











## RESEARCH ARTICLE

# Scrub encroachment promotes biodiversity in temperate European wetlands under eutrophic conditions

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**Abstract**

Wetlands are important habitats, often threatened by drainage, eutrophication, and suppression of grazing. In many countries, considerable resources are spent combatting scrub encroachment. Here, we hypothesize that encroachment may benefit biodiversity—especially under eutrophic conditions where asymmetric competition among plants compromises conservation targets. We studied the effects of scrub cover, nutrient levels, and soil moisture on the richness of vascular plants, bryophytes, soil fungi, and microbes in open and overgrown wetlands. We also tested the effect of encroachment, eutrophication, and soil moisture on indicators of conservation value (red-listed species, indicator species, and uniqueness). Plant and bryophyte species richness peaked at low soil fertility, whereas soil fertility promoted soil microbes. Soil fungi responded negatively to increasing soil moisture. Lidar-derived variables reflecting the degree of scrub cover had predominantly positive effects on species richness measures. Conservation value indicators had a negative relationship to soil fertility and a positive to encroachment. For plant indicator species, the negative effect of high nutrient levels was offset by encroachment, supporting our hypothesis of competitive release under shade. The positive effect of soil moisture on indicator species was strong in open habitats only. Nutrient-poor mires and meadows host many rare species and require conservation management by grazing and natural hydrology. On former agricultural lands, where restoration of infertile conditions is unfeasible, we recommend rewilding with opportunities for encroachment toward semi-open willow scrub and swamp forest, with the prospect of high species richness in bryophytes, fungi, and soil microbes and competitive release in the herb layer.

**KEYWORDS**

biodiversity conservation, eDNA, fen, meadow, nutrients, succession

**TAXONOMY CLASSIFICATION**

Biodiversity ecology, Botany, Community ecology, Conservation ecology, Restoration ecology

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## 1 | INTRODUCTION

Open fens and meadows are characteristic wetland habitats listed on the EU Habitats Directive and targets for conservation (Council Directive 92/43/EEC, 1992). They are species-rich and host large numbers of rare and threatened species (Bedford & Godwin, 2003; Grootjans et al., 2006; van Diggelen et al., 2006; Wassen et al., 2005). Since the mid-20th century, 80% of European wetlands have been degraded or lost due to, for example, encroachment following abandonment of traditional extensive grazing, and eutrophication (Joyce, 2014; Middleton et al., 2006; Verhoeven, 2014). Scrub encroachment is part of the natural succession process; open habitats grow into a late successional forest in the absence of disturbances, such as lightning-ignited fire, flooding, and grazing (e.g., Bond et al., 2005; Van Wieren, 1995; White, 1979). However, because of human interference, natural disturbances have diminished overall (e.g., Brunbjerg et al., 2014; Middleton et al., 2006; Scholes & Archer, 1997) and the resulting succession has caused widespread scrub encroachment across habitat types and biomes from savannas and steppes to arctic tundra (Naito & Cairns, 2011). In Europe, an increase in vegetation density in the period 2001–2015 has been documented and the vegetation change is likely caused by woody regrowth after the abandonment of livestock grazing (Buitenwerf et al., 2018). Likewise, in Denmark, 17% of the area registered as a meadow in 1992 has now undergone encroachment (Levin & Nainggolan, 2016), however, most of the historical encroachment has happened in the period 1945–1992 (Finderup Nielsen et al., 2021). The pattern is likely to be the same for fens and meadows. When fens and meadows are overgrown with scrub, they lose their legal EU Habitats Directive protection until the scrub eventually grows into late successional swamp forest, which is also protected by the directive (bog woodland 91D0 or Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* 91E0). In Denmark, nearly 27 million € are spent annually on agri-environmental supplements for livestock grazing and mowing in nature areas to combat encroachment and conserve open habitats (Ministry of Food and Environment, 2015). Despite the effort, only approximately 20% of the semi-natural grasslands, including wet meadows and moors, are currently under active management (Ejrnæs et al., 2021). This means that most mires are abandoned and subject to free succession. Besides the abandonment of extant fens and mires, many historical fens and meadows have been actively drained, fertilized, and ploughed and are today arable fields and leys. As part of the green transition, a large share of this low-lying farmland is projected to be abandoned and rewetted to avoid further carbon loss from the organic soils. While abandonment from agriculture implies a potential for biodiversity, these areas often have large nutrient pools and strongly modified hydrology due to decades of agricultural use. Eutrophication is a threat to species-rich open meadow plant communities due to severe asymmetric competition among plant species increasing with high soil fertility (Grime, 1973; Wassen et al., 2005). However, increased shading from encroachment may be hypothesized to relax competition for light among herbs and reduce the competitive exclusion in the

field layer as compared to open meadows. Grazing may also partly counterbalance the negative effects of eutrophication (Brunbjerg et al., 2014), but is unlikely to fully compensate (Ejrnæs et al., 2006). Moreover, the full positive effect of disturbances may depend on the restoration of natural hydrology (Kołos & Banaszuk, 2013, 2018). The combined effects of nutrients, hydrology, and disturbance regimes in restored wetlands are difficult to predict, but recent studies indicate that the transformation from arable fields to wetlands often fails to restore the species-rich vegetation consisting of stress-tolerant forbs and bryophytes characteristic for wetlands (Baumane et al., 2021; Kreyling et al., 2021).

Wetland-restoration success is often evaluated on the basis of plants and birds, while important knowledge obtained from other organism groups, for example, arthropods and fungi, is ignored. In fact, the diversity of heterotrophic organisms, such as arthropods and fungi, is expected to increase with the structural complexity of vegetation and diversity of carbon sources in ecosystems (Brunbjerg et al., 2017; Elton, 1966; Pihlgren & Lennartsson, 2008). In a recent large-scale study, the presence of a shrub layer was the most important variable explaining variation in species richness of fungi and arthropods (Brunbjerg et al., 2020). Heterotrophic organisms gain from the increased biomass following encroachment, as shrubs provide resources and habitats for a large suite of species including herbivores, pollinators, decomposers, and epiphytes (Bruun et al., 2022).

In meadows, eutrophication and groundwater level may control the encroachment process and yield different vegetation structures at different combinations of hydrology and eutrophication. Field observations had led us to hypothesize that thickets and woodlands on wet, nutrient-poor soils grow more heterogeneous in structure, leaving many canopy openings, compared to thickets and woodlands on more nutrient-rich and/or less wet soils. This complexity of vegetation structure can be measured using light detection and ranging (lidar), which is a cost-effective way of gaining fine-resolution data on vegetation structure as compared to field measurements (Lefsky et al., 2002) and which has been shown to capture aspects of vegetation structure that are important and otherwise overlooked for biodiversity (Moeslund, Zlinszky, et al., 2019; Thers et al., 2019). A range of variables representing vegetation structure can be derived from lidar, although the translation to and correlation with well-understood properties is not always straightforward. Despite this, lidar has been used to get detailed information on shrub biomass and cover and has been found to be a “promising tool to map and monitor grassland shrub dynamics at the landscape scale” (Madsen et al., 2020).

In this paper, we investigate the variation in biodiversity along gradients of soil moisture, soil fertility, and scrub cover. We further test the hypothesis that the occurrence of indicators of high conservation value can be promoted by allowing encroachment to take place—especially in restored wetlands on highly eutrophic former agricultural soils. We suggest two mechanisms for such a positive effect: (a) shrubs and trees provide habitat and food resources for large numbers of heterotrophic species, (b) a shrub and tree layer may invoke a competitive release in the herb layer reducing the competitive exclusion of typical wetland plants.

## 2 | METHODS

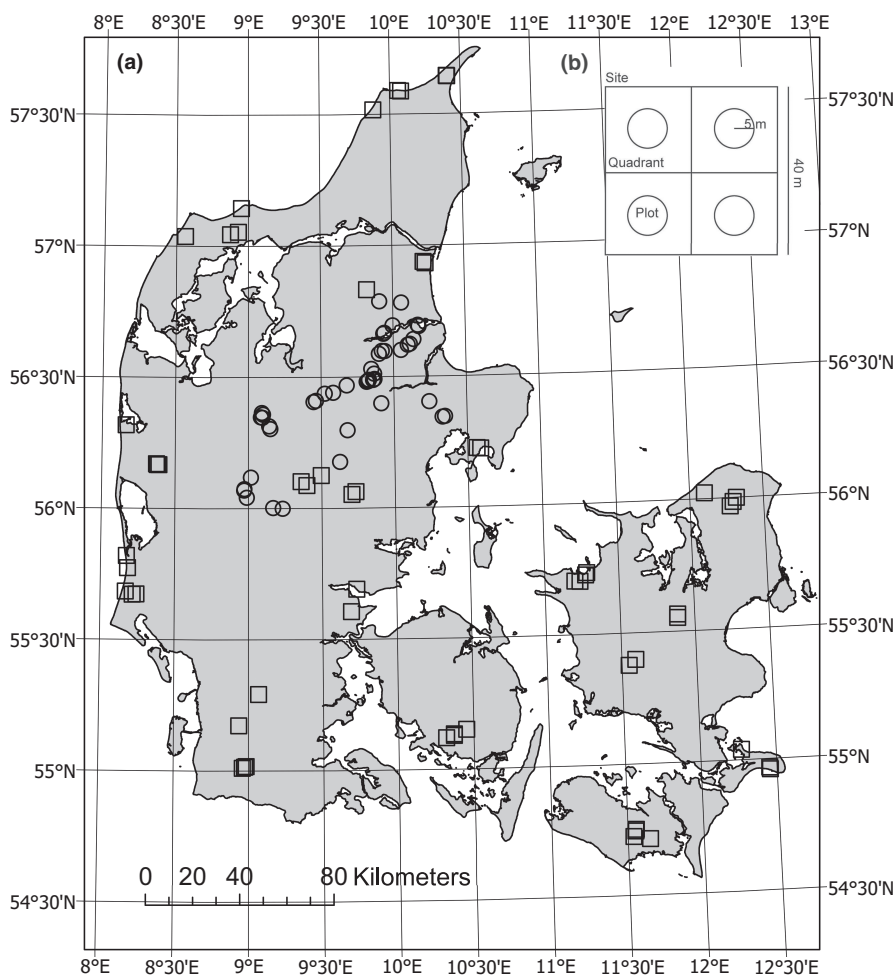
As part of the present study, we conducted field inventories at 44 wetland sites. The data collection was designed to supplement data collected in the Biowide project, a nation-wide survey of biodiversity in Denmark (Brunbjerg et al., 2019). Biowide included a total of 130 study sites (40×40m), of which we included all 58 sites evaluated as moist or wet based on plant species composition and soil moisture measurements. The Biowide sites varied in woody species cover from open vegetation over heterogeneous scrub to closed-canopy forest. The Biowide sites also varied in nutrient status from infertile to fertile soils, but were selected to foremost include natural and semi-natural habitats. The additional 44 sites were chosen to increase data coverage of former agricultural land, semi-natural meadows, and agriculturally improved meadows, as well as different levels of scrub encroachment. We did a stratified selection of sites according to succession/light availability (open, tall herb, shrubs, closed canopy), nutrient status (high/fertilized, mid, low/natural), and soil moisture (moist, wet). Sites were located across Denmark, preferably with a minimum distance of 500m between sites (except two set of sites, where distances were 252m and 491m, Figure 1a). The geographic dispersal of sites ensured sites from both carbon-rich soils, clay, and sand. Each site (40×40m)

consisted of four 20×20m quadrants, each with a 5m circular plot in the center (Figure 1b).

To illustrate the coverage of the soil moisture, nutrient, and encroachment gradients covered by the combined data, we compared site mean Ellenberg F, N, and L values (Ellenberg et al., 1992) for all 5m circle plots with a reference dataset from national monitoring, using identical 5m circular plots (59,227 sites from agricultural, semi-natural, natural open, and forest vegetation <http://www.naturdata.dk>) (Nygaard et al., 2017). Mean Ellenberg values were calculated for plots with more than five species present. In scatterplots of plot-mean Ellenberg values, 95 percentile convex hull polygons were drawn for the reference dataset as well as the Biowide and wetland dataset to visually evaluate the representativity of our data (Appendix A).

### 2.1 | Biowide data collection

Collection of vascular plants and bryophyte presence/absence data: Vascular plants and bryophytes were inventoried by a trained botanist and exhaustive lists for the four 5m circle plots were made for each site. In addition, all additional species in the quadrat, but outside the 5m circles, were recorded. The inventory was done in summer



**FIGURE 1** (a) Map of Denmark showing the location of the 102 sites (circle: wetland sites,  $n = 44$ ; square: Biowide sites,  $n = 58$ ). (b) Site layout with four 20×20m quadrants each containing a 5m radius circle (plot).

2014 with a few early spring vascular plant species added in May 2015 (Brunbjerg et al., 2019). We aggregate the four 5 m circle plots and additional species list to obtain a site species list for analyses.

### 2.1.1 | Collection of soil eDNA data

As alternative measures of biodiversity, we used the richness of operational taxonomic units, that is, OTUs (Blaxter et al., 2005) of fungi and (eukaryotic) soil microbes obtained from metabarcoding of soil-extracted eDNA (Frøslev et al., 2017, 2019). We collected soil samples from all sites for the eDNA inventory. Samples were taken in October–November 2014. At each site, we sampled 81 equally distant soil samples from the top c. 15 cm and pooled them after the removal of coarse litter. We homogenized the soil by mixing with a drilling machine mounted with a mixing paddle. A subsample of soil for DNA extraction and metabarcoding was taken from the homogenized sample.

### 2.1.2 | Soil moisture

Soil moisture was measured using a FieldScout TDR 300 Soil Moisture Meter. Sixteen equally distanced measurements were taken in each 40×40 m site in May 2016 (spring/wet period). To cover the temporal variation in moisture the measurements were repeated in August 2016 (summer/dry period) (Brunbjerg et al., 2020).

## 2.2 | Wetland sites data collection

All additional data collection specifically for the present study was done according to Biowide protocols (Brunbjerg et al., 2019), with the exception of the following: (1) presence/absence of early-spring plants species was not recorded on a separate visit, (2) soil samples were collected during the plant inventory in July–August 2018, that is, not in November. Soil moisture was measured as in the Biowide project in July–August 2018.

The present dataset spans 102 sites covering most types of wetlands including agriculturally improved meadows, natural meadows, fens, bogs, reed swamps, meadows dominated by large herbaceous perennials, open wetlands with scattered willows and birches, willow thickets, birch forests, and swamp forests (Appendix B).

## 2.3 | DNA extraction and metabarcoding

For Biowide and wetland soil samples, DNA was extracted and subjected to eDNA metabarcoding through DNA extraction, PCR amplification of genetic marker regions (DNA barcoding regions), and massive parallel sequencing on the Illumina MiSeq platform as described in Brunbjerg et al. (2019). For this study, we used high-throughput sequencing data from marker genes amplified with primers targeting eukaryotes (mainly soil microbes) (18S) and fungi (ITS2).

OTU tables were constructed following the overall pipeline in Frøslev et al. (2017). For both fungi and eukaryotes, this consisted of an initial processing with DADA2 (ver. 1.8) (Callahan et al., 2016) to identify exact amplicon sequence variants (ESVs) including removal of chimeras. The preparation of the Biowide-fungal (ITS2) and Biowide-soil microbe (18S) eDNA datasets have been published in Fløjgaard et al. (2019) and Frøslev et al. (2019), respectively, although the fungal dataset was re-sequenced for this study (a detailed description of the sequencing data can be seen in Appendix C).

## 2.4 | Lidar-based measures

We calculated 23 lidar variables to represent encroachment in the 102 sites. We used the same procedure as in Valdez et al. (2021). The calculations were based on the National lidar-based point cloud (recorded leaf-off, springs, and autumns 2014–2015; light wavelength: 1550 nm; point density = 4–5 points/m<sup>2</sup>, vertical uncertainty: 5–10 cm) that is freely available from [www.dataforsyningen.dk](http://www.dataforsyningen.dk). The lidar point cloud was converted to a canopy height model by subtracting the terrain model (DTM) from the surface model (DSM). The final set of variables had a resolution of 1.5 m (except one, see below). For all lidar processing and calculation, we used the OPALS tools (Pfeifer et al., 2014) version 2.3.1 in a Python 2.7 environment, and we used the supplier classification of points into terrain and vegetation that came with the dataset originally. The means and standard deviations of the following lidar variable were calculated for a 30 m radius circle centered in each study site to reflect actual levels and their variability within sites and their immediate surroundings. For further details on calculation of lidar variables, see Valdez et al. (2021). The set of lidar variables encompassed: potential solar radiation (mean and std), adjusted solar radiation (i.e., solar radiation adjusted for vegetation cover; mean and std), amplitude (uncalibrated, but corrected for aircraft type and seasonality, see Valdez et al., 2021), vegetation height (mean and std), vegetation cover (mean, std), mean vegetation density at 0–100 cm, 1–3 m, 3–10 m, and 10–50 m, canopy openness (mean, std), terrain openness (mean, std), terrain slope (mean, std), echo ratio (i.e., canopy complexity; mean, std), heat load (std), and mean fine-scale (0.5 m) terrain roughness (Appendix D).

## 2.5 | Response variables

### 2.5.1 | OTU richness

As alternative measures of biodiversity, we used the richness of operational taxonomic units, that is, OTUs (Blaxter et al., 2005) of fungi and soil microbes from metabarcoding of soil eDNA (Frøslev et al., 2017, 2019). Classical data collection of fungi is time consuming and OTU richness has been found to resemble classical observed species richness at least for groups of macrofungi that are feasible to include in field inventories (Frøslev et al., 2019). We used OTU richness of fungi and soil microbes as response variables to reflect diversity of species groups not monitored otherwise in this project.

**Red-listed species:** Site richness of red-listed species (belonging to the categories *Critically endangered*, *endangered*, *vulnerable*, *near-threatened*, and *data deficient*) was calculated for vascular plants and bryophytes based on the current national red list (Moeslund, Nygaard, et al., 2019, [Redlist.au.dk](https://redlist.au.dk)).

**Indicator species:** Indicator species include vascular plant species considered moderately to very sensitive to habitat alteration (Fredshavn et al., 2010, see [Appendix E](#)). The list of indicator species (Fredshavn et al., 2010) was developed to indicate favorable conservation status according to the Habitats Directive (European Commission, 2007). Common to these indicator species is a preference for infertile habitats (low Ellenberg N and high Grime's S values, Grime, 1979).

**Biotic uniqueness:** Uniquity is a scale-dependent metric of biodiversity reflecting how unique the biodiversity of a given site is compared to the gamma diversity across the containing region or collection of sampled sites (Ejrnæs et al., 2018). Uniquity can be calculated based on both observational data as well as non-annotated DNA data (e.g., OTUs) and hence can reflect both species uniqueness and genetic uniqueness. Contrary to other biodiversity metrics, uniqueness accounts for sampling bias and spatial scale. Due to the built-in weighting method, uniqueness of non-annotated DNA-data can be calculated corresponding to, for example, red-listed species (Ejrnæs et al., 2018). Here, we calculated fungi and soil microbe uniqueness according to Ejrnæs et al. (2018) in order to reflect the unique site contribution of fungi and soil microbe DNA to the gamma diversity of the collection of sites. Uniquity calculations were based on fungal and soil microbe OTU matrices, site habitat classes, and weights from the full Biowide dataset ( $n = 130$ , Brunbjerg et al., 2019) combined with the wetland dataset ( $n = 44$ ). The parameter X was set to 1000.

## 2.6 | Explanatory variables

### 2.6.1 | Soil moisture

The trimmed mean of 16 measures per site was used to reflect site soil moisture. For Biowide sites, we used the trimmed mean in August. We detected a systematic discrepancy between moisture in Biowide sites (measured in 2016) and wetland sites (measured in 2018), which could be accounted for by the summer of 2018 being extremely dry. We therefore interpolated the soil moisture trimmed mean values for wetland sites using the predicted values from a k nearest neighbor regression using soil moisture trimmed mean in Biowide sites ( $n = 130$ , Brunbjerg et al., 2019) as response variable, Ellenberg F values as explanatory variable and  $k = 11$ .

### 2.6.2 | Soil fertility

Good and reliable field-based measures of nutrient availability are difficult to obtain, as nutrient availability is extremely variable across time and space (Andersen et al., 2013 and references herein). In contrast, the nutrient ratio (mean site Ellenberg N/mean

site Ellenberg R, Ellenberg et al., 1992) has been found to reflect eutrophication in wetlands and be highly correlated with the number of typical species in fens (Andersen et al., 2013). For each site, we calculated mean Ellenberg N and Ellenberg R values (plant-based bioindication of nutrient status and soil pH, respectively) (Ellenberg et al., 1992). The Ellenberg nutrient ratio was used to reflect eutrophication and the idea of the ratio is to account for the fact that natural nutrient availability in wetlands increases with pH. To avoid circularity in analyses, plant species included in the plant-based conservation indicators (red-listed plants, typical plants) were removed before calculating the nutrient ratios for each model in question.

### 2.6.3 | Encroachment

We made a rough classification of sites into two groups (open vegetation and scrub/forest vegetation) based on field photos. The encroachment variable was coarse, that is, "open" represented mainly open and herb-dominated vegetation but sites with scattered small shrubs were also categorized as "open" plots as long as the shrubs did not dominate the plot. The two-level factor variable was used as explanatory variable.

## 2.7 | Statistical analyses

### 2.7.1 | Richness models

We built generalized linear models with Poisson errors using a set of biodiversity indicators in turn as response variable: vascular plant species richness, bryophyte species richness, fungal OTU richness, and soil microbe OTU richness. Soil moisture, nutrient ratio, and the 23 lidar-derived variables were used as explanatory variables. Standardized plant species richness was used as co-variable in models using OTU richness as response. Negative binomial errors were used if overdispersion was detected in Poisson models (Hilbe, 2011). We allowed for interaction between lidar variables and nutrient ratio, and lidar variables and soil moisture, respectively. We included a quadratic term of nutrient ratio and moisture variables if the full model significantly improved according to the  $\Delta AIC < 2$  criterion (Burnham & Anderson, 2002). The residuals of full models were checked for model misfit and overdispersion and spatial autocorrelation using correlograms from the R package *ncf* (Bjørnstad, 2020). Because of the large number of explanatory variables we used stepwise forward selection using the  $\Delta AIC < 2$  criterion (Burnham & Anderson, 2002). We used the variation inflation factor (VIF) to test for co-variability among selected explanatory variables and accepted values  $< 3$ .

### 2.7.2 | Conservation models

In order to test the effect of encroachment on conservation value, we built generalized linear models using a set of biodiversity indicators as response variables: the presence of red-listed vascular plants

(binomial error), presence of red-listed bryophytes (binomial error), indicator species as defined by Fredshavn et al. (2010) (Poisson error), and fungal uniqueness and soil microbe uniqueness (Gaussian error). Soil moisture and nutrient ratio were used as explanatory variables, and encroachment was tested as a binomial variable discriminating between scrub (woodland with bushes and trees) and open meadows, mires, and fens. Negative binomial errors were used if overdispersion was detected in Poisson models (Hilbe, 2011). We allowed for interaction between encroachment and nutrient ratio, and encroachment and soil moisture, respectively. We included a quadratic term of the explanatory variables if the full model significantly improved according to the  $\Delta\text{AIC} < 2$  criterion. The residuals of full models were checked for model misfit and overdispersion and spatial autocorrelation using correlograms from R package ncf (Bjørnstad, 2020). We used backwards elimination of explanatory variables using the  $\Delta\text{AIC} < 2$  criterion (Burnham & Anderson, 2002) to reduce full models to final models.

We checked if soil eDNA data for the two datasets could be pooled despite different sampling season (Biowide soil eDNA sampled in November, wetland soil eDNA sampled in July–August), and analyses of similar data showed no marked impact of season on biodiversity measures (fungal and soil microbe OTU richness and uniqueness) on the scale where these analyses are concerned.

### 3 | RESULTS

The study sites covered the full gradient in nutrient availability of the reference data, but only the wetter part of the reference moisture gradient as expected (Appendix A). The encroachment gradient (Ellenberg L) was also almost fully covered. The wetland dataset supplemented Biowide data nicely by adding drier and more nutrient-rich sites. Of the 102 sites, we classified 37 as woodland or scrub.

Vascular plant species richness ranged from 12 to 141 species per site. Red-listed plants were found at 45 sites including both open and scrub/forest sites, with a maximum of 18 red-listed species per site. Red-listed plant species pertained to both the open (in 49% of open sites) and scrub-forest category sites (in 35% of scrub-forest sites). Site bryophyte species richness ranged from 0 to 50 species. Only 21 sites held red-listed bryophytes (1–2 species). Indicator plant species were found in all 102 sites (2–89 species per site). Variation in ecological conditions and species richness across the 102 sites are given in Appendix F.

#### 3.1 | Richness models

We found a negative effect of nutrient ratio on plant species richness. However, only c. 7% of the variation in plant species richness could be accounted for by the model (Table 1). The final model for bryophytes explained 50% of the variation in bryophyte species richness with a strong negative effect of nutrient ratio and a strong positive effect of vegetation cover on bryophyte richness when

looking at effect sizes. The model of soil fungal OTU richness explained c. 45% of the variation in OTU fungal richness with a strong positive effect of plant species richness. Potential solar radiation and variation in vegetation cover also had a positive effect on fungal OTU richness, while increasing soil moisture, canopy openness, and vegetation density at 3–10 m had negative effects. Potential solar radiation had the largest positive effect on the number of soil microbe OTUs, while fine-scale terrain roughness also affected soil microbe OTU richness positively (Table 1). Nutrient ratio and vegetation density at 3–10 m (impenetrability) interacted, indicating a positive effect of nutrient ratio and a positive effect of encroachment on soil microbe OTU richness except in very eutrophic sites where encroachment had a negative effect (Figure 2). We found no indication of significant spatial autocorrelation in any of the final models, when checking correlograms.

#### 3.2 | Testing the effect of encroachment on conservation interest

The explanatory strength of conservation models ranged from 9.5% for the soil fungal uniqueness model to 41.3% for the model for indicator species (Table 2). Across all response variables, high degree of eutrophication (represented by Ellenberg N/R) affected biodiversity of conservation interest negatively. Encroachment, on the contrary, seemed to have a positive effect on biodiversity indicators. Soil moisture had a positive effect on the number of indicator species and soil microbe uniqueness (Table 2). Interactions between encroachment and nutrient ratio and moisture were only detected in the model for indicator species indicating that encroachment could counterbalance the negative effect of high nutrient levels and dry conditions at least to some degree (Figure 3). We found no indication of significant spatial autocorrelation in any of the final models when checking correlograms.

### 4 | DISCUSSION

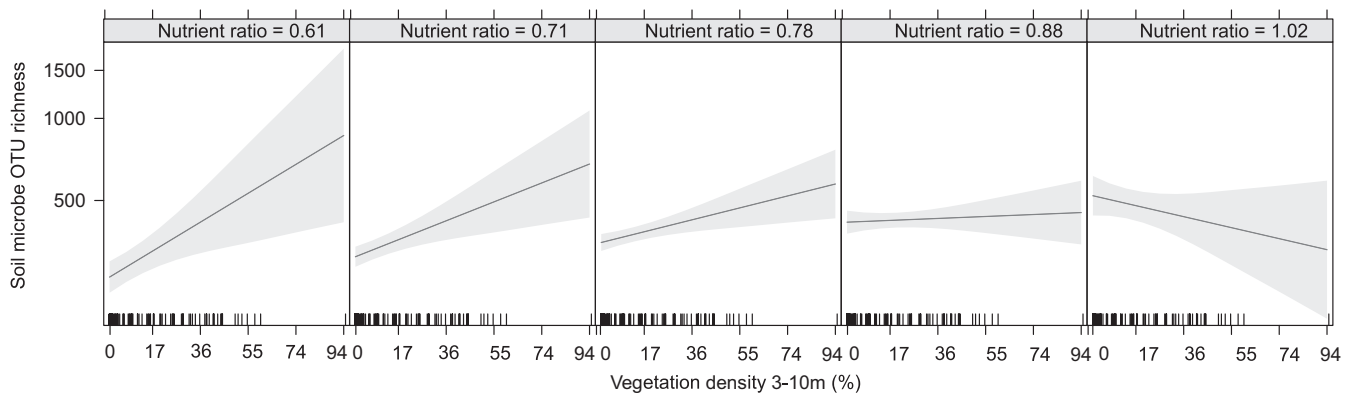
As expected our study confirmed a rather strong negative effect of soil fertility on the biodiversity of freshwater wetlands, including indicators for conservation status. More surprisingly, we found encroachment by shrubs and trees to have a positive effect on red-listed bryophyte richness, indicator plant species richness, and uniqueness of fungi and soil microbes. We propose a competitive release following encroachment as the negative response of indicator plants to soil fertility was only present in open wetlands and not in scrub and woodland.

Encroachment is often considered a threat to open-landscape biodiversity (Ratajczak et al., 2012; Stoate et al., 2009). In a conservation management perspective, focus is often on maintaining early successional vegetation, for example, by grazing and mowing of fens (van Diggelen et al., 2015) to ensure favorable conditions for especially rare plant species sensitive to encroachment (Bart, 2021).

**TABLE 1** Modeling output for GLM negative binomial models using vascular plant species richness, bryophyte species richness, fungal OTU richness, and soil microbe OTU richness as response variables and nutrient ratio (Ellenberg N/R), soil moisture, and the set of 23 lidar variables as explanatory variables.

	Dependent variable			
	Plant richness	Bryophyte richness	Fungal OTU richness	Soil microbe OTU richness
	Negative binomial	Negative binomial	Negative binomial	Negative binomial
	DE = 7.3%	DE = 50.0%	DE = 44.7%	DE = 39.9%
(Intercept)	3.826*** (0.046)	2.651*** (0.060)	5.689*** (0.028)	5.979*** (0.028)
Nutrient ratio	-0.134*** (0.046)	-0.596*** (0.074)		0.096*** (0.031)
Soil moisture			-0.076*** (0.028)	
Plant species richness			0.180*** (0.029)	
Vegetation cover		0.672*** (0.080)		
Canopy openness			-0.117*** (0.042)	
Vegetation density 3–10m			-0.100** (0.041)	0.076** (0.030)
Potential solar radiation		0.169*** (0.060)	0.118*** (0.029)	0.115*** (0.027)
Complexity (amplitude)		-0.171** (0.077)		
Fine-scale terrain roughness				0.074*** (0.028)
Vegetation cover variability			0.115*** (0.039)	
Nutrient ratio:vegetation density 3–10m				-0.084** (0.035)

Note: Estimates,  $p$ -values (\*\*<.05, \*\*\*<.01) and standard errors (in parentheses) are given. DE = deviance explained calculated as (null.deviance-deviance)/null.deviance.



**FIGURE 2** Model output for soil microbe OTU richness illustrating the interaction between nutrient ratio (Ellenberg N/R) and vegetation density at 3–10m (%).

Traditionally, there has been a focus on vascular plants, when defining habitat types, evaluating conservation status, and planning management, for example, within the framework of the EU Habitats Directive (Brunbjerg et al., 2018). However, this plant-focus may create a biased perception of the effect of encroachment on biodiversity as this effect at least depends on the habitat type and the response group in question (Eldridge et al., 2011). To our surprise, we did not find a negative effect of encroachment in our models for red-listed vascular plant richness or indicator plant richness. This is not to say that light-demanding species are not replaced by invading shrubs, but either the loss is relatively weak or losses are off-set

by gains of equally rare species. For the indicator plant species, the highest richness is observed in infertile and open habitats (Figure 3) pointing to the need for protecting these against encroachment.

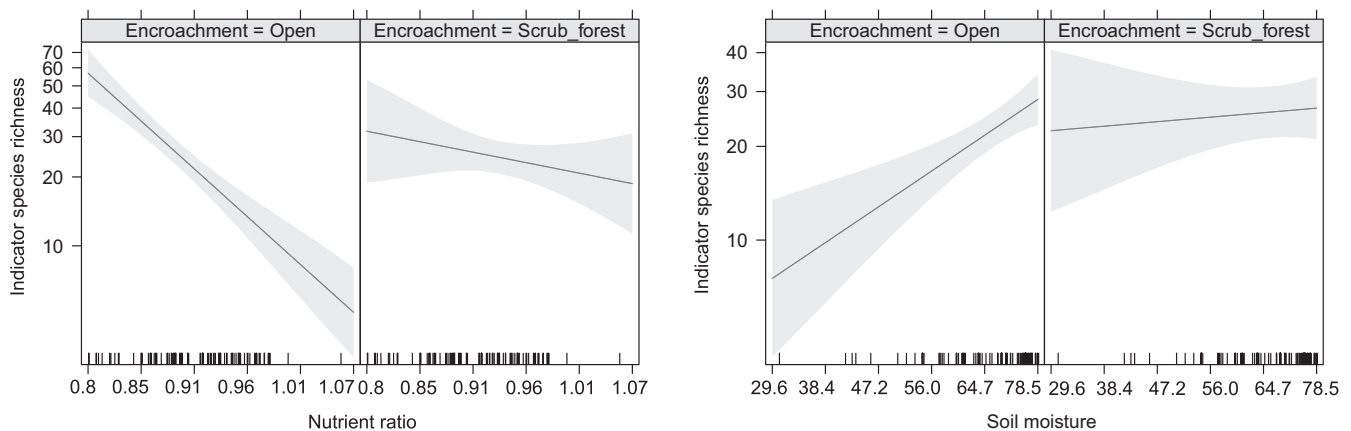
We found a positive effect of encroachment on bryophyte species richness and presence of red-listed bryophytes. Bryophyte richness was higher in sites with relatively high vegetation cover and low lidar amplitude. A high vegetation cover (measured by lidar with 5 points/m<sup>2</sup>) does not necessarily mean that the vegetation is dense (recall that the lidar was recorded during leaves-off and hence it is unlikely to capture the herb layer reliably). Instead, it likely means that bryophyte richness is highest where the vegetation includes

**TABLE 2** Modeling output for models using number of red-listed vascular plants (logistic), red-listed bryophytes (logistic), number of indicator species (GLM negative binomial), fungal uniqueness (Gaussian), and soil microbe uniqueness (Gaussian) as response variables and nutrient ratio (Ellenberg N/R), soil moisture, and encroachment as explanatory variables.

	Dependent variable				
	Red-listed plants	Red-listed bryophytes	Indicator species	Fungal uniqueness	Soil microbe uniqueness
	Logistic	Logistic	Negative binomial	Gaussian	Gaussian
	DE = 14.7%	DE = 17.8%	DE = 41.3%	DE = 9.5%	DE = 16.7%
(intercept)	-0.260 (0.221)	-2.835*** (0.581)	3.078*** (0.066)	52.577*** (5.218)	28.424*** (1.849)
Nutrient ratio <sup>a</sup>	-1.020*** (0.254)	-0.804** (0.390)	-0.481*** (0.064)	-14.103*** (4.540)	
Soil moisture			0.265*** (0.070)		6.064*** (1.487)
Encroachment		2.951*** (0.831)	0.167 (0.115)	21.110** (9.396)	6.552** (3.078)
Nutrient ratio:Encroachment			0.376*** (0.117)		
Soil moisture:Encroachment			-0.231** (0.100)		

Note: Estimates, *p*-values (\*\*<.05, \*\*\*<.01) and standard errors (in parentheses) are given. DE = deviance explained calculated as (null.deviance-deviance)/null.deviance.

<sup>a</sup>Plant species belonging to the response variable (red-listed plants, typical plants) were removed when calculating the Ellenberg nutrient ratio for each of these model, respectively.



**FIGURE 3** Model output for richness of indicator species illustrating the interaction between (a) nutrient ratio (Ellenberg N/R) and encroachment and (b) soil moisture and encroachment.

small shrubs and trees, which also seem to be the case for the richness of red-listed bryophytes in our study.

Heterotrophic organisms are likely to benefit from the expansion of niches linked to build-up and diversification of organic carbon following encroachment with shrubs and trees (Brändle & Brandl, 2001; Bruun et al., 2022). While the positive effect on bryophytes could be linked to additional substrate for epiphytic species, the beneficial effect of this expansion of ecospace (ss. Brunbjerg et al., 2017) on heterotrophic biodiversity is due to organic plant material for herbivores, symbionts, and decomposers as supported by our findings of positive effects of encroachment on fungi and soil microbes. OTU fungal richness showed a complex response with positive effect of canopy closure but negative effect of the density of low trees (i.e., the 3–10 m vegetation layer). In addition, there was a positive effect

of solar radiation indicating preference for an open park-like ecosystem. Only few studies have been conducted successfully linking fungal diversity to lidar variables (but see Thers et al., 2017; Moeslund, Zlinszky, et al., 2019) with variables like vegetation structure, successional stage, steep terrain, dead wood, and a dense shrub layer being important for fungal species richness. However, the results may not be readily comparable as both of the mentioned studies used macro-fungi from field inventories as response variables instead of OTU fungal richness from soil samples (for a comparison see Frøslev et al., 2019). We could only reproduce the positive effect of tree density on soil microbes under low soil fertility, indicating that in nutrient-poor wetlands, trees contribute to ecospace expansion (Brunbjerg et al., 2017), maybe in the form of substrate (i.e., falling leaves or root sap). Lastly, soil microbe richness was higher at



relatively high potential radiation and fine-scale terrain roughness, indicating that some abiotic variation in terms of microtopography and vegetation structure promotes richness.

For soil fungi and microbe communities, we found more unique assemblages with encroachment, but for indicator species of vascular plants in contrast, we found the classic peak of high species richness in open, nutrient-poor fens (Wassen et al., 2005). Others have also found complex richness responses to shrub encroachment, including a hump-shaped relationship (Kesting et al., 2015), indicating that encroachment of scattered shrubs in open grasslands may cause increased habitat heterogeneity which benefit species richness, while complete overgrowth will lead to reduced vascular plant species richness on the scale of small sample plots (Dierschke, 2006; Galvaneke & Lepš, 2008; Ratajczak et al., 2012; Teleki et al., 2020), probably due to light extinction and leaf litter cover inhibiting seedling establishment (Jensen & Schratzler, 1999).

Eutrophication is well-documented as a major threat to freshwater meadow biodiversity. Eutrophication causes a shift in species composition from slow-growing, light demanding vascular plants and bryophytes to more competitive and fast-growing species (Bobbink, 2004; Bobbink et al., 1998; Hogg et al., 1995)—a more rapid shift than the vegetation changes due to natural succession (Hogg et al., 1995). Our results are aligned with the negative effect of soil fertility for both species richness of vascular plants and bryophytes, but show a more complex interaction for soil microbial OTU richness. Hence, the diversity of soil microbes increased with eutrophication in open meadow sites, possibly reflecting increased available carbon to use as substrate. This effect is absent from encroached sites, possibly because the litter and rhizosphere of shrubs and trees add diverse carbon sources, irrespective of soil fertility. Species of conservation interest and uniqueness of soil fungi similarly showed a negative response to soil fertility. The number of red-listed plant and bryophyte species decreased with increasing soil fertility. Negative effects of eutrophication has been found to be more severe for rare species due to their initial low abundance—at least in grasslands and wetlands (Clark & Tilman, 2008).

We hypothesized that in eutrophic sites, competitive release (Keddy & Maclellan, 1990) may be a positive consequence of scrub encroachment and the resulting vertical differentiation of vegetation layers. The competitive release hypothesis is underpinned by the notorious depauperate plant species richness in eutrophic herbaceous vegetation due to asymmetric competition for light and nutrients (e.g., Crawley et al., 2005). We found the mentioned strong negative effect of eutrophication on plant species richness in open herb communities, but a much smaller effect under canopy cover for the number of indicator species (Figure 3), supporting the hypothesis of competitive release.

Rewetting and recreating natural hydrology is a well-established management recommendation for fen and meadow systems (Kofos & Banaszuk, 2013, 2018). However, we did not find a general positive effect of soil moisture on species richness and indicators of conservation value in our study, the effect was only positive for indicator

species in open habitats but not after encroachment. We suspect that the effect of soil moisture can be partly confounded with both eutrophication and encroachment because leached nutrients in the watershed are transported with the water and released into the wetland communities and also the wettest areas tend to be abandoned first and generally exhibit heavier encroachment than less wet sites.

Anecdotal evidence from our own surveys of aerial photographs indicates that willow scrub and alder swamps predominantly occur in the wettest parts of river valleys, for example, in places where historical small-scale peat extraction has left inundated pits and rendered the tract unsuitable for cultivation.

In our study, plant species richness and the number of red-listed plants were solely affected by soil fertility and the models only had limited predictive power (c. 7% and c. 14%, respectively). The predictive power of these models may seem low when compared to c. 60% explained variation in plant species richness in the full Biowide dataset in Brunbjerg et al. (2020). Although the two studies are not directly comparable, several explanations may be suggested for this discrepancy, for example, our encroachment variable is rather crude and also variables representing disturbance (e.g., grazing) and historical events related to former cultivation not included in this study could possibly be important for the plant species richness in our wetland sites.

Because of the need for reducing greenhouse gas emissions (the Paris agreement, United Nations, 2015), for example, by agricultural abandonment of organic soils/peat lands to decrease CO<sub>2</sub>-emissions, Denmark is now planning to abandon 100.000 ha of carbon-rich low-lying cultivated areas. These areas are likely to have high nutrient status from former cultivation and restoring natural low nutrient status as a basis for developing protected, open habitat types are costly and tedious. Instead, allowing encroachment when abandoning is expected to further increase CO<sub>2</sub>-sequestration because of the accumulation of carbon in shrub biomass and will furthermore permit synergies between climate and biodiversity goals. While setting aside cultivation of these potential wetlands implies a great potential for ecological restoration, our study shows that notably, earlier eutrophication caused by decades of arable farming will almost inevitably hamper the restoration target of species-rich meadows and fens. Based on our results, we recommend to combine a relaxed attitude to encroachment with reintroduction of natural disturbances (e.g., widespread rewilding of large herbivores) in order to promote semi-open scrub and woodland communities. Scattered bushes and thickets are natural elements in grazing systems, as many shrub species are vigorous resprouters, for example, *Salix* species (Klimkowska et al., 2010). In areas not suitable for year-round grazing, so-called “passive rewilding” (i.e., natural processes are allowed to restore themselves, Svenning et al., 2016) may be a superior solution compared to mechanical harvesting or intensive summer meadow grazing. This strategy for restoration of set aside of former cultivated fields should not supplement and not replace the critical conservation of unique fens and meadows of high conservation value relying on a long an unbroken historical continuity and naturally low nutrient status.

## AUTHOR CONTRIBUTIONS

**Ane Kirstine Brunbjerg:** Formal analysis (lead); methodology (equal); project administration (supporting); validation (equal); writing – original draft (lead); writing – review and editing (lead). **Camilla Fløjgaard:** Conceptualization (equal); investigation (equal); methodology (equal); project administration (lead); validation (equal); writing – review and editing (equal). **Tobias Guldborg Frøslev:** Data curation (equal); validation (equal); writing – review and editing (equal). **Dagmar Kappel Andersen:** Investigation (equal); validation (equal); writing – review and editing (equal). **Hans Henrik Bruun:** Conceptualization (equal); validation (equal); writing – review and editing (equal). **Lars Dalby:** Data curation (equal); validation (equal); writing – review and editing (equal). **Irina Goldberg:** Investigation (equal); validation (equal); writing – review and editing (equal). **Louise Juhl Lehmann:** Investigation (equal); validation (equal); writing – review and editing (equal). **Jesper Erenskjold Moeslund:** Data curation (equal); validation (equal); writing – review and editing (equal). **Rasmus Ejrnæs:** Conceptualization (equal); formal analysis (supporting); funding acquisition (lead); methodology (equal); validation (equal); writing – original draft (supporting); writing – review and editing (equal).

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## DATA AVAILABILITY STATEMENT

The dataset is available at Dryad Digital Repository (<https://doi.org/10.5061/dryad.bcc2fqzgr>).

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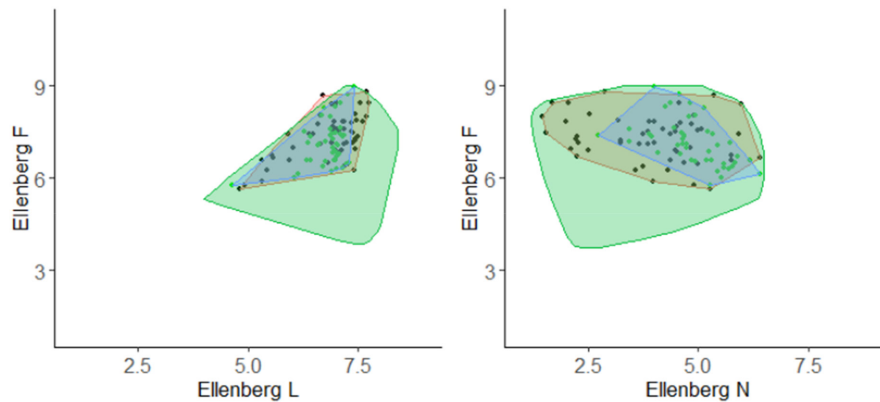
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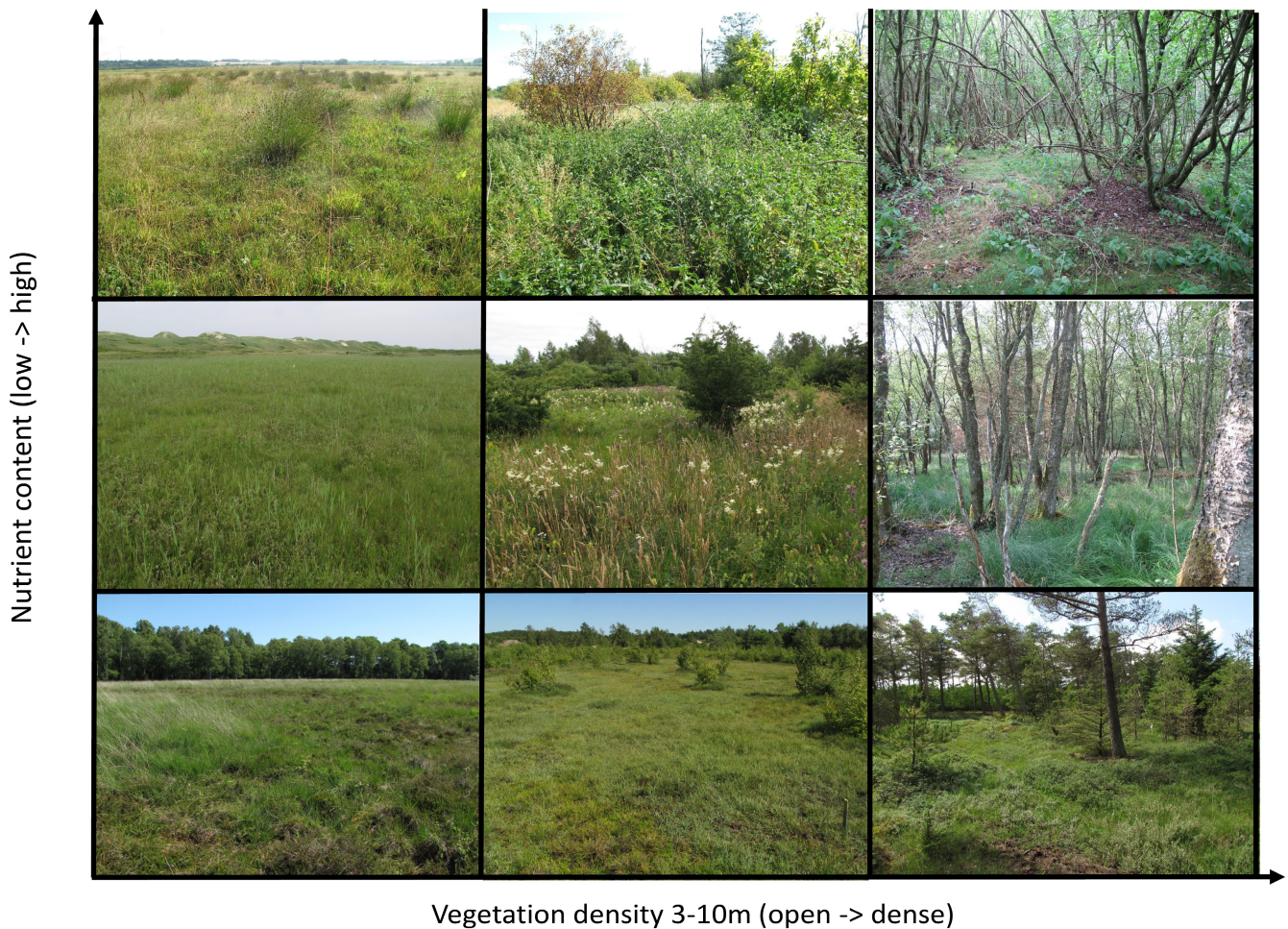
## APPENDIX A

95 percentile convex hull plots of Ellenberg F, L, and N values from a reference dataset (<http://www.naturdata.dk>) of open and forest habitat types (green,  $n = 59,227$ ) as well as the datasets used in this study: Biowide sites (orange bounding box and black dots,  $n = 58$ ), wetland sites (blue bounding box and green dots,  $n = 44$ ). Black and green dots represent site mean Ellenberg values of the 102 sites based on 406.5 m circle plots with  $\geq 5$  vascular plant species.



## APPENDIX B

Pictures of nine of the 102 sites and their approximate position in an ecological space of encroachment represented by vegetation density at 3–10m (x-axis) and productivity/nutrient status represented by Ellenberg N (y-axis).



## APPENDIX C

### Detailed information on molecular data

#### DNA extraction (44 study sites)

DNA was extracted from 4 g of soil using the PowerMax Soil DNA Isolation kit (Qiagen), following the manufacturer's procedure. DNA extract was purified using the PowerClean DNA Clean-Up Kit (Qiagen), and DNA was normalized to 1 ng/μl after fluorometric quantification using the Qubit™ dsDNA HS Assay Kit (Thermo Fischer). The 44 samples were extracted in smaller batches with one negative control for each batch.

#### Sequence data

For both the 44 study sites and the 130 Biowide sites, we amplified and sequenced molecular marker regions for fungi and eukaryotes. For fungi, the internal transcribed spacer region 2 (nrITS2) was amplified using primers gITS7 (5'-GTGARTCATCGARTCTTG-3') (Ihrmark et al., 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). For eukaryotes, the primers TAREuk454FWD1 (5'-CCAGCASCYCGGTAATTCC-3') and TAREukREV3 (5'-ACTTTCGTTCTTGATYRA-3') (Stoeck et al., 2010) were used to amplify the V4 rRNA loop of the small nuclear ribosomal subunit (18S nrDNA).

PCR amplifications contained 0.04 U μl<sup>-1</sup> AmpliTaq Gold (Life Technologies), 0.6 μM of each primer, 0.8 mg ml<sup>-1</sup> bovine serum albumin (BSA) and, 1X Gold Buffer, 2.5 mM of MgCl<sub>2</sub>, 0.2 mM each of dNTPs and 1 μl (1 ng) DNA extract, in 25 μl reaction volume. For every batch of PCR reactions, three PCR blanks and extraction blanks were included. For fungi was used an initial denaturation step of 5 min at 95°C, followed by 31 cycles of denaturation of 30 s at 95°C, 30 s annealing at 55°C, 60 s elongation at 72°C, and a final elongation at 72°C for 7 min. For eukaryotes was used an initial denaturation step of 7 min at 95°C, followed by first 15 cycles of 30 s at 95°C, 30 s at 53°C, 45 s at 72°C, then 20 cycles of 30 s at 95°C, 30 s at 48°C, 45 s at 72°C, and a final elongation at 72°C for 10 min. The presence and size of DNA fragments were verified on 2% agarose gel, stained with GelRed™ (Biotium, CA, USA).

Primers were designed with 96 unique tags (MID/barcodes) of 6 bp at the 5' end, preceded by 1, 2 or 3 N's. No primer tag was used more than once in any sequencing library and no combination of forward

and reverse primer tags was reused in the study. PCR products were pooled into pools with approximately the same number of samples, with no tag reused in any pool. Each pool was cleaned with the MinElute purification kit (Qiagen) and built into separate sequencing libraries using the TruSeq DNA PCR-Free Library Preparation Kit (Illumina). This was done following the manufacture procedure, except that all suggested bead purifications were replaced with MinElute purification. Before and after library building, pools were checked on an Agilent BioAnalyzer 2100 to verify the length of the products. Adapter dimers were removed using Agencourt AMPure XP beads, with a 1,5 bead:sample ratio. The libraries were mixed in equal proportions and sequenced together on one 300 paired-end run on the Illumina MiSeq (3000) at the Danish National Sequencing Centre. Eukaryote sequence data for 44 study sites were produced for this study, and bioinformatically combined with data for the 130 Biowide samples which was produced in an earlier study (Fløjgaard et al., 2019). Fungi sequence data for 44 study sites were produced for this study including all above steps, whereas the data for the 130 Biowide sites were produced by resequencing the libraries (from Frøslev et al., 2019) with 300 bp PE sequencing, and bioinformatically combining the two datasets.

#### Sequence processing

The bioinformatic processing of the sequence data followed the strategy outlined in Brunbjerg et al. (2019). Demultiplexing of samples was done with custom scripts that keeps R1 and R2 separate for DADA2 processing (Frøslev et al., 2019), and is based on Cutadapt (Martin, 2011)—and also Sickle (Joshi & Fass, 2011) for fungi—as in Frøslev et al. (2019) searching for a sequence pattern matching the full-length combined tag and primer allowing for no errors, and removing possible remnants of the other primer at the 3' end. We used DADA2 v 1.8 (Callahan et al., 2016) to identify OTUs (also known as exact amplicon sequence variants, ESVs) and for removal of chimeras (bimeras). For eukaryotes, the OTU tables were used directly. The fungal data were clustered at 98.5% and filtered to contain only ingroup data—that is, kingdom Fungi. For the fungal dataset, taxonomy was assigned by matching against the UNITE database (Nilsson et al., 2019), and for the eukaryotic data using a custom script based on BLASTn searches against genbank (Altschul et al., 1990).

Sequence data and analytical documentation can be obtained by contacting the first author.

## APPENDIX D

Lidar-based explanatory variables and interpretation. If the standard deviation of a variable was calculated, in addition to its mean, the variable is denoted with an asterisk.

	Explanatory variable	Explanation
Light/heat	Potential solar radiation*	Potential solar radiation input only considering the terrain
	Adjusted potential solar radiation*	Same as above but adjusted for vegetation cover
	Heat load index (only standard deviation)	Variation in south-westness
	Canopy openness*	How open the vegetation is (also low vegetation)
	Vegetation cover*	Fraction of vegetation points to ground point
	Terrain slope*	

	Explanatory variable	Explanation
Microtopography	Terrain openness*	Site-scale flatness of terrain
	Fine-scale terrain roughness	Fine-scale (0.5 m) terrain heterogeneity
Encroachment	Vegetation height*	
	Vegetation density at 0–1 m, 1–3 m, 3–10 m, and 10–50 m	Fraction of vegetation points in given layer compared to that in all other vegetation layers
	Amplitude	Succession state (high amplitude = high reflectance/flat and simple vegetation, low amplitude = low reflectance/complex vegetation). The measure is corrected for aircraft type and seasonality, (see Valdez et al., 2021)
	Echo ratio*	Canopy cover and complexity

## APPENDIX E

Indicator species considered moderately to very sensitive toward habitat changes as defined by Fredshavn et al., 2010. The list of indicator species was developed to indicate favorable conservation status cf. the Habitats Directive (European Commission, 2007). Species name and family are given.

Species name	Family
<i>Acer campestre</i>	Sapindaceae
<i>Agrostis canina</i>	Poaceae
<i>Agrostis vinealis</i>	Poaceae
<i>Aira praecox</i>	Poaceae
<i>Ajuga pyramidalis</i>	Lamiaceae
<i>Ajuga reptans</i>	Lamiaceae
<i>Alisma plantago-aquatica</i>	Alismataceae
<i>Alnus glutinosa</i>	Betulaceae
<i>Anacamptis morio</i>	Orchidaceae
<i>Andromeda polifolia</i>	Ericaceae
<i>Anemone nemorosa</i>	Ranunculaceae
<i>Anemone ranunculoides</i>	Ranunculaceae
<i>Angelica archangelica</i>	Apiaceae
<i>Angelica sylvestris</i>	Apiaceae
<i>Antennaria dioica</i>	Asteraceae
<i>Anthoxanthum odoratum</i>	Poaceae
<i>Anthyllis vulneraria</i>	Fabaceae
<i>Arabis hirsuta</i>	Brassicaceae
<i>Armeria maritima</i>	Plumbaginaceae
<i>Arum cylindraceum</i>	Araceae
<i>Astragalus danicus</i>	Fabaceae
<i>Avenula pubescens</i>	Poaceae
<i>Berula erecta</i>	Apiaceae
<i>Betula pubescens</i>	Betulaceae
<i>Bidens cernua</i>	Asteraceae
<i>Blechnum spicant</i>	Blechnaceae
<i>Blysmus compressus</i>	Cyperaceae
<i>Blysmus rufus</i>	Cyperaceae
<i>Botrychium lunaria</i>	Ophioglossaceae
<i>Botrychium simplex</i>	Ophioglossaceae
<i>Brachypodium sylvaticum</i>	Poaceae

Species name	Family
<i>Briza media</i>	Poaceae
<i>Calluna vulgaris</i>	Ericaceae
<i>Caltha palustris</i>	Ranunculaceae
<i>Campanula rotundifolia</i>	Campanulaceae
<i>Cardamine amara</i>	Brassicaceae
<i>Cardamine bulbifera</i>	Brassicaceae
<i>Cardamine flexuosa</i>	Brassicaceae
<i>Cardamine pratensis</i>	Brassicaceae
<i>Carex acuta</i>	Cyperaceae
<i>Carex appropinquata</i>	Cyperaceae
<i>Carex arenaria</i>	Cyperaceae
<i>Carex buxbaumii</i>	Cyperaceae
<i>Carex canescens</i>	Cyperaceae
<i>Carex caryophyllea</i>	Cyperaceae
<i>Carex cespitosa</i>	Cyperaceae
<i>Carex demissa</i>	Cyperaceae
<i>Carex diandra</i>	Cyperaceae
<i>Carex dioica</i>	Cyperaceae
<i>Carex distans</i>	Cyperaceae
<i>Carex echinata</i>	Cyperaceae
<i>Carex elongata</i>	Cyperaceae
<i>Carex flacca</i>	Cyperaceae
<i>Carex flava</i>	Cyperaceae
<i>Carex hostiana</i>	Cyperaceae
<i>Carex lasiocarpa</i>	Cyperaceae
<i>Carex lepidocarpa</i>	Cyperaceae
<i>Carex leporina</i>	Cyperaceae
<i>Carex nigra</i>	Cyperaceae
<i>Carex oederi</i>	Cyperaceae
<i>Carex panicea</i>	Cyperaceae
<i>Carex paniculata</i>	Cyperaceae

Species name	Family
<i>Carex pilulifera</i>	Cyperaceae
<i>Carex pulicaris</i>	Cyperaceae
<i>Carex remota</i>	Cyperaceae
<i>Carex rostrata</i>	Cyperaceae
<i>Carex sylvatica</i>	Cyperaceae
<i>Carlina vulgaris</i>	Asteraceae
<i>Centaurea jacea</i>	Asteraceae
<i>Centaurium erythraea</i>	Gentianaceae
<i>Centaurium littorale</i>	Gentianaceae
<i>Cerastium semidecandrum</i>	Caryophyllaceae
<i>Ceratocapnos claviculata</i>	Papaveraceae
<i>Chrysosplenium alternifolium</i>	Saxifragaceae
<i>Chrysosplenium oppositifolium</i>	Saxifragaceae
<i>Cicuta virosa</i>	Apiaceae
<i>Circaea alpina</i>	Onagraceae
<i>Circaea lutetiana</i>	Onagraceae
<i>Cirsium acaule</i>	Asteraceae
<i>Cirsium palustre</i>	Asteraceae
<i>Cladium mariscus</i>	Cyperaceae
<i>Comarum palustre</i>	Rosaceae
<i>Convallaria majalis</i>	Asparagaceae
<i>Corallorhiza trifida</i>	Orchidaceae
<i>Corydalis cava</i>	Papaveraceae
<i>Corylus avellana</i>	Betulaceae
<i>Crataegus laevigata</i>	Rosaceae
<i>Crataegus monogyna</i>	Rosaceae
<i>Crepis paludosa</i>	Asteraceae
<i>Cuscuta epithymum</i> var. <i>epithymum</i>	Convolvulaceae
<i>Cynosurus cristatus</i>	Poaceae
<i>Dactylorhiza incarnata</i>	Orchidaceae
<i>Dactylorhiza maculata</i>	Orchidaceae
<i>Dactylorhiza majalis</i>	Orchidaceae
<i>Danthonia decumbens</i>	Poaceae
<i>Drosera intermedia</i>	Droseraceae
<i>Drosera rotundifolia</i>	Droseraceae
<i>Dryopteris carthusiana</i>	Dryopteridaceae
<i>Dryopteris cristata</i>	Dryopteridaceae
<i>Dryopteris dilatata</i>	Dryopteridaceae
<i>Dryopteris filix-mas</i>	Dryopteridaceae
<i>Eleocharis multicaulis</i>	Cyperaceae
<i>Eleocharis palustris</i>	Cyperaceae
<i>Eleocharis quinqueflora</i>	Cyperaceae
<i>Eleocharis uniglumis</i>	Cyperaceae
<i>Elymus caninus</i>	Poaceae
<i>Empetrum nigrum</i>	Ericaceae
<i>Epilobium lamyi</i>	Onagraceae
<i>Epilobium palustre</i>	Onagraceae

Species name	Family
<i>Epilobium parviflorum</i>	Onagraceae
<i>Epipactis helleborine</i>	Orchidaceae
<i>Epipactis palustris</i>	Orchidaceae
<i>Epipactis purpurata</i>	Orchidaceae
<i>Equisetum fluviatile</i>	Equisetaceae
<i>Equisetum hyemale</i>	Equisetaceae
<i>Equisetum palustre</i>	Equisetaceae
<i>Equisetum sylvaticum</i>	Equisetaceae
<i>Equisetum telmateia</i>	Equisetaceae
<i>Erica tetralix</i>	Ericaceae
<i>Eriophorum angustifolium</i>	Cyperaceae
<i>Eriophorum latifolium</i>	Cyperaceae
<i>Eriophorum vaginatum</i>	Cyperaceae
<i>Erysimum strictum</i>	Brassicaceae
<i>Euphrasia nemorosa</i>	Orobanchaceae
<i>Festuca ovina</i>	Poaceae
<i>Frangula alnus</i>	Rhamnaceae
<i>Fraxinus excelsior</i>	Oleaceae
<i>Gagea spathacea</i>	Liliaceae
<i>Galium boreale</i>	Rubiaceae
<i>Galium odoratum</i>	Rubiaceae
<i>Galium palustre</i>	Rubiaceae
<i>Galium saxatile</i>	Rubiaceae
<i>Galium sternerii</i>	Rubiaceae
<i>Galium uliginosum</i>	Rubiaceae
<i>Galium verum</i>	Rubiaceae
<i>Genista anglica</i>	Fabaceae
<i>Gentiana pneumonanthe</i>	Gentianaceae
<i>Gentianaella uliginosa</i>	Gentianaceae
<i>Geranium sanguineum</i>	Geraniaceae
<i>Geum rivale</i>	Rosaceae
<i>Gymnocarpium dryopteris</i>	Cystopteridaceae
<i>Hedera helix</i>	Araliaceae
<i>Helichrysum arenarium</i>	Asteraceae
<i>Herminium monorchis</i>	Orchidaceae
<i>Hieracium vulgatum</i>	Asteraceae
<i>Hierochloë odorata</i>	Poaceae
<i>Honckenya peploides</i>	Caryophyllaceae
<i>Hordelymus europaeus</i>	Poaceae
<i>Hottonia palustris</i>	Primulaceae
<i>Hydrocharis morsus-ranae</i>	Hydrocharitaceae
<i>Hydrocotyle vulgaris</i>	Araliaceae
<i>Hylotelephium telephium</i>	Crassulaceae
<i>Hypericum tetrapterum</i>	Hypericaceae
<i>Illex aquifolium</i>	Aquifoliaceae
<i>Iris pseudacorus</i>	Iridaceae
<i>Jasione montana</i>	Campanulaceae



Species name	Family
<i>Juncus anceps</i>	Juncaceae
<i>Juncus articulatus</i>	Juncaceae
<i>Juncus balticus</i>	Juncaceae
<i>Juncus bulbosus</i> subsp. <i>bulbosus</i>	Juncaceae
<i>Juncus compressus</i>	Juncaceae
<i>Juncus filiformis</i>	Juncaceae
<i>Juncus gerardii</i>	Juncaceae
<i>Juncus inflexus</i>	Juncaceae
<i>Juncus ranarius</i>	Juncaceae
<i>Juncus squarrosus</i>	Juncaceae
<i>Juncus subnodulosus</i>	Juncaceae
<i>Juniperus communis</i>	Cupressaceae
<i>Knautia arvensis</i>	Caprifoliaceae
<i>Lamium galeobdolon</i>	Lamiaceae
<i>Lathraea squamaria</i>	Orobanchaceae
<i>Lathyrus japonicus</i> var. <i>glaber</i>	Fabaceae
<i>Lathyrus palustris</i>	Fabaceae
<i>Leontodon saxatilis</i>	Asteraceae
<i>Linum catharticum</i>	Linaceae
<i>Liparis loeselii</i>	Orchidaceae
<i>Lotus corniculatus</i>	Fabaceae
<i>Lotus pedunculatus</i> var. <i>pedunculatus</i>	Fabaceae
<i>Lotus tenuis</i>	Fabaceae
<i>Luzula campestris</i>	Juncaceae
<i>Luzula congesta</i>	Juncaceae
<i>Luzula multiflora</i>	Juncaceae
<i>Luzula pilosa</i>	Juncaceae
<i>Luzula sylvatica</i>	Juncaceae
<i>Lychnis flos-cuculi</i>	Caryophyllaceae
<i>Lycopodiella inundata</i>	Lycopodiaceae
<i>Lycopodium clavatum</i>	Lycopodiaceae
<i>Lysimachia europaea</i>	Primulaceae
<i>Lysimachia maritima</i>	Primulaceae
<i>Lysimachia nemorum</i>	Primulaceae
<i>Lysimachia thyrsoiflora</i>	Primulaceae
<i>Maianthemum bifolium</i>	Asparagaceae
<i>Melampyrum pratense</i>	Orobanchaceae
<i>Menyanthes trifoliata</i>	Menyanthaceae
<i>Mercurialis perennis</i>	Euphorbiaceae
<i>Milium effusum</i>	Poaceae
<i>Monotropa hypopitys</i>	Ericaceae
<i>Montia fontana</i> subsp. <i>fontana</i>	Montiaceae
<i>Montia minor</i>	Montiaceae
<i>Myosotis laxa</i> subsp. <i>caespitosa</i>	Boraginaceae
<i>Myosotis scorpioides</i>	Boraginaceae
<i>Myrica gale</i>	Myricaceae

Species name	Family
<i>Nardus stricta</i>	Poaceae
<i>Narthecium ossifragum</i>	Nartheciaceae
<i>Nasturtium officinale</i>	Brassicaceae
<i>Neottia ovata</i>	Orchidaceae
<i>Odontites vernus</i>	Orobanchaceae
<i>Oenanthe fistulosa</i>	Apiaceae
<i>Ononis spinosa</i> subsp. <i>spinosa</i>	Fabaceae
<i>Ophioglossum vulgatum</i>	Ophioglossaceae
<i>Orchis mascula</i>	Orchidaceae
<i>Oxalis acetosella</i>	Oxalidaceae
<i>Oxybasis rubra</i>	Amaranthaceae
<i>Paris quadrifolia</i>	Melanthiaceae
<i>Parnassia palustris</i>	Celastraceae
<i>Pedicularis palustris</i>	Orobanchaceae
<i>Pedicularis sylvatica</i>	Orobanchaceae
<i>Persicaria lapathifolia</i> subsp. <i>lapathifolia</i>	Polygonaceae
<i>Peucedanum palustre</i>	Apiaceae
<i>Phegopteris connectilis</i>	Thelypteridaceae
<i>Pilosella officinarum</i>	Asteraceae
<i>Pinguicula vulgaris</i>	Lentibulariaceae
<i>Pinus sylvestris</i>	Pinaceae
<i>Plantago maritima</i>	Plantaginaceae
<i>Plantago uniflora</i>	Plantaginaceae
<i>Platanthera bifolia</i> subsp. <i>bifolia</i>	Orchidaceae
<i>Platanthera chlorantha</i>	Orchidaceae
<i>Poa palustris</i>	Poaceae
<i>Polygala serpyllifolia</i>	Polygalaceae
<i>Polygala vulgaris</i>	Polygalaceae
<i>Polygonatum multiflorum</i>	Asparagaceae
<i>Polypodium vulgare</i>	Polypodiaceae
<i>Populus tremula</i>	Salicaceae
<i>Potamogeton polygonifolius</i>	Potamogetonaceae
<i>Potentilla erecta</i>	Rosaceae
<i>Potentilla verna</i>	Rosaceae
<i>Primula elatior</i>	Primulaceae
<i>Primula veris</i>	Primulaceae
<i>Prunella vulgaris</i>	Lamiaceae
<i>Prunus padus</i>	Rosaceae
<i>Prunus spinosa</i>	Rosaceae
<i>Pulmonaria obscura</i>	Boraginaceae
<i>Pulsatilla pratensis</i>	Ranunculaceae
<i>Pyrola minor</i>	Ericaceae
<i>Pyrola rotundifolia</i> subsp. <i>rotundifolia</i>	Ericaceae
<i>Quercus robur</i>	Fagaceae
<i>Ranunculus auricomus</i>	Ranunculaceae

Species name	Family
<i>Ranunculus bulbosus</i>	Ranunculaceae
<i>Ranunculus flammula</i>	Ranunculaceae
<i>Ranunculus lingua</i>	Ranunculaceae
<i>Ranunculus sardous</i>	Ranunculaceae
<i>Rhinanthus angustifolius</i>	Orobanchaceae
<i>Rhinanthus minor</i>	Orobanchaceae
<i>Rhynchospora alba</i>	Cyperaceae
<i>Rhynchospora fusca</i>	Cyperaceae
<i>Ribes nigrum</i>	Grossulariaceae
<i>Ribes spicatum</i>	Grossulariaceae
<i>Rorippa amphibia</i>	Brassicaceae
<i>Rosa canina</i>	Rosaceae
<i>Rosa mollis</i>	Rosaceae
<i>Rosa spinosissima</i>	Rosaceae
<i>Rubus chamaemorus</i>	Rosaceae
<i>Rubus saxatilis</i>	Rosaceae
<i>Rumex aquaticus</i>	Polygonaceae
<i>Rumex conglomeratus</i>	Polygonaceae
<i>Rumex hydrolapathum</i>	Polygonaceae
<i>Rumex maritimus</i>	Polygonaceae
<i>Sagina nodosa</i>	Caryophyllaceae
<i>Salix aurita</i>	Salicaceae
<i>Salix caprea</i>	Salicaceae
<i>Salix hastata</i> subsp. <i>vegeta</i>	Salicaceae
<i>Salix pentandra</i>	Salicaceae
<i>Salix repens</i> var. <i>repens</i>	Salicaceae
<i>Samolus valerandi</i>	Primulaceae
<i>Sanicula europaea</i>	Apiaceae
<i>Saxifraga granulata</i>	Saxifragaceae
<i>Scheuchzeria palustris</i>	Scheuchzeriaceae
<i>Schoenoplectus tabernaemontani</i>	Cyperaceae
<i>Schoenus nigricans</i>	Cyperaceae
<i>Scirpus sylvaticus</i>	Cyperaceae
<i>Scorzonera humilis</i>	Asteraceae
<i>Scrophularia umbrosa</i>	Scrophulariaceae
<i>Sedum acre</i>	Crassulaceae
<i>Selaginella selaginoides</i>	Selaginellaceae
<i>Selinum carvifolia</i>	Apiaceae
<i>Silene nutans</i>	Caryophyllaceae
<i>Sorbus aucuparia</i>	Rosaceae
<i>Sparganium natans</i>	Typhaceae
<i>Spergularia media</i>	Caryophyllaceae
<i>Stachys palustris</i>	Lamiaceae
<i>Stachys sylvatica</i>	Lamiaceae

Species name	Family
<i>Stellaria alsine</i>	Caryophyllaceae
<i>Stellaria graminea</i>	Caryophyllaceae
<i>Stellaria holostea</i>	Caryophyllaceae
<i>Stellaria nemorum</i>	Caryophyllaceae
<i>Stellaria palustris</i>	Caryophyllaceae
<i>Suaeda maritima</i>	Amaranthaceae
<i>Succisa pratensis</i>	Caprifoliaceae
<i>Teesdalia nudicaulis</i>	Brassicaceae
<i>Thalictrum flavum</i>	Ranunculaceae
<i>Thalictrum minus</i>	Ranunculaceae
<i>Thelypteris palustris</i>	Thelypteridaceae
<i>Thymus serpyllum</i>	Lamiaceae
<i>Tilia cordata</i>	Malvaceae
<i>Trichophorum alpinum</i>	Cyperaceae
<i>Trichophorum cespitosum</i>	Cyperaceae
<i>Triglochin maritima</i>	Juncaginaceae
<i>Triglochin palustris</i>	Juncaginaceae
<i>Trollius europaeus</i>	Ranunculaceae
<i>Turritis glabra</i>	Brassicaceae
<i>Ulmus glabra</i>	Ulmaceae
<i>Utricularia australis</i>	Lentibulariaceae
<i>Utricularia intermedia</i>	Lentibulariaceae
<i>Utricularia minor</i>	Lentibulariaceae
<i>Utricularia ochroleuca</i>	Lentibulariaceae
<i>Vaccinium myrtillus</i>	Ericaceae
<i>Vaccinium oxycoccos</i>	Ericaceae
<i>Vaccinium uliginosum</i>	Ericaceae
<i>Vaccinium vitis-idaea</i>	Ericaceae
<i>Valeriana dioica</i>	Caprifoliaceae
<i>Valeriana sambucifolia</i>	Caprifoliaceae
<i>Valerianella locusta</i>	Caprifoliaceae
<i>Veronica beccabunga</i>	Plantaginaceae
<i>Veronica montana</i>	Plantaginaceae
<i>Veronica officinalis</i>	Plantaginaceae
<i>Veronica scutellata</i>	Plantaginaceae
<i>Viburnum opulus</i>	Adoxaceae
<i>Vicia cracca</i>	Fabaceae
<i>Vicia lathyroides</i>	Fabaceae
<i>Vicia sepium</i>	Fabaceae
<i>Viola canina</i>	Violaceae
<i>Viola palustris</i>	Violaceae
<i>Viola reichenbachiana</i>	Violaceae
<i>Viola riviniana</i>	Violaceae
<i>Viscaria vulgaris</i>	Caryophyllaceae

**APPENDIX F**

Variation in ecological conditions and species richness across the 102 sites (mean and standard deviation are given).

Variable	Mean	SD
Plant species richness	46.29	22.82
Bryophyte species richness	17.53	12.53
Red listed plant species richness	1.32	2.76
Red listed bryophyte species richness	0.22	0.44
Indicator species richness	25.73	16.06
Ellenberg F	7.17	0.67
Ellenberg N	4.33	1.10
Ellenberg L	6.82	0.58
Nutrient ratio (Ellenberg N/R)	0.81	0.10
Soil moisture	64.74	8.79
Vegetation cover mean	0.27	0.26
Vegetation density 3–10m	0.17	0.19
Canopy openness mean	0.77	0.56
Solar radiation mean	0.79	0.01
Amplitude	0.01	99.05
Fine-scale terrain roughness	0.01	0.00