A house of cards: Patterns of aquatic invertebrate diversity in agricultural ditches
Whatley, M.H.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
Elodea nuttallii
Chapter 2

Macrophyte loss drives decadal change in benthic invertebrates in peatland drainage ditches

Abstract

Agricultural peatlands and their associated drainage systems are often highly managed and exposed to anthropogenic pressures, such as eutrophication and stable water tables, maintained via drainage during periods of high rainfall and inlet of, alkaline rich, waters during dry periods. These pressures promote peat degradation, resulting in the accumulation of fine degraded peat particles which dramatically alter aquatic habitats by smothering surfaces and decreasing water quality. Consequential effects on benthic communities are expected but have not been investigated so far. We hypothesized that peat degradation can lead to the decline of submerged macrophytes, which are of critical importance to sustaining biodiversity of benthic invertebrate communities. To investigate this we analysed decadal (1985 – 2007) changes in benthic species richness in 29 peat ditches in The Netherlands and, to determine patterns of macroinvertebrate habitat occupancy, carried out a complementary field experiment with submerged artificial macrophytes, natural sediments and emergent bank vegetation. Results from long-term monitoring indicate that chemical conditions in agricultural peat ditches have improved slightly over the last decades; however there has been a simultaneous decline in benthic invertebrate species richness and densities corresponding to a decline in the numbers of submerged macrophytes. The apparent dependence of macroinvertebrates on macrophytes was reinforced by our field experiment which revealed that invertebrate density was highest in submerged artificial plants, while invertebrate species richness was highest in natural emergent vegetation. Conversely, degraded peat sediments supported extremely few invertebrates. Our results clearly illustrate the strong influence of submerged macrophyte loss on macroinvertebrate assemblages in peatland waters. Furthermore, this suggests that improvements in water quality alone will not benefit invertebrates in the absence of suitable vegetative habitats.

Introduction

Peatlands are valuable ecosystems, recognised for their natural, social and economic resources, but these habitats have been greatly reduced. Centuries of human induced modifications including peat extraction, intensive agriculture and nutrient inputs have resulted in the degradation of peatlands. It has been estimated that peatlands once covered approximately 24% of The Netherlands (850AD), while today they account for less than 2% of its area (van Eerden et al. 2010). All natural peat ecosystems have been lost and those remaining have been drained for agriculture and are, to some extent, degraded (van Dam 2001; Lamers et al. 2002; van Eerden et al. 2010).

Agricultural peatlands are often intersected by networks of drainage ditches, which can provide valuable habitat for aquatic organisms (Painter 1999;
Armitage et al. 2003; Herzon & Helenius 2008; Verdonschot et al. 2011). Smaller ditches (< 20 m wide) in particular, being the most numerous and spatially heterogeneous aquatic habitats in these peatlands, facilitate the occurrence of a wide range of benthic invertebrate species. For example (Williams et al. 2003) noted that, despite having low local invertebrate species richness, the ditches supported more rare species than rivers and streams. The communities of small ditches, however, are often exposed to eutrophication, due to the leaching of nutrients from the adjacent agricultural soils (Janse & van Puijenbroek 1998) and changes in water quality prompted by hydrological management (Roelofs 1991; Lamers et al. 2002; Smolders et al. 2006).

Water tables in Dutch peatland areas are maintained within strict limits by pumping excess water out during wet periods and the inlet of external waters, originating from the River Rhine via Lake IJsselmeer, during dry periods. Inlet waters have higher concentrations of sulphate and carbonate than area specific peatland waters. This can increase alkalinity of surface waters and cause degradation of peat soils and subsequent release of fine organic particles into the aquatic environment (Roelofs, 1991). Abiotic conditions in the resulting sludge layer cause the release of nutrients from organic material previously conserved in peat soils; i.e. internal eutrophication (Fig. 2.1). Subsequent algae blooms and growth of floating species (Lemma and Azolla), coupled with the aforementioned sludge accumulation, increase turbidity and light attenuation in ditches, consequently submerged macrophyte coverage declines dramatically (Wood & Armitage 1997; Lamers et al. 2002; Harrison et al. 2007; Verberk et al. 2007). Changes in water quality including pH, dissolved oxygen, alkalinity and macro-ions (e.g. Cl\(^{-}\), K\(^{+}\), Mg\(^{2+}\) and Ca\(^{2+}\)), can strongly influence invertebrate assemblages (van der Hammen 1992; Verbruggen et al. 2011), while nutrient enrichment predominantly influences invertebrates indirectly via a reduction in dissolved oxygen and loss of macrophyte habitat (Verbruggen et al., 2011).

![Fig. 2.1. Relationship between physiochemical and biological components in agricultural peatland ditches under degradation. Schematic adapted from Verberk et al. (2007).](image-url)
Submerged macrophytes provide essential habitat for macro-invertebrates (Scheffer et al. 1984; Kovalenko et al. 2010), since they offer protection from fish predation (Goyke & Hershey 1992) and provide a range of food sources (Underwood & Thomas 1990; Newman 1991), therefore macrophyte decline can negatively impact invertebrate assemblages. Although it is known that invertebrate communities are influenced by deterioration in water quality the impact of submerged macrophyte loss resulting from peat degradation has not been widely studied.

We tested the role of macrophytes in driving benthic invertebrate community composition in North Holland peatland drainage ditches. To investigate the relationship between submerged macrophyte communities and benthic invertebrates we reviewed long-term trends in benthic biodiversity and abiotic characteristics from regional monitoring data. In support of this analysis, a field experiment was run in agricultural peatland ditches in which we sampled macroinvertebrates from natural and artificial substrata (macrophytes) to determine patterns of invertebrate occupancy in different habitats. We expect to observe a strong influence of habitat structure on invertebrates and a higher species richness and density in vegetated habitats (both natural and artificial) compared to bare sediments.

Methods

Long-term trends in benthic richness – regional monitoring data

We investigated temporal trends in water quality, submerged macrophytes and macroinvertebrates by reviewing monitoring data collected over 22 years (1985 to 2007) by the North Holland Water Authority, Hoogheemraadschap Hollands Noorderkwartier (HHNK). All monitoring sites were ditches (width < 16 m, water depth < 1.2 m) situated in peat areas. A complete overview of the HHNK monitoring data covering this period and sample collection details can be found in van Dam (2009).

Monitoring data, including benthic invertebrates, submerged macrophytes and abiotic variables were reviewed. The majority of monitoring locations were visited at least 3 times during the 22 year period with some locations sampled more than others (Fig. 2.2a). Observations for invertebrates were available for more locations than for macrophytes while abiotic variables (total nitrogen (mg L\(^{-1}\)), total phosphorus (mg L\(^{-1}\)), chloride (mg L\(^{-1}\)), chlorophyll-\(a\) (\(\mu\)g L\(^{-1}\)), total sulphate (SO\(_4^{2-}\), mg L\(^{-1}\)) and water transparency (cm)) were available for all locations but not during all time periods.

The invertebrate dataset comprised of 198 samples derived from 29 locations and 17 sampling years between 1985 and 2007. Macroinvertebrates (adult body length > 1 mm) were sampled in either spring or late summer/autumn and were identified to species, with the exception of worms.
(Oligochaeta) which were not identified beyond this subclass and were included only for density analysis. Samples were collected with a dip-net using a multi-habitat sampling technique whereby different habitats (i.e. submerged macrophytes, sediments, open water and emergent vegetation) were subsampled and combined to make one sample. The combined sampled length of the ditch covered approximately 5 m, equivalent to an area of 1.5 m² within a 100 m section. Invertebrate abundance was standardized to density (individuals m⁻²). The macrophyte dataset consisted of 72 samples derived from 22 locations and 11 sampling years between 1990 and 2007. Macrophyte species were sampled over a 50 m transect covering the width of the entire ditch in late summer/autumn and included all floating and submerged aquatic plants.

Invertebrate habitat occupancy – 2010 field experiment

To test the responses of macroinvertebrates to the presence of submerged macrophytes, artificial substrata were deployed within the Wormer and Jisperveld (52°30’N, 4°50’E), The Netherlands (Fig. 2.2b). The Wormer and Jisperveld is a low-lying peat meadow of approximately 2500 ha, comprising low intensity agricultural land intersected by drainage ditches (Janssen et al. 2005). Submerged macrophytes have completely disappeared due to rapid peat degradation and accumulation of extremely fine (< 200 μm) peat particles, which has created a thick layer of amorphous mud and very turbid conditions.

Fig. 2.2. Map showing the position of HHNK monitoring locations and 2010 sampling locations. The small insert map indicates the position of the sample locations within The Netherlands. a) Monitoring locations were sampled from 1985 to 2007; symbol size represents the number of samples collected for invertebrates (triangles) and macrophytes (circles) at each location. b) Position of the three ditches in the Wormer and Jisperveld (North Holland, the Netherlands) where artificial plants were placed and dip-net sampling undertaken in 2010. Image adapted from Janssen et al. (2005).

Sampling was undertaken over a three week period from late July to early August 2010. Three separate ditches with a similar morphology (width <
17 m, water depth < 1 m) were selected for invertebrate sampling and water quality measurements (Fig. 2.2b). Morphological parameters were measured in situ and, due to the highly mobile nature of the degraded peat material, sediment (degraded peat sediments) and water depths were averaged from 18 measurements taken in each ditch.

Samples of undisturbed overlying water were analysed three times over the sampling period. Measurements of conductivity, turbidity, dissolved oxygen (DO, measured between 10 am – 2 pm, 5 cm below the surface and at the sediment-water interface), and pH were taken in the field. Conductivity was measured with a WTW LF 92 meter and Tetracon 96 cell (Weilheim, Germany) and oxygen (DO) and pH were measured with a WTW pH/Oxi 340i/set meter (Weilheim, Germany). Turbidity was measured with a WTW TURB 350 IR meter (Weilheim, Germany) and subsequently converted to water transparency (cm) using the conversion table developed by Kevin Fermanich (University of Wisconsin 2010). Analysis of nitrogen (TN), phosphorus (TP and PO₄³⁻), sulphate (SO₄²⁻), total iron (Fe), carbonate (CO₃²⁻), chloride (Cl⁻) and chlorophyll-a were carried out in the laboratory using standardised national protocols accredited by the Netherlands Standards Institute (NEN).

To determine patterns of invertebrate habitat occupancy, macroinvertebrates (adult body length > 1 mm) were sampled from three different habitats within each ditch; emergent bank-side vegetation, bare sediments and submerged artificial plants. From this point forward the habitats will be referred to as Bank, Sediment and Plastic Plant, respectively. Bank vegetation was dominated by reed species characteristic of ditches in the region (*Phragmites australis* and *Typha angustifolia*).

![Fig. 2.3. Individual components (left) and the assemblage of the artificial substrata (right). Substrata consisted of a Plastic Plant mounted on a non-buoyant plastic base, placed on plant fibre (Hessian cloth) to simulate root structure and housed inside a galvanized steel cage.](image)

Artificial substrata, consisting of a Plastic Plant (resembling the common water plant hornwort, *Ceratophyllum demersum*) mounted on a non-
buoyant plastic base and placed on plant fibre (Hessian cloth) to simulate root mass, were housed in galvanized steel cages (mesh 1 cm, base 15 x 15 cm, height 25 cm) (Fig. 2.3). In each ditch four cages were deployed adjacent to the bank and approximately 5 cm above the sediment-water interface (Fig. 2.4). Substrata were left in the field for 56 days prior to sampling to allow time for colonization (Wise & Molles 1979; Higler & Verdonschot 1989).

Invertebrates were collected over a 3 week period from late July to early August, 2010. This sampling period is in accordance with current and long-term monitoring protocols (Bijkerk 2010). Bank and Sediment samples were collected with a dip-net (mesh, frame, bag depth; 900 μm, 25 x 29 cm, 25 cm) by sweeping the net continuously along a 5 m transect. This sampling method was adopted to maintain distinct samples for each habitat and is comparable to standard monitoring practices in The Netherlands. Plastic Plants were sampled by placing a dip-net under the substrata before removing them from the water, to reduce invertebrate loss. Invertebrate density from Plastic Plant substrata were standardized assuming a 5% macrophyte coverage area, equivalent to the median macrophyte coverage recorded during the early monitoring period (1990 – 1991).

Fig. 2.4. Ditch sampling scheme for 2010 macroinvertebrate field experiment, showing the positioning of Plastic Plants and where Bank and Sediment samples were collected with a dip-net along a continuous 5 m length taken parallel to the shoreline.

Four replicate invertebrate samples were taken in each habitat type (Bank, Sediment and Plastic Plants) in each ditch (Fig. 2.4), giving a total of 12 samples per ditch (with the exception of ditch 3 for which one Sediment sample was lost). Invertebrate samples were taken back to the laboratory and sorted live and subsequently preserved in 70% ethanol for later determination. Where possible, invertebrates were identified to species, with the exception of Chironomid larvae (Diptera), which were identified to subfamily. The following taxonomic groups were identified with the corresponding keys: Ephemeroptera
(Elliott & Humpesch 2010), Heteroptera (Savage 1989), case-bearing Trichoptera (Wallace et al. 2003), caseless Trichoptera (Edington & Hildrew 1995), Hirudinea (Elliott & Mann 1979), Gastropoda (Macan 1977), Coleoptera and Odonata (Nilsson 2005) and Diptera (Nilsson 2005). Amphipoda, Isopoda and Mysida were each represented by a single common species, and Bivalvia were grouped by genus. Mites (Arachnida) and worms (Oligochaeta) were not identified to species but abundance was recorded for density calculations.

Statistical Analysis

Long-term monitoring data (1985 – 2007) were collected repeatedly from 29 locations. Due to the monitoring design, sequential observations of abiotic variables, invertebrates and macrophytes taken from the same location were not statistically independent from one another. For this reason Generalized Estimating Equations (GEE) were used to analyse the long-term trends and correlations between variables (Liang & Zeger 1986). The basic model was defined with sampling location as the clustering variable, season (in case of invertebrates) as the predictor variable, location as the factor and either time (year), macrophyte species richness or abiotic variables as covariables.

Changes in invertebrate species richness over time were investigated at the local scale ($\alpha$ diversity), equivalent to the average species richness per sample, the regional scale ($\gamma$ diversity), equivalent to the regional species pool over a two year period, and by calculating an additive model of beta diversity ($\beta_{Add}$), equivalent to $\gamma - \alpha$, to determine the variation among communities during the monitoring period (Lande 1996). In this study we used additive $\beta_{Add}$ diversity because it had the advantage of being in the same units as $\alpha$ and $\gamma$ diversity and, thus, straightforward comparisons between these different measures of diversity were possible (Lande 1996; Anderson et al. 2011).

GEE model predictions were plotted with observed species richness (i.e. $\alpha$ diversity) of macrophytes and invertebrates over time to determine temporal trends and between invertebrate richness against macrophyte richness to ascertain the relationship between these two groups.
The predictions were calculated by the following equations:

\[ y_j = \beta_{0,j} + \beta_1 x \]  

\[ \beta_{0,j} = u_j + \beta_0 \]

where: 
- \( y \) = species richness at location \( j \); 
- \( \beta_{0,j} \) = model intercept for location \( j \); 
- \( \beta_0 \) = component of model intercept which is independent of location; 
- \( u_j \) = location-specific component of intercept; 
- \( \beta_1 \) = slope of \( x \); 
- \( x \) = value of the covariable, either year, macrophyte species richness or abiotic variable;

In this study we will report the form of this model which is aggregated over the different locations:

\[ y = \beta_0 + \beta_1 x \]  

\[ \beta_0 = \sum_j \left( \frac{n_j \beta_{0,j}}{N} \right) \]

where the summation is a weighted average using the relative number of observations per location \( (n_j/N) \) to determine the relative importance of each intercept \( \beta_{0,j} \). The general form of a GEE (Equation 2) can then be used to predict at locations different from those where measurements were collected. By doing so, the term \( u_j \) disappears from the model and the variance of the values \( u_j \) adds to the model error.

Models run on invertebrate species richness and macrophyte richness were tested against all covariables, while invertebrate densities (individuals m\(^{-2}\)) were analysed over time. We applied an autoregressive correlation structure to our model to correct for correlations between observations from the same location in close temporal succession to one another (Quinn & Keough 2002). The distributions of species richness and abiotic variables were normal with an identity link function, i.e. the dependent variable was not transformed within the GEE model. Densities of dominant invertebrate taxa displayed skewed residual distributions, therefore a gamma distribution with a log link function was applied in the GEE model to analyse temporal trends of individual density. GEE models were run in IBM SPSS Statistics (v. 20).

Data collected during the 2010 field experiment were analysed to compare species richness and densities of benthic invertebrates between the three ditch habitats. Invertebrate abundances were converted to density (individuals m\(^{-2}\)). As two different sampling methods were used during the field study (artificial substrata and net samples of naturally occurring substrata)
species richness was rarefied against invertebrate abundance to standardise samples (Gotelli & Colwell 2001) using EcoSim version 7.72 (Gotelli & Entsminger 2011). Since the three different habitats were located within each ditch, nested-ANOVAs (habitat nested within ditch) were run in IBM SPSS Statistics (v. 20) to test for significant differences in species richness and invertebrate density between ditches and habitats. In the case of a significant test result, a Tukey HSD post hoc test was run.

Normality of both the monitoring and 2010 field experiment data were checked with a Shapiro-Wilk test and QQ-plots were used to assess homogeneity of variances. If these assumptions were not met data were log$_{10}$ transformed or, in the case of density data, log$_{10}$ ($x + 1$) transformed prior to statistical analysis.

![Fig. 2.5. Overview of invertebrate species richness during the monitoring period (1985 – 2007). Three diversity indices were derived: $\gamma$ = regional diversity, i.e. total number of species collected over two years; $\alpha$ = local diversity, i.e. mean species richness of a single sample (± 95% CI); $\beta_{Add}$ = $\gamma$ - $\alpha$, i.e. difference in species richness between regional and local scales. Grey bars indicate the number of samples collected within each time period. No diversity indices were calculated for 1987 – 1989 since only one sample per year was available for this period.

\textbf{Results}

\textit{Long-term trends – regional monitoring data}

There was a significant reduction in total nitrogen ($\beta_1 = -0.006$, S.E. = 0.002, $P = 0.005$, $r^2 = 0.97$, $n = 98$), total phosphorus ($\beta_1 = -0.011$, S.E. = 0.005, $P = 0.038$, $r^2 = 0.75$, $n = 193$), total sulphate ($\beta_1 = -0.015$, S.E. = 0.002, $P < 0.001$, $r^2 = 0.71$, $n = 181$) and chloride ($\beta_1 = -0.008$, S.E. = 0.002, $P < 0.001$, $r^2 = 0.76$, $n = 193$) in surface waters during the monitoring period. Although water transparency declined over time ($\beta_1 = -1.83$, S.E. = 0.77, $P = 0.018$, $r^2 = 0.81$, $n$
Transparency was not significantly related to surface water chlorophyll-
$\alpha$ concentration, due in part to temporal variation, but was negatively correlated
with total phosphorus ($\beta_1 = -26.68, \text{S.E.} = 11.73$, $P = 0.023, r^2 = 0.80, n = 112$). Total sulphate covaried with several other variables, chlorophyll-$\alpha$ ($\beta_1 = -0.397, \\
\text{S.E.} = 0.189, P = 0.036, r^2 = 98, n = 88$) total nitrogen ($\beta_1 = -0.20, \text{S.E.} = \\
0.090, P = 0.026, r^2 = 98, n = 88$), chloride ($\beta_1 = 0.599, \text{S.E.} = 0.083, P < \\
0.001, r^2 = 0.63, n = 181$) and transparency ($\beta_1 = 73.38, \text{S.E.} = 20.18, P < \\
0.001, r^2 = 0.86, n = 112$). Further interactions between abiotic parameters
relating to seasonal dynamics and complex non-linear relationships were also
probable but such analysis was beyond the scope of this study.

All measures of macroinvertebrate diversity ($\alpha$, $\gamma$ and $\beta_{\text{Add}}$) decreased
over time and $\beta_{\text{Add}}$ diversity was positively affected by the number of samples
collected in each time period (Fig. 2.5). Therefore, $\alpha$ diversity (species richness
from this point forward) was considered to be a representative proxy of
invertebrate diversity within the monitoring dataset.

Between 1990 and 2007 macrophyte species richness declined
significantly in 83% of monitoring locations (GEE, $\beta_1 = -0.38, \text{S.E.} = 0.06, P < \\
0.001, r^2 = 0.68, n = 72$) with an overall loss of eight macrophyte species during
this period (Fig. 2.6a). Macrophyte species richness was not related to nutrients,
however it was negatively correlated with chlorophyll-$\alpha$ concentration (GEE, $\beta_1$
$= -31.71, \text{S.E.} = 15.21, P = 0.023, r^2 = 0.82, n = 33$) and positively correlated
with water transparency, although this was on the boundaries of statistical
significance (GEE, $\beta_1 = 0.15, \text{S.E.} = 0.08, P = 0.050, r^2 = 0.84, n = 31$).

Invertebrate species richness declined simultaneously with macrophyte
richness, a significant decline was observed in 79% of monitoring locations
between 1985 and 2007 (GEE, $\beta_1 = -0.014, \text{S.E.} = 0.002, P < 0.001, r^2 = 0.43, \\
n = 198$), with an overall loss of 43 species over this period (Fig. 2.6b). Invertebrate species richness was not affected by sampling season or any
measured abiotic parameter but was significantly positively correlated with
macrophyte richness (GEE, $\beta_1 = 0.024, \text{S.E.} = 0.006, P < 0.001, r^2 = 0.35, n = \\
72$) (Fig. 2.7).
Fig. 2.6. Species richness of a) macrophytes and b) invertebrates plotted over time. Grey dots represent field observations and the black line is the GEE prediction, calculated from the model formula. Dashed lines show 95% confidence belts for the GEE prediction.

Fig. 2.7. Invertebrate species richness plotted against macrophyte species richness. Grey dots represent observations in the field and the black line is the GEE prediction, calculated from the model formula. Dashed lines show 95% confidence belts for the GEE prediction.
Table 2.1. Median densities (No. m^{-2}) at the beginning and end of the 22 year monitoring period for dominant invertebrate taxa in each order with at least 24 observations or 12% of all samples and Generalized Estimating Equation model results of densities against time. Ranges in density of each taxon are given in parentheses. Values were calculated for 2006 because only one observation was available for 2007. Taxa are listed in order of least to most number of observations, N/A – too few observations for statistical analysis, n.s. – not significant.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Order</th>
<th>Median density (range)</th>
<th>GEE model output</th>
<th>No. Obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oecetis furva</td>
<td>Trichoptera</td>
<td>7 (2 - 11) N/A</td>
<td>β: -0.07 S.E.: 0.01 P: &lt; 0.001 r^2: 0.62</td>
<td>24</td>
</tr>
<tr>
<td>Ischnura eugans</td>
<td>Odonata</td>
<td>7 (3 - 10) N/A</td>
<td>β: -0.07 S.E.: 0.02 P: &lt; 0.001 r^2: 0.39</td>
<td>43</td>
</tr>
<tr>
<td>Haliphus ruficolli</td>
<td>Coleoptera</td>
<td>18 (17 - 18) N/A</td>
<td>β: -0.05 S.E.: 0.04 P: n.s. r^2: n.s.</td>
<td>45</td>
</tr>
<tr>
<td>Sidae lataa</td>
<td>Megaloptera</td>
<td>4 (1 - 41) 1 (1 - 3)</td>
<td>β: -0.04 S.E.: 0.02 P: n.s. r^2: n.s.</td>
<td>55</td>
</tr>
<tr>
<td>Neomysis integra</td>
<td>Mysida</td>
<td>2 (1 - 70) N/A</td>
<td>β: -0.09 S.E.: 0.03 P: 0.009 r^2: 0.43</td>
<td>65</td>
</tr>
<tr>
<td>Oligochaeta</td>
<td>Oligochaeta</td>
<td>120 (17 - 84) 11 (5 - 16)</td>
<td>β: -0.08 S.E.: 0.04 P: 0.043 r^2: 0.55</td>
<td>100</td>
</tr>
<tr>
<td>Helobdella stagnalis</td>
<td>Hirudinea</td>
<td>15 (12 - 18) 4 (1 - 15)</td>
<td>β: 0.05 S.E.: 0.02 P: 0.008 r^2: 0.30</td>
<td>107</td>
</tr>
<tr>
<td>Planorbi planorbi</td>
<td>Gastropoda</td>
<td>15 (38 - 50) 12 (5 - 43)</td>
<td>β: -0.04 S.E.: 0.02 P: n.s. r^2: n.s.</td>
<td>108</td>
</tr>
<tr>
<td>Caenis sp.</td>
<td>Ephemeroptera</td>
<td>8 (1 - 25) 1 (1 - 23)</td>
<td>β: -0.01 S.E.: 0.03 P: n.s. r^2: n.s.</td>
<td>112</td>
</tr>
<tr>
<td>Asellus sp.</td>
<td>Isopoda</td>
<td>4 (3 - 4) 1 (1 - 5)</td>
<td>β: -0.02 S.E.: 0.02 P: n.s. r^2: n.s.</td>
<td>125</td>
</tr>
<tr>
<td>Chironomus sp.</td>
<td>Diptera</td>
<td>207 (1 - 300) 3 (1 - 14)</td>
<td>β: -0.11 S.E.: 0.02 P: &lt; 0.001 r^2: 0.28</td>
<td>145</td>
</tr>
<tr>
<td>Gammarus tigrinus</td>
<td>Amphipoda</td>
<td>8 (2 - 62) 6 (1 - 13)</td>
<td>β: 0.02 S.E.: 0.02 P: n.s. r^2: n.s.</td>
<td>154</td>
</tr>
<tr>
<td>Arrenurus sp.</td>
<td>Arachnida</td>
<td>4 (1 - 319) 3 (1 - 9)</td>
<td>β: -0.08 S.E.: 0.03 P: 0.002 r^2: 0.18</td>
<td>163</td>
</tr>
<tr>
<td>Sigara sp.</td>
<td>Heteroptera</td>
<td>62 (10 - 70) 2 (1 - 22)</td>
<td>β: -0.06 S.E.: 0.02 P: 0.002 r^2: 0.32</td>
<td>165</td>
</tr>
<tr>
<td>All taxa</td>
<td></td>
<td>1412 (1284 - 2084) 114 (33 - 179)</td>
<td>β: -0.03 S.E.: 0.01 P: &lt; 0.001 r^2: 0.39</td>
<td>198</td>
</tr>
</tbody>
</table>
Total invertebrate density (number of individuals m\(^{-2}\)) declined significantly over time. Changes in the median densities and the ranges of the dominant taxa in each order are shown alongside GEE model outputs reflecting temporal trends in the densities of taxa (Table 2.1). The majority of taxonomic groups declined significantly, including worms (subclass: Oligochaeta), Arrenurus sp. (Arachnida), Oecetis furva (Trichoptera), Ischnura elegans (Odonata) and Sigara sp. (Heteroptera). However, the greatest rate of decline was observed in nonbiting midges Chironomus sp. (Diptera) followed closely by the crustacean Neomysis integer (Mysida). The predatory leech Helobdella stagnalis (Family: Glossiphoniidae) was the only species to increase significantly in density over the 22 year monitoring period.

Invertebrate habitat occupancy – 2010 field experiment

The three ditches in the Wormer and Jisperveld were comparable in salinity (chloride), water transparency, pH and nutrients (Table 2.2). Furthermore, all ditches had a steep oxygen gradient, with surface water DO ranging between 12.9 – 4.6 mg L\(^{-1}\), while DO at the sediment-water interface ranged between 0.1 – 0.6 mg L\(^{-1}\). Ditch 1 had the highest concentrations of TN and TP and chlorophyll-\(a\), although these differences were not significant.

*Fig. 2.8. Non-rarefied species richness (Non-rar.) and cumulative species richness of habitats rarefied against 20 (Rar. 20) and 100 individuals (Rar. 100). The results were pooled from 12 samples of each habitat over the three ditches (with the exception of the habitat Sediment which comprised of 11 samples). The rarefaction process was computed repeatedly 1000 times. Error bars represent ± 95% CI.*
Table 2.2. Morphological and chemical characteristics of the three ditches sampled in the Wormer and Jisperveld in 2010. All values are calculated from the mean of three measurements (unless otherwise noted) ± 95% CI.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Ditch 1</th>
<th>Ditch 2</th>
<th>Ditch 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ditch width (m)</td>
<td>7 ± 3</td>
<td>10 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Water depth (cm)</td>
<td>30.3 ± 4.8</td>
<td>23.0 ± 1.8</td>
<td>63.0 ± 9.9</td>
</tr>
<tr>
<td>Sediment depth (cm)</td>
<td>14.8 ± 4.1</td>
<td>16.5 ± 2.5</td>
<td>30.6 ± 4.1</td>
</tr>
<tr>
<td>DO 5cm water depth (mg L⁻1)</td>
<td>9.3 ± 2.6</td>
<td>10.1 ± 2.8</td>
<td>5.8 ± 1.2</td>
</tr>
<tr>
<td>DO SWI (mg L⁻1)</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>38 ± 4</td>
<td>29 ± 7</td>
<td>45 ± 14</td>
</tr>
<tr>
<td>Transparency (cm)</td>
<td>22 ± 2</td>
<td>28 ± 5</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>Chlorophyll-α (µg L⁻¹)</td>
<td>147 ± 160</td>
<td>24 ± 7</td>
<td>91 ± 68</td>
</tr>
<tr>
<td>pH</td>
<td>8.7 ± 0.9</td>
<td>8.8 ± 1.4</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹)</td>
<td>814 ± 123</td>
<td>1061 ± 107</td>
<td>1014 ± 35</td>
</tr>
<tr>
<td>Chloride (mg Cl⁻ L⁻¹)</td>
<td>140 ± 30</td>
<td>177 ± 31</td>
<td>170 ± 23</td>
</tr>
<tr>
<td>Carbonate (mg CO₃²⁻ L⁻¹)</td>
<td>157 ± 45</td>
<td>200 ± 30</td>
<td>190 ± 20</td>
</tr>
<tr>
<td>Sulphate (mg SO₄²⁻ L⁻¹)</td>
<td>50.0 ± 8.6</td>
<td>85.3 ± 4.3</td>
<td>83.7 ± 5.4</td>
</tr>
<tr>
<td>Total Iron (µg Fe L⁻¹)</td>
<td>183 ± 17</td>
<td>123 ± 69</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>Total P (mg P L⁻¹)</td>
<td>0.35 ± 0.12</td>
<td>0.17 ± 0.08</td>
<td>0.2 ± 0.07</td>
</tr>
<tr>
<td>Orthophosphate (mg P L⁻¹)</td>
<td>N/A</td>
<td>0.03 ± 0.02</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Total N (mg N L⁻¹)</td>
<td>5.3 ± 1.5</td>
<td>3.5 ± 0.5</td>
<td>3.2 ± 0.7</td>
</tr>
</tbody>
</table>

*Averaged from 18 measurements per ditch

**Dissolved Oxygen (DO)**

*Sediment-Water Interface (SWI)

*Converted from turbidity according to University of Wisconsin (2010)

The 2010 field experiment revealed that non-rarefied invertebrate species richness was significantly higher in Plastic Plants compared to Sediment samples (nested ANOVA, $F_{(5,3)} = 34, P < 0.001$). However, the greatest number of species were recorded in Bank samples, which were significantly higher than Plastic Plants, rarefied against 200 individuals (nested ANOVA, $F_{(5,3)} = 49, P < 0.001$) and Sediment samples, non-rarefied data (nested ANOVA, $F_{(5,3)} = 54, P < 0.001$) (Fig. 2.8).

The majority of species found in Plastic Plants (non-rarefied) were from the taxonomic groups Gastropoda, Hirudinea and Trichoptera (6 – 10
sp.), while emergent Bank vegetation had the highest number of species in the Coleoptera, Diptera and Gastropoda (11 – 15 sp.) followed by Hirudinea, Heteroptera and Trichoptera (6 – 10 sp.). The number of species was low in all taxonomic groups in Sediments (1 – 5 sp.) (Fig. 2.9).

Total invertebrate density (individuals m$^{-2}$) differed significantly between habitats (nested ANOVA, $F_{(8,6)} = 196, P < 0.001$), being highest in Plastic Plants (mean density ± 95% CI, 816 ± 171), intermediate in Bank vegetation (303 ± 100) and lowest in Sediments (34 ± 11). In Plastic Plants the most numerous taxa were *G. tigrinus* (Amphipoda) and *Chironomus* larvae (Diptera) (> 100 m$^{-2}$), followed by *S. lutaria* (Megaloptera) (51 – 100 m$^{-2}$) then *Asellus aquaticus* (Isopoda), *Bithynia tentaculata* (Gastropoda), *H. stagnalis* (Hirudinea), *Caenis sp.* (Ephemeroptera), *Cyrnus flavidus* (Trichoptera) and Oligochaeta (21 – 50 m$^{-2}$). Emergent Bank vegetation supported the highest density of *N. integer* (Mysida) (< 100 m$^{-2}$) followed by *G. tigrinus* (Amphipoda) and Chironomidae larvae (Diptera) (51 – 100 m$^{-2}$) and, finally, *Ischnura elegans* (Odonata) (21 – 50 m$^{-2}$) (Fig. 2.9). Invertebrate density was exceptionally low in sediments, with over 50% of all taxonomic groups present at a density < 1 m$^{-2}$ and predominantly consisted of the most common taxa, namely *Chironomus* larvae, *G. tigrinus* and *N. integer*.

**Discussion**

The aim of this study was to determine the role of submerged macrophyte habitats in driving benthic macroinvertebrate community composition in agricultural peatland waters. The combined analysis of long-term monitoring data and the complementary 2010 field experiment data draws attention to the strong relationship between submerged macrophytes and invertebrate species richness. Over the 22 year monitoring period invertebrate and macrophyte species richness declined synchronously. Moreover, the prominent colonization of artificial submerged macrophytes (Plastic Plants) within two months after placement in the field experiment demonstrated the rapid response of invertebrate communities to macrophyte habitat availability. Invertebrate densities recorded in Plastic Plant substrata were similar to natural densities recorded at the beginning of the HHNK monitoring period (1985), before macrophytes had been lost from these peat ditches. These changes were highlighted by a shift from clear water conditions, in which a range of macrophyte species were present, to a turbid state characterised by high concentrations of suspended particles and dominated by algae. The driver for this shift was probably long-term degradation of peat soils prompted by oxygenation and the inlet of sulphate rich alkaline waters, causing subsequent particle accumulation in the aquatic environment (Roelofs 1991; Lamers *et al.* 2002).
Physicochemical processes underlying declining benthic species richness in North Holland peatlands

In recent years, measures have been taken to reduce nutrient inputs into North Holland peatland ditches although, while TN, TP and SO$_4^{2-}$ concentrations have declined over time, concentrations remain high and the majority of waters are still eutrophic to hypertrophic. The effects of reducing external nutrient loadings to surface waters may have been masked by nutrient recycling in these historically enriched peatlands. Under anoxic conditions, microbial breakdown of organic material and the further release of nutrients is exacerbated by high concentrations of ions like sulphate and nitrate which can act as electron acceptors (Jones 1979; Caraco, Cole & Likens 1989), promoting bacterial degradation of peat. Sulphate reduced to sulphide can bind to reduced iron, generating iron sulphides (FeS and FeS$_2$) (Holmer & Storkholm 2001). This chemical competition for iron causes iron bound phosphates (Fe-PO$_4$) to be released into the water column (Smolders et al. 2006). Roelofs (1991) demonstrated that this process was further accelerated in Dutch peatlands under alkaline conditions, causing a positive feedback of continued peat degradation and high nutrient availability in ditches.

The monitoring data indicates that high nutrient concentrations have persisted for several decades, coincident with a reduction in water transparency in North Holland’s agricultural peatlands, compromising submerged macrophyte growth and favouring algae and floating macrophytes (such as Lemna sp.) (Barko et al. 1991; Søndergaard et al. 2003; Geurts et al. 2009). The
loss of submerged macrophytes in these shallow, soft sediment ditches can lead to the establishment of an alternative stable state via mechanisms similar to those found in productive shallow lakes (Scheffer et al. 1993) characterized by turbid conditions under which light attenuation increases, retarding the growth of submerged vegetation (Scheffer 1990). The decline in water clarity due to fine sediments combined with high nutrient availability initiates macrophyte decline while growth of algal and floating plants, alongside unstable sediments, constrains macrophyte re-establishment (Janse & van Puijenbroek 1998; Schutten et al. 2005).

Consequences of submerged macrophyte loss for benthic invertebrates

The importance of macrophyte habitat structure is well established and, in line with our study, has been identified previously as an important driver of the spatial distribution of invertebrates within ditches (Scheffer et al. 1984). Macrophytes provide habitats required for various invertebrate life stages e.g. oviposition and emergence (McLaughlin & Harris 1990; Orr & Resh 1992), and hence their structure and complexity are central to promoting invertebrate diversity and overall density (Jeffries; Higler & Verdonschot 1989; Lucena-Moya & Duggan 2011). For example, Kovalenko et al. (2010) and Hansen et al. (2011) reported a positive relationship between macrophyte complexity and invertebrate abundance. In a similar study conducted in eutrophic agricultural ditches, Hinojosa-Garro et al. (2010) found that increased vegetation complexity supported more invertebrate species and higher densities of predators (Coleoptera, Hemiptera and Odonata) and grazers (Gastropoda). Comparable results were obtained in our field study in which Plastic Plants also supported higher densities of grazing snails and the predatory alderfly S. lutaria (Megaloptera) and caddisfly Cynthus flavidus (Trichoptera).

Sediments composed of degraded peat have a semi-liquid structure providing no support for infauna (e.g. Chironomidae and Oligochaeta) and no refuge from predation by benthivorous fish (Moss & Timms 1989). Moreover, the positive influence of macrophytes on sediment oxygen conditions (Carpenter & Lodge 1986) and stability (Moss & Timms 1989) results in feedback mechanisms, whereby the loss of vegetation exacerbates physical and chemical conditions within degraded peat sediments.

We observed both long-term negative effects of declining macrophyte habitat on invertebrate species richness and density, as well as short-term positive effects of introduced artificial macrophytes on the invertebrate community. The decline in invertebrate richness over the monitoring period was observed over both local and regional scales, as reflected by changes in $\gamma$ (gamma) and $\beta$Adl (beta) diversity (Fig. 2.5). These findings suggest that a reduction in macrophyte habitat, driven by the aforementioned peat degradation process, underlies the regional decline in invertebrate richness over
the last two decades. During the monitoring period densities of *Chironomus sp.* (Diptera), *Oecetis furva* (Trichoptera) and Oligochaeta declined significantly while, in our 2010 field study, densities of these same taxa were greater in submerged artificial macrophytes than in natural vegetation. Observed differences in species richness between Plastic Plants and natural Bank vegetation could be explained by the greater habitat complexity provided by natural vegetation, containing a range of species compared to Plastic Plants, which represented a single plant species.

Aside from providing a physical habitat for organisms, differences in invertebrate densities between artificial and natural substrata could be partly explained by increased food availability to grazers via growth of epiphytic algae (Carpenter & Lodge 1986; Underwood & Thomas 1990) or by protection of prey species from benthivorous fish, known to be present in the area (Hofman 2007). Invertebrate densities in artificial plants, however, were comparable to densities seen in North Holland ditches in 1985 (when macrophytes were present) and within the ranges of those found in other lentic freshwaters with submerged macrophytes (Viljoen, Cyrus & Wepener 2001; Storey 2007; Verdonschot et al. 2011). Additionally, it takes time for periphyton communities to develop on Plastic Plants and although the artificial substrata were left in the field for more than 50 days before sampling, we assume this food source is more readily available on natural vegetation. This suggests that food availability and predation were not the predominant factors determining the extensive and rapid colonisation of artificial macrophytes by invertebrates.

Benthic macroinvertebrate assemblages in degraded peatland ditches are stressed by hypoxia (caused by eutrophication), fine particle accumulation and habitat loss. The continued decline in benthic species richness and water transparency in recent years, despite decreases in external nutrient loading, suggests that sediment resuspension and internal nutrient remobilization, exacerbated by peat degradation, are underlying this trend. In our study we observed the regional shift from macrophyte to algae dominated systems caused by eutrophication and sediment loading. The results of our long-term monitoring data analysis and field experiment highlights the importance of macrophyte habitat structure to invertebrate communities. The underlying negative effects of peat degradation on the benthic invertebrate community therefore appeared to be largely indirect through the resulting loss of macrophytes.

**Management implications**

It is evident that benthic invertebrate species richness would improve in agricultural peat ditches if submerged macrophytes were re-established. In waters devoid of submerged plants invertebrates are concentrated along zones of emergent bank vegetation. Thus, importance should be placed on
maintaining and expanding the existing vegetation. The combination of fine particle accumulation, eutrophication and increased alkalinity causes multiple stressor effects on both invertebrates and submerged vegetation (Lamers et al. 2002; Verberk et al. 2007). Due to the potentially high rate of internal nutrient release in agricultural peat areas, peat degradation needs to be addressed by removing nutrient-rich sediment (e.g. by dredging) in combination with a reduction of external nutrient loading and the inlet of alkaline and SO\(_4^{2-}\)-enriched waters (Lamers et al. 2002; Smolders et al. 2006). This could be facilitated by allowing more flexible water tables, moving towards an integrative terrestrial-aquatic management approach (Janssen et al. 2005) aimed at reducing overall peat oxidation and mineralization.

Ultimately a clear-water phase is necessary to allow submerged macrophytes to re-establish. To achieve this re-establishment, nutrients and particularly light attenuation need to be reduced to values lower than when the system switched initially (van Nes et al. 2002). To facilitate an increase in transparency sediment resuspension must be reduced. This may be achieved through biomanipulation to reduce the numbers of benthic and planktivorous fish, in combination with dredging to remove nutrient-rich fine sediments and to reduce sediment resuspension (Lamers et al. 2002; Verberk et al. 2007). In a field study run in several hydrologically isolated ditches in the Wormer and Jisperveld, Hofman (2007) found this combination of dredging and fish removal increased zooplankton numbers, improved water clarity and led to the re-establishment of submerged vegetation. This demonstrates that rehabilitation of smaller, isolated ditches is achievable, although applying such measures to the whole peat area would be very costly and may not give the same results, particularly in larger water bodies that are more exposed to the wind.

In conclusion, this study demonstrates the strong influence of submerged macrophyte loss and introduction of artificial macrophytes on benthic invertebrate assemblages. High nutrient concentrations and oxidation of peatlands has resulted in the degradation of peaty soils, leading to the release of fine particles into the water. Amorphous sediments composed of degraded peat particles provide unsuitable habitats for benthic invertebrates while eutrophication, coupled with increased alkalinity and low dissolved oxygen, have additional detrimental effects on the invertebrates. The accumulation of degraded peat particles in the aquatic environment predominantly has an indirect negative effect on invertebrates, by triggering the loss of macrophytes, underlying the need to address both physicochemical and biological components in the management and restoration of agricultural peatlands.
Acknowledgments

We would like to thank Gert van Ee, Emile Nat and Ron van Leuken for their constructive advice throughout this project, Ivo Roessink, José van Diggelen and Fons Smolders for orientating us at the start of the project, Nigel Upchurch and Thijs de Boer for their help with the maps and diagrams, Ellard Hunting for his feedback on the manuscript, Pim Koelma, Coen Wagner and Alejandra Goldenberg for their assistance during fieldwork and Andre Timmer and Ed Zijp of Natuurmonumenten, for allowing us to access the Wormer and Jisperveld. This research was funded by Stichting Waterproef and Hoogheemraadschap Hollands Noorderkwartier.