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RESEARCH ARTICLE

The Influence of Time on the Soil Seed Bank and Vegetation across a Landscape-Scale Wetland Restoration Project

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Abstract

Wicken Fen National Nature Reserve (NNR) in Cambridgeshire, U.K. is a wetland of international importance isolated in a landscape dominated by arable farming. The prospect of species extinctions within the NNR led to the creation of the Wicken Fen Vision, an ambitious project that will eventually expand the reserve boundary by the purchase and restoration of c.50 km^2 of arable land. We sampled three fields from each of three distinct age-categories of restoration land (5, 15, and 60 years post-arable), and three fields within the adjacent, undrained NNR, to determine (1) differences in seed bank composition across age-categories, (2) relationships between restoration age, the seed bank and standing vegetation, and (3) changes in species traits across age-categories. Historic arable management contributed to an apparent "vertical mixing" effect in the seed bank of the youngest two age-categories, with associated and significant differences in species functional traits across the study area. Almost all plants associated with NNR vegetation were absent from restoration area seed banks and standing vegetation. Seed bank species common to all ages-categories exhibited a bias toward moderate to high Ellenberg F (moisture) values, persistent seed banks, and lateral vegetative spread. Relatively short (c. 6 years) periods of drainage and plowing impact heavily upon seed bank diversity and soils, resulting in a lack of predrainage vegetation, even after decades of subsequent restoration adjacent to intact, species-rich habitat. However, the seed banks of highly degraded fields can contribute toward the creation of novel wetland vegetation assemblages over time and under suitable environmental conditions.

Key words: **fen, lateral vegetative spread, natural regeneration, plant traits, restoration, seed bank, standing vegetation, wetland, Wicken Fen.**

Introduction

In Britain, as in other parts of Europe, fen meadow and lowland wet grassland habitats have declined dramatically in the past century due to land drainage and agricultural intensification (Anonymous 1998; Manchester et al. 1999). This trend has been particularly marked in the Fens of East Anglia (U.K.) where a huge expanse of topogenous and ombrogenous mire habitat once covering an area of 3,850 km^2 now totals only 7.13 km^2. Here rapid habitat loss began in the seventeenth century with drainage and considerable re-alignment of river courses to create grazing pastures. Technological advances since the mid-nineteenth century have led to suitable conditions for crop production and ultimately the intensive arable land use that is prevalent today. The remaining undrained habitat is now located within a few isolated nature reserves on the southern fringes of the original fen basin (Moore 1997).

The dramatic decline in undrained habitat has promoted research into the potential for the restoration of fen and wet grassland vegetation alliances through the utilization of the soil seed bank (Thompson & Grime 1979; Grootjans & van Diggelen 1995; Bekker et al. 1998a; Jensen 1998; Wagner et al. 2003). The composition and resilience of the seed bank are known to play an important role in the process of habitat restoration (Roberts 1981; Bekker et al. 1997; Thompson et al. 1997; Pakeman & Small 2005), although the value of the seed bank to restoration varies greatly according to the type and duration of degradation activities.

Investigations examining fen meadow and wet grassland have generally concluded that the seeds of the main constituent species of undrained habitats are transient in nature, and are not viable in the seed bank after a relatively short time period (Jansen et al. 2000; Matus et al. 2003; Blomqvist et al. 2003; but see Jensen 2004). Under this scenario, re-establishing species based on predegradation assemblages must initially

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rely upon the restoration of dispersal vectors which were historically present (Middleton 1999) or upon artificial introduction through direct seeding, transplanting donor hay (Klimkowska et al. 2009) or the planting of propagated plants (Wells 1983; Galatowitsch & van der Valk 1994; McDonald et al. 1996). However, these approaches, even if successful in restoring wetland function, are unlikely to restore the former wetland ecosystem because peat wastage/degradation of soils, hydrological fragmentation and habitat isolation have all combined to create a novel starting point for restoration (Hughes et al. 2005).

Increasingly, wetland restoration projects are being designed at a landscape scale (e.g. Oostvaardersplassen, The Netherlands; Wicken Fen Vision, U.K., www.wicken.org.uk/vision; Great Fen Project, U.K., www.greatfen.org.uk) and often include management based on the concept of "re-naturation"; allowing ecosystem change to a future natural state through minimal anthropogenic intervention (Pfadenhauer & Klötzli 1996). Such a future natural state incorporates the historic changes that will have occurred in the hydrology and soils as well as the biota of highly degraded systems. Consequently, restoration in this context does not imply replicating complex species assemblages that were present historically, because many of these species have traits (sensu Grime 1979) that effectively filter them from all sites that no longer have intact soils or hydrological processes. As a result, novel assemblages may become established through a combination of the availability of viable seeds in the soil, natural dispersal of seed and plant material, and suitable conditions for germination and establishment. It follows that analysis of the traits of plants available in the restoration soils can be seen as a necessary step in helping to predict future natural states.

The main purpose of this study was to evaluate the influence of the seed bank on wetland habitat development across a project area containing land in three distinct restoration age-categories, located adjacent to Wicken Fen National Nature Reserve (NNR) in East Anglia, U.K. Through the collection of seed bank and standing vegetation data from within a landscape-scale restoration project and the bordering NNR, the following three hypotheses were addressed:

(1) The seed bank of highly degraded fields change with time under a wetland restoration regime characterized by natural regeneration and extensive grazing.
(2) The relationship between the seed bank and standing vegetation changes with restoration age.
(3) The range and type of species traits in seed banks and standing vegetation will change with restoration age.

Methods

Location of Study Site

The study site was situated 16 miles north of Cambridge (U.K.) (52.3°N, 0.3°E) and encompasses both Wicken Fen National Nature Reserve and the 'Wicken Fen Vision,' a landscape-scale wetland restoration initiative set up by the National Trust (the NGO that owns the site) adjacent to Wicken Fen NNR. The area receives an average annual rainfall of 530 mm. Average annual potential evapotranspiration rate in the area is 594 mm, and exceed rainfall during much of the growing season (McCartney et al. 2001; McCartney & de la Hera 2004).

Wicken Fen NNR and the Wicken Vision

Wicken Fen NNR, one of the oldest nature reserves in the United Kingdom, comprises 159 ha of undrained alkaline peat, and supports nationally scarce fen grassland and tall herb communities associated with moderate to low fertility floodplain fens with moderate to high pH (McCartney & de la Hera 2004). The site is of European importance for its *Molinia caerulea-Cirsium dissectum* community, and it has a remarkably diverse flora and fauna, with close to 8,000 species recorded (Warrington et al. 2009). For the past century, the reserve has been surrounded by drained and intensively farmed arable land, effectively isolating the NNR and its associated species and habitats. It is now perched 2–3 m above the agricultural land due to peat drainage and wastage.

The Wicken Fen Vision aims to purchase *ca* 50 km^2 of land, stretching from the boundary of Wicken Fen NNR to the northern boundary of the city of Cambridge. The restoration land, currently encompassing 9.3 km^2 (18.6%) of the proposed project area, is located on former intensively farmed arable fields that grew a wide variety of crops, and is managed by natural regeneration, hydrological manipulation where practicable, and an extensive grazing regime employing hardy breeds of Highland cattle and Konik ponies. This low-intensity management strategy allows for the potential formation of a constantly changing mosaic of habitats rather than a targeted set of habitats and vegetation alliances in fixed locations, and may be viewed as a more natural, cost-effective (Primack 1996) and adaptable form of landscape-scale conservation management.

Seed Bank and Vegetation Sampling

As a result of the staggered nature of land purchase, it was possible to select three distinct restoration age-categories for sampling across the project area: 5, 15, and 60 years post-arable (Table 1). In addition to these three age-categories, a fourth area was sampled from within the undrained Wicken Fen NNR to provide a reference area. Although the remnant soils in all the restoration areas consist of shallow, highly degraded peats (Morgan 2005), the historical variations in duration, location, and intensity of arable farming have contributed to differences in soil profiles for each of the three restoration age-categories (Table 1).

Soil seed banks were sampled in November 2007 using an auger of 6 cm diameter and 10 cm depth. Three compartments (fields surrounded by wet ditches) were sampled within each of the three age-categories of restoration land and the reference area. In each compartment, soil was taken from two transects of 50 m length located at distances of 2 m and 32 m from a

Table 1. Description of prerestoration management and *in situ* soil characteristics for each age-category.

Years in Restoration	*Historical Management*	*Soil Profile*	*% Soil Moisture*	*% Soil Organic Matter*
5	Drainage and intensive agricultural management arable regime for a continuous period of >70 years, leading to substantial peat wastage	Peat depth > 46 cm, directly overlying Gault clay bedrock	−10 cm: 32 −30 cm: 34 −50 cm: 48	−10 cm: 37 −30 cm: 33 −50 cm: 04
15	Drainage and intensive agricultural arable management regime for a continuous period of >70 years, leading to substantial peat wastage	Peat depth > 34 cm, with silt and gravel deposits above the Gault clay	−10 cm: 43 −30 cm: 40 −50 cm: 37	−10 cm: 34 −30 cm: 33 −50 cm: 04
60	Drainage and agricultural management arable regime for a continuous period of 6 years, leading to peat wastage	Peat depth > 70 cm, overlying silty loam and gravel deposits on Gault clay	−10 cm: 70 −30 cm: 68 −50 cm: 62	−10 cm: 38 −30 cm: 57 −50 cm: 23
Reference habitat	Intact peat within undrained habitat under nature conservation management for >100 years	Continuous sedge peat to depths of >200 cm	−10 cm: 76 −30 cm: 74 −50 cm: 82	−10 cm: 54 −30 cm: 55 −50 cm: 67

The oldest restoration area (60 years) was drained and plowed during the early 1940s under the Ministry of Agriculture's "Dig for Victory" campaign (Ennion 1942), before restoration by natural regeneration commenced in the late 1940s and early 1950s. Mean values taken from Morgan (2005) are displayed for % soil moisture and % soil organic matter.

chosen ditch edge and later pooled. Two bulk samples (each consisting of 10 soil cores taken at regular intervals from each transect) were divided into two depths (0–5; 5–10 cm) to investigate the vertical distribution of seeds. This generated four samples (i.e. two depths for each bulk sample) for each transect, eight samples for each compartment, and 24 samples for each age-category and the reference area. The soil volume for each bulked sample was 1,411 cm^3, which exceeds the volumes of 400–600 cm^3 (Hayashi & Numata 1971) and 1–1.2 L (Hutchings & Booth 1996) recommended to accurately detect species composition in a grassland seed bank. Immediately following collection, samples were stored in the dark at a constant 3°C for 4 weeks to mimic natural stratification, and then passed through a 10 mm diameter wire sieve to extract plant debris. Each sample was then mixed thoroughly and spread to an even depth of 4 cm (following Roberts 1981; Heard et al. 2003) above a 1 cm layer of sterilized sharp sand in a germination tray. Trays were randomly placed in an unheated greenhouse on 5 January 2008 and watered from below using an automated system. Preset light controls allowed for a daily constant of 16 hours light and 8 hours darkness. Germination was recorded for a 12 month period, with seedlings identified, counted and extracted every three weeks. To avoid permanent burial of potentially viable seeds, periodic mixing of the samples took place every three months. Species that were not readily identifiable at an early stage were removed and grown on until diagnostic features were visible. Five control trays filled with sterilized peat were included to test for possible contamination of samples by airborne seeds.

The seedling emergence method has the potential to give a biased assessment of the seed bank due to differences between greenhouse and field conditions (van der Valk 1992; Fallinska 1999). However, it is the most appropriate method for comparing the seed bank with above ground vegetation (Brown 1998) as long as caution is used regarding interpretation of species that have not germinated due to the absence of that species or the lack of suitable germination cues.

Standing vegetation was recorded in July 2007 using five 4-m^2 quadrats randomly placed along each 50 m seed bank transect, with species (nomenclature follows Stace 1997) and percentage abundance recorded.

Data Analysis

For the examination of seed bank and standing vegetation composition, Detrended Correspondence Analysis (DCA) was performed using the package CANOCO for Windows 4.5 (ter Braak & Ŝmilauer 1997–2002). Data were log $(x + 1)$ transformed, and rare species downweighted to prevent both very common and rare species from unduly influencing the ordination. For both vegetation and seed bank data, hierarchical analysis of variance (ANOVA) using the package MINITAB v.14 (www.minitab.com) was employed to test for differences between (1) age-categories and (2) the soil depth (seed bank only) and their interaction on the first and second DCA axes. "Treatment" effects were tested against the appropriate error term; age in the field stratum and depth at the soil core stratum, followed by Tukey's HSD to compare categories when tests were significant.

The potential for the seed bank to influence standing vegetation under a range of biophysical conditions (for example different hydroperiods) was addressed through identifying species functional traits. Species were classified to C-S-R and Regeneration Strategy types according to Grime et al. (2007) and Thompson et al. (1997) and were categorized for their tolerance to varying hydrological conditions using Ellenberg's F (moisture) values (Hill et al. 2004).

In the C-S-R analysis, C = Competitor, S = Stress-tolerant, R = Ruderal (with CR, CS, SR, and CSR employed as intermediate strategies). Four main Regeneration Strategy types were present in the seed bank and standing vegetation: V = vegetative expansion; S = seasonal regeneration; W = numerous widely dispersed seeds; B_s = persistent seed bank, with many species having more than one association to a strategy type. Comparison of C-S-R strategy types across restoration age-categories and between core depths was made for seed bank species by calculating a cover-weighted mean for each bulked soil core sample, with one-way ANOVA used to test for differences between age and depth categories. Regeneration Strategies for each restoration age were calculated for seed bank species and standing vegetation using a cover-weighted mean at the field scale. Ellenberg values for F (moisture) were calculated for seed bank and standing vegetation following the same procedure. Additional details of plant traits are given in Tables 4 and 5.

Sørenson's similarity coefficient [$S_s = 2c/(a + b)$, where a = number of species in seed bank, b = number of species in vegetation, and c = number of species common to both seed bank and vegetation] was used to determine the similarity of the seed bank and standing vegetation for each restoration age-category and the reference site based on presence-absence data using the statistical package MVSP (Kovach 1993). Predictions on the potential for the seed bank to influence vegetation assemblages were made by pooling seed bank species which were present across all age-categories sampled (termed 'constant species') and those which were specific to one of the seed bank age-categories (termed 'exclusive species').

Results

Seed Bank and Standing Vegetation Composition

A total of 9,882 seedlings from 135 species emerged from the soil samples. Monocotyledons accounted for 31 species (23.0%) and 39.8% of the total number of seed bank seedlings, while dicotyledons accounted for 104 species (77.0%) and 61.2% of the total number of seedlings. The 60 year age-category produced the most seedlings (33.9%), followed by the reference habitat (28.4%), the 5 year age-category (22.2%) and the 15 year age-category (15.5%). The 60 year age-category also produced the most species (85), although the 5 year (82 species) and 15 year (81 species) categories displayed similar numbers in the seed bank. The reference seed bank contained the fewest number of species (63). The most common species in the seed bank were *Poa trivialis* (11.7%), *Urtica dioica* (8.3%), *Eupatorium cannabinum* (6.9%), *Juncus inflexus* (6.6%), *Samolus valerandi* (6.2%), *Carex hirta* (3.2%), and *Agrostis stolonifera* (3.4%). Of the species found within the NNR which could be considered constituent species, only three were present within the restoration land: *Juncus subnodulosus*, *Calamagrostis canescens*, and *Cladium mariscus*. All three species were found in the 60 year age-category, with the latter two present only in the seed bank and in very small numbers.

The mean number of species, as determined by the Tukey HSD, did not vary significantly with depth in the 5 year or 15 year age-categories ($p = 0.245$ and $p = 0.176$, respectively). However, depth was a significant factor for the 60 year ($p < 0.001$) and the reference age-categories ($p < 0.001$), with the upper soil layer (0–5 cm) containing more species on average than the lower soil layer (5–10 cm).

In the seed bank ordination (Fig. 1), there were highly significant differences between age-categories on both the first ($F_{[3,8]} = 70.51$, $p < 0.001$) and second ($F_{[3,8]} = 62.74$, $p < 0.001$) DCA axes. The standing vegetation ordination (Fig. 2) displayed highly significant differences between all four ages on the first ($F_{[3,8]} = 71.89$, $p < 0.001$) but not the second ($F_{[3,8]} = 0.70$, $p = 0.58$) DCA axes.

The Sørenson similarity coefficient (S_s) for the standing vegetation and seed bank (Table 2) increased from the youngest to the oldest restoration age-category and was highest in the reference site.

Functional Traits

Ellenberg F (E_F) values indicated differences in the standing vegetation across the restoration areas, with the 5 year (average $E_F = 5.255$) and 15 year (average $E_F = 5.795$) age-categories significantly drier ($p = 0.003$ and $p < 0.001$, respectively) than the 60 year habitat (average $E_F = 7.353$). There was no significant difference in E_F values for standing vegetation between the 60 year and the reference habitat ($p = 0.346$).

In the seed bank, the 5 year category (average $E_F = 5.762$) comprised species indicating significantly drier conditions than in the 60-year age-category ($p = 0.017$; average $E_F = 7.694$), but the 15-year age-category was not significantly different from the 60-year age-category ($p = 0.285$). As in the standing vegetation, there was no significant difference in the seed bank between the 60 year age-category and the reference habitat ($p = 0.596$).

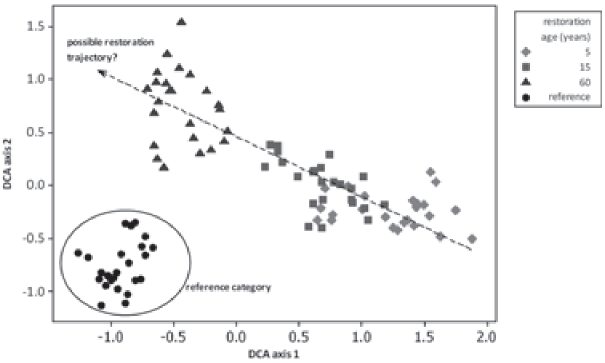

Figure 1. Sample scores of individual seed bank samples on the first and second axes of the Detrended Correspondence Analysis of the seed bank data. Symbols used to differentiate the four age-categories. The separation of the reference and restoration age-categories reflects the general absence of species typical of undrained, intact peat which were found within the NNR seed bank but not within the restoration land seed banks. Separation of the restoration categories along axis 1 principally appears to reflect time in restoration, as well as contrasting soil condition, hydrology and historical management. The two axes explained 19.6% (axis 1) and 8.9% (axis 2) of the variation in the data. Possible restoration trajectory superimposed.

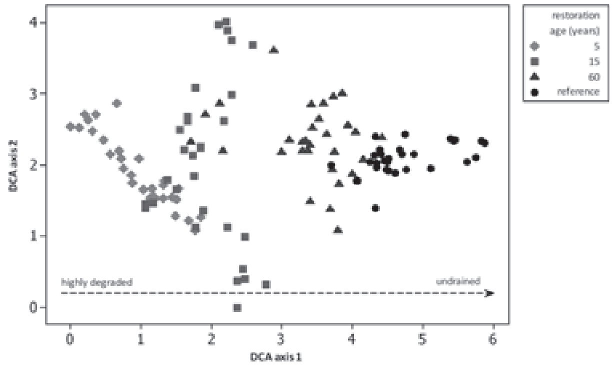

Figure 2. Sample scores of individual quadrats on the first and second axes of the Detrended Correspondence Analysis of the vegetation data. Symbols used to differentiate the four age-categories. Separation along Axis 1 reflects environmental conditions and the progression from recently degraded land to undrained reference habitat, with species associated with highly disturbed situations prevalent to the left of Axis 1 and species associated with intact fen meadow present only to the right of Axis 1. This trend is also apparent in the C-S-R analysis. The two axes explained 26.5% (axis 1) and 7.0% (axis 2) of the variation in the data. Possible restoration trajectory superimposed.

Table 2. Similarity of the seed bank and standing vegetation.

Age-category	*Veg*	*Sb*	*Veg + Sb*	*S_s veg-sb*
5	43	82	29	0.41
15	44	81	32	0.51
60	61	85	42	0.57
Reference	69	63	43	0.65

Columns display number of species found in the standing vegetation (Veg), the seed bank (Sb), species common to the vegetation and seed bank (Veg + Sb) and the Sørenson coefficient score ($S_S$veg-sb) for each age-category sampled.

Only one Regenerative Strategy (S) showed a significant difference between age-categories within the seed bank, whereas four regenerative strategies (S, VBs, VW, WBs) showed significant differences between age-categories for standing vegetation. The reference age-category was significantly different from all restoration ages for two of these strategies (VW and WBs); the 5-year age-category showed a significant difference from all other age-categories for the S regeneration strategy; and the 60 year age-category was significantly different from all other age-categories for the VBs regeneration strategy.

The seed bank C-S-R analysis revealed marked differences between restoration age-categories: most species were ruderal in the 5 and 15 year categories, while most were competitors in the 60 year and reference sites (Table 3).

Table 3. The relative proportions of seed bank species identified as Competitors (C), Stress Tolerators (S), or Ruderals (R).

Age-categories	*Mean C*	*Mean S*	*Mean R*
5	$0.4^{\mathbf{a}}$	$0.1^{\mathbf{ab}}$	$0.51^{\mathbf{ab}}$
15	$0.4^{\mathbf{a}}$	$0.1^{\mathbf{b}}$	$0.51^{\mathbf{b}}$
60	$0.46^{\mathbf{a}}$	$0.26^{\mathbf{a}}$	$0.29^{\mathbf{a}}$
Reference	$0.51^{\mathbf{b}}$	$0.22^{\mathbf{a}}$	$0.27^{\mathbf{a}}$
F value	4.54	56.48	38.65
p value	**< 0.01**	**< 0.01**	**< 0.01**

Columns contain the mean score for each age-category for each C-S-R strategy type. For individual columns differences between age-category means were tested using Tukey's HSD if there was a significant ANOVA *F* value. Means that do not share a common superscript letter can be considered significantly different. C = competitor, S = stress-tolerator, R = ruderal. Bold type denotes significant *p* values <0.05.

Exclusive and Constant Species

The clear separation of seed bank restoration age-categories illustrated in Figure 1 can be demonstrated further by examining the seed bank species present within each age class. Species which were specific to a restoration age-category ('exclusive species') are shown in Table 4. Plants characterized as ruderal, weedy species with an annual life history and a therophytic life form, are prevalent in the exclusive species identified in the 5-year and 15-year age-categories, whereas the 60-year age-category is characterized by a suite of species more associated with wet grassland or a weedy-wet vegetation, a perennial life history and a hemicryptophytic life form. The exclusive species found in the reference seed bank all have affinities to a fen/degraded fen grassland vegetation, but have similar life history, form and regeneration to those exclusive to the 60 year age-category. The standing vegetation ordination displayed a similar pattern to the seed bank, that is, species associated with dry, fertile, disturbed sites (5 years; 15 years) were separated along DCA axis 1 from wet, intact infertile sites (60 years; reference vegetation).

Species which were common to all age classes (termed 'constant species') in the seed bank are shown in Table 5. All constant species have an Ellenberg F (moisture) score of between 6 and 9, indicating a propensity for constantly moist or damp soils, and all apart from one species (*Festuca rubra*) have a persistent seed bank type. It is notable that of the 16 species common to all age classes in the seed bank, nine (including *Juncus articulatus, J. subnodulosus, J. inflexus, Agrostis stolonifera*, and *Epilobium parviflorum*) appear in the standing vegetation in the 60-year age-category. Of these nine species, seven may regenerate by means of extensive lateral spread (as defined in Grime et al. 2007) (highlighted in bold in Table 5).

Discussion

The trend of greater species diversity in the upper (0–5 cm) soil depth for the 60 year and reference study areas is consistent with previous seed bank studies (Maas & Schopp-Gluth 1995; Bekker et al. 1998b; Matus et al. 2003), but the lack of a significant difference between upper and lower soil depths in the 5 and 15-year age-categories is notable. It is possible that this may be attributable to land management practices prior to restoration, when the regular plowing of the soils are likely to have caused a 'vertical mixing' effect within the seed bank. This would help to explain the lack of differentiation between soil depths in the two youngest restoration age-categories.

Table 4. Exclusive species: species specific to a seed bank age-category.

Species	*Rest age*	*SV*	*SbT*	*Lat spread*	*Ellenberg F*	*Regen strategy*
Alopecurus myosuroides	*5*	√	3	1	5	Bs
Lolium perenne	*5*	√	1	3	5	S
Papaver dubium	*5*		3	1	5	Bs
Papaver rhoeas	*5*		3	1	5	Bs
Persicaria maculosa	*5*		4	1	6	Bs
Polygonum aviculare	*5*		3	1	5	Bs
Rumex acetosa	*5*		2	2	5	V, S
Veronica hederifolia	*5*		3	1	5	Bs
Chaenorhinum minus	*15*		3	1	4	S, ?Bs
Conium maculatum	*15*	√	2	1	5	S
Stellaria media	*15*		3	1	5	Bs
Carex hirta	*60*	√	?	5	7	V, ?Bs
Carex otrubae	*60*	√	2	3	8	V, ?Bs
Equisetum arvense	*60*	√	1	5	6	V, W, S
Festuca pratensis	*60*	√	1	2	6	V, S
Galium palustre	*60*	√	3	4	9	V, Bs
Poa pratensis	*60*		3	3	5	V, Bs
Potentilla anserina	*60*	√	2	5	7	V
Potentilla reptans	*60*		3	5	5	V, Bs
Ranunculus repens	*60*	√	3	5	7	(V), Bs
Trifolium repens	*60*	√	3	4	5	(V), Bs
Calamagrostis canescens	*Reference*	√	?2	5	9	V, W
Cladium mariscus	*Reference*	√	?	5	10	V, ?
Galium uliginosum	*Reference*	√	?1	4	9	V, ?Bs
Hydrocotyle vulgaris	*Reference*		2	5	8	V, ?Bs
Molinia caerulea	*Reference*	√	2	4	8	V, ?Bs
Salix caprea	*Reference*	√	1	5	7	(V), W, S
Scutellaria galericulata	*Reference*	√	?	3	8	V, ?

Species found within the soil seed bank which were exclusive to one of the four restoration age-categories. Abbreviations from Grime et al. (2007) and Thompson et al. (1997):
(a) Rest age: number = years since restoration, ref = reference habitat.
(b) SV: species was found in the standing vegetation.
(c) SbT from Thompson et al. (1997) and Grime et al. (2007) seed bank types, that is, 1, transient seed bank present in summer, germinating synchronously in autumn; 2, transient seed bank present in winter, germinating synchronously in winter/spring; 3, small quantity of seed persists in the soil for >5 years, seed concentration high only after seeds just shed; 4, numerous persistent seeds in the soil throughout the year.
(d) Lateral spread (Grime et al. 2007) 1, therophyte (very limited lateral spread); 2, perennials with small, compact and unbranched rhizomes or small tussocks ≤100 mm in diameter; 3, perennials with rhizomatous systems or tussocks attaining 100–250 mm; 4, perennials attaining diameter of 250–1,000 mm; 5, perennials attaining diameter of >1,000 mm. Values ≥4 in bold.
(e) Ellenberg *F* (moisture) value, where 5 = moist-site indicator, (fresh soils of average dampness); 7 = dampness indicator, (constantly moist or damp, but not wet); 9 = wet-site indicator, water-saturated, badly aerated soils (Hill et al. 2004).
(f) Regenerative strategy for species: V, *lateral vegetative spread*; S, *seasonal regeneration by seed in vegetation gaps*; W, *numerous small, wind-dispersed seeds or spores*; Bs, *persistent bank of seeds or spores*; ?, *strategies of regeneration by seed uncertain.*

The seed bank ordination displays a separation of the restoration age-categories along an apparent trajectory from the early stages of restoration through to the oldest of the restoration ages sampled. It is important to note that the position of the reference seed bank category in the ordination is quite separate from the apparent trajectory of the restoration age-categories. This result supports the first hypothesis—that seed banks will change through time under wetland restoration management involving natural regeneration.

The seed bank species composition in the 60-year restoration age-category is particularly instructive when examining the detrimental impact of arable cropping and drainage upon previously intact fen vegetation. This age-category site has had minimal disturbance relative to the 5 and 15 year categories. Even so, the short period (ca. 6 years) of habitat destruction by drainage and arable land use in the 1940s appears to have dramatically altered the seed bank composition when compared to the reference seed bank assemblage. The subsequent six decades of management by natural regeneration, hydrological manipulation, and herbivore grazing have failed to restore a standing vegetation or a seed bank associated with target U.K. fen vegetation communities (see Rodwell 1991), even when such vegetation assemblages are in situ and adjacent to restoration land. This suggests that it is unlikely that a reference-type of fen vegetation could be restored solely through utilization of the seed bank resource once other parts of the habitat, such as soils, have been irreversibly damaged. This conclusion is in agreement with other investigations into the restoration of target wetland vegetation (Brown 1998; Matus et al. 2003; Bossuyt & Honnay 2008), and supports the high priority attached to the retention and protection of undrained fen habitat.

Table 5. Constant species: species present in all seed bank age-categories.

Species	*5 SV*	*15 SV*	*60 SV*	*Ref SV*	*SbT*	*Lat spread*	*F*	*Regen strategy*
Agrostis stolonifera	√	√	√	√	3	**5**	6	V,Bs
Chenopodium rubrum					3	1	7	Bs
Cirsium arvense	√	√	√	√	3	**5**	6	V,W,Bs
Epilobium hirsutum					3	**5**	8	V,W,Bs
Epilobium montanum					3	2	6	(V),W,Bs
Epilobium parviflora			√		3	2	9	(V),W,Bs
Festuca rubra	√	√	√	√	1	**4**	5	V,S
Geranium dissectum	√	√			2	1	5	S
Juncus articulatus			√	√	3	**4**	9	V,Bs
Juncus bufonius					3	1	7	Bs
Juncus inflexus			√	√	3	**4**	7	V,Bs
Juncus subnodulosus			√	√	3	**5**	9	V,Bs
Poa trivialis	√	√	√	√	3	2	6	V,Bs
Samolus valerandi				√	3	**4**	8	?V,Bs
Urtica dioica	√	√	√	√	3	**4**	6	V,Bs
Veronica catenata			√		3	2	10	(V),Bs

Species found within the soil seed bank which were present in all of the four age-categories used in the study.
(a) SV √: indicates species found in the standing vegetation. Numerical prefix equates with years since restoration, ref = reference habitat.
(b) All other defined in Table 4.

Habitats which have a high level of disturbance are more likely to have a high correspondence (Sørenson similarity coefficient (S_s) score) between the composition of the soil seed bank and vegetation (e.g. Bekker et al. 1999). However, an S_s score of 0.41 after 5 years in restoration suggests that recruitment from the seed bank declines rapidly following cessation of high levels of disturbance (Dölle & Schmidt 2009). The increase in Sørenson similarity scores occurring with age since restoration appears to relate to a very gradual recruitment of species into the standing vegetation from the seed bank and supports the second hypothesis. However, interpretation of change through time is complex and likely also to be a consequence of site specific factors. Recruitment is likely to be linked to various environmental filters, including more naturalized hydroperiods and an associated increase in Ellenberg *F* (moisture) scores, disturbance events and the germination strategies of the buried seed bank. In a re-naturation management regime, the recruitment of additional species not present within the standing vegetation is most likely to be linked to seed dispersal vectors such as zoochory (Malo & Suárez 1995; Mouissie 2004), hydrochory and anemochory and/or by sporadic disturbance events promoting germination of species in the seed bank (Pakeman & Small 2005). On the Wicken Vision project area, the self-reliant herds of grazing animals are capable of creating disturbance at a local scale through trampling but at present are not able to move onto the NNR, and so cannot yet act as agents for zoochory between the two sites.

The lack of significant differences in seed bank regenerative strategies is marked across age-categories, and highlights the heterogeneous nature of the seed bank at all stages of habitat restoration. Statistically significant differences between age-categories, regenerative strategies and the standing vegetation may indicate that changes in site conditions determine the opportunities for the germination and establishment of seed bank species.

The bias toward species with a primary regeneration strategy of seasonal regeneration (S) in the 5 and 15 year age-categories for both the seed bank and standing vegetation is consistent with the recent history of agricultural land management and the developing nature of the standing vegetation. By the oldest restoration age (60 years), species which combine strategies of lateral vegetative spread and a persistent seed bank have established in the standing vegetation. This grouping of regenerative strategies is typically associated with meadows which have been severely drained in the past (Grime 1979; Grime 2002). Such habitats are frequently dominated by a few aggressive species, and must rely on temporally unpredictable disturbance events such as poaching and grazing by livestock in order to promote the germination and recruitment of new species (Isselstein et al. 2002).

This pattern of vegetation Regeneration Strategies is also evident in the C-S-R results. After prolonged periods of annual disturbance by plowing, species that can tolerate periods of intense, frequent disturbance (as represented by the high R score) are much more likely to be abundant in the early stages of arable reversion. As the habitat begins to stabilize, so the plants adapted as stress tolerators (S) increase. These findings are in general agreement with recently published work on fen seed bank plant traits in Poland and Germany (Klimkowska et al. 2010). The similarity between the S scores for the 60 year restoration age and the reference habitat and their lower R scores indicate the diminishing influence of the intense, regular and widespread mechanical disturbance maintained during the previous arable regime and suggest that the traits of species found in the seed bank changes through time under restoration as suggested in the third hypothesis.

Ellenberg moisture scores for the standing vegetation in part reflect the gradual restoration of a wetland hydroperiod after drainage, but may also relate to the differences in soil type following agricultural intensification. The Ellenberg *F* results for the seed bank suggest a marked difference in environmental conditions between the 15 and 60 year age-categories. This in turn may have allowed some species associated with a wetter environment, which were present within the seed bank, to establish in the vegetation within 60 years.

The presence of only three exclusive species in the 15 year age category compared to the eight species found in the 5-year age-category and the 10 species found in the 60 year category suggests a gradual change in seed bank species composition over time. The appearance in the 60 year seed bank and standing vegetation of many exclusive species typical of wetland vegetation (e.g. *Carex otrubae, Equisetum arvense*, and *Galium palustre*) and the increased Ellenberg moisture scores suggests a partial restoration of hydrological function and an increased potential for the establishment of wetland vegetation (albeit a species-poor type) through natural regeneration under suitable conditions. However, the clear differences in exclusive species functional traits found in each age-category are likely to reflect the impact and duration of previous arable regimes, and the subsequent length of time in which the seed bank has been able to recover since restoration commenced. In addition, some of the exclusive species found in the 60 year age-category may have survived the short period of intensive agricultural use in either the seed bank or the standing vegetation close to the field boundary.

The relationship between hydrological control, land management and the potential for the restoration of wetland vegetation through the seed bank is perhaps most clearly demonstrated when examining the constant species and their respective functional traits. All ages sampled have the potential to contribute toward a wetland vegetation type, but it is not until the oldest of the restoration ages that the majority of the constant seed bank species appear in the standing vegetation. Restoration relies upon numerous environmental factors promoting germination and establishment (Middleton 1999), including substrate, disturbance, fluctuation in temperature and hydrology. The frequency and timing of disturbance events also contribute to the successful recruitment and retention of vulnerable seedlings (Croft et al. 1997). The functional traits exhibited by the constant species suggest that hydrological control coupled with managed disturbance (through flooding, drawdown or grazing) will best promote the early establishment of species-poor wetland vegetation through natural regeneration following commencement of restoration.

Conclusion

Following six decades spent under conservation management, preceded by just six years of degradation through regular plowing and drainage of the peat soils, even the oldest and most intact of the restoration age-categories is lacking the constituent plant species which are present within the adjacent undrained vegetation of the NNR. The transient nature of undrained fen and wet grassland seed banks coupled with the rapid loss of peat through drainage and oxidation suggests that under natural regeneration, hundreds of years will need to elapse before vegetation diversity returns to predrainage levels. Even then it is likely that historic changes in the depth, structure and biology of the soils will result in novel vegetation assemblages, with the loss of peat depth and quality having a direct impact on the ability of the soils to store and slowly release water over dry periods in the late spring and summer months (Gillman 1994).

However, if the desired outcome of a project is not the replication of historic habitat but rather the development through natural regeneration of potentially novel wet grassland assemblages, then the seed bank can help to achieve this goal provided suitable conditions are present to facilitate the germination of seed bank species and subsequent establishment of seedlings. Such vegetation is not designed to replace what has been lost in the past. Rather, restoration through re-naturation may succeed in creating adaptable wetland vegetation assemblages in the face of predicted climate change scenarios. Such schemes may also begin to contribute to the restoration of ecosystem functions, such as hydrological function at a catchment scale with potentially beneficial effects on remnant intact vegetation. This restoration regime is particularly applicable within unpromising areas with highly degraded soils and hydrology. The restored vegetation is likely to be species-poor relative to undrained habitats, but if structural diversity can be sustained through extensive grazing by herbivores and fluctuating water tables, opportunities will be presented for the recruitment of flora and fauna over time. The introduction of a variety of dispersal vectors is also likely to play a key role in diversification of the sward over time, and should be considered alongside any re-naturation strategy.

Implications for Practice

- The restoration of reference fen vegetation on highly degraded arable land is likely to require the importation of propagules from outside sources.
- Where conditions are viewed as unpromising for restoration of reference habitat at a landscape scale, seed banks may contribute toward the restoration of novel wetland vegetation assemblages over time.
- Such novel assemblages are likely to be dominated by laterally spreading, aggressive species, and restoration site managers may want to consider the removal of biomass by cutting, extensive grazing and/or hydrological manipulation.

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