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High nutrient loads hinder successful restoration of natural habitats in freshwater wetlands

Jesper E. Moeslund1,2, Dagmar K. Andersen3, Ane K. Brunbjerg1, Hans H. Bruun4, Camilla Fløjgaard1, Sebastian N. McQueen5, Bettina Nygaard1, Rasmus Ejrnæs1

Restoration of natural processes in ecosystems is key to mitigate the biodiversity crisis. Here, we evaluate 20 Danish stream-valley restoration projects—mainly by rewetting—in terms of successful restoration of natural wetland habitats. We used quadratic discriminant analysis and generalized linear models to compare 80 vegetation plots from the restoration projects with >60,000 natural or semi-natural wetland reference plots. We modeled the influence of time since restoration, grazing, rewetting, and nutrient availability on (1) the probability that study plots belong to natural habitats and (2) their richness of high-quality-habitat indicator species. The probability of a restored wetland having developed into a natural wetland habitat—such as an alkaline fen—was generally below 10%. Also, we found only half as many indicator species in restored wetlands than in reference wetlands and we demonstrated that the number of characteristic alkaline fen species did not deviate from what could be expected under the prevailing nutrient conditions. We found a negative effect of soil nutrient availability on the number of high-quality-habitat indicator species and the lowest probability of plots being natural wetlands in the most nutrient rich plots. The effect of grazing was only positive in the first years after restoration and only in the most nutrient rich plots, while the effect of rewetting sites to historical hydrological conditions was generally negative. Our findings suggest that unnaturally high nutrient availability is probably the core limiting factor for successful restoration of natural wetlands and their associated plant diversity.

Key words: alkaline fens, eutrophication, grazing, hydrology, indicator species, mires, rewetting

Implications for Practice

- To successfully restore natural and characteristic freshwater wetland habitats, the main focus must be on recreating natural processes and conditions.
- Restoring natural hydrology and grazing is probably not enough, the soil and water must be nutrient poor for successful restoration of stream-valley wetlands.
- Restoration of stream-valley wetlands such as alkaline springs and fens is more likely to be successful in spring-dominated landscapes where clean groundwater diffusely seeps through the soil.

Introduction

Freshwater wetlands are vital ecosystems for the conservation of biodiversity. They are sensitive to even small land-use changes, and they hold a high number of red-listed species (Moeslund et al. 2019; Tickner et al. 2020). In modern times, 54–57% of wetlands globally have been lost and since 1700 AD this number is probably up to 87% (Davidson 2014). Many freshwater wetlands have been lost or degraded as a consequence of altered hydrology due to drainage, drinking water extraction, dam-building, and other human-made changes to the natural water cycle (Brinson & Malvárez 2002; Rolls et al. 2018). Also, in the last century, the natural disturbances by large herbivores—e.g. maintaining open vegetation and leaving dung (and in some cases carcasses)—have dramatically decreased in these important ecosystems, further contributing to biodiversity losses (Svenning et al. 2016; Biró et al. 2019). Ecological restoration aims at reversing these negative trends (Wheeler et al. 1995).
Here, we evaluate 20 different cross-regional restoration projects (mainly restoring hydrology) in Denmark to point out the most important factors for ensuring cost-effective restoration success of natural wetland habitats.

In Northern Europe, stream-valley (i.e. riparian) wetlands typically count habitats such as alkaline fens, Molinia-meadows (i.e. temporarily wet meadows dominated by *Molinia caerulea* [L.] Moench), and spring-fed wetlands. North European alkaline fens are characterized by the seepage of base-rich groundwater, with several *Carex* species and *Schoenus nigricans* L., *Juncus subnodulosus* Schrank, *Liparis loeselli* (L.) Rich., and *Pinguicula vulgaris* L. The often surface-water-affected Molinia-meadows typically hold *M. caerulea* (L.) Moench, and several *Juncus* species, e.g. *Juncus conglomeratus* L. (European Commission 2013). Finally, spring-fed wetlands can vary in species composition depending on the soil characteristics and openness of the vegetation. Natural processes and disturbances such as groundwater seepage, flooding, and grazing combined with a naturally nutrient poor and base-rich environment have historically caused these wetland communities to be rather open swards and rich in plant and bryophyte species (Bergamini et al. 2001; Moran et al. 2008; Biró et al. 2019). Consequently, they have been listed by the European Union as important habitats for the European community to conserve and protect within their natural ranges (Council of the European Communities 1992).

Globally, efforts to restore freshwater wetlands to their former extents and habitat conditions are widespread (Perry 2004; Måslon et al. 2008; Menichino et al. 2016). However, most often restoration has not aimed solely to restore natural habitats and their associated biodiversity, but also or even primarily targeted carbon storage, downstream flood prevention, or promoting nitrogen fixation and phosphorus sedimentation services to lower the impact of nutrient pollution in the recipient lakes and seascapes (Zedler 2000; Hoffmann & Baattrup-Pedersen 2007; Moreno-Mateos & Comín 2010; Audet et al. 2020; Hoffmann et al. 2020). The methods used for restoration of freshwater wetlands varies significantly between projects and can include for instance the restoration of hydrology, landscape terrain, grazing, and nutrient balances as well as reintroduction of typical species (Pfadenhauer & Grootjans 1999; Matthews et al. 2009a; Richardson et al. 2016). However, while there are numerous studies of wetland restoration methods (see references above and references therein), only few freshwater restoration projects are evaluated post-restoration (Taddeo & Dronova 2018; Baumane et al. 2021) and even fewer are systematically monitored and hence cost-effective restoration of freshwater wetlands is still poorly supported by research and empirical evidence.

In this study, we compared 80 vegetation plots from 20 different freshwater-wetland restoration projects in Danish stream valleys with >60,000 vegetation plots in near-natural and human-influenced wetlands to gain insight into what conditions are most likely to yield successful restoration of these wet habitats. More specifically, we addressed the following questions: (1) How similar are the restored wetlands to comparable natural habitats with regard to plant diversity and composition? (2) What is the impact of previous land use, grazing, time since restoration, and nutrient availability on restoration success?

### Methods

#### Study Area

In Denmark, there are several settings (i.e. combinations of former land use and methodology) influencing the outcome of wetland restoration projects. Most projects are conducted on former extensive farmland or improved meadows in drained and ditched stream valleys or drained lakes and some on former fish-farms. These restoration projects differ in type of terrain, original hydrology, soil type and soil chemistry, restoration methods, and so on. In this study, we focused on a broad range of restoration projects grouped by their former land use: (1) groundwater-fed fish farms and (2) extensive farmland areas in low-lying riparian settings and often historically flooded by the nearby stream, typically former intensive or extensive agricultural land. For projects in the first group, we expected that the water quality is relatively high, since this mainly consists of groundwater, although there can be nutrient residue as a legacy from former aquaculture. Hence, we hypothesized that restoration on former spring-fed fish-farms could result in a relatively high habitat quality. For projects in the second group, the water for rewetting typically comes from flooding by stream water or drainpipes and consequently we expected such sites to be more nutrient rich, possibly affecting the restoration of habitat quality negatively. We included 20 restored wetlands in Jutland, Denmark, equally covering former spring-fed fish-farms and former extensive farmland areas mainly influenced by surface water or water from drainpipes (Fig. 1). The 20 sites were selected to cover a large region (Eastern Central Jutland, approximately 5,500 km²), but still located in a way that allowed us to visit two to three sites a day. The sites were selected to cover a broad range of different restoration settings as indicated above and included, e.g. projects that partly or mainly aimed to improve stream water quality and projects that were more aimed at restoring stream or wetland biodiversity. We required all sites to have at least a brief description of the restoration process, e.g. former land use and year of restoration completion. While we do not know with absolute certainty—as the information is not available for our restoration sites, we believe all the restored sites had a drastically altered hydrology prior to restoration as this is the typical setting for such projects in Denmark.

#### Field Data

In a first step, we mapped the wetness of each of the 20 restoration sites by visual inspection in the wet, wet-moist, moist, moist-dry, and dry. The wet, moist, and dry categories corresponded to the legend of a historical map that we used for modeling (see section “Effect of Practice on Restoration Success”), that is, wet corresponded to bog or swamp, moist to meadow, and dry to a dry grassland. The intermediate categories (wet-moist and moist-dry) were used in areas with mosaics of these categories. Then, in each site, we laid out four 5-m radius circular vegetation plots (80 in total) as described in the following. In cases where plots already existed from previous projects these were resurveyed (18 plots) to contribute to time series data for these projects. If not, we selected plots randomly from a
10 × 10 m grid avoiding plots with >50% lake or pond coverage, >35% woody canopy cover, and plots with dry patches. We operated with these constraints as we were only interested in open terrestrial wetland habitats. Additionally, we skipped randomly selected plots that were <30 m away from other plots. Within each plot, we recorded all vascular plant and bryophyte species. Finally, we recorded whether plots were affected by grazing by horses or cattle (i.e. bites on the vegetation, dung, trampling, or presence of animals). Field work was conducted from May to June 2019. The final field-dataset contained the above-mentioned environmental and species data for the 80 study plots comprising a total of 2,308 records of 201 species.

Reference Data
To answer our study questions, we needed reference data from more or less natural wetland habitats of the same type as our study sites. In Europe, habitat types deemed important by the EU community through the Habitats Directive (Annex I; Council of the European Communities 1992)—are considered more or less pristine nature with intact natural processes and hence generally the aim for natural-habitat restoration projects. From the program for monitoring Danish Annex I habitats—the National Monitoring and Assessment Programme for the Aquatic and Terrestrial Environments (NOVANA, described by Fredshavn et al. 2018 and The Environmental Protection Agency 2022)—we extracted plant species lists from 40,144 wetland plots covering all Denmark (Table 1). However, to put the restored plots into a broader context we also needed data from more degraded areas and plots with clear human-influence. Therefore, we also extracted plant species lists from 24,572 non-Annex-I wetland plots that were recorded through Danish municipalities’ inventories of natural and semi-natural protected nature (Fredshavn et al. 2010). These plots encompassed: “improved meadows” (clear signs of earlier agricultural use, e.g. nutrient enrichment), “other meadows” (meadows with lower nutrient availability and more natural flora than improved meadows), “poor fens” (rather acidic nutrient poor fens, but not bogs or wet heathland), “nitrophile tall-herb communities” (e.g. reed swamps and riparian tall herb societies), and “nutrient poor meadows” (e.g. meadows affected by human activities but with no clear signs of artificial nutrient enrichment). In the next step, we removed plots from both datasets having less than five species, as too low species numbers can introduce arbitrary mean indicator values in subsequent analyses (see section “Data Preparation”). Finally, we lumped taxa to the species level and merged the two datasets resulting in a reference dataset comprising 1,375,925 records of 1,345 vascular and bryophyte species covering 64,716 plots and 17 wetland habitat types (Table 1).

Data Preparation
All vegetation plots used here, both the newly recorded and the reference dataset were inventoried following the same method. The plots were 5-m radius circular plots inventoried in the growing season by botanically trained fieldworkers. Prior to statistical analysis, the datasets were processed through several steps to add relevant information and to make sure data were consistent. First, we standardized plant nomenclature, that is, renamed 22 species synonyms in the field-dataset to make
names fully match those in the reference dataset. Then, we retrieved the updated Ellenberg indicator values (EIVs) for plants from Hill et al. (1999) containing values indicating plants’ preferences for soil nitrogen (EIV_N), reaction (EIV_R), moisture (EIV_F), and light availability (EIV_L) covering 1,791 taxa. Species names were matched by use of the Taxonomic Name Resolution Service (Boyle et al. 2021; 100 names) combined with a manualy check of synonyms for 52 species in the Danish taxon checklist (allearter.dk, accessed 4 October 2021). After lumping taxa in the EIV-dataset at the species level, we extracted indicator values for the 942 species in the EIV-dataset that were present in our study plots and in the alkaline fen reference plots using a Wilcoxon rank sum test (variables were not normally distributed). The number of characteristic alkaline fen species in our study plots had zero alkaline fen indicator species, we also used a broader set of high-quality-habitat indicator species (Fredshavn & Ejrnæs 2007, vascular plants, see species list in Table S1). These high-quality-habitat indicator species are selected to indicate favorable conservation status in the sense of the EU Habitats Directive (Council of the European Communities 1992) and include vascular plant species considered moderately to very sensitive to habitat destruction (Fredshavn & Ejrnæs 2007).

### Statistical Analysis

All statistics described below was conducted in the R statistical programming environment (R Core Team 2021).

### Comparing Natural Wetland Habitats with Restored Habitats

To gain insight into the floristic differences between restored wetland sites and natural alkaline fens, we compared the number of characteristic alkaline fen species in our study plots and in the alkaline fen reference plots using a Wilcoxon rank sum test (variables were not normally distributed). The choice of alkaline fens for comparison habitat relies on the fact that the restored sites had highest probability of being alkaline

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### Table 1. Overview of the habitat types included together with number of plots and the Annex I habitat code. Habitats within the same habitat group were lumped together for analysis. Habitats with poor coverage or lacking ecological meaning (i.e. not defined by the species living there) were left out of analysis (marked with *). The estimated land cover of each habitat group is given in the final column with priors used for quadratic discriminant analysis in parentheses (see main text).

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Annex I Code</th>
<th>No. of Plots</th>
<th>Habitat Group Name</th>
<th>Est. % Land Cover (Prior)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active raised bogs</td>
<td>7110</td>
<td>2,416</td>
<td>Acidic bogs and fens</td>
<td>0.1 (0.026)</td>
</tr>
<tr>
<td>Degraded raised bogs still capable of natural regeneration</td>
<td>7120</td>
<td>2,326</td>
<td>Acidic bogs and fens</td>
<td></td>
</tr>
<tr>
<td>Transition mires and quaking bogs</td>
<td>7140</td>
<td>6,120</td>
<td>Acidic bogs and fens</td>
<td></td>
</tr>
<tr>
<td>Poor fen</td>
<td></td>
<td>2,465</td>
<td>Acidic bogs and fens</td>
<td></td>
</tr>
<tr>
<td>Calcareous fens with Cladium mariscus and species of the Caricion davallianae</td>
<td>7210</td>
<td>1,130</td>
<td>Alkaline fens</td>
<td>0.2 (0.052)</td>
</tr>
<tr>
<td>Alkaline fens</td>
<td>7230</td>
<td>14,627</td>
<td>Alkaline fens</td>
<td></td>
</tr>
<tr>
<td>Improved meadows</td>
<td></td>
<td>3,003</td>
<td>Improved meadows</td>
<td>1.5 (0.389)</td>
</tr>
<tr>
<td>Molinia meadows on calcareous, peaty, or clayey-silt-laden soils (Molinion caeruleae)</td>
<td>6410</td>
<td>7,118</td>
<td>Molinia meadows</td>
<td>0.2 (0.052)</td>
</tr>
<tr>
<td>Other meadows</td>
<td></td>
<td>9,548</td>
<td>Other meadows</td>
<td>1.0 (0.260)</td>
</tr>
<tr>
<td>Nitrophile tall-herb communities</td>
<td></td>
<td>9,556</td>
<td>Tall herbs</td>
<td>0.8 (0.208)</td>
</tr>
<tr>
<td>Northern Atlantic wet heaths with Erica tetralix</td>
<td>4010</td>
<td>5,096</td>
<td>Wet heathlands</td>
<td>0.05 (0.013)</td>
</tr>
<tr>
<td>Depressions on peat substrates of the Rhynchosporion</td>
<td>7150</td>
<td>1,311</td>
<td>Wet heathlands</td>
<td></td>
</tr>
<tr>
<td>Humid dune slacks*</td>
<td>2190</td>
<td>6,482</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligotrophic to mesotrophic standing waters with vegetation of the Littorelletea uniflorae and/or of the Isoeto-Nanojuncetet*a</td>
<td>3130</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels*</td>
<td>6430</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petrifying springs with tufa formation (Cratoneurion)*</td>
<td>7220</td>
<td>5,354</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient poor meadows*</td>
<td></td>
<td>205</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
fens (see the section “Effect of Practice on Restoration Success”).

To investigate the degree to which soil fertility influences restoration success, we tested if our study plots held as many alkaline fen characteristic species as we would expect from the prevailing soil fertility. To do this we constructed a generalized linear model (GLM, Poisson distributed errors) with number of characteristic alkaline-fen species (see “Reference Data”) as response and mean $\text{EIV}_N/\text{EIV}_R$ ratio as explanatory variable. This model included only alkaline fen plots from our reference dataset (recall that these are the most natural alkaline fens in the country, see above). Then we used it to predict the number of characteristic alkaline-fen species in our restoration plots and finally that number was compared to the actual observed number of these species by a Wilcoxon rank sum test. To avoid circularity in this analysis, we used a mean $\text{EIV}_N/\text{EIV}_R$-ratio calculated without the characteristic alkaline-fen species.

**Effect of Practice on Restoration Success.** To enable prediction of the most likely habitat types of our field plots and to enable analysis of restoration practice on restoration success, we used quadratic discriminant analysis (QDA) to create a classification model for classifying plots into habitat types based on environmental conditions (EIVs, see above). While multinomial logistic regression (MLR) or neural-network-based models could be optional alternatives, QDA is—in our experience—a robust and reproducible supervised classification method suitable for the classification analysis we wished to conduct here. Our classification model was based on the reference dataset. However, to construct a biologically sound classification model, we first excluded plots with poor habitat representation (i.e. low number of plots; Table 1). We also excluded plots belonging to habitat types with poor ecological meaning (i.e. not defined by the species living there): Petrifying springs (EU habitat code: 7220) are defined by the hydrology while dune slacks (habitat code: 2190) are defined by topographic position and can vary considerably in soil moisture, soil nutrient availability, and successional stage (Table 1). Second, we grouped plots belonging to habitats with more or less the same plant communities (see Table 1). Then, we ran a QDA using the “qda” function in the MASS package (Venables & Ripley 2002) to predict these grouped habitat types from the plants’ overall preferences for nitrogen (plot mean $\text{EIV}_N$), soil reaction ($\text{EIV}_R$), moisture level ($\text{EIV}_M$) and light availability ($\text{EIV}_L$; Fig. 2). Since the number of plots in the reference dataset for each habitat group does not reflect their actual abundance in the landscape, we used priors weighted according to the

![Figure 2. The predicted distribution of reference plots (small points) and study plots (larger points) along the moisture (y-axis) and nutrient (x-axis) gradients in this study (see main text). The mean Ellenberg indicator values summarizes the plants’ overall preference for moisture level and nutrient level in a given plot. The different wetland types in the legend are explained in the main text. Generally, they span acidic to calcareous and moist to wet (but not permanently flooded) wetland types on the gradient from rather human disturbed to relatively natural. Habitat types marked with asterisks are Annex I habitats in the European Habitats Directive (Council of the European Communities 1992), that is, the most natural of the wetlands included.](image-url)
estimated national land cover of each habitat group, to avoid overfitting to habitat groups that are overrepresented in the dataset, e.g. alkaline fens. These estimates and the priors reflecting these and hence used in the QDA is given in Table 1 (Annex I habitat coverage was taken from Fredshavn et al. 2019 and the remaining from supplementary table 1 in Ejrnæs et al. 2018).

Subsequently, we used the classification model to predict which habitat types were most likely for the restored wetland plots in our study. In all but two cases, our study plots had highest probability (posterior) for belonging to the habitat type “alkaline fens” (Fig. 3). While one never knows for sure in which direction the vegetation in a restored plot is developing, we believe the best estimate is the (semi-)natural habitat type that it resembles the most some years past restoration. Hence, in the remaining analyses we assumed that the restored wetlands are developing towards becoming alkaline fens and considered this the main target habitat type.

We checked that the explanatory variables were roughly normally distributed within each habitat type as this is required for QDA. In some cases, the distributions were somewhat skewed but that is not an issue of concern since QDA is relatively robust with respect to skew (McCune & Grace 2002). Despite this, we tested the robustness of the QDA approach described above, by running a similar (to the QDA) MLR model. This parallel procedure enabled us to check that results were independent from model choice. This MLR modeling is described in Supporting Information.

We used GLMs to investigate the effects of restoration practice—such as rewetting, grazing and time—on restoration success. We constructed four separate statistical models (1 through 4 in the following) with four different response variables. First, to analyze these factors in relation to how close a given restoration plot is to Annex I wetlands we used the posteriors from the QDA as response variables; (1) both the posteriors for alkaline fens only (i.e. the probability that a restored plot is an Annex I alkaline fen, coded 7230) and (2) the summed posteriors (i.e. the probability that a restored plot is any of the included Annex I wetland habitats). Then, we used (3) the number of high-quality-habitat indicator species as response instead.

Table 2. Overview of explanatory variables used for evaluating the effect of restoration practice on restoration success.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Former land use</td>
<td>Two classes</td>
<td>Tells whether a site was a spring-fed fish farm or an extensive farmland</td>
</tr>
<tr>
<td>No. of years since</td>
<td>Continuous</td>
<td>The number of years since completion of restoration</td>
</tr>
<tr>
<td>restoration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing</td>
<td>Binary</td>
<td>Tells if there were signs of grazing during fieldwork or not</td>
</tr>
<tr>
<td>Plot-scale rewetting</td>
<td>Binary</td>
<td>Tells if the plot is as wet or wetter than it used to be historically</td>
</tr>
<tr>
<td>Site-scale rewetting</td>
<td>Continuous (%)</td>
<td>The percent of the area that is as wet or wetter than historically</td>
</tr>
</tbody>
</table>

Figure 3. Posteriors from the quadratic discriminant analysis (QDA) described in the main text for the habitats used in this study and listed in Annex I in the European Habitats Directive (Council of the European Communities 1992). The posteriors represent a given vegetation plot’s probability of belonging to a given habitat type. In this figure, we only show most natural of the wetland habitat types included in this study as these are often the aim of wetland restoration.
Figure 4. Predicted effects of the interactions and squared terms on (1) the likelihood of restored wetlands being classified by our model as an alkaline fen (A–D) and (2) the number of high-quality habitat indicator species (E). Where uncertainty is shown (semi-transparent shading around lines) this corresponds to the 95% CI. Panel D shows a contour plot of the interaction between two continuous variables and hence the contours correspond to the predicted quadratic discriminant analysis (QDA) posterior probability (Pred. prob.) shown in the y-axes of the other graphs. A contour plot extrapolates the data and hence is invalid far outside the areas where the actual probabilities (Act. prob.) for each plot is shown by dots (e.g. the upper right corner of the plot).
Table 3. Explanatory variable importance given as the residual deviance after the explanatory variable in question has been removed from the model. The higher the residual deviance after variable removal the more important is the explanatory variable considered. In cases with interactions, these also count in the importance (i.e. interactions with the variable in question was also removed from model for calculation of variable importance). For comparison, model null deviance and residual deviance is given along with deviance explained and the number of observations used in the model (N).

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>Prob. of Alkaline Fen</th>
<th>Prob. of Annex I Wetland</th>
<th>No. of High-Quality Habitat Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Former land use</td>
<td>923.0</td>
<td>1,131.6</td>
<td>105.0</td>
</tr>
<tr>
<td>Grazing</td>
<td>917.8</td>
<td>1,092.6</td>
<td>115.2</td>
</tr>
<tr>
<td>No. of years since restoration</td>
<td>932.3</td>
<td>1,111.4</td>
<td>104.8</td>
</tr>
<tr>
<td>Site-scale rewetting</td>
<td>853.4</td>
<td>1,030.5</td>
<td></td>
</tr>
<tr>
<td>Former land use × grazing</td>
<td>835.5</td>
<td>1,014.3</td>
<td></td>
</tr>
<tr>
<td>Grazing × no. of years since restoration</td>
<td>915.0</td>
<td>1,090.0</td>
<td></td>
</tr>
<tr>
<td>Former land use × site-scale rewetting</td>
<td>861.9</td>
<td>1,045.1</td>
<td></td>
</tr>
<tr>
<td>No. of years since restoration × site-scale rewetting</td>
<td>863.6</td>
<td>1,042.2</td>
<td></td>
</tr>
<tr>
<td>Mean N/R ratio</td>
<td></td>
<td></td>
<td>256.8</td>
</tr>
<tr>
<td>Grazing × mean N/R ratio</td>
<td></td>
<td></td>
<td>104.5</td>
</tr>
<tr>
<td>Null deviance</td>
<td>1,016.5</td>
<td>1,225.4</td>
<td>274.0</td>
</tr>
<tr>
<td>Full model deviance</td>
<td>825.0</td>
<td>1,004.1</td>
<td>94.1</td>
</tr>
<tr>
<td>Deviance explained</td>
<td>18.8%</td>
<td>18.0%</td>
<td>65.7%</td>
</tr>
<tr>
<td>N</td>
<td>76</td>
<td>76</td>
<td>76</td>
</tr>
</tbody>
</table>

These species are considered vulnerable to habitat deterioration by agricultural intensification (Fredshavn & Ejrnæs 2007) such as tilling, fertilization, drainage, and cropping (e.g. grass leys). Finally, we used (4) the mean EIVN/EIVR ratio as response variable in a fourth model. We expected that analyzing this variable could give insight into how soil nutrient availability varies under different restoration settings.

In our modeling, we used the five explanatory variables detailed in the following and summarized in Table 2. Former land use was represented as a binary explanatory variable denoting whether a study site (and thereby the four plots within it) was either a restored former spring-water fed fish farm or a former extensive farmland in which drainage has been reduced as part of the wetland restoration (see further explanation in the section “Study Area”). To test the effect of time since restoration, we calculated the number of years since restoration (i.e. since the completion of the restoration projects). To study effects of large herbivores, we created a binary variable, grazing, denoting whether a plot was within a site that had signs of grazing by horses or cattle (see section “Field Data”) or not. To analyze the effect of rewetting, that is, to which degree historical hydrology was restored to historic hydrological conditions, we created two explanatory variables accounting for the fact that the effect of rewetting is spatial-scale dependent (Rolls et al. 2018); plot-scale and site-scale rewetting. To do this we first digitalized historical hydrological conditions at our study sites by delineating areas that used to be wet, moist, and dry in the first large-scale mapping of Danish territory in 1862–1899 (“Højre målebordsblade,” freely available through www.datatilsyn.dk). The accuracy of these old maps is relatively low and hence the variables based on this digitalization also come with some uncertainty. On the other hand, the field-mapped corresponding current wetness of each site also comes with uncertainty as it is quite difficult to draw exact limits between different degrees of wetness even in the field. Subsequently, for all study plots, we determined the wetness of the majority of the plot (recall: plots are 5-m radius circles) historically and currently. For example, if 60% of the plot was wet and 40% was moist, we considered the plot wet in this context. Based on this, we constructed a binary plot-scale rewetting variable holding a yes if a plot was as wet or wetter than historically, otherwise a no. At site-scale, we calculated the areal percentage that were as wet or wetter than it used to be historically. For this calculation, we did not consider areas which were dry historically as this study concerns restoration of wetlands. We weighted these percentages for each wetness-category by its original area and then summed them. The result was a variable reflecting to what degree historically moist or wet study sites are contemporarily at least as moist or wet as they were in the late 1800s. In our modeling (see below), the site-scale rewetting consistently gave better models (Akaike’s information criterion [AIC]; Akaike 1974) and the plot- and site-scale variables gave the same results, so we did not use the plot-scale rewetting variable in our final models.

For each of the four response variables, we constructed a GLM having errors following either a binomial (QDA posteriori), Poisson (number of indicator species) or Gaussian distribution (EIVN/EIVR) to test the effects of (1) former land use, (2) number of years since restoration, (3) presence or absence of grazing, (4) the degree to which hydrology was restored to historical conditions, and (5) the mean EIVN/EIVR ratio. The latter was only used in the model of indicator species, as the QDA was based on EIV’s, and hence using it in the other models would cause circularity. In the binomial modeling of posteriors from the QDA, R allows to use a two-column matrix format having number of successes and number of failures for each plot as columns. To create this response-variable format, we used the posteriors in integer percent as “number of successes” and 100%—“number of successes” as “number of failures.”

We used a stepwise backwards model selection procedure based on AIC (Akaike 1974), removing the variable causing the highest drop in AIC in each step and stopping when ΔAIC did not change more than 2 (Burnham & Anderson 2002). As part of the model...
selection procedure, we tested all two-way interactions and kept those that improved the model significantly (same ΔAIC criteria as above). As previous studies have shown that the effect of time after restoration is not necessarily linear (Matthews et al. 2009b), we also included a quadratic term of this variable to allow for a nonlinear fit. After modeling we checked all models’ residuals for spatial autocorrelation using Moran’s I (Paradis 2015), and found none (p >0.05). We checked the final models for model misfit by inspecting predicted versus actual residual plots, and we checked that the Poisson model was not overdispersed.

Results

Our results showed that the probability that a restored wetland is an Annex I wetland habitat (including alkaline fens) is generally below 10%. Only four plots reached a probability of more than 50% (Fig. 3). Also, we only found half as many species characteristic of alkaline fens in restored wetlands (median = 1 species) than in reference fens (median = 2 species, Wilcoxon rank sum test p-value <<0.001). In addition to this, we demonstrated that the number of characteristic alkaline fen species did not deviate from what could be expected under the prevailing soil nutrient availability in the restored sites (Wilcoxon rank sum test p-value = 0.17).

Our two models of probabilities (i.e. QDA posteriors) that a given plot is either an Annex I alkaline fen or any Annex I wetland habitat were almost identical and explained about 18–19% of the variation in data. Therefore, we report and discuss the results of the alkaline fen model only, since almost all plots were closest to this habitat type. Results for the model for all Annex I wetlands can be found in Figure S1.

Generally, the plots’ probability of belonging to the habitat type “alkaline fens” were higher in former extensive farmland compared to former spring-fed fish farms (Table 4) notably in nongrazed plots (Fig. 4A). The effect of time since restoration was the most important explanatory factor for this probability (Table 3). It peaked after approximately 12–15 years in grazed areas and after approximately 30 years in nongrazed areas (Fig. 4B) and appeared to be highest in the plots with poor rewetting (Fig. 4D). Generally, rewetting had a negative influence on the probability of a plot resembling an alkaline fen wetland habitat, but this was most pronounced in former extensive farmlands (Fig. 4C). Full modeling results are shown in Table 4. All significant interactions are shown in Figure 4.

Second, our results showed that soil nutrient availability was the most important factor for the number of high-quality-habitat indicator species (Table 3) with a general negative impact. 

Table 4. Modeling results overview. The three main groups of columns represent results for each of the three models having the given response variable. OR, odds ratio; CI, confidence interval; IRR, incidence rate ratio; Prob., probability; red., reduced; drain., drainage; mean N/R ratio, the ratio between mean Ellenberg indicator values for soil nitrogen (N) and soil pH (R), a measure of soil nutrient availability.

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>Prob. of Alkaline Fen</th>
<th>Prob. of Annex I Wetland</th>
<th>No. of High-Quality Habitat Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log(OR)</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>Former land-use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring-fed fish farm</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Extensive farmland</td>
<td>2.6</td>
<td>1.8, 3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grazing</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Yes</td>
<td>2.2</td>
<td>2.8, 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>0.38</td>
<td>0.25, 0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of years since restoration</td>
<td>—0.01</td>
<td>—0.01, 0.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site-scale rewetting</td>
<td>3.9</td>
<td>2.6, 5.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former land-use × grazing</td>
<td>0.56</td>
<td>0.22, 0.91</td>
<td>0.001</td>
</tr>
<tr>
<td>Extensive farmland × no.</td>
<td>——</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Grazing × no. of years since restoration</td>
<td>—0.17</td>
<td>0.13, 0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former land-use × site-scale rewetting</td>
<td>—2.9</td>
<td>—3.9, —2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Extensive farmland × site-scale rewetting</td>
<td>—0.35</td>
<td>—0.46, —0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean N/R ratio</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Grazing × mean N/R ratio</td>
<td>—5.6</td>
<td>—7.5, —4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. × mean N/R ratio</td>
<td>—6.0</td>
<td>—7.5, —4.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
on this richness. The effect of grazing was most pronounced in the most nutrient rich plots and in these it had a positive effect on the number of high-quality-habitat indicator species (Fig. 4E) while time since restoration generally appeared to have a negative influence (Table 4). Figure 5 shows two examples of study plots with a high number and two with a low number of high-quality-habitat indicator species and their soil nutrient availability and grazing status.

Third, we found that the mean soil nutrient availability in the restored wetlands was only affected by the former land use with former extensive farmlands having a lower nutrient availability ($\beta = -0.04, p = 0.010$) than former spring-fed fish farms.

The MLR analysis gave similar results as the QDA (see Supplement S1), and we therefore consider our QDA classification results robust and reliable.

**Discussion**

In this study, we showed that restored wetlands generally hold a vegetation that is far from reference Annex I wetlands—both with regard to characteristic species and the more general plant species composition—and this was also true for sites restored >20 years ago. Our results confirm several previous studies reporting the same tendency (Målson et al. 2008; Matthews...
et al. 2009b; Baumane et al. 2021). We also showed that relatively nutrient rich plots had significantly lower richness of high-quality-habitat indicator species and that—given the prevailing nutrient availability—the current richness of characteristic alkaline-fen species corresponds to predictions based on a large reference dataset. These findings suggest that the main barrier to restoration success is nutrient load rather than dispersal or establishment limitation. If restoration of naturally infertile soils is not ensured, we predict that Annex I wetland habitats in stream valleys cannot be restored successfully. Instead, restored areas will likely develop into species poor wetlands with dominance of highly competitive plant species (Naja et al. 2017). An effective way to achieve a natural nutrient balance is to remove the nutrient-rich topsoil, possibly combined with addition of lime where appropriate (Målson & Rydin 2007; Målson et al. 2008). In fact, on former farmland complete topsoil removal is sometimes the only way to achieve near-natural nutrient conditions in wetland restoration (Smith et al. 2011).

Time since restoration had an overall positive influence on the probability of a study plot resembling an alkaline fen, but only in the first 12–30 years after restoration. In grazed areas this effect was significantly lower, and the effect of time wore off earlier. One possible explanation for this pattern is that some plant species characteristic in alkaline fens—e.g. Carex nigra (L.) Reichard, Lychinis flos-cuculi L., and Menyanthes trifoliata L.—may establish rather quickly making the vegetation resemble a poorly developed alkaline fen. However, after some years—if the nutrient load is too high as indicated in this study (see above)—the characteristic alkaline fen species could disappear again due to competitive exclusion (Borer et al. 2014). Matthews et al. (2009b) found a similar pattern for several wetland-restoration success-indices including one based on plant species’ conservation value. They also considered dominance of tall growing herbs a few years after restoration a plausible explanation for this finding. This proposed explanation could also explain why we found a small but significant negative effect of time on the number of high-quality-habitat indicator species. Such a phenomenon could be aggravated by finishing wetland restoration with a nutrient rich topsoil layer and seeding of agricultural grass cultivars, which was a common practice in the first generation of wetland restoration projects in Denmark but now less frequently used (e.g. Andersen et al. 2005). These grass cultivars are highly competitive and quickly establishes a dense monospecific sward. A broader explanation could be that the oldest restoration projects were undertaken with different targets than the younger ones, that is, with less focus on re-creating optimal conditions for wetland plant diversity. In a recent study, Baumane et al. (2021) found no effect of time since restoration on the vegetation in riparian wetlands. They suggested this to be a consequence of too high nutrient input but could not rule out dispersal limitation. We did not find evidence that dispersal limitation is causing the observed slow or stalled recovery in restored wetlands because the number of characteristic alkaline fen species do not deviate from the expected number given environmental conditions. Our results suggest that one cannot expect any further development into more characteristic wetland nature with time (see discussion above on nutrients).

Our study showed that previous land use is decisive for restoration success. Contrary to our expectations, the probability of a plot resembling an alkaline fen was higher in former extensive farmlands than in former spring-fed fish farms. However, when analyzing nutrient differences between the two former land-use types we found that former fish-farms had higher soil nutrient availability than former extensive farmlands, possibly explaining why these former extensive agricultural areas appeared more similar to alkaline fens than the former fish farms. In addition to this, when we controlled for soil nutrient availability in our analysis of high-quality indicator species this pattern changed; the number of indicator species was higher in former spring-fed fish farms than in former farmland areas. This raises an important point in need for confirmation from future studies, namely that—soil nutrient availability being equal—restoration of alkaline fens probably has the highest potential in sites where nutrient poor and calcareous groundwater (in contrast to often nutrient rich surface flood water) seeps diffusely from the underground and possibly washes away nutrients and in some cases immobilizes the phosphorus pool (Boomer & Bedford 2008; Venterink et al. 2009; Audet et al. 2015; Kooijman et al. 2020). In our study sites, results indicate that the nutrient rich sludge from former fish-farms was not consistently removed or that fishponds were filled with nutrient rich soil during restoration. Finally, there may be other differences in restoration practice between the two former land uses, e.g. whether practitioners attempted to restore natural microtopography which is known to be important for natural wetland plant diversity (Moeslund et al. 2013).

Grazing by cattle or horses had a positive effect on the probability of a plot resembling an alkaline fen or any Annex I wetland habitat, but only in the first years after restoration. We also found a positive effect of grazing on the number of high-quality habitat indicator species, but only in the most eutrophic of our study sites. On the other hand, grazing did not improve the probability of a plot being an alkaline fen in the later time periods after restoration, but this negative effect was only clear in former extensive farmland areas and was relatively small in the oldest sites. Generally, grazing is considered important for freshwater wetland biodiversity as it creates heterogenous vegetation and terrain and thereby numerous microhabitats for specialist species (Biró et al. 2019 and references therein), and our study also partly supports this. The lack of a consistent and clear positive effect of grazing may also reflect the low precision and relatively broad spatial scale (i.e. site level) of our grazing data, possibly confounding with other activities such as sward improvements or perhaps an overriding effect of eutrophication.

The degree to which an area was rewetted to resemble historical wetness generally had a negative impact on the probability of a plot being an alkaline fen, notably in former extensive farmlands. This is counterintuitive because clearly, restoring hydrology should have a positive impact on wetland restoration as these habitat types cannot be restored without water (Pfadenhauer & Grootjans 1999; Målson et al. 2008). In wetlands, different species have specialized in different hydrological niches, and all these niches can only be present if hydrology is near-intact (Rolls et al. 2018). Here, we only see such a positive effect of rewetting in the first approximately...
10–15 years. Based on our results (see discussion about nutrients above), we suggest that this could be a consequence of too high a soil or water nutrient availability in the restored sites generally. In other words, we believe the most plausible explanation is that the sites benefit from successful wetting in the first few years, after which the nutrient balance short-circuits this positive development and becomes the main determinant of the final restoration outcome. An alternative explanation could be that the terrain has lowered because of degradation of soil organic matter following more aerobic soil conditions after drainage. This could result in longer and deeper flooding events than historically. Finally, yet another possible explanation could be the fact that our hydrological data was rather coarsely measured (see “Model Uncertainties”).

Model Uncertainties
In our models, the results regarding wetting and time since restoration comes with uncertainties. Determining contemporary wetness in the field and subsequently historical wetness based on old maps is a difficult task and the resulting variables will probably vary slightly depending on the person undertaking the task. Here, we carefully conducted this task and only one person was involved in the historic map interpretations to ensure consistency. Also, we believe that site-scale hydrology acts at a relatively large spatial scale further reducing the impact by the uncertainties associated with this variable. Regarding time since restoration, we only had three sites (12 plots) that were older than 20 years and this clearly causes some uncertainty regarding the effect of time in the later years after restoration. That said, we believe our results are solid and trustworthy.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. List of the high-quality-habitat indicator species from Fredshavn & Ejrnæs (2007) used in the study.

Figure S1. Predicted effects of the interactions and squared terms on (1) the likelihood of restored wetlands being classified by our model as any Annex I wetland habitat type (A, B, C, D).

Supplement S1. Testing multinomial logistic regression as an alternative to quadratic discriminant analysis.

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