Nibble, cut, stomp and burn

Biodiversity effects of disturbances in fen grassland

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Nibble, cut, stomp and burn: Biodiversity effects of disturbances in fen grassland

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Abstract

Aims: We assessed biodiversity effects of disturbance in meadows and rich fens. We hypothesized that disturbances and their timing affect plant and bryophyte species richness and other indicators of conservation success. More specifically, we expected conservation status to improve with disturbances that mimic natural grazing processes, that is, that extensive year-round grazing with trampling had a more positive effect than intensive summer grazing or mowing.

Location: Kastbjerg wetlands, Jutland, Denmark.

Methods: In a three-year field experiment, we applied trampling, season-specific defoliation with biomass removal and burning in a randomized design in nine wetland sites. We recorded species richness and community composition. Bayesian generalized linear mixed-effects models were built with treatment as fixed effect, site as random effect and species richness or species composition as responses. Leaf N and P, and soil moisture were included as covariables. Further, a quadratic discriminant analysis (QDA) was applied to test for discrimination between treatments based on a set of biodiversity indicators.

Results: Environmental and biotic differences among sites were considerable and significant indicating a considerable effect of historical contingency (local species pool). We found only minor and mostly insignificant effects of disturbance on vegetation. However, a QDA revealed significant differences among treatments based on five indicators for conservation status. Simulated grazing and trampling were generally associated with higher vascular plant richness, bryophyte richness, number of indicator species and stress-tolerant species and decreasing abundance of competitive species.

Conclusion: We found small, but positive effects of disturbance on biodiversity indicators of wetland vegetation after three years of experimental treatments. Initial site differences explained most variation, indicating strong historical contingency. Our results support the need for restoration of disturbances in fens and meadows, and the importance of prioritizing areas with near-natural biotas.

KEYWORDS

burning, conservation grazing, disturbance, grazing ecology, mowing, natural processes, riverine wetlands, species composition, species richness, year-round grazing
1 | INTRODUCTION

Open fens and meadows are characteristic wetland habitats listed on the EU Habitats Directive and targeted for conservation (European Commission 1992). Open fens are predominantly groundwater-fed, characterized by natural hydrology and low nutrient input, and host a large number of species including rare and threatened ones (Bedford & Godwin, 2003; Wassen et al., 2005; Grootjans et al., 2006; Van Diggelen et al., 2006). Historically, many European fens and meadows were slightly drained and used as pastures for grazing and/or haymaking (Van Diggelen et al., 2006). In particular since the mid-20th century, 80% of European wetlands have been degraded or lost due to abandonment and encroachment, eutrophication, agricultural intensification, including deep drainage, direct fertilization or conversion to rotational field (Middleton et al., 2006; Joyce, 2014; Verhoeven, 2014). The combination of agricultural intensification and lack of natural processes, notably natural hydrology and natural grazing processes, caused an increase in productivity, biomass and litter and a decrease in biodiversity — especially loss of characteristic stress-tolerant, small-statured fen vascular plant species at the expense of competitive and fast-growing species (Diemer et al., 2001; Joyce, 2014; Lamers et al., 2015). In general, Europe has been subject to an increase in vegetation density in the period 2001–2015 (Buitenwerf et al., 2018), most likely because of abandonment of extensive, traditional livestock husbandry. In Denmark, 17% of the semi-natural grasslands, including wet meadows and moors, are dominated by scrub due to lack of management and natural disturbance (Levin & Nainggolan, 2016) and the conservation status of Danish fens and meadows is reported as poor (Fredshavn et al., 2019). To restore open fens and recreate the natural habitats for endangered species, reintroduction of natural processes is necessary (Joyce, 2014). Restoring natural hydrological conditions and reintroducing natural disturbances or management alternatives such as burning, mowing or grazing are key to biodiversity conservation (Kotowski & van Diggelen, 2004; Andersen et al., 2013; Seer & Schrautzer, 2014; Lamers et al., 2015). Restoring natural hydrology may be necessary to gain the full positive effect of disturbances (Kołos & Banaszuk, 2013, 2018).

Disturbance improves the light conditions in fens and meadows and thereby changes competition among species. Low-statured species that would otherwise have been lost to asymmetric competition for light may benefit from extensive disturbances (Grime, 1973; Lepš, 1999) and reintroducing disturbance can cause a shift in species characteristics toward less competitive and more stress-tolerant species of herbs, bryophytes and sedges (Menichino et al., 2016).

Mowing and harvesting or hay-cutting is widely used in fen management and obviously removes biomass from the area and eventually nutrients will be depleted (Middleton et al., 2006; Török et al., 2021). In a long-term experiment, Sand-Jensen et al. (2019) found annual hay-cutting in fens to cause a drop in nutrient status (reflected by Ellenberg N values), an increase in light availability (reflected by Ellenberg L) and a drastic increase in vascular plant species richness. Because of the efficiency of nutrient removal, mowing may be preferred over grazing as a management tool (Joyce, 2014). Grazing, like mowing, improves light penetration, but grazing also creates bare soil for germination and establishment of vascular plants and bryophytes (Middleton, 2016) and higher habitat heterogeneity compared to mowing, because grazing is patchy in space and time (Bergamini et al., 2001; Middleton et al., 2006). Trampling is one of the noticeable differences between grazing and mowing and the effects of trampling can be prominent especially in wet habitats such as fens and meadows where trampling creates bare soil for germination and hummocks contributing to heterogeneity in microtopography.

Burning is a natural process also in fens and mires caused by lightning and may be a cost-effective alternative to mowing and grazing (Valkó et al., 2014). While a common management practice in the United States, burning has generally been viewed as destructive to drained peatlands in Europe (Middleton et al., 2006; Joyce, 2014). However, Pitkänen et al. (1999) found that mire fires in Finland happened naturally with approximately 100–600 year frequency in the Holocene and we consider it a natural, although uncommon, disturbance in fens and mires and also a relevant management practice. In addition to removing biomass, burning also removes accumulated litter but releases nutrients short-term during the burning process (Middleton et al., 2006). Burning and the resulting litter removal have been found to have a positive effect on wetland plant diversity because of the improved conditions for seed germination and establishment, for example, higher soil temperatures (Heim et al., 2021). However, the positive effect may be short-term (Heim et al., 2019). A review of the effects of prescribed burning on European grassland concludes that the frequency of burning is highly important as annual burning may cause a decrease in species richness and increased cover of competitive species (Valkó et al., 2014). Long-term, high-frequency burning has been found to increase the forb:graminoid ratio (Heim et al., 2019), a biodiversity indicator of pollinator resources and of good conservation status (Nygaard et al., 2019).

In this study, we examined the effects of simulated disturbances on species richness and community composition in a controlled field experiment along gradients of soil moisture and soil fertility in semi-natural and natural meadows and rich fens in Denmark. We consider whole-year grazing a natural ecological process, where large herbivores remove biomass year-round and also contribute to other ecosystem processes, including trampling. In the widespread lack of natural grazing processes, agri-environmental practices, that is, summer grazing (both intensive and extensive) and mowing replace the natural grazing process but presumably with a loss of some ecosystem processes. It is our hypothesis that the more natural, whole-year grazing has a more positive effect on biodiversity. Here, we simulate these three types of grazing from more natural whole-year grazing to intensive summer grazing. We also simulate trampling and use a multifactorial design that allows us to investigate the separate effects of trampling and “biting/eating” of the grazing process on vegetation. We evaluated the effects on total species richness and species richness of vascular plants, bryophytes and indicator species,
on two ordination axes and on the biodiversity indicators Grime C (competitive dominance in the plant community) and forb:gram-inoid ratio. We expected that any kind of reintroduced disturbance (summer-intensive/extensive grazing, year-round grazing, mowing and trampling) will have a positive effect on species richness across the environmental gradients, but that year-round grazing in combination with trampling is the treatment closest to mimicking natural grazing and therefore will have the most positive effect on species richness and biodiversity indicators. A wide range of management methods are used in contemporary nature restoration. However, more knowledge on the effect of the various types of management is still needed. Our assessment allows us to evaluate if disturbances are necessary for the restoration of natural meadows and rich fens; if grazing or mowing is more beneficial for biodiversity; and if low-density year-round grazing benefits biodiversity more than medium-density summer grazing. We expect plant communities to change in response to disturbances, and that site nutrient status and wetness will be key in determining the presence and immigration of indicator species of fens and mires, but also potentially the magnitude of impact from disturbances. Some of the study sites qualify as priority habitats of the European Habitats Directive (7230; alkaline fens and 6410: Molinia meadows, EU Habitats Directive 1992) and knowledge-based management recommendations are paramount to their conservation and to the restoration of degraded habitats currently not qualified as priority habitat.

2 | MATERIALS AND METHODS

2.1 | Study sites

Our study sites were located in Kastbjerg Ådal (river valley) in Eastern Jutland, Denmark (Figure 1a). It is within the Natura 2000 and appointed habitat area no. 223 because of the wide stretch of fens and mires, among other qualities. The water course is in good ecological status according to the Water Framework Directive. Nitrogen deposition in this area is low to moderate, 12.5–14.5 kg N/ha/yr (Ellermann et al., 2021). Meadows and fens dominate the study area, known for “the longest stretch of rich fen” in Denmark. Large parts of the river valley are heavily degraded by drainage, fertilization and scrub encroachment, but there have also been recent efforts to restore the watercourse and the valuable rich fens in the valley. Most fens and wet meadows have been abandoned and are now increasingly dominated by tall grasses, tall forbs and willow scrub, but summer grazing occurs in some areas and efforts are made to ensure grazing in the most valuable fens. The drier meadows are typically mown by heavy machinery. The sites were selected to represent gradients in soil moisture from moist to wet and gradients in nutrient status or productivity from poor to rich (Figure 1b) and included rich fens with characteristic species (3, 7), fens dominated by Juncus subnodulosus (8) and by Equisetum fluviatile (5), drained fens encroached by Phragmites australis (2) and natural meadows with characteristic species (1) and encroached by Epilobium hirsutum (9) and meadows characterized by clovers and cultural grasses (4, 6) (see also Appendix S2 for site species lists).

Due to the patchy nature of the fens in good conservation status, there was a trade-off between site area and homogeneity when selecting the study areas. We settled on nine sites of 10 m², each with 10 1-m² plots. The sites were selected to ensure within-site homogeneity and to cover the major gradients in soil moisture (from moist to wet) and fertility between sites. The 10 plots within each site had treatments assigned randomly. Despite the location in the same river valley, the sites were considered independent because of their different management history and starting conditions and a typical inter-site distance of approximately 225 meters. The experiment was established in June 2017 and treatments were repeated monthly during summer and bimonthly during winter, depending on treatment. Responses were recorded in July 2019.

2.2 | Experimental setup and treatments

Each of the nine sites were divided into 10 plots of 1 m × 1 m each with an inner square of 0.5 m × 0.5 m and a surrounding plot buffer zone with a control and the following treatments: burning, mowing, trampling, intensive summer grazing (SI), intensive summer grazing with trampling (SIT), extensive summer grazing (SE), extensive summer grazing with trampling (SET, year-round grazing (YR), and year-round grazing with trampling (YRT). Treatments were allocated randomly to each plot with the restriction that the control plot was always in one corner. The experiment was multifactorial with respect to grazing and trampling, whereas burning and mowing were stand-alone treatments. Initial biomass in each plot was estimated at the beginning of the experiment in June 2017 as follows: all standing biomass and litter was removed from the plots by manual cutting at the soil surface and following the microtopography. Bryophytes were harvested by hand plucking. Biomass, litter and bryophytes from the plot buffer zone were cut separately from the inner square. To estimate the species abundances, a representative sample of the inner square was sorted into litter and live biomass (including bryophytes) by species as sorting the complete biomass was not feasible. All species, litter and biomass from the buffer zone was dried at 55°C and weighed. Using the relative abundance of species in the representative sample and with respect to the weight of the total biomass in the inner square, we estimated the abundance of the species in the inner square.

Burning was simulated in March 2018 and 2019. We used wooden boards to shield and adjacent areas were watered before burning the focal plot with a gas weed burner. We burned on a calm day following a dry period with frost to ensure minimum risk of igniting underlying peat and fire spreading over the ground, but ensuring that the standing biomass and litter would be dry enough to ignite. This is not a simulation of a naturally occurring wildfire, but corresponds to the conditions that managers would prefer for prescribed conservation burning at larger scales. We simulated mowing as a biomass removal in June 2018. Biomass was removed uniformly
across the whole plot at a height of approximately 5 cm depending on microtopography. This corresponds to conservation mowing in management but without the added disturbance and pressure from machines. Trampling disturbance was applied using short stilts that could be attached to the field biologist’s boot. The surface of the stilt was 49 cm² which corresponds to a pressure of 1.3–1.5 kg/cm² with the added weight of the field biologist. This again corresponds to the pressure of a hoof of cattle weighing approximately 300–400 kg. Trampling was applied by stepping into the field randomly 60 times once every month from May to September and was the same treatment in combination with intensive, extensive and year-round grazing. Grazing was simulated by cutting the above-ground biomass using a 1-m² frame divided into a 10-cm coordinate system using the letters A–J on the x-axis and the numbers 1–10 on the y-axis. We cut tufts of biomass within the coordinate system using a list of random combinations of letters and numbers. This system enables “ungrazed” individuals to flower and set seeds. Based on our experience with grazing as an agri-environmental management practice in Denmark, we defined intensive summer grazing as taking place between May and September with the goal of removing all standing biomass by September. Extensive summer grazing also takes place from May to September, but we carried this out at half the intensity as intensive summer grazing. Year-round grazing obviously takes place during the whole year (here administered May–September and November, January and March) with the goal of removing all standing biomass by the end of winter (March) before the beginning of a new growing season. We used the initial standing biomass (June 2017) as a measurement of plot productivity and estimated the amount of biomass to be removed during “grazing” as approximately 20% of the initial productivity each month from May to
September in intensive plots and with all standing biomass “grazed” in September. For extensive plots, we estimated removed biomass as approximately 10% of the initial productivity each month from May to September, leaving some standing biomass in September. Year-round grazing biomass removal was estimated as approximately 10% of yearly productivity removed every month from May to September and in November and 20% removed in January and March resulting in no standing biomass at the end of the winter. As expected plot productivity changed as a result of the treatments, the amount of biomass removed had to be adjusted throughout the experiment. In practice, we aimed for removing twice the amount of biomass in intensive plots relative to extensive plots within the same site and always ensuring that no standing biomass was left in intensive plots in September, approximately 50% of the standing biomass was left in extensive plots in September and no standing biomass was left in year-round grazing plots in March (see actual removed biomass by treatment in Appendix S1). All treatments were applied to the whole plot (1 m × 1 m), while the biomass response was only measured in the inner square (0.5 m × 0.5 m), leaving a buffer zone between plots with different treatments.

2.3 | Response variables

A full plot (1 m × 1 m) species list was recorded in the field at the end of the experiment. From this total plot richness, vascular plant plot richness, bryophyte plot richness and number of indicator species per plot were calculated. Indicator species of conservation status are species considered moderately to very sensitive toward habitat degradation as defined by Fredshavn et al. (2010, see Appendix S3). Indicator species are often adapted to relatively infertile habitats revealed by low Ellenberg N values and high Grime’s S values reflecting tolerance to nutrient shortage.

Mean plot Grime’s C and S values (Grime et al., 1989) were calculated based on vascular plant species lists. We converted Grime’s life strategies to numerical values based on Ejrnæs and Bruun (2000).

We performed a non-metric multidimensional scaling analysis (NMDS) on the presence/absence of vascular plant and bryophyte species at the end of the experiment using the function “metaMDS” in R package vegan (Oksanen et al., 2017) in R version 4.0.3 (R Core Team, 2017), using Sørensen dissimilarity and a four-dimensional solution (k = 4). The plot coordinates at the three first NMDS axes were extracted (NMDS4 was discarded as noise) and these, along with the four richness variables as well as Grime’s C and S values, were used as response variables in linear mixed models (LME) as described in the subsection Statistical analyses.

Supplementary to regression models of single response variables we carried out a quadratic discriminant analysis (QDA) as described in the subsection Statistical analyses using the change in six indicators during the course of the experiment. The difference between plot species richness at the beginning and end of the experiment was calculated based on the species lists from sorted initial biomass and end biomass (0.5 m × 0.5 m). Start – end differences were also calculated separately for vascular plant species richness, bryophyte species richness, richness of indicator species, the ratio between biomass of forbs and graminoids (grasses, sedges and rushes) and Grime’s C and S mean site values.

2.4 | Explanatory and covariables

Leaf nitrogen, carbon and phosphorus were determined from plot-level sampling of leaf plates of grasses, that is, the most abundant species group across sites. Fresh leaf plates were collected at the beginning and end of the project and then dried, ground and analyzed in the lab. Soil moisture (% volumetric water content) was measured as the mean of four measurements per plot at the beginning and end of the project using a FieldScout TDR 300 Soil Moisture Meter (Spectrum Technologies, Aurora, USA).

The total number of species found at each site was used as a covariable in species richness models reflecting the local species pool.

2.5 | Statistical analysis

All modeling was performed in R version 4.0.3 (R Core Team, 2017). Data exploration prior to modeling followed the protocol in Zuur et al. (2010). The variables local pool, average moisture level and N/P ratio were standardized by subtracting the mean and dividing by the standard deviation before modeling.

Generalized linear mixed modeling (GLMM) was used to assess the effect of treatments, moisture and nutrients on a series of response variables. To account for local differences among sites and the geographically nested design we used site (n = 9) as random intercept. We used a Poisson distribution and log link function for species richness responses and a Gaussian distribution for NMDS ordination coordinates. We checked for overdispersion. The GLMM models were specified as follows: The local species pool was used as covariable in the four species richness models; average moisture level at end and N/P ratio in leaves at beginning were used as explanatory variables and allowed to interact with the treatment variable (factor variable with nine levels: burning [F], mowing [M], trampling [T], intensive summer grazing [SI], SI with trampling, extensive summer grazing [SE], SE with trampling, year-round grazing [YR], and YR with trampling). GLMM models were fitted using approximate Bayesian inference in the inla package (Rue et al., 2009). Marginal posterior distributions were summarized by 95% Bayesian credible intervals (BCI) corresponding to the 0.025 and 0.975 quantiles of the posterior distribution (Zuur et al., 2017). We did not use model selection but considered variables to be statistically important if the 95% BCI did not overlap zero (Zuur et al., 2017). To compare model predictions we calculated Bayesian $R^2$ values using the “r2_bayes” function in the performance package (Lüdecke et al., 2021). We verified model assumptions by plotting residuals versus fitted values.
We used QDA, a classification method, to test for significant discrimination of treatments based on a selected set of biodiversity indicators (function “qda” in the R package MASS – Venables & Ripley, 2002) and using leave-one-out cross-validation. The set of biodiversity indicators included changes (over the course of the experiment) in mean plot Grime’s S, mean plot Grime’s C, number of indicator species, forb:graminoid ratio, bryophyte species richness and vascular plant species richness.

3 | RESULTS

3.1 | Environmental variation and biomass

N/P ratio ranged from 0.6 to 2.3 at the beginning of the experiment and 0.4 to 2.3 at the end (Pearson correlation: 0.70, $t = 9.16$, $df = 89, \ p < 0.001$), while plot mean soil moisture ranged from 48.8% to 82.9% volumetric water content at the beginning of the experiment and 47.8% to 84.0% at the end (Pearson correlation: 0.70, $t = 9.12$, $df = 88, \ p < 0.001$). Thus, moisture and nutrient levels did not change significantly during the experiment. Start values were used for analyses. The 10 plots within each of the nine sites were similar regarding moisture and nutrient levels, indicating homogeneity within sites (Figure 2). The nine sites covered the variation in moisture and nutrient status along the gradients they were selected to represent (Figure 2). The initial biomass across sites varied according to environmental variation but was also heavily influenced by management history. The biomass harvested across treatments varied with intensity and timing of disturbances, that is, mowing and extensive summer grazing removed the least biomass and summer-intensive and year-round grazing removed the most biomass. Harvested biomass decreased when defoliation treatments were combined with trampling (Table 1).

3.2 | Species richness and species composition

The number of plant species in plots of 1 m × 1 m ranged from 11 to 41, with vascular plant richness ranging between nine and 35 species and bryophyte richness from one to eight species. The number of indicator species in each plot ranged from one to 24. The most abundant vascular plant species were Festuca rubra (found in 83 of 90 plots), Cirsium palustre (77 plots), Poa trivialis (69 plots) and Lotus pedunculatus (63 plots) while the most abundant bryophytes were Brachythecium rutabulum (77 plots) and Calliergonella cuspidata (71 plots).

The ordination based on vascular plant and bryophyte presence/absence (final stress = 0.09) accounted for 83.5% of the variation in species composition, when correlating the original distance matrix with distances in ordination space. Axis 1 (NMDS1) captured 45.6% of the variation, axis 2 (NMDS2) 19.0% and axis 3 (NMDS3) 15.9%. Moisture, N/P ratio, Grime’s C and S were highly correlated with NMDS1, while Grime’s C was correlated with NMDS2 and NMDS3 (Appendix S4).

3.3 | Effects of treatment, moisture and nutrients

When using GLMMs to model species richness and composition the effects of treatments were absent or small at most. The local species pool was significant in all GLMM models. In models using vascular plant species richness, bryophyte species richness, number of indicator species, mean Grime’s S or NMDS1 as response variable, treatments were not significant. For forb:graminoid ratio, the interaction between moisture and fire was significant. For Grime’s C, all treatments except burning were significant as well as the interaction between intensive summer grazing with trampling and moisture and extensive summer grazing and moisture (Figure 3). For NMDS2 the interaction with moisture and intensive summer grazing as well as year-round grazing with trampling were significant. For NMDS3 burning, mowing, extensive

![Figure 2](image-url) Productivity (total live standing biomass, g) at the beginning of the experiment (size of points) along the gradient of leaf N (% leaf N, x-axis) and soil moisture (%Volumetric Water Content, y-axis). Colors indicate the nine sites.
grazing with trampling and year-round grazing were significant as well as the interaction between trampling and moisture (Figure 3).

For the four models in which treatments were significant the random variable (site) explained more variation than the fixed variables (range: 8.2%-22.6%).

### 3.4 | Change in biodiversity indicators

The QDA shows that there is a general and highly significant pattern in the changes in biodiversity indicators from start to end of the experiment that allows the plots to be grouped according to the treatments (cross-validated Pearson's chi-squared test: $\chi^2 = 234.1$, $df = 81$, $p$-value = 4.476028e−14). Overall, the QDA was successful in predicting treatments (accuracy rate = 53.2%, Table 2).

The means of the biodiversity indicators used in the QDA generally indicated a positive effect of disturbance on biodiversity. Grazed and trampled plots were generally characterized by increased stress tolerance, vascular plant species richness, bryophyte species richness and number of indicator species (Figure 4). The control, that is, leaving the vegetation undisturbed, consistently had a negative effect on all biodiversity indicators except for a small positive effect on bryophytes. Contrary to our expectations grazing and trampling did not increase the forb:graminoid ratio, except for a minor increase for year-round grazing with trampling.

### 4 | DISCUSSION

Open fens and meadows are of high conservation value and several habitats are acknowledged and protected under the EU Habitats Directive. However, the lack of natural processes such as grazing, together with eutrophication and land-use intensification, has led to poor conservation status in more than 50% of European fens, mires and bogs (European Commission 2020).

Encroachment of shrubs and tall herbs is a widespread symptom of lack of natural disturbances and currently, two remediation trajectories are in play: (1) encroachment can be countered by agri-environmental practices such as mowing and summer grazing; or (2) it can be mitigated by ecological restoration or rewilding that aims to restore natural ecological processes to create self-sustaining biodiversity ecosystems (Perino et al., 2019). Our experimental treatments were designed to span from the absence of disturbance (control) to agri-environmental practices, such as mowing, prescribed burning and summer grazing, to restoring natural grazing by year-round grazing at natural densities.

### 4.1 | Local species pool versus treatment effects

The local species pool was highly important for species richness and combined with the overall variation in moisture and nutrient contents across sites this overshadowed the effect of treatments, supporting the species pool theory (Zobel, 1997, 2016) or Hubbell's ideas of functional redundancy and prevailing neutral effects in plant communities (Hubbell, 2005). The strong effect of historical contingency should therefore be taken into account when planning and evaluating restoration projects. We found only minor (and few significant) effects of treatments on vegetation in GLMM models after three years. This contradicts previous studies with significant effects in field experiments involving disturbances in grassland and dune experiments of similar duration (Ejrnaes et al., 2006; Brunbjerg et al., 2014). The delayed response of the system could be attributed to the dominance of long-lived perennial plant species that respond
(a) Total species richness
Total $R^2=75.1$ (68.5–81.0)
Fixed $R^2=64.3$ (54.4–71.9)

(b) Vascular plants
Total $R^2=73.6$ (66.0–79.3)
Fixed $R^2=62.8$ (53.0–71.0)

(c) Indicator species
Total $R^2=84.8$ (80.4–88.5)
Fixed $R^2=27.0$ (10.6–43.7)

(d) Bryophytes
Total $R^2=78.1$ (49.0–66.7)

(e) Grime C
Total $R^2=93.9$ (92.7–95.1)
Fixed $R^2=8.2$ (5.4–11.2)

(f) Forb:graminoid ratio
Total $R^2=71.8$ (66.5–76.9)
Fixed $R^2=22.6$ (15.0–30.7)

(g) NMS2
Total $R^2=91.2$ (89.3–92.8)
Fixed $R^2=9.2$ (5.1–14.3)

(h) NMS3
Total $R^2=88.6$ (86.1–90.8)
Fixed $R^2=10.5$ (6.0–15.4)
slower to changes and the short timeframe for noticeable effects of nutrient depletion through the disturbances of mowing and grazing. Also, all plots were subjected to an initial biomass harvest intended to have all sites starting equal. This implied a total biomass harvest for weighing and sorting, but it also removed the most obvious effect of disturbance on abandoned tall-herb vegetation.

Comparable studies have been able to detect changes in species richness after four and six years of treatment (Lepš, 1999; Liira et al., 2009). In order to fully understand the long-term effects of disturbances and to make recommendations for restoration we need to do long-term experiments and monitoring (Hellström et al., 2006; MacDougall & Turkington, 2007; Sand-Jensen et al., 2019; Török et al., 2021). The reverse process — that wetland vegetation changes after abandonment — has been detected after three years (Joyce, 2014).

### 4.2 Grazing and trampling

Grazing may benefit bryophytes due to the removal of biomass and reduced accumulation of litter causing an increase in light availability and areas of bare soil to be colonized by bryophytes or other less competitive species (Boch et al., 2018). Trampling in this experiment was not unambiguously positive, nor was the added effect of trampling to grazing. Trampling is important, however, in making room for small-statured vascular plants and bryophytes because of the disturbance of the soil surface and the creation of bare soil (Boch et al., 2018). We only trampled during summer months, even in the year-round grazing treatment with trampling (YRT), for a consistent trampling treatment across the experiment. We hypothesize that winter trampling may affect grasses differently than summer trampling. Grasses as well as forbs are highly tolerant of grazing, but a recent study highlights the difference between tolerance in terms of growth rate and tolerance in terms of fitness (Bråthen et al., 2021). Bråthen and coworkers hypothesized that grasses increase their growth (biomass) in response to disturbance, whereas forbs increase in richness. This corresponds to the mostly negative change in forb:brominoid biomass that we see in response to grazing as well as a positive change in species richness and characteristic species (mostly forbs). The effect of grazing on grass cover may also depend on site nutrient conditions as suggested by Henning et al. (2017), who observed a decrease in grass cover and an increase in forb cover in nutrient-poor dry grassland sites after seven years of low-intensity year-round grazing. Lastly, undisturbed vegetation may favor long-lived forbs that store nutrients compared to grasses, as has been found in calcareous grasslands (Köhler et al., 2005).

### TABLE 2 Contingency table based on predictions from the cross-validated (leave-one-out) quadratic discriminant analysis with treatment as response variable and a set of indicators for conservation as predictor variables. Each row presents how many sites of each treatment (Observed) were predicted to belong to the different treatments (Predicted)

<table>
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<th>C</th>
<th>F</th>
<th>M</th>
<th>T</th>
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<th>SE</th>
<th>YR</th>
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Note: Indicators for conservation: difference (experiment_end – experiment_start) in Grime’s S, Grime C, richness of indicator species, forb:graminoid ratio, richness of bryophytes and richness of vascular plants as explanatory variables. Treatments: control (C), burning (F), mowing (M), trampling (T), intensive summer grazing (SI), extensive summer grazing (SE), year-round grazing (YR), intensive summer grazing with trampling (SIT), extensive summer grazing with trampling (SET), year-round grazing with trampling (YRT).
characteristic, stress-tolerant species (often less competitive and small forbs) appear to have increased in the grazed and trampled plots, their biomass was probably small and “unmeasurable” compared to that of grasses.

Contrary to the simulated grazing used in this experiment, natural grazing by large herbivores is selective, causing a heterogeneous grazing impact including patchy deposition of dung and urine, trampling and variation in grazing intensity and vegetation structure contributing to biodiversity at scales larger than the plot size of this experiment (Dumont et al., 2007; Mikola et al., 2009; Gilhaus et al., 2014). These differences may also have contributed to the difficulties in discerning and ranking the effect of various levels of grazing intensities. The inclusion of other biodiversity indicators for conservation success, such as pollinator richness or richness of insect herbivores, may further increase our understanding of the biodiversity effects of disturbances. For example, mowing and intensive grazing may negatively affect flower abundance and consequently the abundance and richness of pollinators (Tadey, 2015; Lázaro et al., 2016; Johansen et al., 2019). Likewise, heterogeneity of vegetation structure as found in low-intensity grazing may benefit butterflies and grasshoppers (Jerrentrup et al., 2014).

4.3 | Mowing

Contrary to expectations (e.g., Liira et al., 2009), we found no significant effect on forb:graminoid ratio by treatments in GLMMs, but a high positive change in forb:graminoid ratio in the mown plot in the QDA. Mowing has traditionally been used in fen and meadow management as it provides winter fodder for animals and the extensive mowing with light machinery or by scythe can reduce encroachment and have a positive effect on biodiversity. However, mowing is increasingly done by heavy machinery that creates a more homogeneous vegetation and destroys microtopography (Kotowski et al., 2013). Mowing often implies harvesting and 100% removal of biomass as opposed to grazing where the main part of the nutrients in the consumed biomass (N and P ~ 75%, K ~ 80%–90%) is returned to the area as dung (Petersen et al., 1956). This implies opportunities for depleting excess nutrients by mowing but also a removal of plant biomass from the ecosystem and thus also host plants and flowers for insect herbivores and pollinators (Johansen et al., 2019). Our mowing simulation was very extensive and conserved microtopography. Modest biomass removal in the growing season may have favored competitive forbs over graminoids as indicated by the relatively high values for Grime’s C and low values for Grime’s S of the mown plots. The positive effect of mowing on forb:graminoid ratio support the finding of Valkó et al. (2012), that mowing supports the conservation of characteristic meadow forbs, but contrasts the experience that many machine-mown meadows stand out as grass-dominated.

Other studies have shown the effect of disturbance (mowing) to depend on the original plot species richness (Menichino et al., 2016). Menichino et al. (2016) found species-poor sites to respond
to mowing with a reduction of shrub and grass cover and an increase in species richness, while no effect on species richness was found in species-rich sites. Hellström et al. (2004) found no effect of mowing on species richness in dry meadows. They suggested that the lack of grazing herbivores acting as dispersal vectors also meant a lack of seed dispersal. The same may be true in our study, as simulated grazing does not provide any seed dispersal.

Comparative studies of the effect of grazing and mowing on plant species richness are ambiguous. Some studies find grazed fens to have higher bryophyte richness than mown fens (Bergamini et al., 2001) while the results of other studies assessing vascular plants and bryophytes are opposite (e.g., Stammel et al., 2003) or with no difference in species richness or number of endangered species between mown and grazed sites (Seer & Schrautzer, 2014). Such ambiguous results also reflect differences in environmental conditions, management history and treatments applied.

4.4 | Burning

Burning appeared to be negatively associated with indicator species and otherwise without strong links to indicators. Despite a low management cost, prescribed burning is only rarely used for fen management in Europe. Our results are in line with those of Liira et al. (2009) who found spring burning, as used in our study, to have very small effects on plant species richness and sward height. Summer burning may have much stronger effects but is also more challenging to apply and control in practice.

5 | CONCLUSIONS

In this study we tested the hypothesis that restoring natural processes in fens and meadows increases biodiversity, that is, richness of vascular plants, bryophytes and indicator species, and changes plant community composition to improve conservation status. We did not find a clear pattern supporting the hypothesis that the more natural, simulated year-round grazing treatment would lead to improving biodiversity indicators over, for example, intensive summer grazing or mowing. The majority of variation in species composition could be attributed to variation among sites and treatments were not significant for species richness measures. The disturbances had significant effects on species composition (notably NMDS3 as response variable in GLMMs) and the biodiversity indicator mean values revealed that simulated grazing and trampling were generally associated with higher vascular plant richness, bryophyte richness, number of indicator species and stress-tolerant species and decreasing abundance of competitive species. The results also indicate a competitive release (Grime, 1973) with disturbance causing a decrease in mean Grime’s C values (Figure 3) that over time could have led to more noticeable changes in species richness and composition. Given the overall positive effects of disturbance, but also the differences in effects between treatments, this study indicates that biodiversity would benefit from a restoration of natural processes where disturbances are patchy and heterogeneous in space and time.

The weak effect of treatments may be linked to the relatively short duration of the study. We recommend more than three years of treatment in field studies of disturbance effects in wetlands to increase the chance of detecting significant ecological responses.

AUTHOR CONTRIBUTIONS

Rasmus Ejrnæs, Hans Henrik Bruun and Camilla Fløjgaard conceived the research idea and study design. Camilla Fløjgaard, Louise Juhl Lehmann and Ane Kirstine Brunbjerg collected the field data. Lars Dalby curated data. Rasmus Ejrnæs, Camilla Fløjgaard and Ane Kirstine Brunbjerg planned and performed the analyses. Rasmus Ejrnæs and Ane Kirstine Brunbjerg led the writing of the paper. All authors contributed to writing and revising the paper.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data set is available at 10.5281/zenodo.6500628.

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REFERENCES


SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

Appendix S1. Biomass removed monthly by simulated grazing in treatments summer extensive (SE), SE with trampling (SET), summer intensive (SI), SI with trampling (SIT), year-round grazing (YR) and YR with trampling (YRT).

Appendix S2. Site species list.

Appendix S3. Indicator species of open habitat types moderately to highly sensitive to habitat changes as defined by Fredshavn et al. (2010).

Appendix S4. Non-metric multidimensional scaling (NMDS) ordination for vascular plant and bryophyte presence/absence at the end of experiment.