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## Decomposition of aquatic pioneer vegetation in newly constructed wetlands



Ciska C. Overbeek<sup>a,\*,1</sup>, Harm G. van der Geest<sup>a</sup>, E. Emiel van Loon<sup>b</sup>, Arne D. Klink<sup>a,2</sup>, Seth van Heeringen<sup>a</sup>, Sarah F. Harpenslager<sup>c,3</sup>, Wim Admiraal<sup>a</sup>

<sup>a</sup> Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, The Netherlands

<sup>b</sup> Department of Computational Geo-Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, The Netherlands

<sup>c</sup> Department of Aquatic Ecology and Environmental Biology, Institute for Water and Wetland Research, Radboud University, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands

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### ABSTRACT

Artificial wetlands are constructed around the globe for a variety of services, including wastewater treatment and carbon storage. To become a carbon sink, a newly constructed wetland must have a fully developed vegetation, consisting of species that can produce more organic matter than is being lost through decomposition. However, the effects of environmental conditions on the overall balance between production and decomposition might be complex. In this study, two large-scale field litterbag experiments were performed in a three-year old constructed wetland in the Netherlands, to separate the effects of litter characteristics and environmental conditions on decomposition rates of aquatic pioneer vegetation. Dimension reduction by principal component analysis was used to limit the number of variables for subsequent analyses in linear models. When transplanted to one common environment, litter characteristics alone could explain 52% and 26% of the variation in decomposition after 6 and 12 months, respectively. When both litter characteristics and environmental conditions were tested simultaneously and litter was decomposed in its original environment, 37% and 23% of the variation could be explained after 6 and 12 months, respectively. Both experiments showed two phases of decomposition: the initial leaching phase with an important role for litter characteristics and microbial communities in the model, and the second, slower phase, which is predominantly determined by litter characteristics and environmental conditions such as water quality. Model results could not be extrapolated to a fully developed reference area. Optimization of conditions in order to limit decomposition rates seems difficult and therefore we suggest using management options to influence biomass production and thereby fully exploit the use of newly constructed wetlands for carbon storage.

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### 1. Introduction

Artificial wetlands are constructed around the globe for a variety of services (Zhao et al., 2015), including wastewater treatment (Kivaisi, 2001; Vymazal, 2014) and carbon storage (Klein and Werf,

2014). For optimal functioning of constructed wetlands, a fully developed vegetation is required. In newly constructed wetlands, similar to other pioneer systems, autonomous vegetation development will depend on environmental conditions as well as the seed bank present in the sediment. Characteristic vegetation types can develop in a couple of years, even without the introduction of species (Fennessy et al., 1994; Mitsch et al., 1998; Odland, 1997), but it may take several decades for the wetland to become a stable functioning ecosystem (Mitsch and Wilson, 1996). In the first years after construction, vegetation diversity is generally lower in unplanted than in planted wetlands (Mitsch et al., 2005; Williams and Ahn, 2015), but richness will increase over time (Reinartz and Warne, 1993). In contrast, unplanted wetlands or those with monocultures can be more productive in the initial years after the

\* Corresponding author.

E-mail address: [c.c.overbeek@uva.nl](mailto:c.c.overbeek@uva.nl) (C.C. Overbeek).

<sup>1</sup> Present address: Nelen & Schuurmans, Zakkendragershof 34-44, 3511 AE Utrecht, The Netherlands.

<sup>2</sup> Present address: Eurofins AquaSense, H.J.E. Wenckebachweg 120, 1114 AD Amsterdam-Duivendrecht, The Netherlands.

<sup>3</sup> Present address: School of Biological and Chemical Sciences, Queen Mary University, Mile End Road, E1 4NS, London, United Kingdom.

construction of the wetland than more diverse ones (Means et al., 2016; Mitsch et al., 2005).

A newly constructed wetland built for carbon storage will require production of biomass to exceed decomposition. Autonomous development of such systems can result in a range of environmental conditions and plant and litter characteristics. For example, higher soil organic matter content and lower bulk density stimulate establishment of emergent rather than submerged vegetation (Galatowitsch and Valk, 1996). Such differences most likely have a big impact on both production and decomposition rates in these wetlands. However, the effects of environmental conditions on the overall balance between production and decomposition might be complex. For example, while higher nutrient availability increases biomass production (Fennessy et al., 2008; Sarneel et al., 2010), it will also stimulate decomposition rates (Fennessy et al., 2008; Lee and Bukaveckas, 2002; Rejmánková and Houdková, 2006; Sarneel et al., 2010). Biomass production and nutrient content of plant material increases with increasing nutrient concentrations in the environment (Dee and Ahn, 2014; Fennessy et al., 2008), resulting in changes in the type and activity of the organisms that feed on this plant material (Andersen et al., 2010; Boulton and Boon, 1991; Dimitriu et al., 2010; Reed and Martiny, 2013; Straková et al., 2011; Trinder et al., 2009). These changes could in turn result in altered decomposition rates (Fennessy et al., 2008). In the process of decomposition, different phases can be recognized (Berg and Laskowski, 2006). The most easily degradable water-soluble compounds and non-lignified carbohydrates will be decomposed in the first phase, after which lignified carbohydrates and lignin will be decomposed at a lower rate in the second phase. Decomposition rates in the first phase can be increased by high nutrient availability in the litter, while high tissue concentrations of N can inhibit lignin-degrading enzymes and thereby decrease decomposition rates in the second phase. In the third and last phase, decomposition rates will approach zero.

Still, most studies on constructed wetlands focus on production only, and those studies considering decomposition only quantify the effects of single factors (e.g. nitrogen or phosphorus levels, or pH) on decomposition rates, mostly in a controlled setting (Aerts et al., 2005; Kok and Velde, 1991; Qualls and Richardson, 2000). To improve our understanding of carbon sequestration rates in newly constructed wetlands, it is therefore necessary to determine the combined effect of autonomous vegetation development and environmental conditions (including the presence of a decomposer community) on decomposition rates in these systems. In this study we aim to determine the influence of both plant and litter characteristics and environmental conditions on decomposition rates of aquatic pioneer vegetation in newly constructed wetlands. A large-scale field litterbag experiment was performed in the Volgermeerpolder (the Netherlands), a three-year old constructed wetland consisting of different experimental basins, using aquatic pioneer vegetation from within the basins. In addition, we used vegetation samples from the Weerribben (the Netherlands), as a fully developed reference area. We measured 35 variables, 28 abiotic and 7 biotic, and converted these to single factors for litter characteristics, sediment quality, water quality, microbial community composition and fraction of macroinvertebrate detritivores to determine which predictor variables best explain decomposition rates. To separate the effects of litter characteristics from those of environmental conditions, two experiments were performed. In Experiment 1, aquatic pioneer vegetation of different origin was left to decompose in one environment to determine only the influence of differences in litter characteristics on decomposition, while in Experiment 2 the same aquatic pioneer vegetation was placed in its original growing environment to study all factors simultaneously. This experimental approach will provide us with important insight into the drivers for decomposition of plant material under

different environmental conditions in newly constructed wetlands and may lead to improved design criteria for building wetlands for carbon sequestration purposes.

## 2. Materials and methods

### 2.1. Site description

The experiments were carried out using aquatic pioneer vegetation collected at the Volgermeerpolder (52°25'17"N; 4°59'35"S) and the Weerribben (52°47'30"N; 5°54'37"S), in the Netherlands. The Volgermeerpolder is a newly constructed wetland, which was created in 2011 on a sand covered geomembrane on top of a former waste dump, with the geomembrane separating the waste hydrologically from the wetland, with the aim to initiate peat development (Egbring, 2011). It contains multiple basins ranging in size from 500 to 1600 m<sup>2</sup>, formed by clay dikes and sand substrate. Some basins were complemented with a layer of ~30 cm organic sludge (originating from a nearby peatland area, 52°17'13"N; 4°46'12"S), resulting in a range of organic matter fractions in the sediment from 0.01 to 0.23 in the different basins. Initial vegetation development depended on sediment and water composition and presence of seeds in the sediment. Three years after construction of the wetland, mainly submerged vegetation developed in basins with low fraction of organic matter in the sediment, generally the basins with bare sand sediment without the complementary layer of organic sludge. In basins with a higher fraction of organic matter mainly emergent vegetation developed. The 12 basins used in this study were fed either with nutrient-rich surface water from the surrounding agricultural fields, or with rainwater (collected in a separate storage basin). Water levels were kept at 60 ± 15 cm above the sediment surface.

The Weerribben is a well-developed peatland with many shallow man-made ditches (~60 cm water depth) and sediments with high organic matter fractions (0.61–0.71). Vegetation at our research sites in the Weerribben consisted mainly of floating and occasionally some emergent plants.

### 2.2. Physico-chemical variables

Starting three years after construction, various physico-chemical characteristics of surface water and sediment were measured several times in one year (details in Supplementary Material A). Surface water temperature (T), electrical conductivity (EC) and pH were measured at 10 cm below the water surface using a HQ40D portable meter (HACH-Lange, Tiel, the Netherlands). Alkalinity was determined on unfiltered samples by titration down to pH 4.2 using an auto-burette with accurately determined titer (ABU901, Radiometer, Copenhagen, Denmark, or Metrohm 716 DMS Titrino, Metrohm Applikon, Herisau, Switzerland). Surface water samples were filtered before further analysis in the laboratory. Nitrate (NO<sub>3</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), dissolved organic nitrogen (DON), soluble reactive phosphorus (SRP), potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>) were measured on an auto-analyzer (AA3 system, Bran & Luebbe, Norderstedt, Germany, or San ++ system, Skalar, Breda, the Netherlands). Chloride (Cl<sup>-</sup>), calcium (Ca<sup>2+</sup>), total iron (Fe), total manganese (Mn), total phosphorus (P) and total sulphur (S) were measured using inductively coupled plasma spectrometry (ICP-OES iCAP 6000, Thermo Fisher Scientific, Waltham, MA, USA, or Optima 8000DV, Perkin Elmer, Waltham, MA, USA).

Sediment samples were pooled from five subsamples per basin, using the top 5–10 cm, and stored at 4 °C until further analyses. Fraction organic matter (OM) was determined using loss on ignition (LOI, 4 h at 550 °C). Percentage carbon (C), nitrogen (N) and sulphur (S) were measured on an elemental analyser (Carlo Erba

**Table 1**

Range in sediment and water quality variables (mean (10–90% percentiles)) for basins in a newly constructed wetland (Volgermeerpolder; n = 12) and fully developed wetland (Weerribben; n = 3). FW = fresh weight.

	Volgermeerpolder	Weerribben
<b>SEDIMENT</b>		
Fraction organic matter (mg/mg DW)	0.11 (0.01–0.23)	0.66 (0.62–0.70)
Olsen-P ( $\mu\text{mol/l}$ FW)	119 (74–159)	28 (22–35)
Percentage C (mg/mg DW)	5.33 (0.65–10.04)	26.68 (8.93–41.80)
Percentage N (mg/mg DW)	0.26 (0.03–0.48)	1.30 (0.38–2.14)
Percentage S (mg/mg DW)	0.26 (0.05–0.54)	0.97 (0.38–1.56)
C:N ratio (–)	19.5 (16.7–21.2)	24.6 (19.7–30.6)
K <sup>+</sup> (mmol/l FW)	20 (5–59)	3 (2–4)
Na <sup>+</sup> (mmol/l FW)	4 (1–7)	0 (0–0)
Ca <sup>2+</sup> (mmol/l FW)	298 (79–823)	39 (23–57)
Total Fe (mmol/l FW)	82 (21–221)	16 (11–20)
Total Mn (mmol/l FW)	2 (1–4)	0 (0–0)
Total P (mmol/l FW)	4 (1–10)	1 (1–1)
<b>SURFACE WATER</b>		
Temperature autumn/winter (°C)	7.6 (7.3–7.9)	4.5 (4.4–4.5)
Temperature spring/summer (°C)	14.7 (14.2–14.9)	20.9 (20.3–21.4)
Electrical conductivity ( $\mu\text{S/cm}$ )	779 (593–1017)	287 (284–290)
pH (–)	8.1 (7.7–8.4)	7.9 (7.7–8.0)
Alkalinity (meq/l)	3.4 (2.3–4.1)	2.1 (2.1–2.1)
NO <sub>3</sub> <sup>-</sup> ( $\mu\text{mol/l}$ )	0.43 (0.27–0.75)	0.43 (0.22–0.70)
NH <sub>4</sub> <sup>+</sup> ( $\mu\text{mol/l}$ )	3.6 (1.9–5.9)	2.1 (1.7–2.3)
Dissolved organic N ( $\mu\text{mol/l}$ )	15.9 (7.1–28.9)	46.5 (44.6–48.8)
Soluble reactive phosphorus ( $\mu\text{mol/l}$ )	2.2 (0.2–6.7)	0.3 (0.1–0.5)
K <sup>+</sup> ( $\mu\text{mol/l}$ )	130 (50–186)	12 (10–13)
Na <sup>+</sup> ( $\mu\text{mol/l}$ )	2565 (1075–4268)	647 (644–650)
Cl <sup>-</sup> ( $\mu\text{mol/l}$ )	2490 (605–4505)	683 (665–697)
Ca <sup>2+</sup> ( $\mu\text{mol/l}$ )	2116 (1638–2560)	931 (918–941)
Total Fe ( $\mu\text{mol/l}$ )	2 (1–4)	1 (1–1)
Total Mn ( $\mu\text{mol/l}$ )	2 (1–3)	0 (0–0)
Total P ( $\mu\text{mol/l}$ )	4 (1–10)	0 (0–0)
Total S ( $\mu\text{mol/l}$ )	1528 (699–2243)	187 (180–194)

NA1500, Thermo Fisher Scientific, Waltham, MA, USA, or Vario EL cube, Elementar, Hanau, Germany). Phosphorus readily available for uptake by vegetation (Olsen-P, Olsen et al., 1954) was determined using extraction with 0.5 M NaHCO<sub>3</sub>, all other characteristics were measured as described for the water samples above.

For all measured characteristics, except for water temperature, yearly averages and percentiles were calculated since no seasonal differences were observed. For water temperature, the yearly average temperature was calculated separately for the months of October–December (to represent the autumn and winter period, first 6 months of decomposition) and April–June (to represent the spring and summer period, second 6 months of decomposition) (Table 1).

### 2.3. Litter characteristics

Vegetation was collected from the field at the end of the growing season in September 2013. Plant species were separated wherever possible and collected in proportion to their natural occurrence in the field, excluding the 2 m edges of the ponds to reduce impact of clay sediment on the shore. Collected material was rinsed to remove sediment and dried for several weeks at room temperature in the laboratory to produce litter. Part of the litter was oven dried at 60 °C to estimate a conversion factor between air-dry and oven-dry weight, as well as to prepare for analysis of C, N and S content of the different plant species. Since the S content fell below the detection limit in some samples, these values were set at 0. When the proportional dry weight of a plant species was less than 2% of the total dry weight per basin, the species was excluded from further analyses. Characteristics from all other species were averaged per basin, using the proportional dry weight per species as a weighing factor.

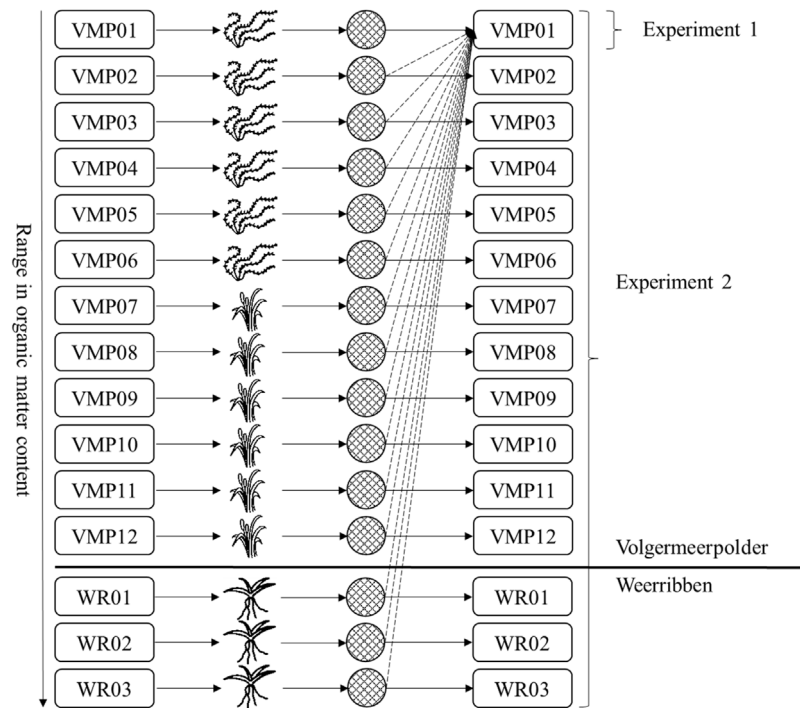
### 2.4. Decomposer community

The decomposer community of the Volgermeerpolder developed from inocula introduced by construction and filling the basins with water, and by colonization from surrounding areas. Furthermore, the open basins serve as a refuge for waterfowl that may have introduced microorganisms and macroinvertebrates.

BIOLOG GN2 plates (BIOLOG Inc., Hayward, CA, USA) were used to determine functional microbial community composition and activity in the sediments three years after wetland construction and at the reference site. For each basin, the top 5 cm of five sediment samples taken in June 2014 were pooled together to get a representative sample per basin and stored at 4 °C until further processing the next day. Sediment samples were diluted with sterilized demi water to obtain a dilution of 1:7, shaken by hand for a minute to detach bacteria from sediment particles and centrifuged at 1000g for 15 min after which the supernatant was diluted with sterilized demi water to a final dilution of 1:87 (adapted from Hench et al., 2004). BIOLOG plate wells were inoculated with 150  $\mu\text{l}$  bacterial suspension and incubated in the dark at 15 °C to simulate natural conditions. Absorption was measured at 590 nm every 24 h for 7 days (VersaMax microplate reader, Molecular devices, Sunnyvale, USA). The absorbance for individual wells was corrected for background absorbance by subtracting absorbance of the control well, subsequently considering negative values as zero. Community metabolic diversity (CMD), a measure for microbial diversity, was calculated by correcting absorbance values to binary data (presence/absence) using a threshold absorbance of 0.25 (Garland, 1996). The maximum slope in the sigmoidal response curve for CMD appeared after three days of incubation, providing the highest distinctiveness between samples. Therefore, measurements taken after three days of incubation were used in further analyses.

To determine the macroinvertebrate community in all basins, samples were collected six months after the start of the experiment at the same time as the litter from Experiment 2 (May/June 2014) by gently but quickly lifting the litterbags (see below) from the sediment using a net to include macroinvertebrates. Litterbags and macroinvertebrates were transported to the laboratory in sealed plastic containers and stored at 4 °C until further processing the next day. The macroinvertebrates found in these samples were assumed to represent the community composition for both experiments and time periods. Collected macroinvertebrates were identified up to family-level, except for chironomids and oligochaetes which were identified up to tribe and class level respectively. This taxonomic information was used to estimate the representation of functional feeding groups (FFGs). It was assumed that individuals found in the basins all originated from source populations in the surroundings, and that data on these source populations could therefore be used to determine the functional feeding guilds (FFGs) of the collected individuals in our study without determination up to species level. Therefore, FFGs were determined for all macroinvertebrate species found in an area stretching 5 km around the Volgermeerpolder in the years 2000–2015 (data provided by local water authority, “HNK-water,” 2015) using the database from Schmidt-Kloiber and Hering (2015). Subsequently, weighted averages were calculated of all FFG fractions per taxonomic family present in the surrounding area, using the total number of times a species was sampled by the water authority in all sampling locations at all times together. Only for chironomids and oligochaetes, weighted averages were calculated per tribe and class respectively (Supplementary Material B). The calculated FFG distribution from the source population was assigned to the sampled individuals. When a sampled individual belonged to a taxonomic family that was not present in the source population, the FFG distribution was assumed to be equal to the one given by Schmidt-Kloiber and Hering (2015). Weighted averages of FFGs per





**Fig. 1.** Schematic overview of Experiment 1 (varying plant characteristics) and Experiment 2 (varying plant characteristics and environmental conditions). Aquatic pioneer vegetation, ranging from submerged vegetation in basins with low organic matter content to emergent vegetation in basins with higher organic matter content and floating vegetation in the Weerribben, is harvested from 12 basins in the Volgermeerpolder (VMP01–VMP12) and 3 sites in the Weerribben (WR01–WR03) and placed in litterbags in one basin with low organic matter content (Experiment 1, dashed lines) or in their basin of origin (Experiment 2, solid lines).

sample were calculated accounting for number of individuals sampled per taxonomic level, without correction for size per individual. Since gatherers, shredders, miners and grazers are all part of the decomposer community, these four FFGs were collectively labeled as detritivores (DET).

## 2.5. Decomposition experiments

To quantify decomposition rates, litterbags filled with dried plant material from the different basins and the Weerribben were used. The conversion factor between air-dry and oven-dry weight was used to calculate initial oven-dry weight of the air-dried litterbags to be able to compare them to oven-dry weight at the time of collection. Litterbags consisted of a plastic petri dish (9 cm diameter) on the bottom to avoid excessive loss of small plant fragments (comparable to Bedford (2004)) with a 4 mm mesh on top (PETEX 07-4000/64, Sefar BV, Lochem, the Netherlands). This mesh size allowed entrance of most macrofauna, meiofauna and microbes.

To separately study the effect of litter characteristics on decomposition rates, litterbags from all environments were placed together in one environment in Experiment 1 (Fig. 1, dashed lines). To study the combined effect of litter characteristics and environmental conditions, all litterbags were placed back in the location where they were collected in Experiment 2 (Fig. 1, solid lines). Litterbags were placed in the field in November 2013 using a random block design with four blocks placing litterbags about 40 cm apart, and secured to the sediment using pins. Upon placement of the litterbags handling loss was determined to be ~6% and ~16% for samples from the Volgermeerpolder and Weerribben, respectively. Half the litterbags were retrieved after 6 months, while the other half was collected after 12 months by gently but quickly lifting the litterbag from the sediment. Litterbags were transported to the laboratory in sealed plastic containers and stored at 4 °C until further processing the next day. Litter was gently rinsed and sieved using a mesh size of 1 mm to exclude sediment particles and dried

for approximately three days at 60 °C, after which remaining litter mass was determined. The weight difference between litter at the start of the experiment (corrected for handling loss) and at time of retrieval was considered to be decomposition and expressed as fraction of the oven-dry start weight to get the fraction of decomposed litter (Frac.D<sub>6</sub> and Frac.D<sub>12</sub> for fraction decomposed after 6 and 12 months, respectively). Fraction remaining litter (Frac.R<sub>6</sub> and Frac.R<sub>12</sub>) was calculated as 1 minus Frac.D<sub>6</sub> and Frac.D<sub>12</sub>, respectively, and used to determine the exponential decay constant  $k$  (day<sup>-1</sup>) with the exponential decay formula

$$-k = \ln(\text{Frac.R}) / (\text{number of days}) \quad (1)$$

The parameter  $k$  was determined for the first and second period of six months of decomposition separately ( $k_{0-6}$ ,  $k_{6-12}$  respectively, number of days = 183), as well as for both periods together ( $k_{0-12}$ , number of days = 365).

## 2.6. Data analysis

### 2.6.1. Dimension reduction

Dimension reduction by principal component analysis (PCA) was applied to the predictor variables, providing a limited number of compound-variables for subsequent analysis. PCA was applied separately to: (a) plant variables (mass percentages of C, N, S, C:N ratio and the fraction emergent vegetation, Supplementary Material A), (b) sediment variables (Table 1) and (c) water quality variables (Table 1). For each variable-group the loading on the first component was retained. In addition to these three compound-variables, the community metabolic diversity for microbes (CMD) and the fraction detritivores in the observed macroinvertebrates (DET) were added to the set of predictor variables (Supplementary Material A).

### 2.6.2. Variable selection

Multiple linear models (Gaussian errors) were formulated, fitted and validated to establish the relative importance of the predictor variables for explaining fractions decomposed (Frac.D<sub>6</sub> and Frac.D<sub>12</sub>). The formulae for the linear models looked like this:

$$\text{Frac.D} \sim \text{LC} + \text{SED} + \text{SW} + \text{CMD} + \text{DET} \quad (2)$$

With Frac.D representing the fraction decomposed after either six months (Frac.D<sub>6</sub>) or twelve months (Frac.D<sub>12</sub>), LC, SED and SW are referring to the first principal components for the plant, sediment and surface water quality (see previous section), CMD refers to the community metabolic diversity and DET to the fraction of detritivores among the observed macroinvertebrates. As a first step in this regression analysis, only the observations in Experiment 1 (see Fig. 1) were used to establish the effect of litter characteristics (LC in Eq. (2)) on decomposition.

Next, in order to determine the combined effects of litter characteristics and environmental conditions on decomposition rates, Experiment 1 and 2 were analyzed together. In this analysis the compound-variable for litter characteristics as used in step 1 was obligatory present, while all combinations of the other predictor variables were added (up to a total of four variables) to form 15 candidate models. Subsequently all models from this candidate model set were ranked based on Akaike's Information Criterion (AIC) (Burnham and Anderson, 2002). The AIC value does not provide the goodness of fit of a model, but deals with the tradeoff between the goodness of fit and the complexity of the model, giving the preferred model the lowest AIC value.

For all models, only those with an AIC-difference less than 2 from the model with the lowest AIC value were considered adequate and retained. For the resulting model ensemble the importance and sign of each predictor variable was determined, as well as mean adjusted R<sup>2</sup> (R<sup>2</sup><sub>adj</sub>).

### 2.6.3. Validation

The models were validated in three steps: (1) by cross-validation using blocks, applying fitted models to predict unseen-data measured at the same time-period (both after 6 or 12 months of decomposition, resulting in R<sup>2</sup><sub>val</sub>), and (2) by extrapolation, applying fitted models (based on data at 6 months of decomposition) to predict unseen-data at 12 months of decomposition (resulting in R<sup>2</sup><sub>val,t2.with.t1</sub>), (3) by extrapolation to a developed wetland, applying fitted models from the Volgermeerpolder to predict unseen-data measured at the same time-period in the developed wetland the Weerribben (both after 6 or 12 months of decomposition, resulting in R<sup>2</sup><sub>val,WR</sub>).

All analyses were performed in R (R Core Team, 2015), using functions from the packages vegan, plyr, reshape and ggplot2 (Oksanen et al., 2017; Wickham, 2016a,b; Wickham et al., 2016).

## 3. Results

### 3.1. Development of vegetation

The broad range in sediment and water characteristics in the constructed basins in the Volgermeerpolder (Table 1) gave rise to a diverse aquatic pioneer vegetation (Supplementary Material A). Three years after construction of the wetland, two types of vegetation had developed in the basins, which was roughly related to the organic matter content of the sediment. Generally, basins with low fraction of organic matter showed dominance of submerged vegetation. The community was composed of *Characeae*, *Potamogeton pusillus*, *P. pectinatus*, *Myriophyllum spicatum*, *Elodea nuttallii*, *Ceratophyllum demersum* and *Lemna trisulca* (species ordered from most to least abundant). In basins with a higher fraction of organic matter mainly emergent vegetation developed: *Typha*

**Table 2**

Range in litter characteristics (mean (10–90% percentiles)) for mixed vegetation collected in basins in a newly constructed wetland (Volgermeerpolder; n = 12) and fully developed wetland (Weerribben; n = 3). All variables are dry weight mass fractions.

	Volgermeerpolder	Weerribben
Fraction emergent vegetation	0.40 (0.00–0.99)	0.00 (0.00–<0.02)
Fraction C	0.52 (0.48–0.56)	0.50 (0.37–0.61)
Fraction N	0.03 (0.02–0.03)	0.01 (0.01–0.02)
Fraction S	0.01 (0.00–0.01)	0.00 (0.00–0.00)
C:N ratio	22 (15–29)	46 (31–59)

*latifolia*, *T. angustifolia*, *Alisma plantago-aquatica*, *Bolboschoenus maritimus*, *Glyceria maxima*, *Eleocharis palustris* and *Equisetum fluviatile* (species ordered from most to least abundant). Vegetation in the reference site the Weerribben consisted mainly of floating and occasionally some emergent species: *Stratiotes aloides*, *Hydrocharis morsus-ranae*, *Iris pseudacorus* and *Nuphar lutea* (species ordered from most to least abundant). The difference in vegetation type and community composition between basins resulted in considerable variation in plant nutrient content (Table 2, Supplementary Material A).

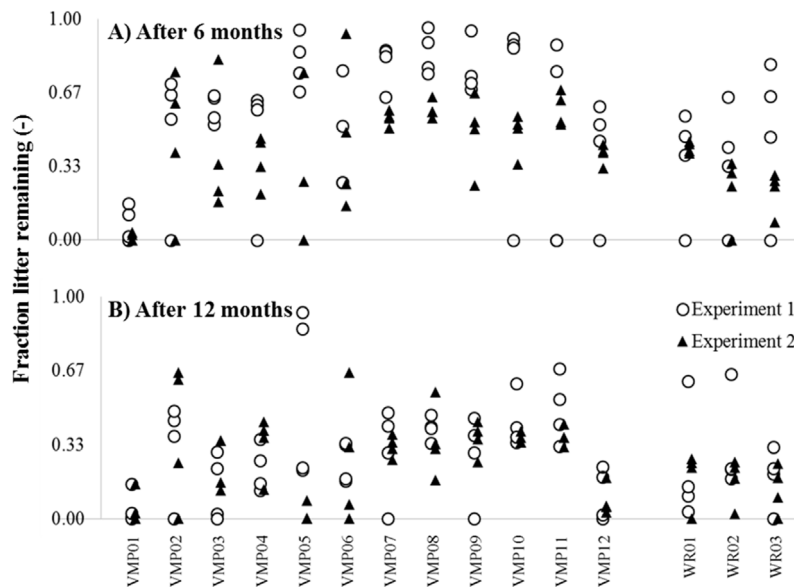
### 3.2. Dimension reduction

The C, N and S content and C:N ratio of the litter, and to a lesser extent the fraction of emergent vegetation, were important in explaining the first principal component for litter characteristics, containing 62% of the total variance in litter characteristics (Supplementary Material C). For sediment quality (Table 1) the first component also contained 62% of the variation. Almost all sediment quality variables were equally important, except for Olsen-P and the sediment C:N ratio which were about half as important (Supplementary Material C). Since water temperature differed between t<sub>6</sub> and t<sub>12</sub>, PCA for water quality variables was performed for both time steps separately. However, both produced almost equal results. With regard to water quality, the first component contained 43% of the variation, both after 6 and 12 months of decomposition. The variables T, EC, K, DON, Na, Ca, Alk and Mn contributed most to explaining the variation, whereas all other water quality variables explained less (Supplementary Material C).

### 3.3. Effect of litter characteristics on decomposition

The fraction of litter remaining for Experiment 1 (equal environmental conditions and decomposer community, varying litter characteristics) decreased over time, with 66 ± 23% and 33 ± 20% of the litter remaining after 6 and 12 months, respectively (Fig. 2A and B). Decomposition rates after 6 and 12 months of decomposition were correlated (r = 0.566). When litter from the Weerribben was placed in the same basin in the Volgermeerpolder, 53 ± 15% and 27 ± 20% of the litter remained after 6 and 12 months, respectively (Fig. 2A and B). Corresponding decomposition rates were k<sub>0-6</sub> = 0.0031 ± 0.0040 day<sup>-1</sup>, k<sub>6-12</sub> = 0.0059 ± 0.0063 day<sup>-1</sup> and k<sub>0-12</sub> = 0.0041 ± 0.0036 day<sup>-1</sup> for the Volgermeerpolder, and k<sub>0-6</sub> = 0.0037 ± 0.0015 day<sup>-1</sup>, k<sub>6-12</sub> = 0.0054 ± 0.0036 day<sup>-1</sup> and k<sub>0-12</sub> = 0.0044 ± 0.0023 day<sup>-1</sup> for the Weerribben.

The compound-variable for litter characteristics derived from the first PCA-component was the only variable present in Experiment 1 and therefore the only predictor variable in the linear decomposition model. This compound-variable could explain 52% of the variation in fraction litter decomposed after 6 months (Frac.D<sub>6</sub>, R<sup>2</sup><sub>adj</sub> = 0.519, R<sup>2</sup><sub>val</sub> = 0.486, Supplementary Material D.1) and 25% of the variation in fraction litter decomposed after 12 months (Frac.D<sub>12</sub>, R<sup>2</sup><sub>adj</sub> = 0.246, R<sup>2</sup><sub>val</sub> = 0.183, Supplementary Material D.2). When predicting Frac.D<sub>12</sub> with the model from Frac.D<sub>6</sub> 26% of the variation could be explained (R<sup>2</sup><sub>val,t2.with.t1</sub> = 0.264).



**Fig. 2.** Fraction litter remaining (fraction dry weight) (A) after 6 months and (B) after 12 months of decomposition, ranked according to sediment organic matter content. VMP01–VMP12 represent basins in the Volgermeerpolder, WR01–WR03 represent sampling sites in the Weerribben. Each data point represents one measurement, with open circles for Experiment 1 and closed triangles for Experiment 2.

When predicting decomposition of Weerribben litter transplanted to the newly constructed wetland Volgermeerpolder, no variation could be explained after either 6 or 12 months ( $R^2_{WR,t1} = 0.000$ ,  $R^2_{WR,t2} = 0.046$ ).

#### 3.4. Combined effect of litter characteristics and environmental conditions on decomposition

The fraction of litter remaining for Experiment 2 (aquatic pioneer vegetation placed in its original environment, with varying litter characteristics and environmental conditions) decreased over time, with  $47 \pm 20\%$  and  $31 \pm 16\%$  remaining after 6 and 12 months, respectively (Fig. 2A and B). Decomposition rates observed after 6 and 12 months of decomposition were correlated ( $r = 0.487$ ). For litter from the Weerribben  $31 \pm 11\%$  and  $20 \pm 8\%$  remained after 6 and 12 months, respectively (Fig. 2A and B). Corresponding decomposition rates were variable ( $k_{0-6} = 0.0051 \pm 0.0040 \text{ day}^{-1}$ ,  $k_{6-12} = 0.0036 \pm 0.0031 \text{ day}^{-1}$  and  $k_{0-12} = 0.0039 \pm 0.0022 \text{ day}^{-1}$  for the Volgermeerpolder, and  $k_{0-6} = 0.0068 \pm 0.0026 \text{ day}^{-1}$ ,  $k_{6-12} = 0.0025 \pm 0.0019 \text{ day}^{-1}$  and  $k_{0-12} = 0.0049 \pm 0.0022 \text{ day}^{-1}$  for the Weerribben).

After applying an all-possible-subsets regression (with up to four predictor variables), four models described  $\text{Frac.D}_6$  best (the averaged model over these four was characterized by  $R^2_{\text{adj}} = 0.368 \pm 0.004$  and  $R^2_{\text{val}} = 0.346 \pm 0.006$ , Supplementary Material D.3). The compound-variable for litter characteristics was obligatory present in all models. Microbial Community Metabolic Diversity (CMD) was also present in all models, while the compound-variables for water and sediment quality and fraction detritivorous macroinvertebrates (DET) were each present in one of the models. When predicting  $\text{Frac.D}_{12}$  with the model ensemble from  $\text{Frac.D}_6$  about 19% of the variation could be explained ( $R^2_{\text{val,t2,with,t1}} = 0.187 \pm 0.008$ ), while only 4% of the variation could be explained when predicting the fraction of decomposition of vegetation originating from the Weerribben ( $R^2_{WR,t1} = 0.039 \pm 0.020$ ).

Also four models were present in the model ensemble with  $\text{Frac.D}_{12}$  as response variable ( $R^2_{\text{adj}} = 0.232 \pm 0.003$ ,  $R^2_{\text{val}} = 0.178 \pm 0.028$ , Supplementary Material D.4). The compound-variable for litter characteristics was still obligatory present in all models. The compound-variable for water quality was also

present in all models. In this case the variables DET, CMD and the compound-variable for sediment quality were each present in one of the models. When predicting the decomposition rate in the Weerribben only 3% of the variation could be explained ( $R^2_{WR,t2} = 0.025 \pm 0.006$ ).

#### 4. Discussion and conclusion

In this study we have quantified decomposition rates of aquatic pioneer vegetation in a newly constructed wetland. Decomposition rates in our study were within the wide range of decomposition rates reported for other newly constructed as well as fully developed wetlands (e.g. Álvarez and Bécares, 2006; Bragazza et al., 2008; Fennessy et al., 2008; Gingerich et al., 2015; Rejmánková and Sirová, 2007). Previous research by Rejmánková and Houdková (2006) already showed that both litter characteristics and environmental conditions can have a significant impact on decomposition rates. Here, we separated the influence of these factors on decomposition rates of aquatic pioneer vegetation in newly constructed wetlands using an experimental approach and linear model predictions.

Under equal environmental conditions, litter characteristics alone explained 52% of the variation in decomposition rates after 6 months of decomposition. Other studies similarly show that, for example, increased litter nutrient content results in higher decomposition rates (Rejmánková and Houdková, 2006; Rejmánková and Sirová, 2007; Sarneel et al., 2010), although studies exist in which differences in litter quality could not explain variation in decomposition rates very well (Moore et al., 2007). Under different environmental conditions, only 37% of the variation in decomposition rates could be explained after 6 months of decomposition, predominantly by litter characteristics and microbial Community Metabolic Diversity. This inclusion of the microbial community as an explanatory variable was to be expected since especially in the first phase of decomposition, leaching of water-soluble compounds and non-lignified carbohydrates are known to be related to microbial community composition and activity (Andersen et al., 2010; Berg and Laskowski, 2006; Straková et al., 2011; Trinder et al., 2009). In the 3 years since construction, we believe that the microbial communities in the experimental basins will be well



developed, allowing them to quickly adapt to changes in environmental conditions (Dang et al., 2005; Dimitriu et al., 2010; Lazzaro et al., 2011; Reed and Martiny, 2013), and play a role in decomposition and leaching of easily degradable compounds.

In the second 6 months of our study, the explanatory power of our models decreased and our models could not accurately predict decomposition after 12 months using data from the first 6 months. In the in-situ experiment, water quality variables became dominant predictors (together with litter characteristics) in this second phase, explaining 23% of the variation in decomposition rates. After the initial stage of decomposition, the remaining litter was likely to be more recalcitrant and more uniform than the initial material. The impact of differences in community composition of pioneer vegetation and subsequent differences in litter quality on decomposition seems therefore to be most prominent during early stages of decomposition.

Models based on data from the constructed wetland Volgermeerpolder could not predict decomposition rates in the developed wetland Weerribben for both time periods and experiments. Since the explanatory power of the models for extrapolation within the newly constructed wetland was significantly higher than that for extrapolation to the developed wetland, it is most likely that some processes involved in decomposition in the developed wetland differ from those in newly constructed wetlands. For example, macroinvertebrate density and biomass, and thereby their decomposing activities, can still show large variation in newly constructed wetlands (Stewart and Downing, 2008), even while community composition might be very similar to well-developed wetlands (Gingerich et al., 2015). Mitsch and Wilson (1996) suggest that it may even take up to two decades before the functioning of the wetland can be determined after the initial stabilization phase.

Indeed, three years after construction, the basins in the newly constructed wetland Volgermeerpolder still showed a high variation in vegetation development, sediment quality, water quality and the decomposer community. Still, three years after construction, decomposition rates could reasonably well be explained by litter characteristics and environmental conditions with linear models, and predictions with these models for wetlands with similar conditions (i.e. different basins in the Volgermeerpolder) was possible. Similar to other pioneer systems, the constantly changing environment of newly constructed wetlands will influence the development of vegetation and decomposer communities, resulting in changes in decomposition rates during different successional stages. It will therefore be very difficult to optimize conditions to limit decomposition in order to maximize carbon storage. We therefore conclude that in the initial years after construction of a wetland it may be beneficial to optimize production instead of minimizing decomposition rates when designing newly constructed wetlands for carbon sequestration purposes. This can for example be done by introducing highly productive plant species or adding nutrients. However, it remains important to consider the balance between increased production rates and increased decomposition rates by addition of nutrients over longer time scales.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2017.06.046>.

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