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Decomposition of Standing Litter Biomass in Newly Constructed Wetlands Associated with Direct Effects of Sediment and Water Characteristics and the Composition and Activity of the Decomposer Community Using *Phragmites australis* as a Single Standard Substrate

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Abstract

In a large-scale field experiment in 18 basins in a three-year old constructed wetland (6 ha) in the Netherlands, we analyzed a wide range of environmental variables, grouped into variable groups, to determine the combined direct effect of the environmental variables and the resulting decomposer community on decomposition rates of standing litter biomass in newly constructed wetlands. The variability among the experimental units could only to a limited degree be explained by linear combinations of all 54 possible predictor variables (30 and 23% of variation explained after 6 and 12 months of decomposition). Moreover, models for decomposition after 6 months could not predict decomposition after 12 months. The poor predictions by our models are probably due to (sometimes large) variations in the predictor variables but small differences in decomposition rates between the different basins. Based on our results it seems that decomposition of standing litter biomass in newly constructed wetlands is relatively uniform when considered in time and space, with low explanatory power by variable groups from biotic and abiotic variables. Normally one would expect differences in decomposition rates with differing environments, but counterintuitively in newly constructed wetlands these differences are small.

Keywords *Phragmites australis* · Decomposer community · In situ experiment · All-possible-subsets regression

Introduction

Most newly constructed wetlands are used for water treatment and habitat restoration for wildlife (Fennessy et al. 1994;

Vymazal 2007; Zhao et al. 2015). To understand the functioning of newly constructed wetlands in terms of nutrient cycling, it is important to quantify production and decomposition rates. However, most studies focus on aboveground plant production rather than on decomposition of this material (as indicator number of results in Web of Science topic search: “constructed wetland” – 3623, “constructed wetland” AND production AND (macrophyte OR plant OR vegetation OR biomass) – 240, “constructed wetland” AND decomposition AND (macrophyte OR plant OR vegetation OR biomass) – 61). Furthermore, since newly constructed wetlands show large variability in both abiotic and biotic conditions, following successional patterns over time, the drivers of decomposition rates in these systems may differ from those in developed systems.

When designing and constructing new wetlands, one of the well-known driving forces for production and decomposition, i.e., nutrient availability, can be influenced by selecting specific types of sediment and water, for example rainwater with

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lower nutrient availability or more nutrient-rich water from nearby agricultural fields. A high availability of nutrients from surface water or eutrophic sediments potentially leads to high biomass production and higher availability of nutrients in the litter (Tanner 1996; Hoagland et al. 2001; Lee and Bukaveckas 2002; Fennessy et al. 2008; Trinder et al. 2009; Emsens et al. 2016a, b). However, decomposition rates will also be high (Rejmánková and Houdková 2006; Sarneel et al. 2010; Emsens et al. 2016a), since high plant tissue nutrient levels will increase decomposition (Serna et al. 2013) of the most easily degradable water-soluble compounds and non-lignified carbohydrates (Berg and Laskowski 2005). In contrast, high concentrations of N can also decrease decomposition rates of lignified carbohydrates and lignin, which are decomposed in later stages, due to their inhibiting effect on lignin degrading enzymes (Berg and Laskowski 2005). When environmental conditions are highly eutrophic, decomposition rates can become uncoupled from litter quality indicators (Emsens et al. 2016a, b), possibly coinciding with a shift in microbial decomposer community composition from a specialization in degradation of recalcitrant organic matter to dominance of opportunistic competitors (Moorhead and Sinsabaugh 2006).

This other important driving factor, the composition of the decomposer community of invertebrates and microorganisms, will also be influenced by the way in which a new wetland is designed. In newly constructed systems, macroinvertebrates colonize the area from the surroundings and will settle if suitable habitats are present (Stanczak and Keiper 2004; Stewart and Downing 2008). Over time, density and diversity will increase when the wetland habitats become more heterogeneous (Voshell and Simmons 1984; Christman and Voshell 1993; Heino 2000). Bacteria and fungi are functionally complementary and degrade different fractions of organic matter in different stages of succession (Findlay et al. 2002; Thormann et al. 2003; Fischer et al. 2006). Microbial community composition and activity can be influenced by litter nutrient availability and abiotic conditions (Trinder et al. 2009; Andersen et al. 2010; Straková et al. 2011), and can in turn influence food quality for macroinvertebrates (Boulton and Boon 1991; Whatley et al. 2014). Since both microbes and macroinvertebrates interact in the decomposition process (Hieber and Gessner 2002), changes in the functional community composition during different stages in succession after the construction of a new wetland need to be considered when studying decomposition rates in such systems.

Most studies focus on the effect of only a few variables on decomposition (e.g., Qualls and Richardson 2000; Aerts et al. 2005; Chimney and Pietro 2006; Álvarez and Bécares 2006; Sarneel et al. 2010; Voellm and Tanneberger 2014), mainly in developed, stabilized wetlands. However, since newly constructed wetlands show such large heterogeneity and variability in abiotic and biotic conditions, it is likely that multiple

variables influence decomposition rates simultaneously or in interaction. Some studies do include the complexity of multiple variables in the field (Thullen et al. 2008), but mainly using artificial substrates like cotton strips (Harrison et al. 1988; Mendelssohn et al. 1999; Tiegs et al. 2013), thereby ignoring the chemical complexity of natural litter biomass. We hypothesize that the mineral composition of both sediment and water with the resulting sediment food quality, and the availability of micronutrients, macronutrients and trace elements in the sediment and water all influence decomposer community composition and activity (e.g., Heino 2000; Stewart and Downing 2008; Straková et al. 2011; Andersen et al. 2013; Whatley et al. 2014). Furthermore, we hypothesize that those abiotic and biotic factors influence decomposition rates of standing litter biomass (e.g., Fischer et al. 2006; Rejmánková and Houdková 2006; Thullen et al. 2008; Fennessy et al. 2008; Sarneel et al. 2010; König et al. 2014; Ping et al. 2017) both directly and indirectly through the indirect effect of abiotic conditions on litter quality (Fennessy et al. 2008; Sarneel et al. 2010; Emsens et al. 2016a, b). To be able to focus on the direct effects of abiotic conditions on aboveground decomposition rates and separate them from the indirect effects, this study used common reed (*Phragmites australis*) as a single standard substrate. To add to the existing knowledge, this study aims to (1.) determine the relative importance of sediment and water characteristics on the composition and activity of the decomposer community, and to (2.) determine the combined direct effect of the environmental variables and the resulting decomposer community on decomposition rates of standing litter biomass in newly constructed wetlands.

To meet this aim, we performed a large-scale field decomposition experiment in different basins at the Volgermeerpolder. This three-year old constructed wetland (6 ha) in the Netherlands targets peat formation and contains 27 basins with a range in substrates, water regimes, and resulting pioneer vegetation and decomposer communities. Fifty-four variables, of which 45 were abiotic and 9 biotic, were measured to determine which predictor variables, either combined in groups or separately, best explain functional decomposer community composition and activity as well as aboveground litter decomposition in newly constructed wetlands.

Materials and Methods

Site Description

All experiments were carried out in 2013–2014 in the Volgermeerpolder (52°25'17"N; 4°59'35"S), the Netherlands, a newly constructed wetland on top of a former waste dump site that was completed in 2011. Multiple basins

ranging from 550 to 1600 m² were created in this wetland to initiate peat development on top of a sand-covered geomembrane. Basins were formed by clay dikes and the sand substrate in some basins was complemented with clay or organic sludge. The application of different mixtures of sand, clay and organic sludge resulted in a range of organic matter fractions in the sediments (0.01 to 0.23, Fig. 1a, Table 1 and Online Resource 1) in the different basins. The 18 basins used in this study were fed either with rain water (collected in a dedicated basin), or with nutrient-rich surface water from surrounding agricultural fields. Water levels were kept at 60 ± 15 cm above the sediment surface. As a result of the combination of different sediment mixtures with the two water regimes, the basins each developed a unique mineral

composition with resulting sediment food quality and availability of micronutrients, macronutrients and trace elements. All experiments and measurements in this study were performed in 2013/2014, three years after the construction of the wetland was completed. During the initial years vegetation in the basins developed depending on the sediment and water composition. Basins with sand sediments remained mainly unvegetated or covered by submerged species (77 ± 7%, mean ± SD), with a helophyte coverage of only 14 ± 6% after 3 years. Basins with addition of clay or organic sludge showed higher coverage by helophytes (40 ± 23 and 71 ± 16%, respectively). *Typha angustifolia* was the dominant species in basins with added clay sediment, covering around 26 ± 21% of the basins, while *Typha latifolia* was the dominant species in

Fig. 1 Variation in selected response variables between basins in standing litter biomass decomposition experiment in the Volgermeerpolder, ranked according to sediment organic matter content. a) fraction organic matter, b) decomposer community with b1) Community Metabolic Diversity, b2) Average Well Color Development, b3) macroinvertebrate FFG distribution, c) aboveground litter remaining (fraction dry weight) with c1) fraction remaining after 6 months and c2) fraction remaining after 12 months of decomposition. In all graphs one data point represents one measurement, except in b3) where it represents four measurements each

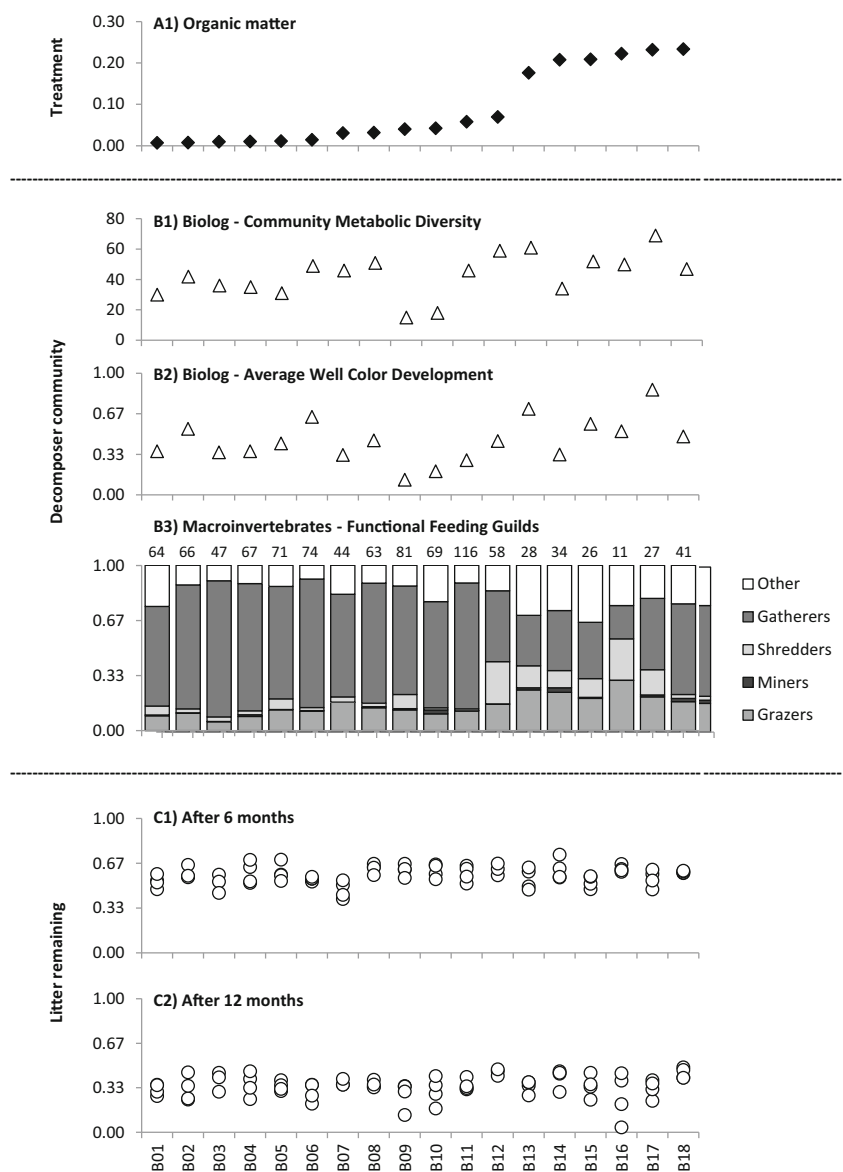


Table 1 Range in sediment and water quality variables (10 – 90 % percentiles (mean)) for basins in Volgermeerpolder ($n=18$)

| Variable | Unit | Surface water | | | | Pore water | | | |
|-------------------------------|-----------|---------------|---|------|--------|------------|---|-------|---------|
| | | 10% | – | 90% | (Mean) | 10% | – | 90% | (Mean) |
| Temperature May | °C | 14.4 | – | 15.7 | (14.9) | | | | |
| Temperature November | °C | 7.3 | – | 7.9 | (7.6) | | | | |
| Electrical conductivity | μS/cm | 534 | – | 1026 | (766) | | | | |
| pH | – | 7.6 | – | 8.7 | (8.2) | | | | |
| Alkalinity | meq/l | 2.6 | – | 4.4 | (3.5) | 7.4 | – | 24.6 | (16.2) |
| CO ₂ | μmol/l | 45 | – | 418 | (164) | 2250 | – | 4371 | (3137) |
| HCO ₃ [–] | μmol/l | 1901 | – | 4205 | (2710) | 7012 | – | 17994 | (12653) |
| NO ₃ [–] | μmol/l | 0.23 | – | 1.14 | (0.56) | 0.07 | – | 3.47 | (1.66) |
| NH ₄ ⁺ | μmol/l | 2.1 | – | 11.0 | (5.8) | 6.7 | – | 81.7 | (43.8) |
| Dissolved organic N | μmol/l | 8.0 | – | 38.2 | (21.5) | | | | |
| Soluble reactive phosphorus | μmol/l | 0.2 | – | 6.0 | (2.1) | 0.8 | – | 21.2 | (8.5) |
| K ⁺ | μmol/l | 46 | – | 176 | (119) | 23 | – | 187 | (100) |
| Na ⁺ | μmol/l | 957 | – | 4508 | (2662) | 797 | – | 3092 | (2123) |
| Cl [–] | μmol/l | 552 | – | 4521 | (2481) | 408 | – | 3640 | (2029) |
| Ca ²⁺ | μmol/l | 1613 | – | 2523 | (2077) | 3341 | – | 9801 | (6553) |
| Total Fe | μmol/l | 1 | – | 5 | (3) | 98 | – | 483 | (219) |
| Total Mn | μmol/l | 1 | – | 5 | (3) | 40 | – | 200 | (95) |
| Total P | μmol/l | 1 | – | 10 | (4) | 19 | – | 151 | (87) |
| Total S | μmol/l | 627 | – | 2230 | (1455) | 97 | – | 1754 | (728) |
| | | Sediment | | | | | | | |
| Fraction organic matter | – | 0.01 | – | 0.23 | (0.09) | | | | |
| Olsen-P | μmol/l FW | 77 | – | 206 | (146) | | | | |
| Percentage N | mg/mg DW | 0.03 | – | 0.48 | (0.23) | | | | |
| Percentage C | mg/mg DW | 0.74 | – | 9.98 | (4.58) | | | | |
| C:N ratio | – | 16.4 | – | 21.3 | (19.3) | | | | |
| Percentage S | mg/mg DW | 0.05 | – | 0.53 | (0.22) | | | | |

sediments with added organic sludge ($65 \pm 22\%$) (Harpenslager et al. 2018).

Physico-Chemical Variables

Starting three years after construction, various physico-chemical characteristics of surface water (SW), pore water (PW) and sediment (SED) were measured several times in one year, as described in Overbeek et al. (2018, details in Online Resource 1).

Measurements of surface water temperature (T), electrical conductivity (EC) and pH were taken in October – December 2013 and 2014 and April – June 2014 at 10 cm below the water surface using a HQ40D portable meter (HACH-Lange, Tiel, the Netherlands). Surface water samples were taken in November 2013 and February, May, July and December 2014 and filtered before further analysis in the laboratory, using Whatman mixed cellulose ester filters ME24. Pore water samples were taken at the same time as surface water samples at

15 cm depth in the sediment using vacuum syringes attached to ceramic soil moisture cups (Eijkelkamp, Giesbeek, the Netherlands). Alkalinity for surface water of unfiltered samples was determined 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). Carbon dioxide (CO₂) and bicarbonate (HCO₃[–]) were measured on an infrared gas analyser or high-temperature combustion total organic carbon (TOC) (IRGA, ABB Analytical, Frankfurt, Germany, or TOC-V CPH, Shimadzu, Kyoto, Japan), using unfiltered samples. Nitrate (NO₃[–]), ammonium (NH₄⁺), dissolved organic nitrogen (DON), soluble reactive phosphorus (SRP), potassium (K⁺) and sodium (Na⁺) were measured in filtered samples on an auto-analyser (AA3 system, Bran & Luebbe, Norderstedt, Germany, or San ++ system, Skalar, Breda, the Netherlands). Chloride (Cl[–]), calcium (Ca²⁺), total iron (Fe), total manganese (Mn), total phosphorus (P) and total sulphur (S) were measured in filtered samples 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 10 cm in February 2014 and the top 5 cm in June 2014, 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 nitrogen (N), carbon (C) and sulphur (S) were measured on an elemental analyser (Carlo Erba NA1500, Thermo Fisher Scientific, Waltham, MA, USA, or Vario EL cube, Elementar, Hanau, Germany). Phosphorus readily available for uptake by vegetation (Olsen_P) was determined using extraction with 0.5 M NaHCO₃.

For all measured characteristics, except for temperature, yearly averages were calculated per basin since no seasonal differences were observed within basins. To give an indication of variation between basins yearly averages and percentiles of all basins combined are presented in Table 1 (see Online Resource 1 for values per basin).

Decomposer Community

As described in Overbeek et al. (2018), the decomposer community of the Volgermeerpolder has developed on top of the mineral sand bed from inocula introduced during the construction and during the pumping of water from the 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. BIOLOG GN2 plates contain 95 wells with single simple carbon substrates and one control well (for an overview of all carbon sources see Garland and Mills 1991), specifically designed to make rapid community-level physiological profiles which best differentiate gram negative bacteria (Garland 1997). 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 1000 g 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 µl 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 and subsequently considering negative values as zero. Average Well Color Development (AWCD), a measure of microbial activity, was calculated according to Garland (1997), by averaging all 95 corrected response well absorbances. For Community Metabolic Diversity (CMD), a measure for microbial diversity, corrected absorbance values were converted to binary data (presence/absence) using a threshold absorbance of 0.25 (Garland 1996). The maximum slope in the sigmoidal response curve for AWCD as well as 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.

Macroinvertebrates were sampled six months after the start of the experiment at the same time as the standing litter biomass (May 2014, see below) and identified to family-level, except for chironomids and oligochaetes, which were identified 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 to species level. To this purpose, 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, <http://hnk-water.nl>) using the database from Schmidt-Kloiber and Hering (2015) (Online Resource 2). Subsequently, weighted averages, using the number of times a species was sampled by the water authority in all sampling locations at all times together as weight, were calculated for all FFG fractions per taxonomic family present in the surrounding area. For chironomids and oligochaetes, weighted averages were calculated per tribe and class, respectively (Online Resource 2). The calculated FFG distribution from the source population was assigned to the sampled individuals. When a sampled individual belonged to a taxonomic family which 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 sample were calculated accounting for number of individuals sampled per taxonomic level, without correction for size per individual. Gatherers (GAT), shredders (SHR), miners (MIN) and grazers (GRA) are all active in decomposition, therefore those four FFGs together were labeled as detritivores (DET).

Decomposition Experiment

To separate the direct effects of environmental variables on decomposition rates of aboveground litter from the indirect effects of litter quality itself, we used common reed (*Phragmites australis*) as a single standard substrate. Common reed is a common wetland plant with easily distinguishable leaves and culms, therefore making it easy to construct quantifiable substrates for decomposition measurements. By constructing bundles of vegetation, keeping larger fragments together, instead of using litterbags (e.g., Petersen and Cummins 1974; Triska and Sedell 1976; McArthur et al. 1994), we avoided negative influences caused by using litterbags such as altering microclimates within the litterbags or exclusion of certain sizes of decomposer organisms (Boulton and Boon 1991; Bradford et al. 2002; Kurz-Besson et al. 2005; Bokhorst and Wardle 2013).

P. australis was collected from fresh stands growing in a ditch in the Volgermeerpolder at the end of the growing season in September 2013 and dried for several weeks at room temperature in the laboratory. Part of the litter was oven dried at 60 °C to determine the conversion factor between air-dry and oven-dry weight, as well as C, N and S content of the litter ($n = 4$, 45.0 ± 0.2 , 1.7 ± 0.1 and $0.30 \pm 0.03\%$ (mean \pm SD), respectively). Collected reed material consisted of equal amounts of stems and leaves and therefore both were tied together to 10 g bundles in equal proportions. The conversion factor between air-dry and oven-dry weight was used to calculate initial oven-dry weight of the air-dried bundles to be able to compare them to oven-dry weight at the time of collection. Litter bundles were placed in the field in November 2013 using a random block design with four blocks of 1×1 meter each placed in an area of 5.5×5.5 m. Each block contained two litter bundles for collection at two different times, resulting in eight litter bundles per basin in total. The bundles within a block were placed about 40 cm apart, and secured to the sediment using pins. Upon placement of the bundles, handling loss was determined to be $\sim 2\%$. One bundle per block was retrieved after 6 months, while the other half was collected after 12 months by gently but quickly lifting the bundle from the sediment using a net to include macroinvertebrates. Litter bundles and macroinvertebrates were transported to the laboratory in sealed plastic bags and stored at 4 °C until further processing the next day, as described in Overbeek et al. (2018). 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 standing litter biomass 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

aboveground litter (Frac_D₆ and Frac_D₁₂ for fraction aboveground litter loss after 6 and 12 months, respectively).

Data Analysis

Predictor and Response Variables

Linear models (multiple regression, assuming Gaussian errors) were formulated, fitted and validated to establish the relative importance of measured predictor variables for explaining response variables in the early stages of our newly constructed wetland (Burnham and Anderson 2002). To post-process the models, all measured predictor variables were grouped in order to determine the relative importance of specific variable groups, defined as presence of these groups in the model ensemble, in explaining the response variable. Variables were grouped to water (SW), sediment (PW + SED), microbes (CMD + AWCD as measured by BIOLOG GN2 plates) or macroinvertebrates (abundance and FFGs, Online Resource 1), using yearly means for water and sediment variables. Next to these categories, predictor variables were also grouped in macronutrients (all SW and PW N + P), sediment food quality (SED OM + C + N + C_N), or other (see Online Resource 1 for an extensive list). To explain variation in (1.) functional community composition of microbes, (2.) activity of microbes, and (3.) fraction detritivores in macroinvertebrates, these three variables were separately taken as the response variable with all remaining separate variables from water and sediment variable groups after variable selection (see “Variable Selection” section and Online Resource 3a) as potential predictor variables in model formulation. When using (4.) fraction aboveground litter loss after 6 months of decomposition or (5.) fraction aboveground litter loss after 12 months of decomposition as a response variable, all remaining separate variables from all variable groups after variable selection (see “Variable Selection” section and Online Resource 3a) were present as possible predictor variables.

Validation

The models were validated in two steps: (1.) by cross-validation, applying fitted models to predict unseen-data measured at the same time-period (both after 6 or 12 months of decomposition, resulting in R^2_{val}), 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 $R^2_{\text{val}_t2_with_t1}$).

The different response variables were observed in different experimental layouts: macroinvertebrate and decomposition observations were laid out in 4 randomized blocks per basin while microbial data were observed only once per basin. For

that reason the different response variables were validated differently: microbial data was validated using leave-one-out cross-validation (LOOCV), macroinvertebrate and decomposition data was validated using blocks for cross-validation: using three blocks to predict the fourth.

Variable Selection

Formulation of the linear models took place in two steps: (1.) selecting a subset from all predictor variables, using regression trees, and (2.) finding the best linear models using all-possible-subsets regression.

In the first step, regression trees were built using all available scaled predictors. For *Frac_D* and *DET* as response variables, where four-fold cross-validation was applied, the regression trees were based on the different combinations of three out of four blocks, after which all potentially important variables resulting from the four regression trees were combined into one list of potentially important predictor variables per response variable. For *CMD* and *AWCD* as response variables, a regression tree was fitted only once to the entire data set (see Online Resource 3a for an overview of the resulting potentially important predictor variables per response variable). In the second step, all possible combinations of up to four variables were made from the set with potentially important predictor variables, excluding combinations of variables with an absolute correlation higher than 0.7, and including interactions between number of macroinvertebrate individuals and FFGs. Only those models 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 effect of each predictor variable was determined, as well as mean adjusted R^2 (R^2_{adj}), with variable importance representing the fraction of models in the model ensemble in which the variable was present. The predictors were also aggregated into variable groups (Online Resource 1), and the relative frequency at which each variable group was represented in the model ensemble was used to express variable group importance.

All analyses were performed in R (R Core Team 2015), using functions from the packages *plyr*, *reshape*, *rpart* and *ggplot2* (Wickham 2007; Wickham 2009; Wickham 2011; Therneau and Atkinson 2018).

Results

Decomposer Community

Functional microbial community composition varies between basins, with Community Metabolic Diversity (*CMD*) varying between 0.26 and 0.66 (mean 0.44) (10–90% percentiles,

Fig. 1b1 and Table 2). For *CMD* as response variable, a model ensemble of 8 models was found with an adjusted R^2 of 0.711 and cross-validated R^2 of 0.598 (Table 2, Online Resource 3b). Variables belonging to both water and sediment variable groups were present in all models (Fig. 2), with calcium in the surface water and phosphorus from the sediment readily available for uptake by vegetation both present in 88% of the models (see Table 2 for an overview of the importance and effect of predictor variables in predicting the response variable). Sediment food quality variables were present in all models as well, with either fraction organic matter, or percentage N or percentage C in the sediment present in each model. Macronutrients, including phosphorus from the sediment readily available for uptake by vegetation, were present in 88% of the models.

Microbial activity varies between basins, with Average Well Color Development (*AWCD*) varying between 26 and 60 (mean 43) (10–90% percentiles, Fig. 1b2 and Table 2). For *AWCD* as response variable, a model ensemble of 12 models was found with an adjusted R^2 of 0.774 and cross-validated R^2 of 0.704 (Table 2, Online Resource 3c). Like *CMD*, variables belonging to both water and sediment variable groups were used in all models (Fig. 2), with pore water alkalinity present in 83% of the models (Table 2). Either fraction organic matter, or percentage N or percentage C in the sediment were present in each model as sediment food quality variable, resulting in a combined presence of 100%. Macronutrients were present in 75% of the models, with pore water NH_4^+ present in 58%.

In all basins in the Volgermeerpolder 82% of all macroinvertebrates are detritivores (63–97%, 10–90% percentiles, Table 2), with gatherers making up the largest fraction and miners a very low fraction of detritivores (Fig. 1b3). With an increasing fraction of organic matter in the sediment, the fraction of gatherers decreases, while the fraction of shredders, grazers and other FFGs increases. The number of individual macroinvertebrates per block is variable within basins and decreases with a higher fraction of sediment organic matter. Forty models were present in the model ensemble with fraction detritivores (*DET*) as the response variable, with an adjusted R^2 of 0.269 and a cross-validated R^2 of 0.218 (Table 2, Online Resource 3d). Water and sediment variable groups are present in 95% and 98% of the models, respectively (Fig. 2), with the sediment C:N ratio present in 85% and surface water soluble reactive phosphorus present in 58% of the models (Table 2). Sediment food quality variables are present in 85% of the models, with sediment C:N ratio present in all those models. Macronutrients are present in all models, with either P or soluble reactive phosphorus in the surface water present in 83% of the models. When considering surface and pore water together, P and/or soluble reactive phosphorus are present in 90% of the models, while N is only present in 28%.

Table 2 Range in five microbial, macroinvertebrate and decomposition response variables (mean (10 – 90 % percentiles), N) as measured in basins in the Volgermeerpolder, including number of models in model ensemble, adjusted R^2 (R^2_{adj} , mean \pm SD), cross-validated R^2 (R^2_{val} , mean \pm SD, using LOOCV for microbial data, and cross-validation using blocks for macroinvertebrate and decomposition data), and extrapolated R^2 predicting unseen-data after 12 months of

decomposition using fitted models based on data after 6 months of decomposition ($R^2_{val_t2_with_t1}$, mean \pm SD). Importance of predictor variable (sub)groups in model ensemble per response variable (importance representing the fraction of models in the model ensemble in which the variable (sub)group was present), with importance and effect of main predictor variables (present in more than 50 % of model ensemble)

| Response variable mean (10 % – 90 %), N | Predictor variable (sub)group – importance | Main predictor variable – importance and effect |
|---|---|---|
| Community Metabolic Diversity (CMD) | | |
| 0.44 (0.26 – 0.66), N = 18 | Water – 100 % | Surface water Ca – 88 % + |
| 8 models in ensemble | Sediment – 100 % | Sediment Olsen_P – 88 % - |
| $R^2_{adj} = 0.711 \pm 0.009$ | Sediment food quality – 100 % | Either fraction organic matter, percentage N or percentage C in sediment – 100 % + |
| $R^2_{val} = 0.598 \pm 0.025$ | Macronutrients – 88 % | |
| Average Well Color Development (AWCD) | | |
| 43 (26 – 60), N = 18 | Water – 100 % | |
| 12 models in ensemble | Sediment – 100 % | Pore water alkalinity – 83 % - |
| $R^2_{adj} = 0.774 \pm 0.008$ | Sediment food quality – 100 % | Either fraction organic matter, percentage N or percentage C in sediment – 100 % + |
| $R^2_{val} = 0.704 \pm 0.017$ | Macronutrients – 75 % | Pore water NH_4^+ – 58 % + |
| Percentage detritivores (DET) | | |
| 82 (63 – 97), N = 72 | Water – 95 % | Surface water soluble reactive phosphorus – 58 % - |
| 40 models in ensemble | Sediment – 98 % | Sediment C:N ratio – 85 % - |
| $R^2_{adj} = 0.269 \pm 0.007$ | Sediment food quality – 85 % | Sediment C:N ratio – 85 % - |
| $R^2_{val} = 0.218 \pm 0.018$ | Macronutrients – 100 % | Either surface water P or surface water soluble reactive phosphorus – 83 % - |
| | | Surface or pore water P and/or surface or pore water soluble reactive phosphorus – 90 % - |
| Percentage aboveground litter loss after 6 months (Frac_D₆) | | |
| 42 (34 – 51), N = 70 | Water – 100 % | Surface water NO_3 – 100 % - |
| | | Surface water S – 92 % + |
| 12 models in ensemble | Sediment – 67 % | |
| $R^2_{adj} = 0.295 \pm 0.007$ | Macroinvertebrate – 75 % | |
| $R^2_{val} = 0.223 \pm 0.024$ | Microbial – 0 % | |
| $R^2_{val_t2_with_t1} = 0.002 \pm 0.003$ | Sediment food quality – 8 % | |
| | Macronutrients – 100 % | Surface water NO_3 – 100 % - |
| Percentage aboveground litter loss after 12 months (Frac_D₁₂) | | |
| 65 (55 – 75), N = 69 | Water – 100 % | Surface water P – 60 % - |
| 5 models in ensemble | Sediment – 100 % | Pore water S – 100 % - |
| $R^2_{adj} = 0.227 \pm 0.008$ | Macroinvertebrate – 100 % | Grazers – 100 % + |
| $R^2_{val} = 0.072 \pm 0.013$ | Microbial – 0 % | |
| | Sediment food quality – 0 % | |
| | Macronutrients – 100 % | |

A positive effect of a predictor variable on the response variable indicates that the response variable will increase with an increase in the predictor variable, while a negative effect indicates a decrease in the response variable with an increase in the predictor variable. For an overview of the possible predictor variables present in variable (sub)groups, see Online Resource 1

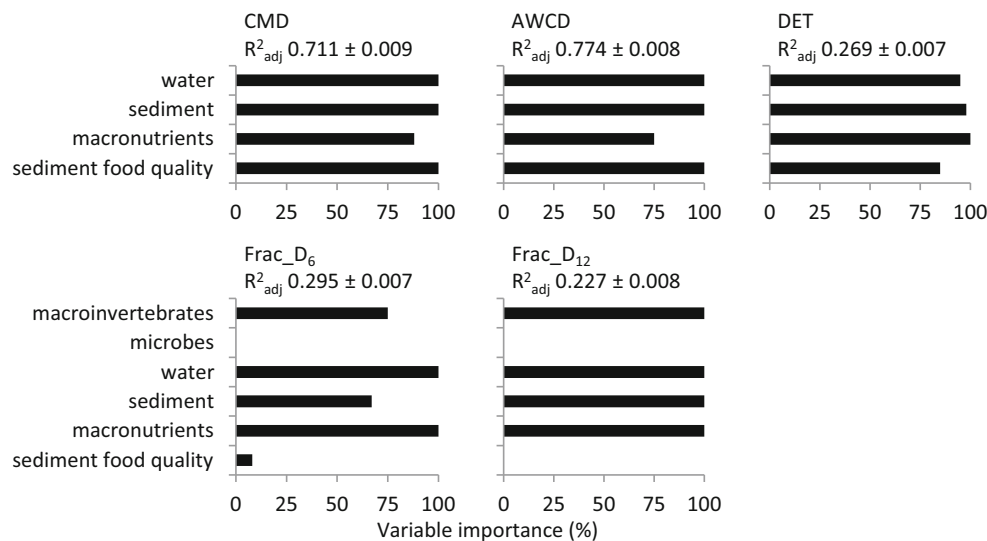


Fig. 2 Presence of predictor variable groups (variable importance (%)) in model ensembles explaining response variables. Response variables: CMD) Community Metabolic Diversity for microbes, AWCD) Average Well Color Development for microbes, DET) fraction detritivores for

macroinvertebrates, Frac_D₆) fraction aboveground litter loss after 6 months of decomposition, Frac_D₁₂) fraction aboveground litter loss after 12 months of decomposition

Decomposition Experiment

The fraction of remaining standing litter biomass decreases over time, with 58 and 35% litter remaining after 6 and 12 months of decomposition compared to starting values, respectively (49–66 and 25–45%, respectively, both 10–90% percentiles, Fig. 1c1 and c2, Table 2), with approximately 0.3% of the standing litter biomass being decomposed daily (see Online Resource 1 for exponential decay constants (k) per basin per period). Variation between basins was relatively small, and comparable with variation within basins after both 6 and 12 months of decomposition. Furthermore, decomposition rates observed after 6 months of decomposition were not correlated to the rates observed after 12 months ($r = -0.076$).

The model ensemble with Frac_D₆ (fraction aboveground litter loss after 6 months) as response variable and all other measured variables (sediment, water, microbes, macroinvertebrates) as potential predictor variables consists of 12 models and has an adjusted R² of 0.295 and a cross-validated R² of 0.223 (Table 2, Online Resource 3e). Water variables are present in all models (Fig. 2), with surface water NO₃ and S present in 100 and 92% of the models (Table 2). Sediment and macroinvertebrate variables are present in 67 and 75% of the models, respectively, while microbe variables are absent in all. Macroinvertebrate variables in the model ensemble comprise the number of individuals, the very small fraction of miners, and the interaction between them. The more dominant fractions of gatherers, shredders and grazers do not show up as predictor variables. Macronutrients are present in all models, with surface water NO₃ present in 100% of the models. Sediment food quality variables are only present in 8% of the models. When predicting Frac_D₁₂ with the model

ensemble from Frac_D₆, almost no variance could be explained ($R^2_{\text{val, t2, with, t1}} = 0.002$, Table 2).

Five models make up the model ensemble with Frac_D₁₂ (fraction aboveground litter loss after 12 months) as response variable, with an adjusted R² of 0.227 and a cross-validated R² of 0.072 (Table 2, Online Resource 3f). Sediment, water and macroinvertebrate variables are all present in each model, while microbe variables are absent (Fig. 2). The macroinvertebrate variable group in the model ensemble is made up solely of grazers. For sediment, pore water S is present in all models. For water, either surface water P or soluble reactive phosphorus is present in all models, with surface water P present in 60%. Macronutrients are present in all models, sediment food quality variables are absent in all models.

Discussion

Using linear models, we aimed to (1.) determine the relative importance of the abiotic conditions (in both the sediment and the water column) on the composition and activity of the decomposer community, and to (2.) determine the combined direct effect of the environmental variables and the resulting decomposer community on decomposition rates of standing litter biomass in newly constructed wetlands. To be able to focus on the direct effects of abiotic conditions on aboveground decomposition rates and separate them from the indirect effects, this study used common reed (*Phragmites australis*) as a single standard substrate. In the model ensembles we have presented here, variables related to the abiotic conditions in water and/or sediment were present in all models, indicating that the abiotic environment is an

important factor influencing decomposition rates of standing litter biomass in newly constructed wetlands. Variables related to the presence of macroinvertebrates were present in most of our models, and are thus also recognized as a driving factor (partly) explaining decomposition rates. These observations corroborate with earlier findings on, for example, the effects of nutrients (Rejmánková and Houdková 2006; Sarneel et al. 2010; Emsens et al. 2016a) and invertebrates (König et al. 2014; Raposeiro et al. 2014) on decomposition rates in established wetland systems. However, sediment and water variables could only explain a small part of variation in the fraction of detritivores. Most likely, macroinvertebrates are still colonizing this newly constructed wetland, therefore their community composition is still variable and heterogeneous in time and space. In most constructed wetlands, especially when constructed basins are directly connected to other water bodies, community composition of macroinvertebrates will be similar to that in wetlands surrounding the area after a couple of years (Christman and Voshell 1993; Stanczak and Keiper 2004; Balcombe et al. 2005), while density and biomass can still show large variation (Stewart and Downing 2008), as was the case in this study. 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. The fact that macroinvertebrates were only identified up to family level instead of species level might partially have led to the low fraction of variance that could be explained with the model ensemble, even though other studies showed that taxonomic resolution at the family level was adequate in determining the effect of environmental factors on macroinvertebrate communities (Bailey et al. 2001; Stewart and Downing 2008).

In contrast to sediment, water and macroinvertebrate variables, the composition of the microbial community was not an adequate predictor of decomposition in any of our models. This result can be explained in two ways; first, it could be that potentially important groups of bacteria involved in decomposition of more complex substrates like lignin and cellulose have been missed in our analysis, because those complex substrates are not present as carbon sources in BIOLOG GN2 plates (Garland and Mills 1991). Moreover, in soils and sediments bacteria interact physically and functionally with fungi in the process of litter degradation (Findlay et al. 2002; Thormann et al. 2003; Fischer et al. 2006; Hervé et al. 2016), specifically targeting the complex compounds using extracellular enzymes (Kirk and Farrell 1987; Thormann 2006). This part of the microbial community is also largely overlooked in the present study since BIOLOG GN2 plates are specifically designed for gram-negative bacteria (Garland and Mills 1991). Second, the microbial community composition and activity were sampled by combining five subsamples per basin, resulting in a sample of the total microbial community present per basin. This sampling procedure is accurate to

determine the influence of sediment and water quality variables on microbial community composition and activity, but probably not to determine the influence of the microbial community composition and activity on decomposition rates of one artificially introduced litter type. On those litter bundles, the microbial communities will most likely have adapted to the conditions present, since microbial communities are very quick in adapting to new conditions (Trinder et al. 2009; Andersen et al. 2010; Flury and Gessner 2011; Weber and Legge 2011), resulting in a different microbial community composition and activity per litter bundle. Even though variables for microbial community composition and activity were absent in the model ensembles for decomposition, we cannot conclude that the microbial community has no influence on decomposition rates, since the microbial community composition and activity were not measured per litter bundle in this study.

Even though the variable groups sediment, water, macro-nutrients and macroinvertebrates were present in all or most of the linear models explaining decomposition rates, our models could only explain a small fraction (30 and 23% after 6 and 12 months of decomposition, respectively) of the variability in decomposition rates for both measurement times. Moreover, the best models were inadequate for extrapolation: the model ensemble using data after 6 months of decomposition could not predict decomposition after 12 months. The poor predictions by our models, and the discrepancies between time points coincide with (sometimes large) variations in the predictor variables but simultaneously small differences in decomposition rates between the different basins. Two studies by Emsens et al. (2016a, b) found that decomposition rates decreased under nutrient rich conditions as compared to nutrient poor conditions, with more uniform decomposition rates between plant species under nutrient rich conditions. Also, decomposition rates were coupled with litter quality indicators under nutrient poor conditions, but under nutrient rich conditions this coupling was absent (Emsens et al. 2016a, b), probably due to a microbial community specialized in decomposition of recalcitrant organic matter (e.g. lignin) under nutrient limiting conditions, and a more opportunistic microbial community under nutrient rich conditions (Moorhead and Sinsabaugh 2006). Since the conditions in all of our basins were nutrient rich, this may have resulted in relatively uniform decomposition rates between basins and accompanying poor predictions by our models, as comparable to the results found by Emsens et al. (2016a, b).

Where in many studies effects of single factors on decomposition rates are quantified experimentally (e.g., Qualls and Richardson 2000; Aerts et al. 2005; Chimney and Pietro 2006; Álvarez and Bécares 2006; Sarneel et al. 2010; Voellm and Tanneberger 2014), our study, including a wide range of variables in a large in situ experiment, later grouped into variable groups, provides scientific insight in the complex interaction between driving factors on decomposition rates. Normally,

one would expect differences in decomposition rates with differing environments, but counterintuitively in newly constructed wetlands these differences are small. However, even though single factors may influence decomposition rates in laboratory studies, we showed that decomposition rates of standing litter biomass in newly constructed wetlands seem to be relatively uniform in time and space, with low explanatory power by variable groups from water, sediment, microbes, macroinvertebrates, macronutrients and sediment food characteristics.

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