



Buried alive: Aquatic plants survive in ‘ghost ponds’ under agricultural fields



Emily Alderton^{a,*},¹, Carl Derek Sayer^a, Rachael Davies^b, Stephen John Lambert^c,
Jan Christoph Axmacher^a

^a Pond Restoration Research Group, Environmental Change Research Centre (ECRC), Department of Geography, University College London, London WC1E 6BT, UK

^b Millennium Seedbank, Kew Royal Botanic Gardens, Ardingly, Sussex RH17 6TN, UK

^c School of Biological Sciences, University of East Anglia, Norwich, Norfolk, NR4 7TJ, UK

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ABSTRACT

The widespread loss of wetlands due to agricultural intensification has been highlighted as a major threat to aquatic biodiversity. However, all is not lost as we reveal that the propagules of some aquatic species could survive burial under agricultural fields in the sediments of ‘ghost ponds’ - ponds in-filled during agricultural land consolidation. Our experiments showed at least eight aquatic macrophyte species to germinate from seeds and oospores, following 50–150 years of dormancy in the sediments of ghost ponds. This represents a significant proportion of the expected macrophyte diversity for local farmland ponds, which typically support between 6 and 14 macrophyte species. The rapid (< 6 months) re-colonisation of resurrected ghost ponds by a diverse aquatic vegetation similarly suggests a strong seed-bank influence. Ghost ponds represent abundant, dormant time capsules for aquatic species in agricultural landscapes around the globe, affording opportunities for enhancing landscape-scale aquatic biodiversity and connectivity. While reports of biodiversity loss through agricultural intensification dominate conservation narratives, our study offers a rare positive message, demonstrating that aquatic organisms survive prolonged burial under intensively managed agricultural fields. We urge conservationists and policy makers to consider utilizing and restoring these valuable resources in biodiversity conservation schemes and in agri-environmental approaches and policies.

1. Introduction

Intensive agriculture has contributed significantly towards global habitat loss and biodiversity declines (Henle et al., 2008; Tschamtko et al., 2012). Agricultural wetlands have particularly suffered in this respect, with huge numbers of agricultural ponds and other small waterbodies lost to drainage and infilling during the last 50 years (Wood et al., 2003; Serran and Creed, 2015). Given the significant contribution of small agricultural ponds and wetlands towards regional aquatic and terrestrial biodiversity (Davies et al., 2016; Sayer et al., 2012), their widespread disappearance poses a considerable challenge for biodiversity conservation and aquatic habitat connectivity.

Many aquatic organisms have evolved strategies for surviving habitat desiccation as dormant propagules. These propagules comprise aquatic macrophyte seeds (de Winton et al., 2000), oospores (Beltman and Allegrini, 1997; Stobbe et al., 2014) and cladoceran ‘resting eggs’ (Hairston, 1996) that can remain viable for centuries and allow rapid species’ re-establishment following habitat restoration (Beltman and

Allegrini, 1997; Kaplan et al., 2014). While long-term viability of propagules has been established for extant aquatic habitats (Bakker et al., 1996; Beltman and Allegrini, 1997; de Winton et al., 2000; Hairston, 1996), their fate in ‘ghost ponds’, ponds that have been in-filled for agricultural land consolidation, has remained unexplored. Ghost ponds are abundant across many agricultural regions, often discernible as damp depressions or by local colour alterations in crops and soil (Fig. 2a). We investigated the restoration potential of ghost ponds, and explored the longevity and germination rates of aquatic plant propagules extracted from their sediments. With around 75% of all ponds lost across large parts of the UK since the start of the 20th century (Rackham, 1986; Williams et al., 2010; Wood et al., 2003), and with similar levels of pond loss recorded in many agricultural regions across the globe (Agger and Brandt, 1988; Curado et al., 2011; Serran and Creed, 2015), ghost ponds could represent a major and overlooked resource for the resurrection of aquatic species ostensibly lost from the agricultural landscape. Both the ‘resurrection’ of ghost ponds, and the translocation of their sediments to newly created sites, could provide

* Corresponding author at: Department of Geography, University College London, Pearson Building, Gower Street, London WC1E 6BT, UK.

E-mail addresses: e.alderton@ucl.ac.uk (E. Alderton), c.sayer@ucl.ac.uk (C.D. Sayer), Rachael.Davies@kew.org (R. Davies), stephen.lambert@uea.ac.uk (S.J. Lambert), j.axmacher@ucl.ac.uk (J.C. Axmacher).

¹ Post (present address): 4 Park Vale Avenue, Apartment 11, Boston, MA, 02134, USA.

highly valuable approaches in aquatic conservation. Ghost ponds have the potential to retain not only historic populations of extant species, but also remnants of flora which have become locally or regionally extinct. Further, ghost pond restoration could help to reinstate the historic landscape connectivity between aquatic habitats.

This study examined the potential viability of dormant propagules buried within the sediments of in-filled ghost ponds. External propagule sources are commonly stated as primary agents of pond colonisation (Mari et al., 2011; Williams et al., 2008), but in restored or resurrected habitats the historic propagule bank may also make a significant contribution. Focusing on three farmland ghost ponds in Norfolk, eastern England, UK, we used a multi-level experimental design to examine the longevity of viable propagules, and indicate the relative importance of the seed bank vs. external propagule sources in mesocosm colonisation. Our work establishes the viability of aquatic plant propagules following burial under intensive agriculture some 45, 50, and ~150 years ago. We show remarkable longevity of aquatic plant propagules beneath cropped agricultural fields, and highlight the great potential of ghost pond restoration for aquatic biodiversity conservation in global agricultural landscapes.

2. Methods

2.1. Experimental design

Our study comprises four complementary approaches (Fig. 1):

- i) The resurrection of three ghost ponds (Fig. 2), following burial

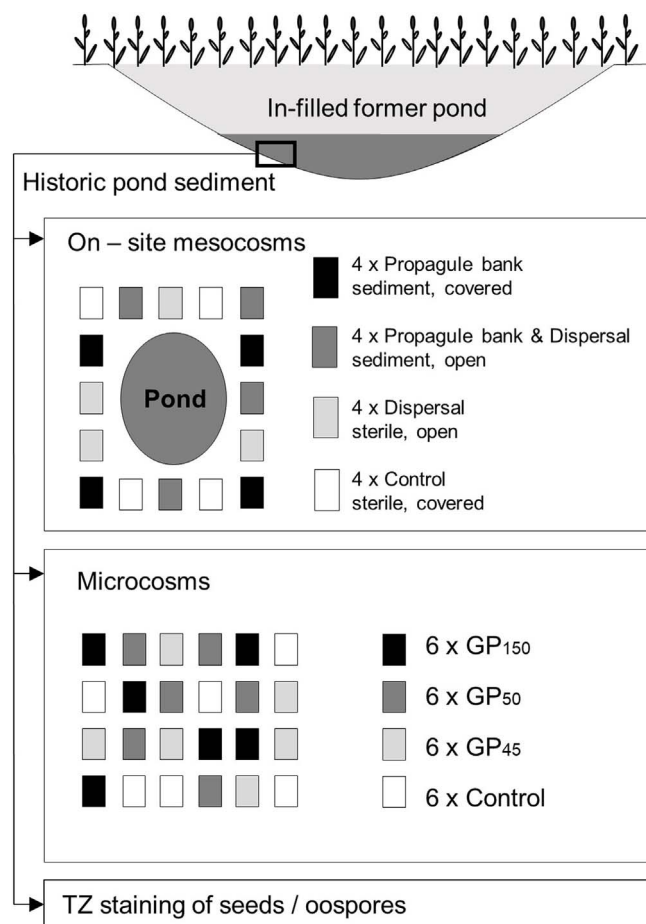


Fig. 1. Study design and experimental treatment. Historic sediment from three ghost ponds (GP₄₅, GP₅₀ and GP₁₅₀) provided the aquatic propagule material for three different experimental treatments; on-site mesocosms (Fig. 3a), sealed microcosms (Fig. 3b), and viability testing using tetrazolium chloride (TZ) stain (Fig. 3c).

50–150 years ago.

- ii) On-site mesocosm experiments (Fig. 3a), comparing macrophyte colonisation of 4 different treatments in four replicates, with mesocosms either open or closed to dispersal, and containing sterile or historic pond sediment.
- iii) Sealed microcosm experiments (Fig. 3b), comparing macrophyte establishment from sterile and historic pond sediment.
- iv) Viability testing of propagules extracted from historic pond sediments using tetrazolium chloride staining (Fig. 3c).

2.2. Locating and excavating ghost ponds

Ghost ponds were identified using historic UK Ordnance Survey (OS) maps and local tithe (1836–1841) maps. Within the study region of Norfolk (5371 km²), eastern England, UK, around 8400 ponds have been lost since the 1950s. The three ghost ponds selected for this study were all located in areas that had experienced relatively high levels of pond loss: within a 3 km radius of each study pond, a further 289 (GP₁₅₀), 275 (GP₅₀) and 147 (GP₄₅) ghost ponds, buried since the early 1950s, were identified (Alderton, 2017). For the three studied ghost ponds, time since burial was estimated from the most recent map demarcation of a pond and from landowner knowledge of pond loss. The oldest ghost pond, GP₁₅₀, was buried sometime between 1839 and 1883. GP₅₀ was in-filled during the late 1960s, and GP₄₅ during the early 1970s. All three ponds were located on land intensively farmed over many decades. Prior to their excavation, pond GP₁₅₀ was situated near a hedgerow, while both GP₅₀ (Fig. 2a) and GP₄₅ were located in the middle of arable fields.

All three ponds were excavated over September–October 2013. Once exact ghost pond locations had been established, a trench was dug through their centre and top soil was removed until dark historic pond sediments were exposed (Fig. 2b). Bulk samples of approximately 30 L of historic pond sediments were collected from multiple locations within the ghost pond basin, and stored in the dark in air-tight bags at 5 °C, prior to use in the mesocosm and microcosm experiments (Fig. 3). Each ghost pond was then fully resurrected following the profile, size and depth of the historic pond basin (Fig. 2c) and given a 6 m + marginal buffer left to natural plant colonisation. The ponds naturally filled with water over winter, and aquatic macrophytes were surveyed at weeks 5, 16, 28, 34 and 40 following excavation.

2.3. On-site mesocosms

Sixteen PVC-lined mesocosms measuring 40 × 30 × 30 cm were placed around each of the ghost pond sites (Fig. 3a). Eight mesocosms were prepared with 2 L of historic ghost pond sediment, each with 4 replicates left open to dispersal ('propagule bank & dispersal') and 4 replicates ('propagule bank') covered with 0.25 mm diameter mesh to prevent the influx of dispersing propagules. The remaining eight mesocosms were prepared with 2 L of a 50/50 mix of steam-treated potting soil and builder's sand (Boedeltje et al., 2002); with 4 again left open ('dispersal') and 4 covered with 0.25 mm mesh ('control'). Despite their small size, the positioning of mesocosms adjacent to the resurrected ghost ponds meant that waterfowl, a key dispersal vector for aquatic macrophyte seeds (Soons et al., 2016), accessed both the ponds and open mesocosms. Mallard (*Anas platyrhynchos*) were directly observed dabbling in the open mesocosms, although other bird species may also have visited the sites. All mesocosms were filled with filtered (53 μm mesh) rainwater and surveyed for aquatic macrophytes at the same time intervals as the ghost ponds.

2.4. Microcosms

Sealed microcosms were set up to corroborate the mesocosm results under strictly controlled conditions (Fig. 3b). Microcosms were set up outside at a central location situated about 25 km from the nearest

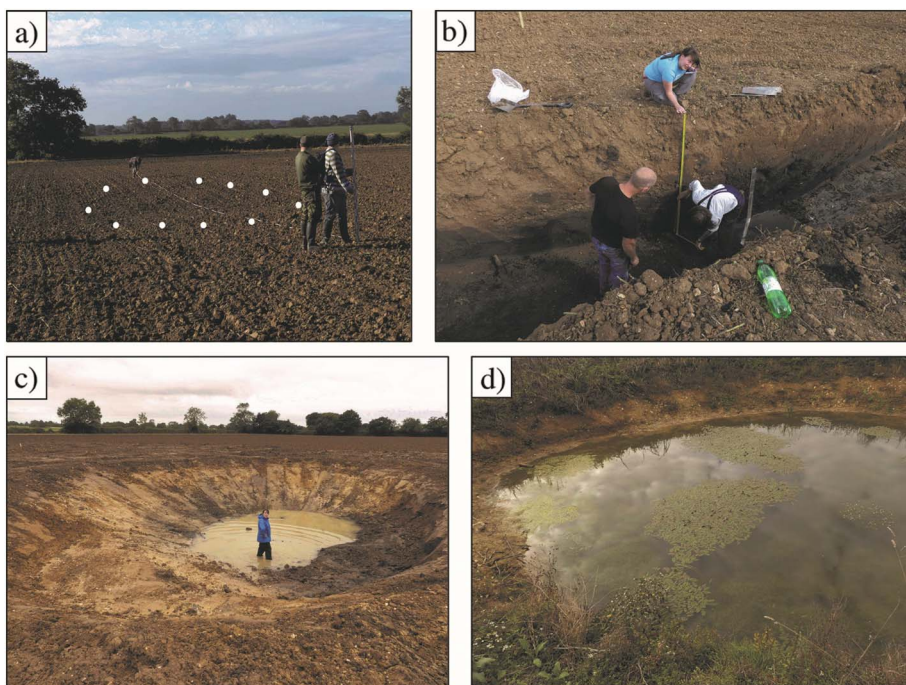


Fig. 2. Photographs of ghost pond GP₅₀ restoration; (a) GP₅₀ prior to excavation (white dots indicate the edge of the ghost pond depression); (b) excavation of a trench through the centre of GP₅₀ showing dark pond sediment layer; (c) GP₅₀ one day after excavation; (d) GP₅₀ one year post-resurrection with abundant beds of *Potamogeton natans* (September 2014).

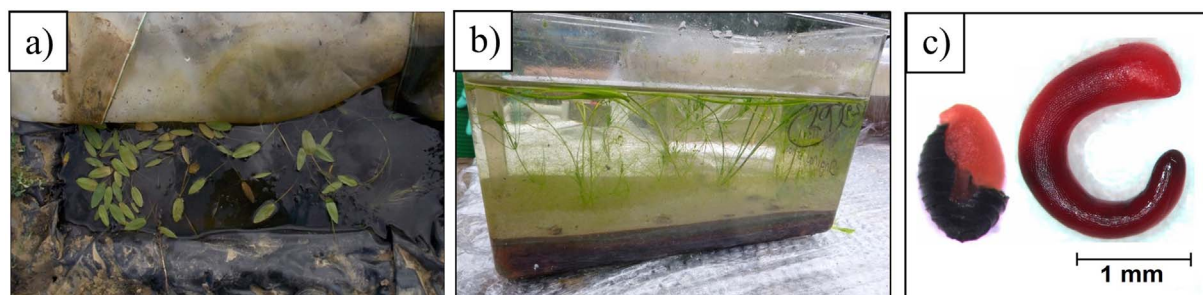


Fig. 3. Photographs of the three experimental components used: (a) *P. natans* growing in GP₅₀ 'propagule bank' treatment mesocosm (July 2014); (b) *P. natans* and *Chara* sp. growing in GP₅₀ microcosm (July 2014); (c) TZ-stained and viable *Chara* sp. oospore from GP₁₅₀ (Left) and *P. natans* embryo from GP₅₀ (Right).

ghost study pond (GP₁₅₀). As such, microcosms experienced similar weather conditions to the study ponds and on-site mesocosms, but without the risk of being exposed to the external input of aquatic propagules. Sediment collected from each ghost pond was homogenized and distributed between 6 plastic microcosms (30 × 20 × 19 cm), in March 2014. Each microcosm was filled with 1 L of sediment. As controls, six additional microcosms were filled again with 1 L of a 50/50 mix of sterile potting soil and builder's sand. All microcosms were filled with filtered rainwater (as for the mesocosms), covered with cling-film, and positioned on outdoor pallets. A frame was constructed to support a thin polythene cover (December–February), or shade netting (June–July), to protect the microcosms from temperature extremes, while allowing for a natural light regime. Aside from protecting the microcosms from winter frost damage and high summer temperatures, we allowed for a wide variation in temperature to break dormancy across species with a range of likely germination triggers (Hay et al., 2008; Proctor, 1967). Microcosms therefore experienced a water temperature range of 0–28 °C. Microcosm germinations were recorded at weeks 8, 13, 18, 22, 29, 37, 47, and 62 between May 2014 and May 2015. After being sealed, filtered rainwater in the microcosms was replaced at weeks 13, 18, 22 and 37 to reduce the effects of stagnation and potential anoxia. During each survey, germinations were counted for each species. To reduce sediment disturbance, and more closely mimic conditions in the on-site mesocosms and ponds, seedlings were not removed from the microcosms. During the time frame of the microcosm study, none of the newly established plants set seed, and as such all

individuals counted must have arisen from the historic propagule bank. By week 62, some charophytes had started to develop oogonium, although these had not ripened before the experiment ended. However, charophytes also propagate vegetatively, and as such it is possible that some 'individuals' may have been counted more than once. To avoid this problem, our results focus on species presence/absence, rather than the number of individuals present.

2.5. Tetrazolium chloride (TZ) staining

TZ staining was conducted at the Millennium Seed Bank, Wakehurst Place, Royal Botanic Gardens, Kew over 30/11/2015–01/12/2015. Sediment collected from the ghost ponds was kept in cold storage for 23 months. For TZ staining, seeds were then extracted from 4 × 50 mL samples of homogenized sediment from each of the three ghost ponds. To maximize the number of examined propagules from the oldest site, GP₁₅₀, an additional 450 mL bulk sample was processed. Sediment was passed through 355 µm and 125 µm sieves to remove all seeds and oospores, which were subsequently transferred to vials of distilled water and returned to cold storage (5 °C) for a further two months before staining. During this period, fungal growth was removed periodically from the propagules. Imbibed cleaned seeds and oospores then underwent an X-ray assay to assess numbers of full, insect infested and empty seeds. For the TZ assay, imbibed seeds were kept at 20 °C for 2–4 days to initiate metabolic processes. *Potamogeton natans* and *Ranunculus aquatilis* seeds were bisected laterally, while *Chara* ssp.

oospores were left intact. Seed embryos and oospores were incubated in 1% TZ solution at 30 °C for 36 and 48 h respectively; the longer time period allowing penetration of the TZ solution through the oospore wall. TZ staining was finally assessed under a light microscope at ×10–20 magnification (Fig. 3c). Seeds/oospores were classed as ‘viable’ if they exhibited complete red staining, and as ‘potentially viable’ if the staining was slightly patchy, or pink in colour. Seeds/oospores were classed as ‘non-viable’ if they did not stain, or exhibited very patchy or pale pink staining.

2.6. Statistical analysis

Profile analysis for the mesocosm treatments was conducted in SPSS. This multivariate technique can be used to test one dependent variable measured at different times, or several different dependent variables measured at the same time. The test is an extension of a repeated measures ANOVA, but avoids multiple comparisons where data are analyzed across more than two time points. Profile analysis is robust to both small sample sizes and violations of normality (Tabachnick and Fidell, 2007; Von Ende, 2001). In this case, it was used to compare three components of the response curves of each mesocosm treatment; the overall level of each curve (levels – whether there is a significant effect of mesocosm treatment), the shape of the response curves relative to each other (parallelism – whether mesocosm treatments differ from each other at different time points), and whether each curve has an average slope different from zero (flatness – the effect of time, irrespective of mesocosm treatment). Graphs were generated in R (package ‘ggplot2’).

3. Results

Ghost pond sediments collected from GP₁₅₀, GP₅₀ and GP₄₅ contained viable propagules from at least 15 aquatic plant species in 10 genera. Twelve of these species rapidly colonized the resurrected ponds within 5–40 weeks, while 8 species also germinated under at least one experimental condition (Table 1).

The sediment of the oldest pond (GP₁₅₀) contained viable propagules of at least 5 aquatic macrophytes; the charophytes *Chara virgata* and *C. vulgaris*, and *Potamogeton natans*, *Ranunculus aquatilis* and *Juncus* sp. (Table 1). When stained, 20% of charophyte oospores from GP₁₅₀ appeared viable (Fig. 3c), with a further 20% classified as ‘potentially viable’. Many oospores, and individual seeds of *Juncus* sp. and *R. aquatilis*, germinated from GP₁₅₀ sediment in cold storage (5 °C) prior to viability testing. Non-viable seeds of *Mentha aquatica* and *Persicaria amphibia* were also recorded in GP₁₅₀ sediment. These species did not germinate in any experimental treatment, but quickly colonized

Table 1

Aquatic macrophyte species found in the study ponds and germinating or viable in at least one experimental treatment. ‘Experimental treatment’ indicates the location and maximum age of the germination/viable propagule; ghost pond (Pond), mesocosm (Meso.), microcosm (Micro.), or viable Tetrzolium chloride (TZ) staining (TZ stain). Details are also provided for oospores and seeds that germinated while in cold storage (Germ. in storage and Germ. age). Viability testing results using 1% TZ stain are shown as the number of seeds/oospores Examined, and the number which were Viable (full stain), and Maybe viable (patchy stain). *Fully-developed charophytes were identified to species level while charophyte oospores were identified to genus only.

Species	Seed/oospore collection			TZ stain			Experimental treatment			
	Number/vol. (ml)	Germ. in storage	Germ. age	Examined	Viable	Maybe viable	TZ stain	Micro.	Meso.	Pond
<i>Chara</i> spp.	740/150	70/740	~150a	160	32	32	~150a	~150a	~150a	~150a
<i>Chara contraria</i>	*	*	*	*	*	*	*	~50a	~50a	~50a
<i>Chara globularis</i>	*	*	*	*	*	*	*	~50a	~50a	~50a
<i>Chara hispida</i>	*	*	*	*	*	*	*	~45a	~45a	~45a
<i>Chara virgata</i>	*	*	*	*	*	*	*	~150a	~150a	~150a
<i>Chara vulgaris</i>	*	*	*	*	*	*	*	~150a	~150a	~150a
<i>Juncus</i> sp.	22/12	1	~150a	22						~150a
<i>Nitella flexilis</i> agg.	0/200							~45a		~45a
<i>Potamogeton natans</i>	69/400	1		69	4	5	~50a	~150a	~50a	~150a
<i>Ranunculus aquatilis</i>	143/550	1	~150a	104						~50a

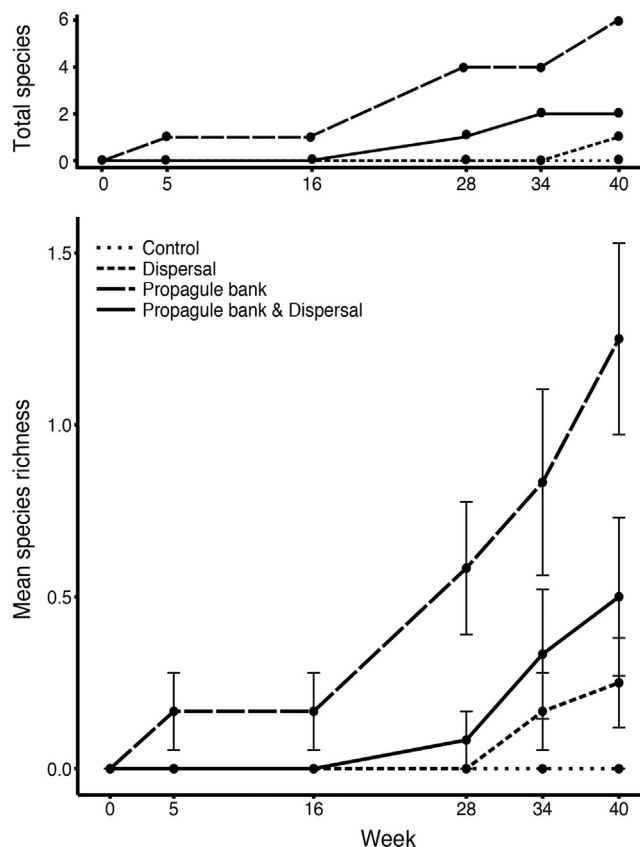


Fig. 4. Total and mean species richness of plants germinating in mesocosms over time. Error bars show standard errors. Treatments as follows: ‘Propagule bank’ (covered mesocosms containing ghost pond sediment); ‘Propagule bank & Dispersal’ (open mesocosms containing ghost pond sediment); ‘Dispersal’ (open mesocosms containing sterile substrate) and ‘Control’ (covered mesocosms containing sterile substrate).

resurrected pond GP₁₅₀.

The propagule bank of GP₅₀ was dominated by viable *P. natans* seeds and non-viable *Lemna trisulca* seeds, with only one viable charophyte oospore identified during TZ staining. Nonetheless, *P. natans* and the charophyte species *C. vulgaris*, *C. globularis* and *C. contraria* all germinated in GP₅₀ and in associated mesocosms and microcosms.

Only empty charophyte oospores and seed cases of *A. plantago-aquatica*, *L. trisulca*, *Potamogeton* spp. and *R. aquatilis* were found in the sediment from GP₄₅. However, *P. natans* grew in the microcosms, mesocosms, and resurrected pond, while *C. hispida* grew in the pond and mesocosms, and *Nitella flexilis* agg. grew in the pond and microcosms.

These findings suggest that GP₄₅ sediment contained much lower densities of viable propagules.

The on-site mesocosm results reflect the importance of dormant propagule reserves for aquatic macrophyte colonisation and diversity (Fig. 4). Germination in covered mesocosms containing ghost pond sediment ('propagule bank' - treatment) was significantly higher ($p < 0.02$), and faster than in other mesocosm types, with macrophytes germinating in the order: *P. natans* (week 5, GP₅₀ sediment), *C. vulgaris* (week 28, GP₁₅₀ and GP₅₀ sediment; week 40, GP₄₅ sediment), *C. contraria* (week 28, GP₅₀ sediment), *C. virgata* (week 34, GP₁₅₀ sediment), *C. globularis* and *C. hispida* (week 40, GP₄₅ sediment). Only *P. natans* (week 28, GP₅₀) and *C. vulgaris* (week 34, GP₁₅₀ and GP₅₀) germinated in the open 'propagule bank & dispersal' treatment, and only *C. vulgaris* (week 34, GP₁₅₀ and GP₅₀) germinated in the open 'dispersal' treatment containing sterile substrate. No germinations occurred under 'control' conditions.

We also observed crustaceans (*Daphnia* spp. and *Cyclops* spp.) in both microcosms and 'propagule bank' mesocosms, indicating the presence of viable 'resting eggs' of crustacean zooplankton, but did not investigate their establishment patterns.

4. Discussion

While agricultural intensification has undoubtedly had highly detrimental impacts on biodiversity, our findings offer a glimmer of hope for the restoration of aquatic habitats and macrophyte communities ostensibly lost to agriculture. We demonstrate that buried ghost pond propagule banks can act as a key source of aquatic macrophytes to ponds and other wetlands in modern agricultural landscapes, where dispersal barriers often limit the effectiveness of conventional habitat creation methods (Raebel et al., 2012; Williams et al., 2008). While temporal dispersal is a known mechanism for the persistence of aquatic organisms in extant habitats (Beltman and Allegrini, 1997; Weyembergh et al., 2004), our study is the first to demonstrate at least centennial-scale survival of aquatic plants in 'extinct' aquatic habitats beneath intensively cultivated agricultural fields.

The observed differences in aquatic propagule viability between our three study sites could suggest that pond conditions pre-burial have a stronger effect upon propagule viability than length of burial. GP₄₅ had dried out prior to its burial during a particularly dry year, whereas pond GP₅₀ was filled in while still wet. Low propagule viability in GP₄₅ corresponds with similar trends observed in drained wetlands linked to desiccation (Brown, 1998; Stroh et al., 2012). Nonetheless, even very low viable propagule densities can enable macrophyte re-establishment (Kaplan et al., 2014) and this likely explains the rapid re-colonisation of aquatic macrophytes in all three resurrected ghost ponds, including in GP₄₅. The mesocosm component of our study directly demonstrates the positive effect of the propagule bank upon both aquatic species diversity and rate of establishment. The unexpected significantly higher macrophyte establishment rates in covered 'propagule bank' mesocosms, compared to open 'propagule bank & dispersal' mesocosms, is most likely due to disturbance by water fowl, especially ducks which were observed to forage in the open mesocosms. This disturbance effect is likely to be less significant within the ghost ponds themselves, due to their much large size.

In contrast to the generally observed loss of viable propagules from wetlands due to drainage and lowered water levels (Brown et al., 1997; Stroh et al., 2012), the rapid burial of pond sediments during in-filling appears to effectively conserve long-term propagule viability. Several of the taxa surviving prolonged burial are keystone species; charophytes provide complex habitat structure and promote water clarity (Schneider et al., 2015), and they have become increasingly scarce in lowland agricultural landscapes (Lambert and Davy, 2011), while floating-leaved species like *P. natans* (Fig. 2d) strongly enhance the diversity of Odonata (Raebel et al., 2012). With UK farmland ponds typically supporting between 6 and 14 aquatic macrophyte species (Davies et al.,

2008; Sayer et al., 2012), the 8 species shown here to survive prolonged burial represent a significant proportion of the expected species diversity in these farmland ponds. Although our study examined the propagule banks of just three sites, their history, origins, and surrounding land use are widely representative of the conditions experienced by many ghost ponds. Intensive farming after pond burial, compaction, fertilizer and herbicide use, and continued intensive farming (outside of a 6 m pond margin) after any potential pond resurrection, are common to many ghost ponds. As such, we believe our study sites provide a realistic demonstration of the potential for using ghost pond propagule banks to aid wetland habitat restoration in European lowland farmland.

Although pond loss has been highlighted as a major challenge across many agricultural regions, current conservation policies offer ponds minimal legislative protection. Further, in regions where pond conservation strategies exist, including the US (Dahl, 2014), and parts of Europe (Céréghino et al., 2008), prevailing approaches focus on the creation of new ponds (Dahl, 2014; Forestry Commission et al., 2016; Freshwater Habitats Trust, 2015). The success of pond creation relies heavily upon plant dispersal from existing habitats (Raebel et al., 2012; Williams et al., 2008), which may be severely hampered in modern-day, highly fragmented agricultural settings. Ghost pond restoration may therefore provide an effective method of returning lost aquatic habitats and their associated macrophyte communities to agricultural landscapes.

Ghost ponds represent biological 'time capsules' whose restoration can facilitate the rapid return of wetland habitat and aquatic plants into the farmland landscape. It is evident, from their high abundance in agricultural landscapes around the globe (Wood et al., 2003; Curado et al., 2011; Dahl, 2014; Serran and Creed, 2015), that ghost ponds represent a rich and highly undervalued conservation resource. In areas where the resurrection of ghost ponds is not a viable option, sediment extracted from known ghost ponds could be analyzed palaeoecologically to identify past aquatic communities (Madgwick et al., 2011), while sediment translocations could facilitate the establishment of lost local species and genotypes in newly created ponds. We believe that ghost pond restoration could play a significant role in reversing some of the dramatic habitat and biodiversity losses caused by the global disappearance of agricultural wetlands, while acting to enhance aquatic habitat connectivity. We urge conservationists to incorporate ghost pond restoration into landscape-scale conservation strategies and evolving agri-environment approaches and policy.

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