between Tubificidae and microorganisms: a full-scale study. Bioresour. Technol. 102 (24), 11132–11136.

McJannet, C., Keddy, P., Pick, F., 1995. Nitrogen and phosphorus tissue concentrations in 41 wetland plants: a comparison across habitats and functional groups. Funct. Ecol. 231–238.

Mermillod-Blondin, F., Lemoine, D.G., 2010. Ecosystem engineering by tubificid worms stimulates macrophyte growth in poorly oxygenated wetland sediments. Funct. Ecol. 24 (2), 444–453.

Mount, D.R., Highland, T.L., Mattson, V.R., Dawson, T.D., Lott, K.G., Ingersoll, C.G., 2006. Use of the oligochaete, Lumbriculus variegatus, as a prey organism for toxicant exposure of fish through the diet. Environ. Toxicol. Chem. 25 (10), 2760–2767.

Peters, G.E., Evans, W.R., Crrist, D.K., Mayne, B.C., Poole, R.E., 1980. Characterization and comparisons of five N2-fixing Azolla-Anabaena associations, I. Optimization of growth conditions for biomass increase and N content in a controlled environment. Plant Cell Environ. 3 (4), 261–269.

Ratsak, C., Koouman, S., Kooi, B., 1993. Modelling the growth of an oligochaete on activated sludge. Water Res. 27 (5), 739–747.

Reddy, K.R., DeLaune, R.D., 2008. Biogeochemistry of Wetlands: Science and Applications. CRC press, Boca Raton.

Renzi, J.J., He, Q., Silliman, B.R., 2019. Harnessing positive species interactions to enhance coastal wetland restoration. Front. Ecol. Evol. 7, 131.

van der Schatte Olivier, A., Jones, L., Vay, L.L., Christie, M., Wilson, J., Malham, S.K., 2020. A global review of the ecosystem services provided by bivalve aquaculture. Rev. Aquac. 12 (1), 3–25.

Shilton, A.N., Powell, N., Guieysse, B., 2012. Plant based phosphorus recovery from wastewater via algae and macrophytes. Curr. Opin. Biotechnol. 23 (6), 884–889.

da Silva Cerozi, B., Fitzsimmons, K., 2016. The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. Bioresour. Technol. 219, 778–781.

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., Müller, R., Moormann, H., 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol. Adv. 22 (1–2), 93–117.

Tamis, J., Van Schouwenburg, G., Kleerebezem, R., Van Loosdrecht, M., 2011. A full scale worm reactor for efficient sludge reduction by predation in a wastewater treatment plant. Water Res. 45 (18), 5916–5924.

Tang, Y., Harpenslager, S.F., van Kempen, M.M., Verbaarschot, E.J., Loeffen, L.M., Roelofs, J.G., Smolders, A.J., Lamers, L.P., 2017. Aquatic macrophytes can be used for wastewater polishing but not for purification in constructed wetlands. Biogeosciences 14 (4), 755–766.

Tchobanoglous, G., Burton, F., Stensel, H., 1991. Wastewater Engineering: Treatment, Disposal and Reuse. 3rd ed. Metcalf & Eddy, McGraw-Hill, New York, pp. 1–13 (McGraw-Hill, New York).
Temmink, R.J., Harpenslager, S.F., Smolders, A.J., Dijk, G., Peters, R.C., Lamers, L.P., Kempen, M.M.J.S.r., 2018. Azolla Along a Phosphorus Gradient: Biphasic Growth Response Linked to Diazotroph Traits and Phosphorus-induced Iron Chlorosis. 8(1) p. 4451.
Thable, T.S., 1984. Einbau und abbau von stickstoffverbindungen aus abwasser in der wurzelraumanlage othfresen. Universität Hessen, Gesamthochschule Kassel.
Ullmann, F., Gerhartz, W., Yamamoto, Y.S., Campbell, F.T., Pfefferkorn, R., Rounsaville, J.F., 1985. Ullmann's Encyclopedia of Industrial Chemistry. VCH publishers.
de Valk, S., Khadem, A.F., Foreman, C.M., van Lier, J.B., de Kreuk, M.K., 2017. Physical and biochemical changes in sludge upon Tubifex tubifex predation. Environ. Technol. 38 (12), 1524–1538.
Van den Brink, P.J., Ter Braak, C.J.J.E.T., Chemistry, 1999. Principal Response Curves: Analysis of Time-dependent Multivariate Responses of Biological Community to Stress. 18(2) pp. 138–148.
Van Hove, C., 1989. Azolla: And Its Multiple Uses with Emphasis on Africa. Food and Agriculture Organization, Rome, Italy.
Vanni, M.J., 2002. Nutrient cycling by animals in freshwater ecosystems. Annu. Rev. Ecol. Syst. 33 (1), 341–370.
Wagner, G.M., 1997. Azolla: a review of its biology and utilization. Bot. Rev. 63 (1), 1–26.
Wang, Q., Wei, W., Gong, Y., Yu, Q., Li, Q., Sun, J., Yuan, Z., 2017. Technologies for reducing sludge production in wastewater treatment plants: state of the art. Sci. Total Environ. 587, 510–521.
Zhang, F., Li, L., 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. Plant Soil 248 (1), 305–312.
Zhang, X., Zeng, H., Wang, Q., Li, J., Ma, C., 2020. Sludge predation by aquatic worms: physicochemical characteristics of sewage sludge and implications for dewaterability. J. Clean. Prod. 258, 120612.
Zhu, X., Yuan, W., Wang, Z., Zhou, M., Guan, J., 2016. Effect of worm predation on changes in waste activated sludge properties. Water Environ. Res. 88 (5), 387–393.
van Zuidam, J.P., van Leeuwen, C.H., Bakker, E.S., Verhoeven, J.T., Ijff, S., Peeters, E.T., van Zuidam, B.G., Soons, M.B., 2019. Plant functional diversity and nutrient availability can improve restoration of floating fens via facilitation, complementarity and selection effects. J. Appl. Ecol. 56 (1), 235–245.