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How to cite:

George, Laura Katharine (2021). The Effects of Hydrological Fluctuation on Grassland Plant Communities in England and Ireland. PhD thesis The Open University.

Version: Version of Record

Link(s) to article on publisher's website:
http://dx.doi.org/doi:10.21954/ou.ro.00013153

The effects of hydrological fluctuation on grassland plant communities in England and Ireland

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A thesis submitted for the degree of Doctor of Philosophy

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January 2021

Abstract

Grassland communities that are subjected to seasonal flooding are highly valuable to society, as they support a wide variety of wildlife and provide numerous other ecosystem services including floodwater and carbon storage. These important habitats are threatened by several anthropogenic pressures and climate change arguably poses the greatest risk to their functioning because of the greater temperature and rainfall variability predicted to occur in the future. The effects of some aspects of water-regime are well studied (e.g. mean water-table depth), but the effect of fluctuating water tables on grassland plant communities is not clearly understood. This study aims to investigate the effects of hydrological fluctuation on wet grassland plant communities. Fine-scale hydrological and botanical data were gathered from a selection of sites representing two habitats which experience differing levels of water-level fluctuation: English floodplain meadows and Irish turloughs. A selection of grassland plant species (*Ranunculus* spp.) were also subjected to fluctuating water levels in a controlled experiment.

This study presents a substantial body of evidence showing that high levels of hydrological fluctuation can result in a decline in plant species richness. Hydrological fluctuation was quantified as the degree of between-year (inter-annual) and within-year (intra-annual) variation in soil waterlogging across the preceding five growing seasons. Both increasing inter- and intra-annual variability correlate with a decline in plant species richness in English floodplain meadows and Irish turloughs. The experimental results suggest that overall wetness could be more important than hydrological variability, and that *Ranunculus acris* may be a better competitor under conditions of stress than *R. repens*. The results of this study have implications for grassland management under a more variable climate; it is proposed that a flexible management approach is required, which takes into account the role of fluctuating hydrology as an important driver of plant communities in seasonally flooded grassland habitats.

Acknowledgements

First of all, I'd like to thank the Eranda Rothschild Foundation for their incredibly generous support in funding this PhD project; it has been a privilege to carry out this research and it simply would not have been possible without the foundation. My sincere thanks also go to my supervision team: David Gowing, Micheline Sheehy Sheffington, Owen Mountford and Kadmiel Maseyk; I could not have done this without your never-ending support and guidance. A particular thank you to David, who had to witness and deal with a few of my melt-downs - thank you for staying calm and always helping me see a way forward. I'm also very grateful to the whole Floodplain Meadow Partnership team, for their brilliant advice and support: thank you Irina and Emma for always listening, thank you Clare for all your advice (especially about my experiment), and thank you Mike for passing on your Leica GPS wisdom.

Thank you to everyone who helped me with my fieldwork on the marvelous, and yet slightly awkward to survey, Irish turloughs. Thank you, Mike Gormally, Paul Johnson, and Owen Naughton, for taking the time to show me around many potential turlough study sites. Also thank you Owen for all your subsequent assistance with the turlough hydrological modelling data. Thank you to my fieldwork buddies: Sam Ham (sorry about all the cattle and horses), Katie Piercy, Carla Gomez, and especially my mother (you were beyond amazing, and I cannot thank you enough). A special thanks goes to Hilary Wallace, for all your help in the field with plant identification, and for your excellent company. I am also very grateful to Mike Prosser (who has since sadly passed away) for identifying many bryophyte specimens.

Thank you Yoseph Araya for your help and advice regarding my experiment, and Rebecca Shepherd, who went above and beyond to help me with a few logistical hiccups along the way. Thank you to all the amazing school administration staff who provided invaluable support, especially Liz Lomas and Michelle Aitken. Thank you to the entire school, it has been such a friendly and supportive place to work these last few years - I could not have coped without all the restorative coffee and biscuit sessions. A shout-out to my amazingly helpful R comrades Matthew Kent, Phil Wheeler, and Julia Cooke. I have had many brilliant office buddies, thank you, and all my fellow PhD students, for making my time at The Open University so much fun.

Last of all, thank you to my dear friends and family, for your constant love and support, even if you don't really understand what my PhD is and/or why I'm doing it. Thank you, Hannah, for always being there to listen. And to Matt, I have no words to express the gratitude I feel - meeting you is the best outcome of this PhD. And last of all, to our son Fred - you haven't exactly helped me finish the PhD, but you have been the most wonderful distraction.

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Chapter 1

Introduction

1.1 Wet grassland habitats

There is a wide variety of wet grassland types across England and Ireland, many of which are home to diverse floral and faunal assemblages. Wet grasslands encompass swards that are either periodically flooded, affected by impeded drainage or overlie groundwater bodies that keep them moist for at least part of the year. Some wet grasslands habitats occur on acidic soils and are typical of species-poor upland pastures, but the focus in this study is the range of species-rich assemblages that occur on base-rich substrates and/or are supplied with base-rich water. These communities appear to be particularly sensitive to small changes in soil hydrology (Silvertown *et al.*, 1999) and therefore provide useful field sites for investigating the relationships between hydrological fluctuations and the response of the vegetation.

1.1.1 Definition

The two wet grassland habitats that have been selected for study because of their biodiversity value and conservation significance are: English floodplain meadows and Irish turloughs. Floodplain meadows and turloughs are types of wetland habitat that are home to grassland communities which are subjected to seasonal flooding, and they are collectively referred to as seasonally flooded, or wet, grasslands throughout this study. Floodplain meadows are areas of grassland used for hay making and aftermath grazing, which are intermittently flooded by an adjacent river or stream, or via groundwater sources (e.g. gravel or sand aquifers) (Rothero *et al.*, 2016). Turloughs are depressions in areas of karst limestone which fill during the autumn via groundwater conduits (or at any other time of year when precipitation is sufficiently high), and in the spring they drain to reveal grass- or sedge-dominated swards which are used for livestock grazing (Sheehy Skeffington *et al.*, 2006). These two fluctuating grassland habitats (i.e. meadows and turloughs) share similar plant assemblages to other habitats such as sand-dune slacks, and draw-down zones at the margins of lakes and other waterbodies.

1.1.2 Importance

Floodplain meadows are one of the richest neutral grassland habitats in the UK, with up to 43 plant species occurring per square metre (Silvertown, 2004; Rothero *et al.*, 2016). One of the most typical NVC (National Vegetation Classification) plant communities of these habitats is the Burnet floodplain meadow (MG4) (Rodwell, 1993). There is, however, a mosaic of other plant communities that are often present alongside the MG4 community, including other mesotrophic grassland assemblages (e.g. Kingcup-carnation sedge meadow, MG8; Foxtail plash, MG13; Knapweed meadow, MG5), as well as less diverse mire and swamp plant communities (Rothero *et al.*, 2016). A number of nationally scarce plant species are found in English floodplain meadows, such as the snakeshead fritillary (*Fritillaria meleagris*), downy-fruited sedge (*Carex tomentosa*) and narrow-leaved water-dropwort (*Oenanthe silaifolia*). Floodplain meadows also provide a vital breeding and feeding habitat for a variety of rare bird species (e.g. snipe, curlew, skylark).

A large percentage of floodplain meadow habitat, which contains MG4 and/or MG8 grassland communities, lies within Sites of Special Scientific Interest (SSSIs). Other meadow sites have been designated as Special Areas of Conservation (SACs) for the presence of the Annex I habitat Lowland Hay Meadows (akin to the MG4 NVC community), and there are Special Protection Areas (SPAs) designated under the European Birds Directive for their international importance for birds (European Community, 2009; Rothero *et al.*, 2016). Turloughs are also Annex I listed habitats in the EU Habitats Directive (European Community, 1992) and have priority status due to their rarity in Europe. They are home to diverse and specialist floral assemblages which are well-suited to the highly variable hydrological conditions characteristic of this habitat. Some of the rare wetland flora found in turloughs include the fen violet (*Viola persicifolia*), shrubby cinquefoil (*Potentilla fruticosa*), and northern yellow-cress (*Rorippa islandica*). There are also some species that have a different growth form when found growing in turloughs and adjacent ‘dry’ areas, such as the creeping buttercup (*Ranunculus repens*; Lynn and Waldren, 2001).

Turloughs support assemblages of crustaceans, beetles and other aquatic and terrestrial invertebrates which have restricted distributions and high conservation value; and it is believed that the absence of fish could be responsible for the success of some rare aquatic invertebrate species in these transitional habitats (Sheehy Skeffington *et al.*, 2006). During their lake phase, turloughs provide vital habitat for over-wintering wildfowl, and this has led to some sites being given SPA status under the EU Birds Directive (European Community, 2009) and one turlough in County Galway (Coole-Garryland complex) has been designated as a Ramsar site (http://irishwetlands.ie/irish-sites/). In addition to the significance of floodplain meadows and turloughs for biodiversity, these fluctuating and transitional habitats provide other essential ecosystem services. For examples, floodplain grassland habitats play a vital role in the regulation of flood events via water retention, and the soils also represent a key carbon store (Lawson *et al.*, 2018). Turloughs also provide extensive holding systems for floodwaters in times of very high rainfall.

1.1.3 Anthropogenic pressures

Grassland plant communities are an important biodiversity resource which is being threatened by multiple anthropogenic pressures. A substantial decline in the extent and quality of Irish turlough and English floodplain meadow habitat began in the mid-nineteenth and mid-twentieth centuries, respectively, largely as a result of agricultural intensification (Sheehy Skeffington *et al.*, 2006; Rothero *et al.*, 2016). The extent of floodplain meadow habitat prior to their degradation is not known, but the major factors leading to the loss of this habitat are the - conversion of hay meadows to arable farming practices, urban development, and mineral extraction (Rothero *et al.*, 2016). Indirect effects of these anthropogenic pressures, such as soil compaction, alterations to hydrological regime, and changes to hay-cutting practices and/or grazing regime, have also resulted in the degradation of meadow habitat (i.e. the loss of desirable plant species and/or communities of conservation interest).

In Ireland, approximately one third of turloughs (with an area of 10 hectares or more) have been irreversibly damaged by large-scale drainage schemes which were undertaken for the reclamation of land for agricultural use (Coxon, 1986; Sheehy Skeffington *et al.*, 2006). Presently, two of the major threats to the biodiversity of turloughs are the nutrient enrichment of groundwater sources and the abandonment of this marginal land by farmers (Sheehy Skeffington *et al.*, 2006). It is, however, the unprecedented scale and rate of climate change which is arguably the most substantial threat to the ecosystem functioning of both floodplain meadows and turloughs. These are hydrologically driven habitats which will therefore be highly susceptible to any further alterations to weather patterns which are predicted to occur as a result of future climate change (see section 1.4).

1.2 Background ecological theory

Classic ecological theory of resource-based competition defines that a species will be a superior competitor within its own ecological niche and will become dominant as a result. However, all plants require the same basic resources and have only a limited number of ways in which to acquire them, which therefore poses a key question in plant community ecology – how do large numbers of species manage to coexist (Silvertown *et al.*, 1999; Silvertown, 2004)? There are several theoretical solutions to this conundrum and the debate amongst community ecologists is ongoing. One possible solution is offered by Hubbell's Unified Neutral Theory (2001) which proposes that species are competitively equivalent and diversity is the result of dispersal limitation, speciation and ecological drift (Rosindell *et al.*, 2011); whereas opposing theories of stable coexistence involve some degree of temporal or spatial niche segregation. There is a growing body of evidence which supports the existence of niche segregation along various environmental axes including soil-moisture status (Silvertown *et al.*, 2015).

Water is needed for almost all functions in terrestrial plants and is often a limiting resource for plant growth (Araya and García-Baquero, 2014), but its subtle importance to plant community structure may have been overlooked in the past. There are a number of studies which confirm the presence of hydrological niche segregation (HNS) in a variety of ecosystems and geographical locations, for example: floodplain meadows in England (Silvertown *et al.*, 1999); fynbos in South Africa (Araya *et al.*, 2011; Guo *et al.*, 2015); and *dehesa* community and alpine grassland in Spain (García-Baquero *et al.*, 2016). HNS has been defined as the partitioning of (i) space on fine-scale soil-moisture gradients (ii) water as a resource and/or (iii) recruitment opportunities among years by the storage effect (Silvertown *et al.*, 2015).

Within general community models, for example those devised by Connell (1978), Huston (1979), and Grime (2006), disturbance is one of the fundamental processes determining species richness. Connell's (1978) Intermediate Disturbance Hypothesis (IDH) and Huston's (1979) Dynamic Equilibrium Model (DEM) similarly predict that the greatest species richness will occur at intermediate levels of disturbance. The IDH predicts that diversity is lowest when disturbances are either frequent or rare. In a high disturbance situation, the community will consist of those few species which are capable of quickly reaching maturity and exclude those with lower powers of dispersal and slower growth; whereas, when disturbances are infrequent, the community will be dominated by fewer, stronger competitors. In the intermediate level of disturbance scenario, more species are able to colonise because there is more time available in the intervals between disturbances.

The DEM, however, predicts that the level of disturbance where greatest diversity is achieved also depends on the level of productivity; therefore, a powerful disturbance is required in order to counteract competitive exclusions at high rates of growth (i.e. high productivity), whereas at lower growth rates only a relatively weak disturbance is needed to prevent competitive exclusion (Huston, 1979). Grime (2006) describes how the degree of disturbance and stress (i.e. low or high productivity) dictate which type of plant strategy is most successful. It is competitors which flourish in highly productive but low disturbance situations, whereas stress-tolerators are suited to conditions of low productivity and disturbance, and ruderals dominate in highly productive and disturbed environments. In this theory there is no viable plant strategy in a low productivity and highly fluctuating situation.

1.3 Effects of water-regime on grassland plant communities

The various components of a water-regime (e.g. periods of flooding and drought) represent an important form of disturbance within grassland communities which can affect the survival of individual plants, community composition and overall richness. Some of the water-regime descriptors commonly used in the ecological analysis of vegetation patterns include: mean (e.g. Jones *et al.*, 2017), median (e.g. Hájek *et al.*, 2013) and maximum (e.g. Aldous and Bach, 2014) water-table depth, amplitude of annual fluctuation in water table (e.g. Kotowski *et al.*, 1998), and exceedance of threshold depths (e.g. Noest, 1994; Gowing *et al.*, 1997).

Current research on the drivers of meadow and turlough plant communities

An early study of HNS used thresholds to demonstrate how hydrologically defined niches were structuring plant communities in two English meadows (Silvertown *et al.*, 1999). Species tolerances were estimated from a range of hydrological conditions in which they were recorded growing at the two sites, and two sum exceedance values (SEV; soil drying and aeration stress) were derived from modelled water-table depths and used as niche axes. However, the effects of water regime on plant species richness were not considered at these sites. There are other European floodplain habitat studies which have found that an increase in flooding can relate to a decline in plant species richness (Maher *et al.*, 2015; Mathar *et al.*, 2015; Garssen *et al.*, 2017). Additionally, one study conducted at a single floodplain in south-east England found that drier conditions can promote species richness (Toogood *et al.*, 2008).

There is a general consensus within the published literature on turloughs, that the development of plant communities in these transitional habitats is largely driven by flood duration, rather than mean flood depth (Goodwillie, 2003; Sheehy Skeffington *et al.*, 2006; Tynan *et al.*, 2007). The earlier research had to rely upon qualitative proxy measures, in lieu of measured hydrological information, to characterise the hydrological regimes of turloughs (e.g. Praeger, 1932; Coxon, 1987a; Goodwillie, 1992). Although some more recent studies have used hydrological data, they have been limited by the amount of data available (e.g. Regan *et al.*, 2007; Moran *et al.*, 2008a; Moran *et al.*, 2008b). To date, there have been no studies which have examined turlough plant communities in relation to fine scale variation in the hydrology, using botanical and hydrological data which are contemporaneous.

Importance of flood timing and 'ecological memory'

The seasonality of fluctuations in water-regime is also an important determinant of grassland community composition. There is evidence to support the notion that the disturbance from flooding during the growing season is the primary driver of species distributions in wet floodplain grasslands (Gowing *et al.*, 1997, 2002; Eck *et al.*, 2004). Conversely, this suggests that flooding events in the winter months are not particularly harmful to plants (Eck *et al.*, 2005). The key mechanism by which flooding controls vegetation is the depletion of oxygen in the soil which can result in the death or inhibition of root growth (Goodwillie *et al.*, 1997). In the UK, the active growing season for plants is within the period March to September (Broad and Hough, 1993) and thus, during the rest of the year the root oxygen demand is much lower and aeration stress is less likely to occur (Gowing *et al.*, 2002). Summer flooding can also have a positive effect on grassland communities by promoting the germination of typical floodplain plant species to colonise gaps in the sward (Mathar *et al.*, 2015).

The important role that preceding hydrological conditions play in the development of plant communities in fluctuating habitats has been demonstrated in some floodplain meadow and dune slack studies (e.g. Noest, 1994; Gowing *et al.*, 2002; Curreli *et al.*, 2013). For instance, the five-year means of a range of hydrological parameters was found to have the greatest explanatory power on species' distribution in a Dutch dune slack study, when compared to the same parameters measured for the year of vegetation recording or the previous year only (Noest, 1994). Additionally, in UK wet meadows, the best explanatory power for changes in vegetation was found for hydrological variables (i.e. SEVs for waterlogging and drought stress) over the preceding three- to seven-year period (Gowing *et al.*, 2002, 2005).

However, despite their importance, there is still a lack of research on the quantification of antecedent conditions and their effect on current ecological processes (Ogle *et al.*, 2015). The notion of 'ecological memory' describes how the effects of current environmental conditions depend upon the conditions of previous years; for instance, the precipitation and temperature patterns of past weeks, months or years can affect many aspects of ecosystem functioning (Ogle *et al.*, 2015). Ecological memory can result from physiological (i.e. plant damage resulting from multiple occurrences of drought, making it vulnerable to more stress) or community-driven (e.g. shifts in competitive dynamics) mechanisms (Harrison *et al.*, 2018).

Fluctuation as disturbance

Fluctuating water levels are another form of disturbance which is thought to be an important process in many wetland types. A number of studies have examined the effects of fluctuating water levels on wetland plant communities, but the evidence presented by these studies is mixed. Some research suggests that hydrological fluctuations have a detrimental impact on plant biomass accumulation (Bakker *et al.*, 2007; Luo *et al.*, 2018), and species richness (Casanova and Brock, 2000). Other studies suggest that intermediate levels of fluctuation have a positive effect on species richness (Pollock *et al.*, 1998; Magee and Kentula, 2005); in contrast, there is some evidence that hydrological fluctuations have little or no effect on plant communities (Day *et al.*, 1988; Kotowski *et al.*, 1998; Nygaard and Ejrnaes, 2009). Despite the characteristic, highly fluctuating nature of Irish turloughs, there has been no research undertaken to examine the effects of hydrological variability on turlough plant communities.

There is evidence that intra-annual variability in precipitation and water-levels have a positive effect on the species richness of USA mesic grassland and of New Zealand lake-margin communities (Knapp *et al.*, 2002; Riis and Hawes, 2002). There are, however, no studies on European floodplain habitats which examine the effects of intra-annual hydrological variability on plant community species richness. Inter-annual fluctuations in water-regime are linked to one form of HNS known as the temporal storage effect. This theory suggests that there is a temporal aspect to recruitment, where established plants (and their seeds) are ‘stored’ during periods of unfavourable conditions until a point in time where the hydrological conditions are suitable for expansion (Silvertown *et al.*, 2015). It is argued that the storage effect is a key driving mechanism of species richness in arid floodplains in Australia (Capon, 2005; Capon and Brock, 2006; Angert *et al.*, 2009) and European floodplain meadow communities along the Upper Rhine in Germany (Hölzel and Otte, 2004; Mathar *et al.*, 2015).

The amount of variability within a water-regime can be difficult to quantify, as opposed to the more traditional hydrological parameters that have been widely used to characterise water-regimes, such as the depth, duration, and frequency of flooding. In the studies mentioned above, which examine the relationship between hydrological fluctuations and plant communities, variability has not been directly measured. Instead it has been estimated using proxies such as differences in flood frequency (Pollock *et al.*, 1998; Hölzel and Otte, 2004; Capon, 2005; Capon and Brock, 2006) and mean number of flooding days per year (Mathar *et al.*, 2015); absolute difference in water level (Magee and Kentula, 2005); and variation in rainfall pattern (Knapp *et al.*, 2002).

1.4 Effects of climate change on grassland plant communities

Wet grassland habitats are an important biodiversity resource which have already been substantially affected by anthropogenic activities (see section 1.1.3). It is clear that the different aspects of a water-regime are an important determinant of grassland plant ecology (see sections 1.2 and 1.3). Climate change is one of the most influential human-induced drivers of hydrological alterations. Each of the last three decades has been consecutively warmer than any preceding decade since 1850, and it is likely that climate change has already increased the frequency and intensity of heavy precipitation events in Europe (IPCC, 2014). Additionally, it is predicted that extreme precipitation and drought events will very likely become more intense and more frequent as global mean surface temperature continues to increase in the future.

Alterations in water regime due to climate change are a significant threat to internationally important wetlands, including floodplain wet grasslands (Brotherton *et al.*, 2019a). Under future climate change, soil-moisture dynamics will increase with greater temperature and rainfall variability; and in combination with increased plant physiological demands for both oxygen and water, this will lead to an increased occurrence of wet and dry extremes of plant stresses. Using downscaled IPCC scenarios to simulate waterlogging and drought stress, it is predicted that both waterlogging and drought stress will increase, on average by ~20% at sites where both stresses occur, in a warmer and more variable future (2050) climate (Bartholomeus *et al.*, 2011).

1.5 Research gaps

In order to manage and conserve sites of high biodiversity value, such as English floodplain meadows and Irish turloughs, it is necessary to understand the mechanisms that drive the assembly of these valuable plant communities. Additionally, it is clear that both current and future climate change represent a significant threat to grassland plant communities via changes in water-regime (see section 1.5). Therefore, there is a need to understand the current mechanisms relating to hydrological variability that affect grassland communities, in order to understand the potential future impacts of changing climate, as well as to inform the management of these habitats and preserve their biodiversity under a more variable climate. The following research gaps have been identified from a literature review (see section 1.3) of published studies examining the effects of water-regime on grassland plant communities:

1. The effects of soil drying and waterlogging on the species richness of plant communities in English floodplain meadows.

2. The examination of Irish turlough plant communities in relation to fine scale variation in the hydrology, using botanical and hydrological data which are contemporaneous.
3. Further research on the quantification of ecological memory and its effect on current ecological processes.
4. The effects of intra-annual hydrological variability on the species richness of wet grassland plant communities.
5. Further research on the effects of inter-annual hydrological variability on the species richness of wet grassland plant communities.
6. It is necessary to explore the ways in which hydrological fluctuations can be directly and meaningfully quantified and studied.

These evidence gaps will be addressed in the current study in order to advance our understanding of English floodplain meadows, Irish turloughs, and other grassland habitats characterised by fluctuating water-regimes (see section 1.6).

1.6 Research aims

The aim of this research is to investigate the effects of hydrological fluctuation on wet grassland plant communities using both field and experimental evidence. The results of this study should clarify what degree of hydrological fluctuation is beneficial or detrimental to plant species richness at the study sites. It is important to note that when 'species richness' is mentioned in this thesis, it is in reference to *plant* species richness only. This research will also provide an indication of how community composition may change in the future under increasingly variable conditions as a result of climate change.

The specific aims of this research project are to:

1. Examine the effects of soil drying and/or waterlogging, using the existing SEV method, on the: i) plant species richness of English floodplain meadows (chapter 3), and ii) plant community assembly (chapter 4) and species richness (chapter 5) of Irish turloughs;
2. To characterise hydrological fluctuations temporally (between and within years) and spatially (fine-scale sampling) in order to investigate their effects on plant species richness in English floodplain meadows (chapter 3) and Irish turloughs (chapter 5);
3. To investigate the effects of different flood durations and frequencies on the growth of a sample grassland plant community, in order to advance the current understanding of the effects of hydrological variability on these species (chapter 6);
4. Comment on the repercussions of findings for the management of the study sites, and similar habitats, under future climate change (chapters 3, 4, 5 &7).

Chapter 2

Site selection and field methods

2.1 Site selection

2.1.1 Site selection criteria

The selection of all study sites was predominantly driven by: i) presence of fluctuating water levels, ii) availability of modelled hydrological data over a minimum period of ten years, iii) distinct zonation of plant communities. In order to meet the aims specified in chapter 1 (section 1.6), this study combines an analysis of both existing data from English floodplain meadows and new data collected from sites with greater levels of hydrological fluctuation.

2.1.2 English floodplain meadows

A range of English floodplain meadow sites was selected for study based upon the criteria outlined above (section 2.1.1) as well as the following conditions: i) availability of botanical data, ii) consistent management regimes, iii) contrasting hydrological characteristics between sites. The name and location of each floodplain meadow site are listed in chapter 3 (section 3.2.1), and the full details can be found in the DEFRA report (Department for Environment, Food and Rural Affairs) from which the data originated (Gowing *et al.*, 2002).

2.1.3 Reconnaissance for new sites

A search for new study sites, which satisfy the conditions outlined above (section 2.1.1), was undertaken in 2016. These sites also had to be hydrologically distinct and experience greater fluctuations in water level than English floodplain meadows, in order to provide a useful and meaningful comparison. Twenty sites located across England, Wales and Ireland were selected as being potentially good comparisons and reconnaissance visits were carried out to assess their suitability for fulfilling the objectives of this research project. Some sites (table 2.1) were discounted because upon reconnaissance it was found that: i) they did not meet the essential criteria (e.g. insufficient hydrological data and/or plant community zonation); ii) there were additional pressures (e.g. intense grazing, nutrient enrichment) present which were affecting the plant communities and potentially masking the effects of fluctuating hydrology; iii) there were unforeseen logistical difficulties (e.g. inability to install monitoring equipment required for study).

Table 2.1: Location, name, underlying geology, and key characteristics of the reconnaissance sites which are not included in the current study; and the reason(s) for their exclusion.

Country	**Site name(s), county**	**Geology**	**Site details and reason(s) for exclusion from current study**
England	Breckland meres, Norfolk	Chalk	- Four fluctuating meres were deemed unsuitable due to insufficient zonation in the marginal vegetation (West Tofts Mere, Devil's Punchbowl) or a high level of grazing and nutrients (Langmere, Ringmere); - Two fluctuating meres (Fowl Mere, Home Mere) had good plant zonation but hydrological data were not readily available; potential for future research.
	Meres and mosses, Shropshire	Sandstone	- Two fluctuating meres were discounted as they lacked any zonation in the marginal vegetation (Cole Mere, Bomere); - Brown Moss (a network of woodland, heath, moss and fluctuating pools) had some interesting plant zonation but was undergoing much disturbance (tree removal at pool edges) and had many invasive non-native species on site (e.g. *Crassula helmsii*).
	Nosterfield Nature Reserve, North Yorkshire	Limestone	- Former quarry with naturally fluctuating water levels now managed as a nature reserve; rich species assemblage with good zonation; a series of dipwells already installed and some (patchy) data available from manual readings and data loggers; - Insufficient hydrological data for current study; potential for future research.
Wales	Nedern Brook SSSI, Monmouthshire	Limestone	- Artificially straightened brook running through agricultural fields which becomes a large lake in winter; a small number of dipwells already installed and some data available; - Insufficient hydrological data for current study; potential for future research.
	Pant-y-llyn, Carmarthenshire		- Wales's only turlough; botanical and hydrological data available; - Discounted due to small size, extremely low species diversity and large mature woodland canopy causing shading problems.
Ireland	Ardkill, Mayo	Limestone	- Relatively small-sized turlough with interesting plant community zonation; discounted due to problems with eutrophication at the site.
	Blackrock/Peterswell, Galway		- Large turlough with flashy hydrological regime; discounted due to high nutrient status and complications with hydrology (river draining into basin).
	Lough Aleenaun, Clare		- Turlough in the Burren; species-poor assemblage and flashy hydrological regime; well-calibrated hydrological model; discounted due to insufficient zonation and high nutrient status.
	Lough Gealáin, Clare		- Another Burren turlough; interesting plant species and zonation; well-calibrated hydrological model; discounted due to logistical difficulties (very shallow soils causing difficulties with dipwell installation) and very low nutrient status (oligotrophic system).

2.1.4 Irish turloughs

Turloughs are well-suited to this research for a number of reasons. Firstly, their distinctive fluctuating hydrology and plant community zonation, first noted by Praeger (1932), make them particularly suitable given the criteria laid out in section 2.1.1. Previous work has been undertaken at a number of turloughs, which aided the selection of study sites through the provision of baseline botanical and hydrological data (see below). Additionally, concurrent work was being undertaken by researchers at Trinity College Dublin, who were open to collaboration with regards to hydrological monitoring and modelling of turloughs. Turloughs also provide a useful comparison to English floodplain meadows because of the overlap in key species and plant-community assemblages present at both habitats.

Preliminary research, and discussions with collaborators, revealed that hydrological monitoring had recently been undertaken at twenty-two turloughs and ten of those sites also had working hydrological models (Naughton, 2011). The results of previous vegetation mapping studies were then examined to find sites which were likely to have distinct plant-community zonation (Goodwillie, 1992; Sharkey *et al.*, 2015); and this led to eight turloughs being short-listed for reconnaissance visits. Subsequently, half of these sites were rejected for the reasons listed in table 2.1. The remaining four sites were selected for study because they satisfied the essential criteria listed in section 2.1.1, and no prohibiting factors were found (as discussed above); these four sites also cover a range of hydrological variability, from relatively steady to flashier hydrographs, allowing the study of different regimes which are representative of the turlough habitat (table 2.2).

Table 2.2: Location, name, underlying geology, and key characteristics of the hydrological regimes of the turlough study sites; hydrological information source: Naughton (2011).

Country	Site name, county	Geology	Key characteristics of hydrological regime
Ireland	Caranavoodaun, Galway	Limestone	Relatively steady; one major flood event per year; small fluctuations in water level throughout year; maximum flood depth 3.8 metres.
	Garryland, Galway		Moderately flashy; multiple flood events per year; maximum flood depth 10.9 metres.
	Lough Coy, Galway		Very flashy; multiple flood events per year; maximum flood depth 10.6 metres.
	Skealoghan, Mayo		Relatively steady; one major flood event per year, but water levels can vary markedly during the flood period; maximum flood depth 3.2 metres.

Unfortunately, part way through the current research project, major changes occurred at Caranavoodaun turlough which meant that it was no longer suitable for study. In August 2016, an area of study was selected (figure 2.1a) and preliminary field work was undertaken. However, water levels at the site remained uncharacteristically high during the multiple visits undertaken by the author (figure 2.1b – d), as well as by colleagues and collaborators, in 2017 and 2018, and no further data were collected as a result. The reason for this drastic change is not known, but one suggestion is that the site's hydrological regime has been altered by nearby motorway construction and additional drainage from this new source may have raised the water levels at Caranavoodaun and/or that the outflow channel has become blocked in recent years (Sheehy Skeffington pers. comm., 2018). The site descriptions of the three remaining turloughs used in this study are detailed below (section 2.1.5).

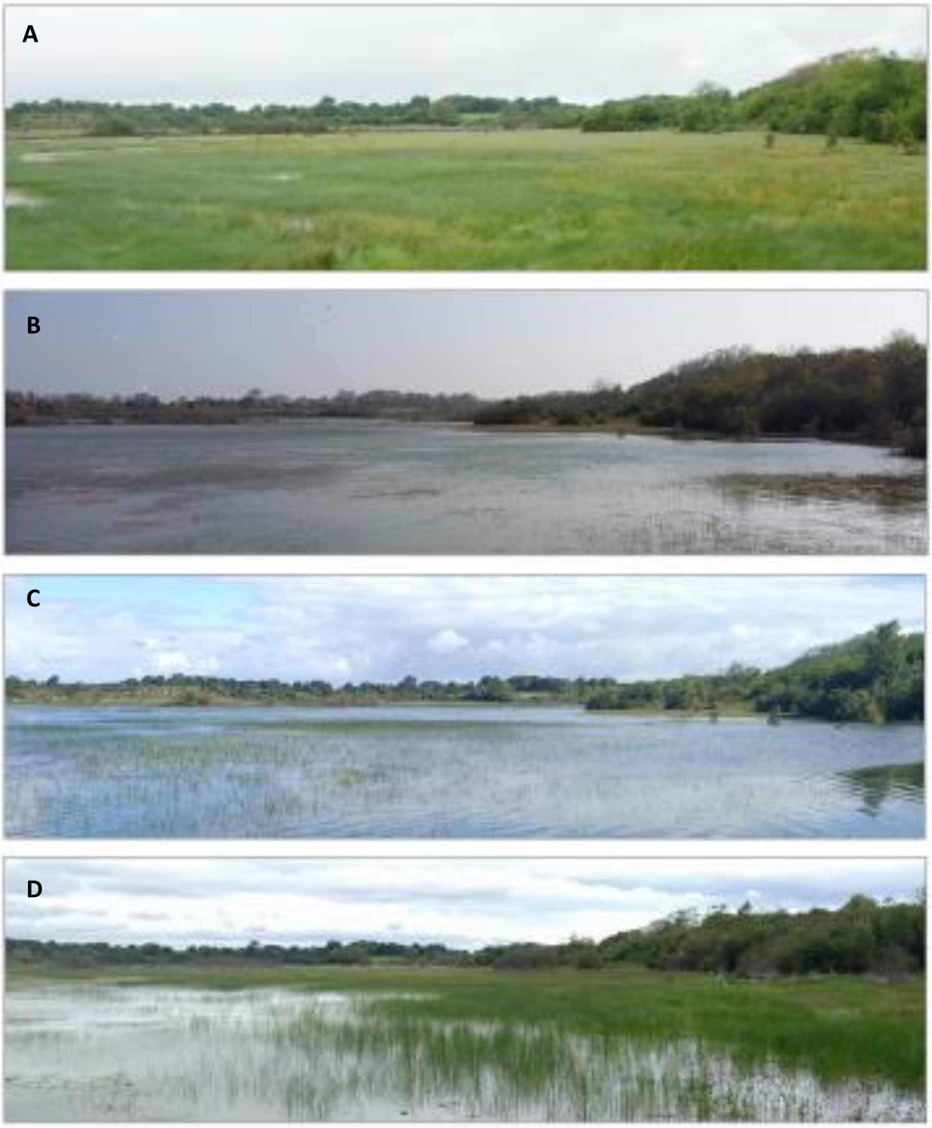


Figure 2.1: Photographs of the study area at Caranavoodaun turlough during visits in: (a) August 2016, (b) June 2017, (c) August 2017, (d) August 2018; fieldwork was only undertaken in 2016 because the site was subsequently inaccessible due to high water levels (photographs B-D).

2.1.5 Turlough site descriptions

Garryland

Garryland turlough basin (20 ha; Irish grid reference M 4104) is located in south-east County Galway, within the Coole-Garryland SAC complex. It is a relatively small basin which is surrounded by woodland, and it has steep slopes with large boulders scattered throughout (figure 2.2). Garryland is one of five turloughs within the Gort lowlands complex, which are hydrologically connected underground; the other four sites within the complex are Blackrock, Caherglassan, Coole and Lough Coy (Gill *et al.*, 2013). Garryland has a relatively flashy hydrological regime, with generally more than one significant flood event occurring per year (Naughton, 2011; Naughton *et al.*, 2015; table 2.2). The turlough is seasonally grazed by sheep, horses and cattle. The soils are moderately acidic and inorganic, with low levels of calcium carbonate, and are composed of shallow, poorly-drained mineral soil types (Kimberley, 2015). A recent vegetation mapping survey found five communities occurring in Garryland, with the *Agrostis stolonifera-Ranunculus repens* community covering the greatest area (Sharkey *et al.*, 2015).

Figure 2.2: Photographs of Garryland turlough showing: (a) the area of study, (b) limestone cliff outcrop in the western part of the basin, (c) black moss *Cinclidotus fontinaloides* growing on a tree at the edge of the turlough which is a characteristic indicator of the (median) maximum winter flood depth.

Lough Coy

Lough Coy (26 ha; M 4907) also forms part of the Gort lowlands turlough complex and is located in south-east County Galway (Gill *et al.*, 2013). It is a relatively deep, bowl-shaped basin with steep-sided slopes dotted with large boulders, and a semi-permanent lake in the centre (figure 2.3). Lough Coy has a flashy hydrological regime, with more than one significant flood event occurring per year (Naughton, 2011; Naughton *et al.*, 2015; table 2.2), it can fill and empty rapidly at any time of year (figure 2.3). The turlough is seasonally grazed by cattle. The soils are moderately acidic and mineral, with low levels of calcium carbonate, and are composed of shallow, poorly-drained mineral and alluvial mineral soil types (Kimberley, 2015). A recent vegetation mapping survey found eight communities occurring in Lough Coy, and dominant communities were *Filipendula ulmaria-Potentilla erecta-Viola sp.* and *Agrostis stolonifera-Potentilla anserina-Festuca rubra* (Sharkey *et al.*, 2015).

Figure 2.3: Photographs of Lough Coy turlough showing: (a) the area of study, (b) the estavelle when the turlough was emptying in August 2016 and then (c) three days later during a rapid filling stage.

Skealoghan

Skealoghan turlough (33 ha; M 2563) is located in south County Mayo and it has SAC status (Moran *et al.*, 2008a). It is a broad site with relatively flat topography (figure 2.4) and limestone outcrops occur in some parts of the basin. Skealoghan has a steady hydrological regime, with generally only one significant flood event occurring per year (Naughton, 2011; Naughton *et al.*, 2015; table 2.2). The majority of the turlough (87%) is under seasonal grazing by cattle and in some fields, by sheep (Moran *et al.*, 2008a; Kimberley, 2015). The soils are circumneutral and peaty, with low levels of calcium carbonate, and are largely composed of fen peats on the basin floor and shallow, well-drained organic soils on the upper slopes (Kimberley, 2015). A recent vegetation mapping survey found twelve communities occurring in Skealoghan, and dominant communities were *P. anserina-Carex nigra*, *Carex nigra-Carex-panicea* and *Lolium* grassland (Sharkey *et al.*, 2015), which, based on an earlier study yielding 13 plant communities (Moran *et al.*, 2008b) can be classified into two main phytosociological associations, the *Cirsio dissecti-Molinietum* and the *Ranunculo-Potentillietum anserinae* (Moran *et al.,* 2008a).

Figure 2.4: Photographs of Skealoghan turlough showing: (a) the area of study facing north and (b) west, and (c) calcified leaves on a *Mentha aquatica* plant.

2.2 Field methods

The complete field methods used to collect the English floodplain meadow data can be found in the DEFRA report from which the data originated (Gowing *et al.*, 2002). In summary, each floodplain meadow was surveyed during the period mid-May to early-July between 1986 and 2002, quadrats measuring 1m × 1m were used and all plant species were recorded with an estimate of their cover. The remainder of this section details the field methods employed to gather data on the Irish turlough sites. For all the methods discussed below, the sampling locations were recorded to an accuracy of 3 ± 1.5 cm using a high precision Leica Zeno 20 GPS.

Site instrumentation and preliminary data collection - 2016

Initial fieldwork was carried out during late July-early August 2016, when each site was instrumented, and preliminary data were collected. An area of study was selected at each site (figure 2.5) and divided into four distinct sampling zones (figure 2.6) based on a visual identification of the plant communities present. The sampling zones are distributed from the base of the turlough basin, where growing conditions are relatively wet (zone 0/1), up to the relatively dry upper slopes (zone 4; figure 2.6).

Figure 2.5: Satellite images of the three turlough sites with the area of study marked by the red boxes (approximately 1.7ha at Garryland, 1.3ha at Lough Coy, 1.4ha at Skealoghan); black arrows at each site show the lines of dipwells installed in an ‘L’ shape along two different gradients; the arrows denote the direction of groundwater flow along slope A (solid arrow) and slope B (dashed arrow) referred to in figure 2.6.

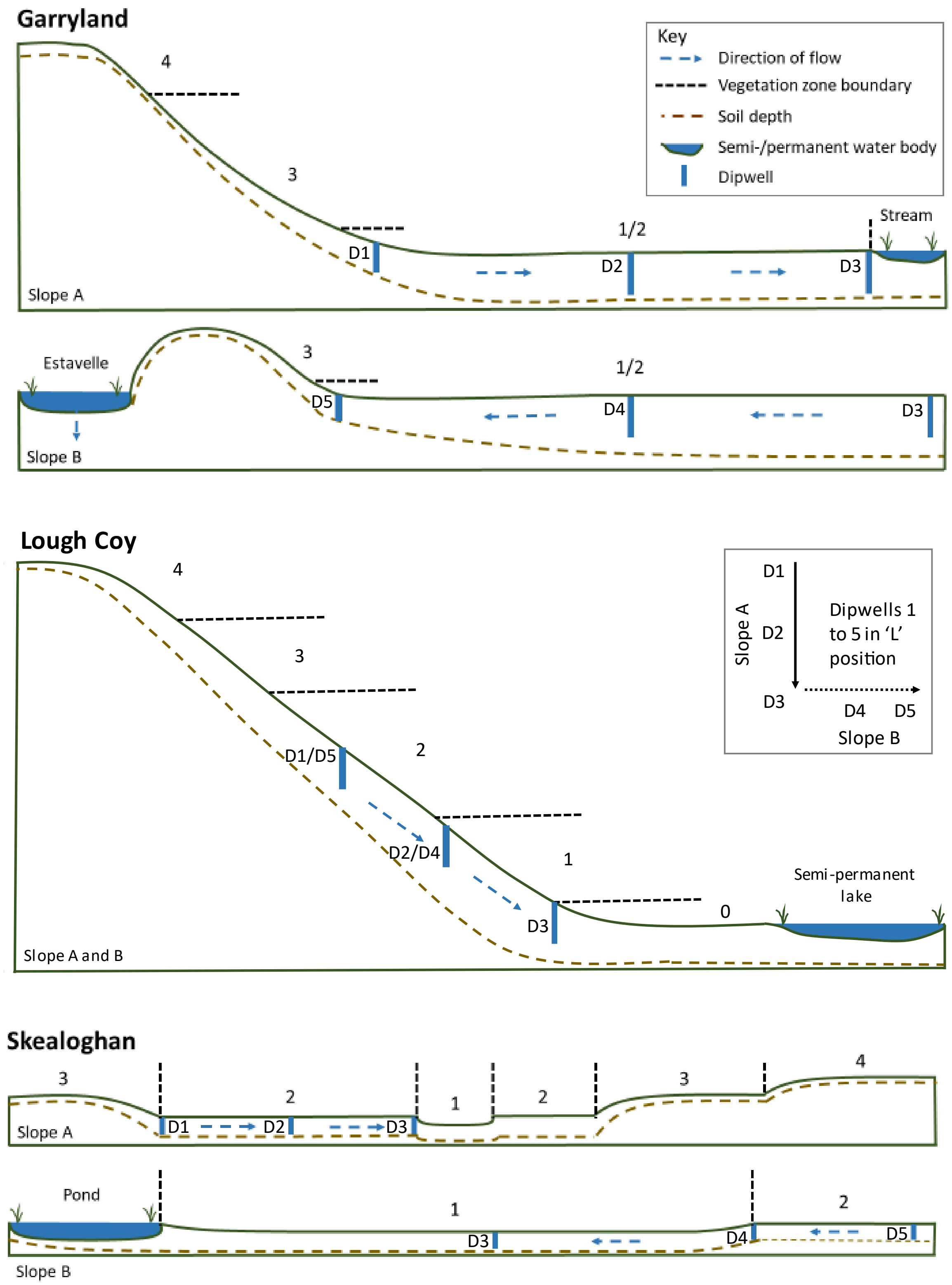


Figure 2.6: Cross-sections of the study area at each turlough, showing the dipwells (labelled D1 to D5) installed in an 'L' shape (see also figure 2.5) along two different gradients (slope A and slope B; both slopes only drawn when the gradient is substantially different); the approximate length of each dipwell transect is: 40m (slope A) and 47m (slope B) at Garryland, 43m (A) and 30m (B) at Lough Coy, 68m (A) and 38m (B) at Skealoghan; the distribution of the vegetation sampling zones from wetter (zone 0/1) to drier (zone 4) conditions is shown, along with approximate soil depth, direction of groundwater flow, and presence of semi- and permanent water-bodies; all drawn by hand, not to scale.

A series of five dipwells were installed at each site in an ‘L’ shape along two different gradients, to monitor ground water levels and establish the direction of flow (figure 2.5 and 2.6). The central dipwell was installed as low as possible in the basin and where it was feasible, the remaining dipwells were placed in the different sampling zones (except for highest and driest areas), to provide as much information as possible on groundwater levels. The holes were excavated by hand using a 5 cm diameter soil auger up to a depth of one metre (minimum 40 cm); sections of PVC pipe were then inserted with the top approximately 3 cm below the ground surface (figure 2.7). The pipe was perforated with holes and covered with woven material to allow water but not silt to enter the dipwell (Rothero *et al.*, 2016). Pressure-transducer water-level loggers (LevelSCOUT, Seametrics, Seattle) were installed in the lowest dipwell at each site (i.e. dipwell 3; figure 2.6) and a metal cap was placed on top of all dipwells to prevent damage by livestock and inundation with sediment. A barometer (BaroSCOUT, Seametrics, Seattle) was installed at Skealoghan for measuring atmospheric pressure, which is used to offset the overall pressure recorded by the LevelSCOUTs. Manual dipwell readings were taken using a ‘buzzing stick’ as often as possible (i.e. when the author was on site and the dipwells were accessible), to provide data for the calibration of the LevelSCOUT instruments.

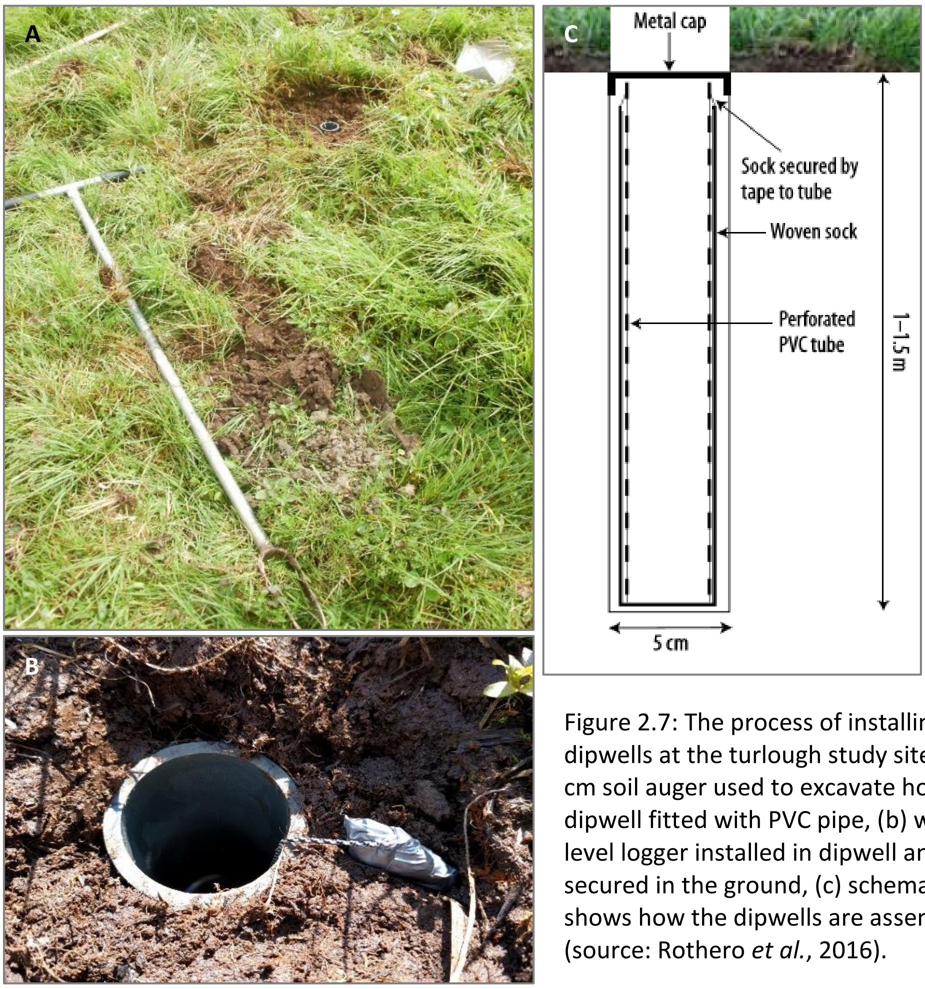


Figure 2.7: The process of installing dipwells at the turlough study sites: (a) 5 cm soil auger used to excavate holes and dipwell fitted with PVC pipe, (b) water-level logger installed in dipwell and secured in the ground, (c) schematic shows how the dipwells are assembled (source: Rothero *et al.*, 2016).

One bulk soil sample (approximately 250 g) was collected per sampling zone (figure 2.8), using a soil corer, from the upper 10 cm of the soil column. The samples were air-dried in Ireland and subsequently tested in the laboratory at the Open University (OU; Milton Keynes, UK) for pH and extractable phosphorus concentration (Olsen *et al.*, 1954; appendix A.1 and A.2).

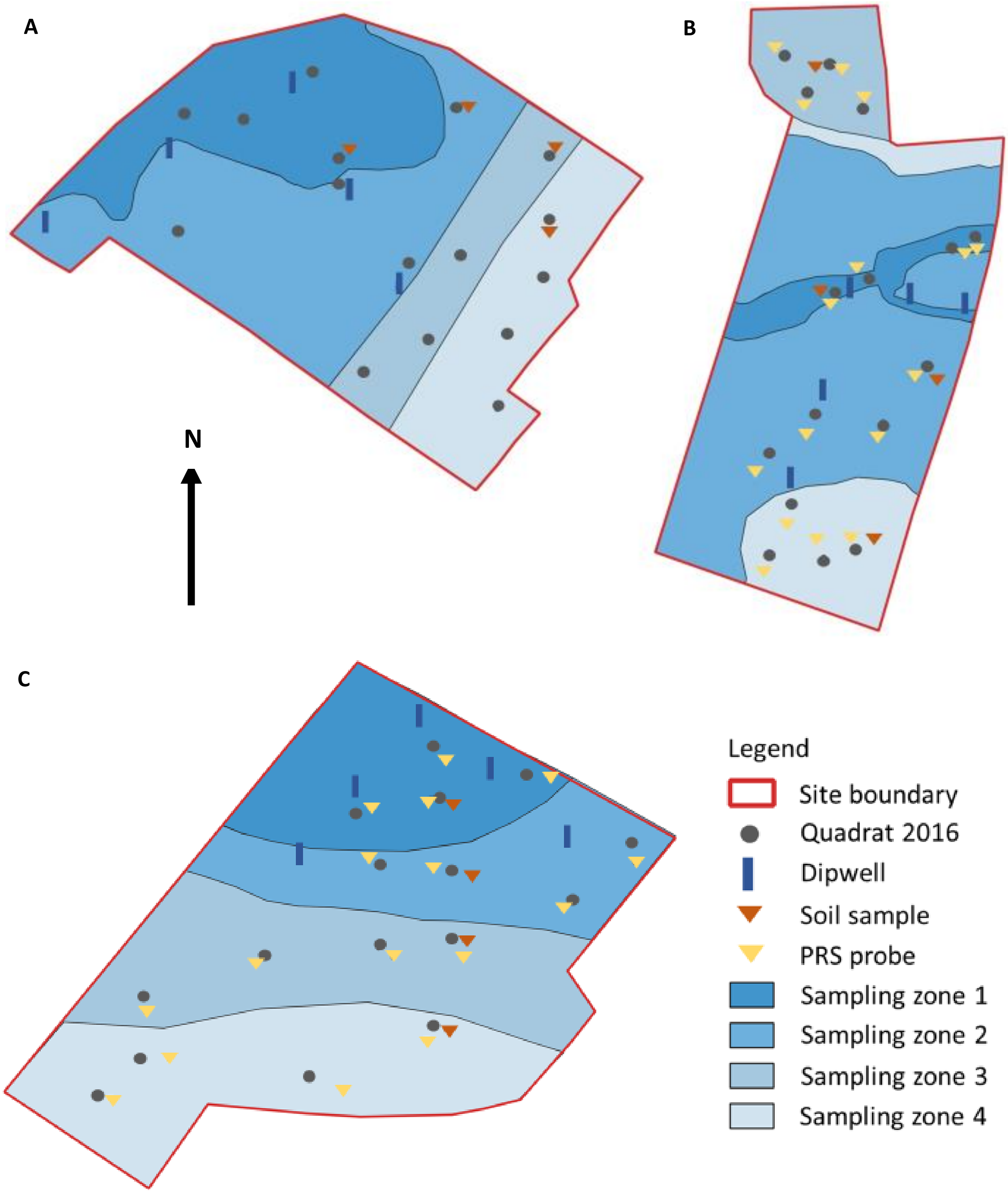


Figure 2.8: Maps drawn in QGIS of the study area of each turlough: (a) Garryland, (b) Skealoghan, (c) Lough Coy; the vegetation sampling zones and dipwell positions are given, as well as the locations of the botanical quadrats surveyed and soil samples collected in 2016, and the Plant Root Simulator (PRS) sample locations from 2017.

A preliminary botanical survey was conducted with four relevés (1 m x 1 m quadrats) randomly distributed in each sampling zone (figure 2.8). In each quadrat all plant species present were given a percentage cover value and identified to species level where possible (figure 2.9), following the nomenclatures of Stace (1991) for vascular plants and Smith (2004) for mosses. Any specimens not identified in the field were preserved for subsequent investigation; identification of the vascular plants and mosses were then undertaken by the author, and experts Hilary Wallace and Mike Prosser, respectively.

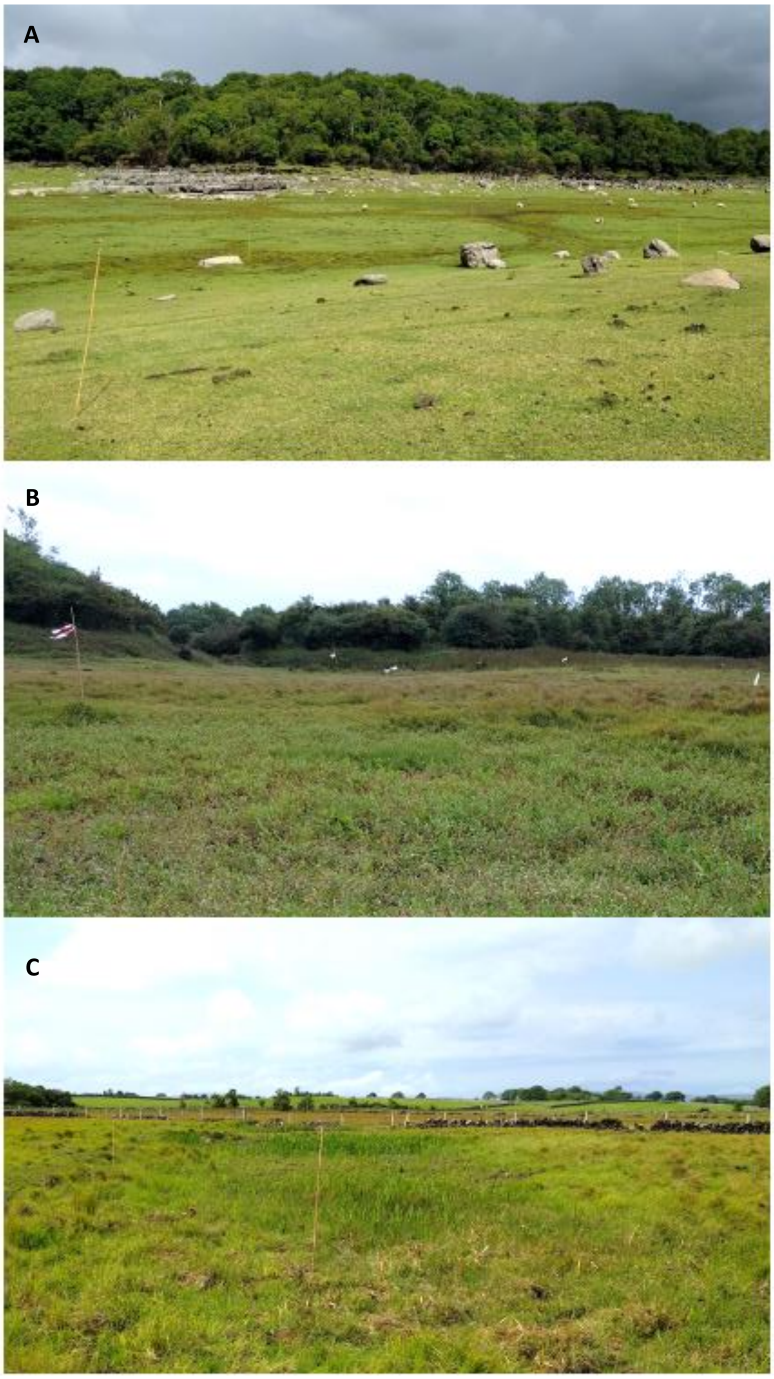


Figure 2.9: Botanical surveys being carried out at (a) Garryland, (b) Lough Coy, (c) Skealoghan in August 2016; the bamboo canes photographed mark sampling location/s (not all locations are in view).

Plant Root Simulator (PRS®) probe installation and retrieval - 2017

Plant Root Simulator (PRS®) probes were used to measure 15 plant-available nutrients (figure 2.10) in the uppermost soil layer (ca 10 cm depth), because this is where most soil nutrients are released and roots are most active (visit www.westernag.ca for full field and laboratory methodologies). After consultation with an experienced PRS Research and Development Coordinator, it was decided that four PRS samples (with four pairs per sample) would be required in each of the four vegetation zones (therefore 128 probes in total per site) in order to capture the variation in the heterogenous soil environment, and that a burial time of 6-8 weeks would be most suitable. The time required for the probes to absorb the nutrients in the soil fully can vary depending on the habitat; for instance, it can take between 1 – 4 weeks in wetlands and 4 – 8 weeks in grasslands. In May 2017, the probes were installed at four locations per vegetation zone at Lough Coy and Skealoghan (figure 2.8 and 2.10); unfortunately, the study area at Garryland turlough was inaccessible due to high water levels (figure 2.11), so no probes were installed there. A slot was made in the soil using a soil knife, the PRS probes were then inserted and an adjacent ‘back-cut’ was made to ensure good contact between the ion exchange membrane and soil (figure 2.10).

Element	Symbol
Nitrogen	NO3-N
Ammonia	NH4-N
Calcium	Ca
Magnesium	Mg
Potassium	K
Phosphorus	P
Iron	Fe
Manganese	Mn
Copper	Cu
Zinc	Zn
Boron	B
Sulfur	S
Lead	Pb
Aluminum	Al
Cadmiun	Cd

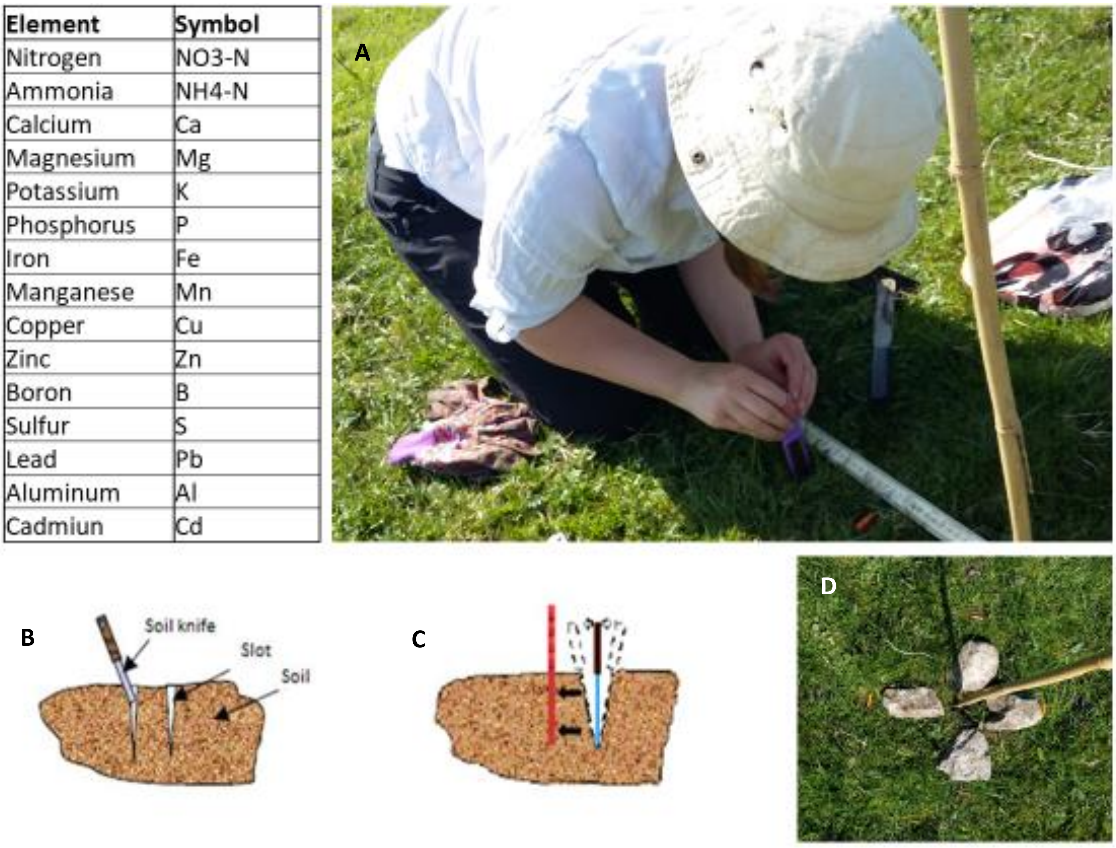


Figure 2.10: Table showing the elements measured by the Plant Root Simulator (PRS) probes, and details of the installation process (a-d): (a) probes being installed by the author at Skealoghan, (b) and (c) show the insertion method (source: www.westernag.ca), and (d) one sample (four pairs of probes per sample) fully installed at Skealoghan.

The probes were recovered in June, having been buried in the ground for 54 days at Lough Coy and 53 days at Skealoghan; all 128 probes were recovered from Lough Coy, and 120 probes were collected from Skealoghan because of some damage and misplacement caused by cattle. The probes were then cleaned in the OU laboratory using distilled water and shipped to Western Ag Innovations in Canada for analysis.

Additionally, in 2017, water-level loggers were installed in dipwells 2 and 4 at Lough Coy and Skealoghan, and in dipwell 2 only at Garryland due to high water levels. Three undisturbed soil cores (5 cm depth x 5 cm diameter) were collected per sampling zone (figure 2.8) in order to calculate waterlogging thresholds (see section 4.2.3 for details). Each sample was collected in the top 10 cm of the soil column (figure 2.11), then transported back to the OU laboratory to be prepared and analysed according to a set protocol (appendix A.3).

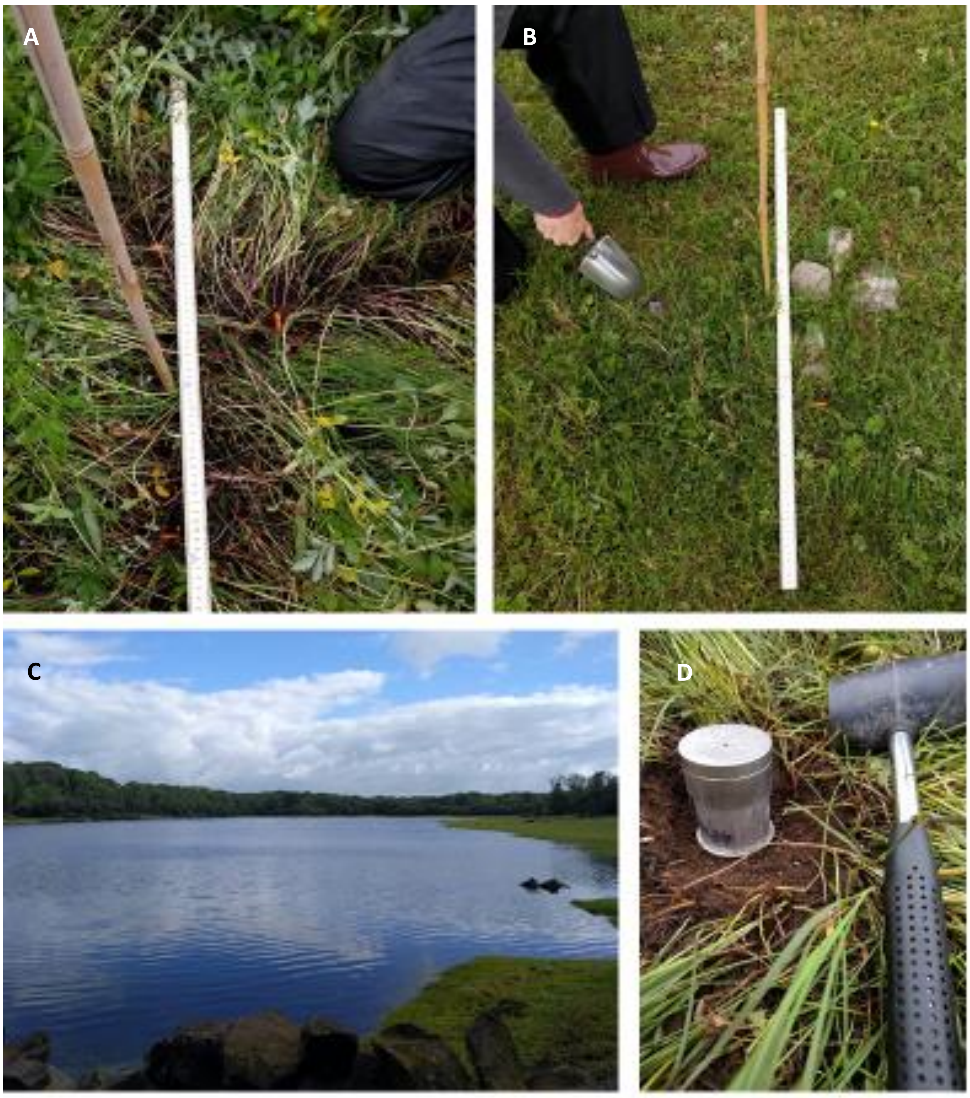


Figure 2.11: Photographs showing the retrieval of Plant Root Simulator (PRS) probes at (a) Lough Coy and (b) Skealoghan; (c) photograph of Garryland in 2017 showing high water levels which prevented the installation of PRS probes at this site; (d) the equipment used to collect the soil cores required for calculating soil waterlogging thresholds in the laboratory (see section 4.2.3 for details).

Botanical surveying - 2018

A more comprehensive botanical survey was conducted in August 2018 as per the method already given above. The original permanently marked quadrats from 2016 were surveyed again, and four new quadrats were also surveyed in each zone (figure 2.12). An additional zone was identified at Lough Coy (zone 0) that had previously been inundated but now comprised a lake-drawdown plant community; eight quadrats were sampled here.

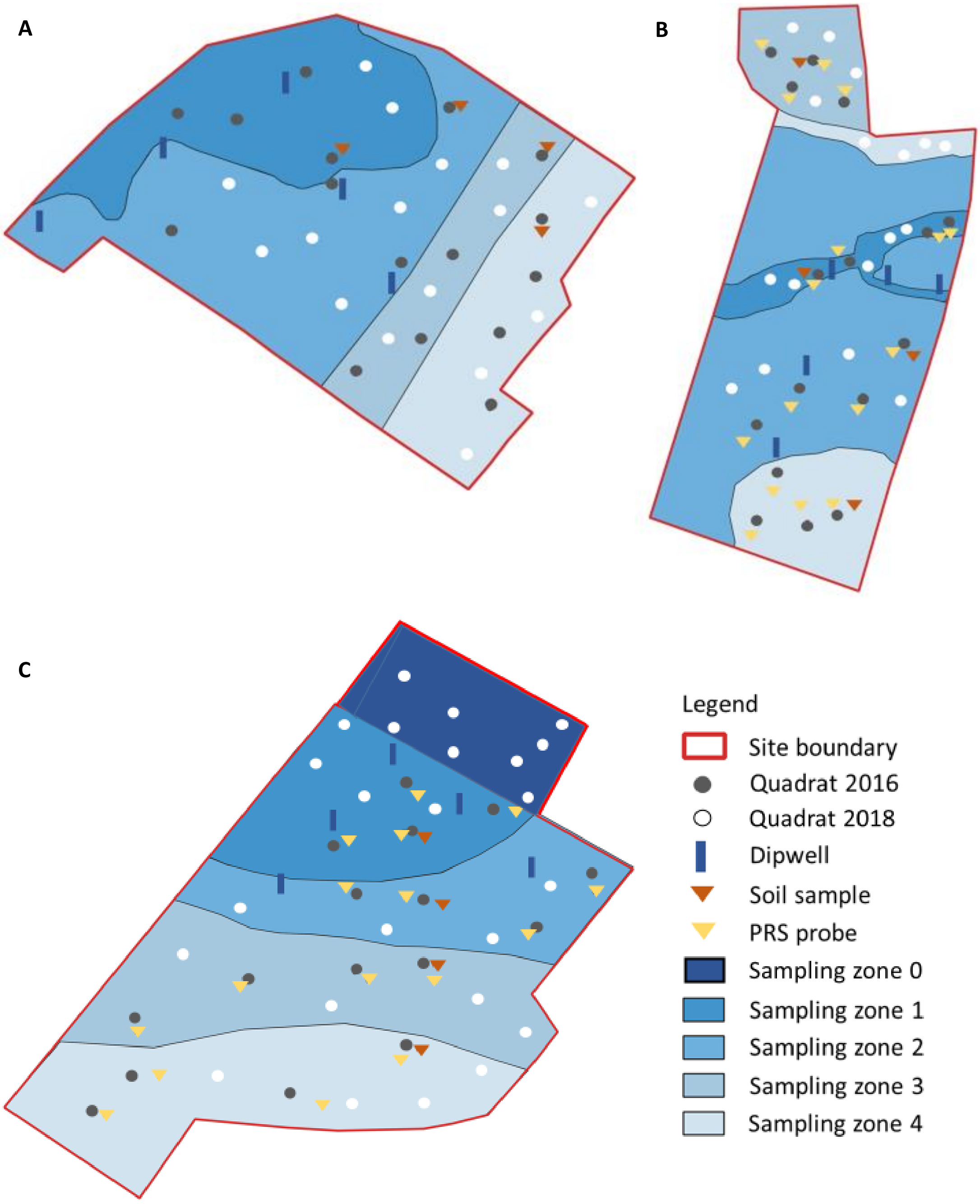


Figure 2.12: Maps drawn in QGIS of the study area of each turlough: (a) Garryland, (b) Skealoghan, (c) Lough Coy; the vegetation sampling zones and dipwell positions are given, as well as the locations of the botanical quadrats surveyed in 2016 and 2018, the soil samples collected in 2016, and the Plant Root Simulator (PRS) sample locations from 2017.

Chapter 3

Effect of inter- and intra-annual hydrological variability on the species richness of vegetation in English floodplain meadows

3.1 Introduction

Grassland habitats in the UK can be highly diverse with up to 40 species per square metre (Silvertown, 2004) and therefore they have been a significant focus of Hydrological Niche Segregation (HNS) research (see chapter 1 for further details). Traditionally managed hay meadows on floodplains, for example, often support diverse plant communities of high conservation value (Gowing *et al.*, 2002). An early study of HNS research revealed the sensitivity of floodplain-meadow plant communities to fine-scale soil-moisture gradients and demonstrated how hydrologically defined niches were structuring plant communities at two English meadows. Species tolerances were estimated from a range of hydrological conditions in which they were recorded growing at the two sites, and two sum exceedance parameters (soil drying and aeration stress) were derived from modelled water-table depths and used as niche axes (Silvertown *et al.*, 1999).

Within general community models, for example those devised by Huston (1979) and Grime (2006), disturbance is one of the fundamental processes determining species richness. The 'Intermediate disturbance' hypothesis by Connell (1978) suggests that the highest diversity in an ecosystem is maintained at intermediate levels of disturbance. If disturbances occur frequently, the community will only consist of those few species which are capable of quickly reaching maturity and exclude those with lower powers of dispersal and slower growth. With larger intervals between disturbances, diversity will begin to increase, because more time is available for the colonisation of more species. Diversity will decrease as the frequency of disturbance events declines further, due to the dominance of fewer, stronger competitors.

The research surrounding the impacts of increased flooding or soil drying (two key forms of disturbance) on plant species richness in floodplain habitats is generally in agreement. For example, a field study conducted along 5 European streams (Garssen *et al.*, 2017) found a decline in riparian species richness after 3 years of increased duration and depth of late winter/early spring flooding. Similar field studies, conducted along the Upper Rhine (Mathar *et* *al.*, 2015) and Shannon (Maher *et al.*, 2015) rivers, found that an increase in flooding reduced species richness in a functional floodplain habitat. Another study looked at the sensitivity of English floodplain grassland communities to water regime alteration using a transplantation field experiment (Toogood *et al.*, 2008); they found that generally, vegetation was responding to drier conditions following major flood events and this 'drying' trend was characterised by an increase in species diversity, with a greater abundance of competitive species and fewer typical wetland plants.

Fluctuating water levels are another form of disturbance which is thought to be an important process in many wetland types. This type of disturbance has both positive and negative effects on individual plants, as well as communities as a whole and overall species richness. There are some species that are reliant on fluctuating water levels to gain a competitive edge over more robust species; for instance the fen violet (*Viola persicifolia*) is only found where there are periodic disturbances, such as fluctuating water levels, which act to stimulate germination and reduce competition from other plants (Palmer, 2006). Conversely, wetland plants which are adapted to waterlogged conditions can suffer when there is a switch between aerobic and anoxic conditions (Bakker *et al.*, 2007); for example, anoxic conditions favour roots with aerenchyma that are capable of oxygen transport, but this root system is less efficient at taking up nutrients (Koncalova, 1990).

Plant physiological and morphological adaptations in response to changing water levels can also be detrimental to growth rates, when compared to more stable conditions. Bakker *et al.* (2007) demonstrated this in an experiment subjecting plants of wet dune slacks to either constant or to fluctuating water tables; fluctuating water levels led to lower total biomass production than constant water levels, indicating that switching from aerated to anoxic soil conditions involved physiological costs. Morphological plasticity can give particular species an advantage, for example *Phalaris arundinacea* is an aggressive invader in wetlands and under different flooding durations it can shift its growth form from a sward (under intermittent and early-season flooding) to a tussock (constant flooding) (Herr-Turoff and Zedler, 2007).

Over time, certain species have developed strategies for coping with hydrological variability but increasingly, anthropogenic activities are disrupting natural temporal variation in water regimes at a rate at which plants cannot adapt. Although many grasslands were created by human activities, environmental perturbations now threaten their biodiversity and functioning (UK National Ecosystem Assessment, 2011). Climate change is one of the most influential human-induced drivers of hydrological alterations; both an increase in the frequency and intensity of extreme events (e.g. drought, floods) is projected (IPCC, 2014). This will sharpen the contrasts between wet and dry seasons and increase unpredictability at all temporal scales, which may have a detrimental impact on the species richness of plant communities. Climate change may also drive a shift in timing of flooding, which, if prolonged into the summer period, can reduce the survival especially of less aquatic species (Maher *et al.*, 2015). There is growing evidence that species will be unable to disperse quickly enough to keep pace with rapid climate change, and this in turn will lead to a decline in plant community diversity (Corlett and Westcott, 2013; Harrison, 2020).

Soil-moisture regime can vary considerably inter-annually (between years), and this is fundamental to one form of HNS known as the temporal storage effect. This theory suggests that there is a temporal aspect to recruitment, where established plants (and their seeds) are 'stored' during periods of unfavourable conditions until a point in time where the hydrological conditions are suitable for expansion (Silvertown *et al.*, 2015). Angert *et al.* (2009) proposed that the storage effect could be the dominant fluctuation-dependent mechanism for organisms in fluctuating ecosystems; the study demonstrated that functional trade-offs between different species of desert annuals result in a diverse utilisation of soil moisture over time, and this explains the population dynamics occurring within the plant community which are driven by inter-annual variation in precipitation.

Large arid floodplains in Australia are amongst the most hydrologically variable river systems in the world, and flooding has an overriding effect on species richness (Capon, 2005). Soil seed banks are linked to vegetation resilience in these hydrologically variable desert floodplains, for example the greatest abundance of viable seed abundance is found in frequently flooded areas and is considerably lower in places that are rarely flooded (Capon and Brock, 2006). It has also been argued that the storage effect is key to the resilience of European floodplain meadow vegetation, as it allows typical species to persist through periods of unfavourable conditions in the soil seed bank (Hölzel and Otte, 2004; Mathar *et al.*, 2015). There is a risk, however, that the seed bank can be diminished when unfavourable conditions persist too long, and this reduces the potential for community diversity to recover once favourable conditions return (Harrison *et al.*, 2018). The dormancy of adult plants, when a perennial herbaceous plant does not produce annual shoots during the growing season, is another important aspect of the storage effect in meadow communities. This mechanism can be prompted by stress, as shown to occur in two species of orchid, allowing plants to "bet-hedge" against potentially catastrophic conditions (Shefferson *et al.*, 2005).

There is some evidence to suggest that species richness is greatest at intermediate flooding frequencies, in agreement with the intermediate disturbance hypothesis. Pollock *et al.* (1998)

developed a model to explain how species richness is influenced by a disturbance regime (flooding) and found that species richness was greatest at intermediate flooding frequencies. There were, however, no highly-productive frequently-disturbed sites in this study, and 57% of 880 micro-plots were either never flooded or permanently flooded; a representation of this variation would be necessary to analyse the relationship between flooding frequency and species richness. Similarly, Casanova and Brock (2000) found that short frequent floods promoted high species richness, where terrestrial species were able to establish during dry phases between short floods, in an experiment where seed bank samples were exposed to 17 different water-level treatments. However, the highest species richness was found in pots that were never flooded and the lowest in continuously flooded pots (i.e. the two low-fluctuation treatments). Additionally, Magee and Kentula (2005) studied the hydrologic requirements of urban wetland plant communities in North America, examining both native and invasive species requirements; they found that the assemblage richest in native species occurred under intermediate water level variability, and that small changes in variability could lead to a shift from native- to invasive-dominated assemblages.

The few studies that directly examine the impact of intra-annual (within year) hydrological variability on plant species richness suggest there is a positive relationship. A study on mesic grassland in the United States found that an increase in intra-annual rainfall variability enhanced plant community diversity, with increased turnover of rare and uncommon species (Knapp *et al.*, 2002). It was argued that variation in resource availability can reduce the effects of competitive exclusion and allow more species to coexist. In New Zealand, intra-annual fluctuations in lake level were found to have a positive effect on the species richness of the littoral plant community, whereas species richness was much lower in lakes with inter-annual level variations (Riis and Hawes, 2002).

There is also some evidence to suggest that plant communities and/or species richness are not influenced by hydrological fluctuation in wetland habitats. For instance, Day *et al.* (1988) found that the three main factors controlling vegetation composition along the Ottawa River wetland were water depth, litter removal and fertility; the impact on species richness was unclear as both the highest and lowest species richness was recorded in the low-fertility high-disturbance grouping. Kotowski *et al.* (1998) examined the behaviour of wetland plant species along a moisture gradient and found that most species were consistent in their response to mean water level rather than fluctuation. Additionally, Nygaard and Ejrnaes (2009) manipulated nutrients, water-table depth and the degree of water-level variance in mesocosms, and found that the effect of fluctuation regimes on species composition and species richness was not statistically significant. In contrast, in another North American field study, Magee and Kentula (2005) found that wetland plant species were abundant over a wide range of water depth, but crucially, they exhibited unique responses (in occurrence and/or abundance) in relation to water level variability.

The current evidence on how hydrological variability affects species richness is unclear. The importance of understanding how hydrological variability influences ecosystem functioning is vital, particularly as the climate is predicted to become more variable. Garssen *et al.* (2015) used a meta-analysis approach to examine the response of riparian plant communities to increased flooding and found a reduction in species richness in those areas where a relatively stable regime became more variable. There is also evidence to suggest that species diversity and/or distributions respond to extreme rather than to mean climate conditions (Knapp *et al.*, 2002; Vervuren *et al.*, 2003; Reyer *et al.*, 2013). Informed guidance on the importance of variability is needed to direct site managers, as current guidelines on the ecohydrological requirements of lowland wetland plant communities only specify the mean, minimum and maximum water table depths and duration of surface flooding (Wheeler *et al.*, 2004).

Therefore, the aim of this study is to examine further the impact of hydrological variability on plant species richness, using a selection of English floodplain meadows. Previous work on these sites has centred on the impact of the duration of soil drying and waterlogging on individual species and communities, but the impact of hydrological variability on species richness has not yet been investigated. The specific objectives of the study are to:

1. Examine the impact of soil drying and waterlogging, individually and combined as measures of overall stress and variability, on plant species richness of the study sites;
2. Investigate the impact of inter- (between year) and intra-annual (within year) variability in waterlogging on plant species richness of the study sites.

3.2 Methods

3.2.1 Site selection

A range of English floodplain meadow sites were selected for analysis in this study based upon the following criteria: i) availability of botanical and modelled hydrological data, ii) consistent management regimes, iii) contrasting physical characteristics (e.g. underlying soil type, geographical location) (figure 3.1, table 3.1).

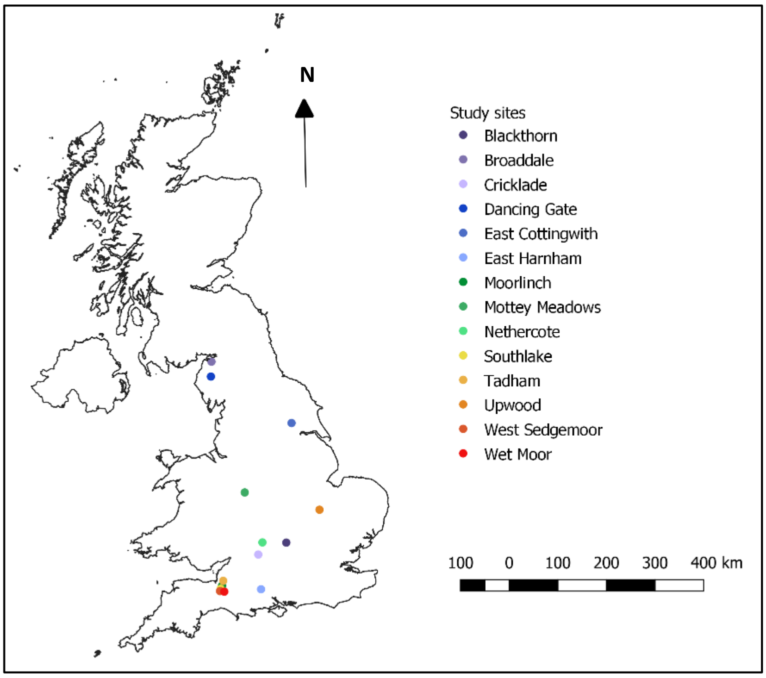


Figure 3.1: Location of the English floodplain meadow study sites.

Table 3.1: Location details and soil types of the English floodplain meadow study sites (adapted from Gowing *et al.*, 2002).

	Site name	**County**	**Grid reference**	**Soil type**
1	Blackthorn	Oxfordshire	SP632190	Clay
2	Broaddale	Cumbria	NY255525	Clay and sandy loams
3	Cricklade	Wiltshire	SU096958	Clay loam over sand
4	Dancing Gate	Cumbria	NY240260	Silty clay over gravel
5	East Cottingwith	Yorkshire	SE700420	Alluvial clay overlying silt
6	East Harnham	Wiltshire	SU151289	Silt overlying gravelly alluvium
7	Moorlinch	Somerset	ST393362	Peaty clay / peat
8	Mottey Meadows	Staffordshire	SJ840134	Loamy soils
9	Nethercote	Gloucestershire	SP175190	Clay loam over gravel
10	Southlake	Somerset	ST364301	Alluvial clay overlying peat
11	Tadham	Somerset	ST416455	Fen peat / oligo-fibrous peat
12	Upwood	Cambridgeshire	TL251825	Clay
13	West Sedgemoor	Somerset	ST352257	Peaty clay / peat
14	Wet Moor	Somerset	ST435245	Alluvial clay overlying peat

All sites are long-established, permanent grasslands under consistent traditional management of a midsummer hay cut followed by grazing of the aftermath growth and have received no inputs of artificial nutrient or pesticide in recent decades. The data were originally used in a study commissioned by the Department for Environment, Food and Rural Affairs (see Gowing *et al.*, 2002 for further details).

3.2.2 Botanical and hydrological data

All sites were surveyed during the period mid-May to early-July between 1986 and 2002; it is assumed that other factors that might affect species richness do not differ between the years. Quadrats measuring 1m × 1m were used to sample the plant community; all vascular plant and bryophyte species were recorded. There is a total of 2218 botanical samples (table 3.2).

Hydrological models were developed and used to simulate the water-table behaviour at the location of each botanical sample within the sites. Four broad hydrological models were used:

1. Ditch-bounded water-table model (Youngs *et al.*, 1989);
2. Shallow aquifer water-table model (Gowing *et al.*, 1998);
3. Water-balance ridge and furrow water-table model (Gowing *et al.*, 1998);
4. Non-bounded water-table model (Gowing *et al.*, 2002).

These models were then tailored to each of the sites using information on local topography and soil properties (see Gowing *et al.*, 2002 for further details).

Table 3.2: English floodplain meadow study site details; the year/s that botanical and hydrological data were collected; the type of hydrological model used for each site (1 to 4; see section 3.2.2); the threshold depths for soil drying and waterlogging (depth below surface in cm) for each site; the number of quadrats per site used in the analyses (data source: Gowing *et al.*, 2002). N.B. hydrological years run from June to May (e.g. the 1994 "hydrological year" refers to June 1994 to May 1995).

	Site name	Hydrological years	Model	Wet threshold	Dry threshold	Botanical survey year	No. of quadrats
1	Blackthorn	1990-1994	3	0.235	0.485	1995	197
2	Broaddale	1991-1995	3	0.304	0.477	1996	49
3	Cricklade	1989-1993	2	0.341	0.446	1994	452
4	Dancing Gate	1990-1994	4	0.359	0.464	1995	45
5	East Cottingwith	1997-2001	3	0.264	0.481	2002	129
6	East Harnham	1994-1998	1	0.443	0.496	1999	90
7	Moorlinch	1995-1999	1	0.273	0.468	2000	192
8	Mottey Meadows	1995-1999	1	0.256	0.464	2000	215
9	Nethercote	1990-1994	4	0.289	0.491	1995	60
10	Southlake	1989-1993	1	0.42	0.487	1994	175
11	Tadham	1981-1985	1	0.356	0.488	1986	299
12	Upwood	1991-1995	3	0.235	0.485	1996	80
13	West Sedgemoor	1988-1992	1	0.447	0.493	1993	60
14	Wet Moor	1994-1998	1	0.427	0.493	1999	175

The depth at which the soil within the densest rooting zone (0-100 mm depth) either becomes waterlogged or dries to a level detectable by plants, was calculated. The soil waterlogging threshold for each site was calculated from a soil-moisture-release curve as the depth that corresponds to 10% air-filled porosity in the upper layer (Gowing *et al.*, 2002). The soil drying threshold was calculated using the Richard's equation (Gardner, 1958) as the depth that gives 0.5 m tension at the surface. The thresholds were based on a 5-year mean as this has been shown to have the greatest explanatory power (Noest, 1994) and previous ecohydrological studies have also used this period to describe water regimes (e.g. Curreli *et al.*, 2013).

3.2.3 Statistical analyses

All data manipulation and analyses were carried out in R (R Core Team, 2013) and Excel (Microsoft, 2013). Species richness was calculated for each of the 2218 quadrats, counting the number of vascular plant and bryophyte species present. The thresholds for soil drying and waterlogging were used to calculate the degree of soil drying and waterlogging at each quadrat, i.e. the number of weeks each threshold was exceeded (using the weekly water table depth data) across a five-year period within the growing season (1st March to 30th September).

Exploratory data analysis was performed to investigate the relative impact of soil drying and soil waterlogging on species richness; as well as overall stress calculated as the number of soil drying and waterlogging weeks added together, variability was calculated by multiplying both stresses. As a result, the significant impact of waterlogging and variability on species richness became apparent and this led to the development of two further measures of hydrological variability. A measure of inter- and intra-annual hydrological variability was calculated, using the data on how often soil waterlogging thresholds were exceeded, as follows (see appendix B for example workings of each measure):

Inter-annual variability: Counted the number of weeks that the waterlogging threshold was exceeded per year for each quadrat (growing season only; max. 31 weeks); calculated the inter-quartile range (IQR) across the 5 years preceding the botanical survey.

Intra-annual variability: Counted the number of weeks that the waterlogging threshold was exceeded in each calendar month for each quadrat (max. 5 weeks); calculated the IQR across each growing season (7 months); calculated the median IQR across the 5 years preceding the botanical survey.

Inter-quartile range (IQR) and median were selected as summary statistics rather than mean and standard deviation because the raw data are not normally distributed (figure 3.2a and 3.2b).

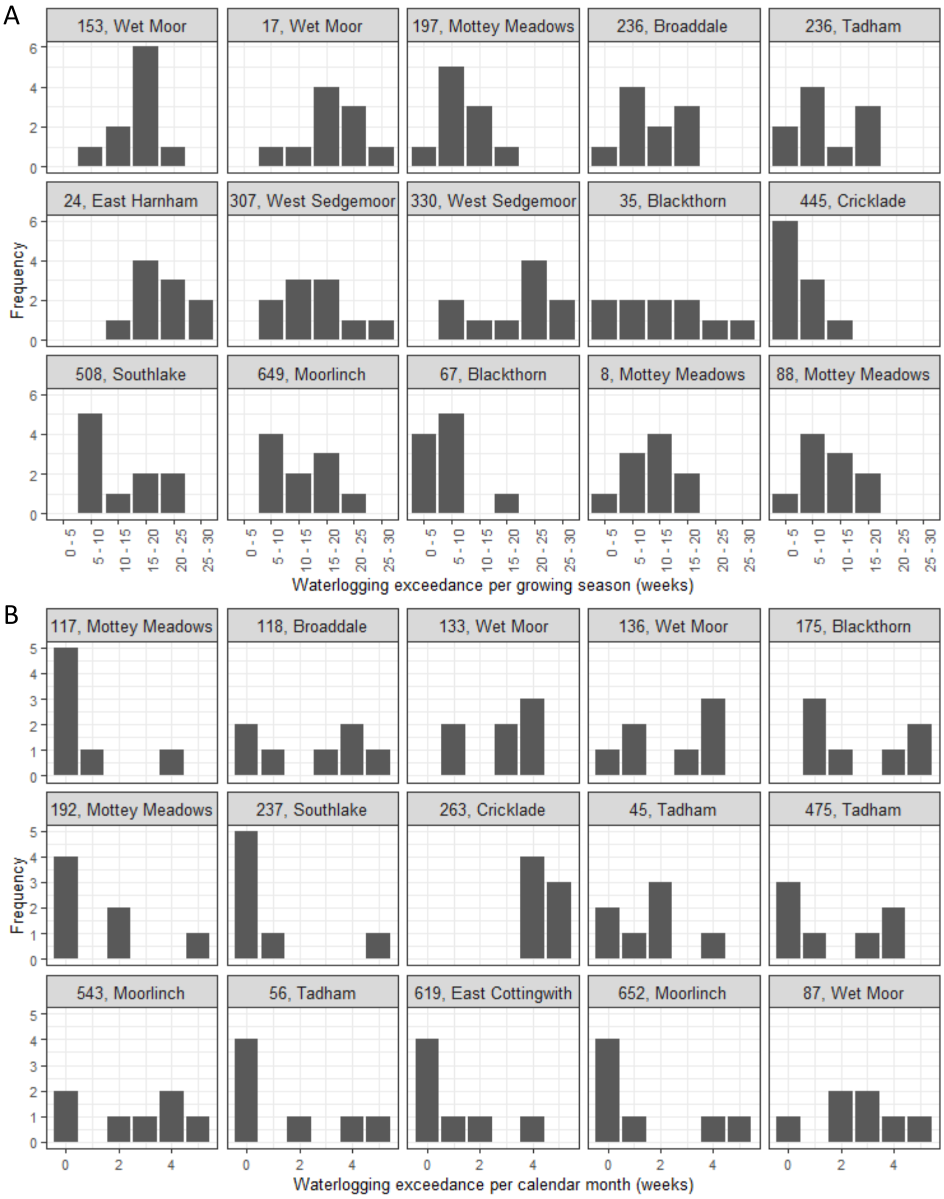


Figure 3.2: Random samples selected to check the distribution of the data used to calculate (a) inter-annual (n=10) and (b) intra-annual (n=7) variability; the quadrat (i.e. sample) number and the site it belongs to are given; see section 3.2.2 and appendix B for details of how inter- and intra-annual measures are calculated.

Scatter plots were generated to compare the species richness of quadrats with these measures of hydrological variability. First order polynomials were used to generate a line of best fit in the scatter plots and linear models were run to calculate the amount of variation explained (R-squared). Linear mixed effect (lme) models were then fitted using the package ‘lme4’ (Bates *et al.*, 2014); species richness being the dependent variable, hydrology the fixed factor, and site a random factor (see appendix C.1 for model outputs). Two types of plot were used to check the suitability of the model; Q-Q plots were used to check for normality, and the residual and fitted values were plotted to check for deviation from linearity. The package ‘lmerTest’ (Kuznetsova *et al.*, 2014) was used to calculate the p-values.

3.3 Results

There is a negative relationship between the total number of weeks a quadrat is waterlogged across five growing seasons, and the species richness of that quadrat (p-value <0.01, R^2 0.02; figure 3.3a). Conversely, there is a positive relationship between the total number of weeks a quadrat experiences soil drying across five growing seasons, and the species richness of that quadrat (p-value <0.01, R^2 0.01; figure 3.3b). When inherent site differences are accounted for using a lme model, both relationships remain highly significant (p-values <0.01).

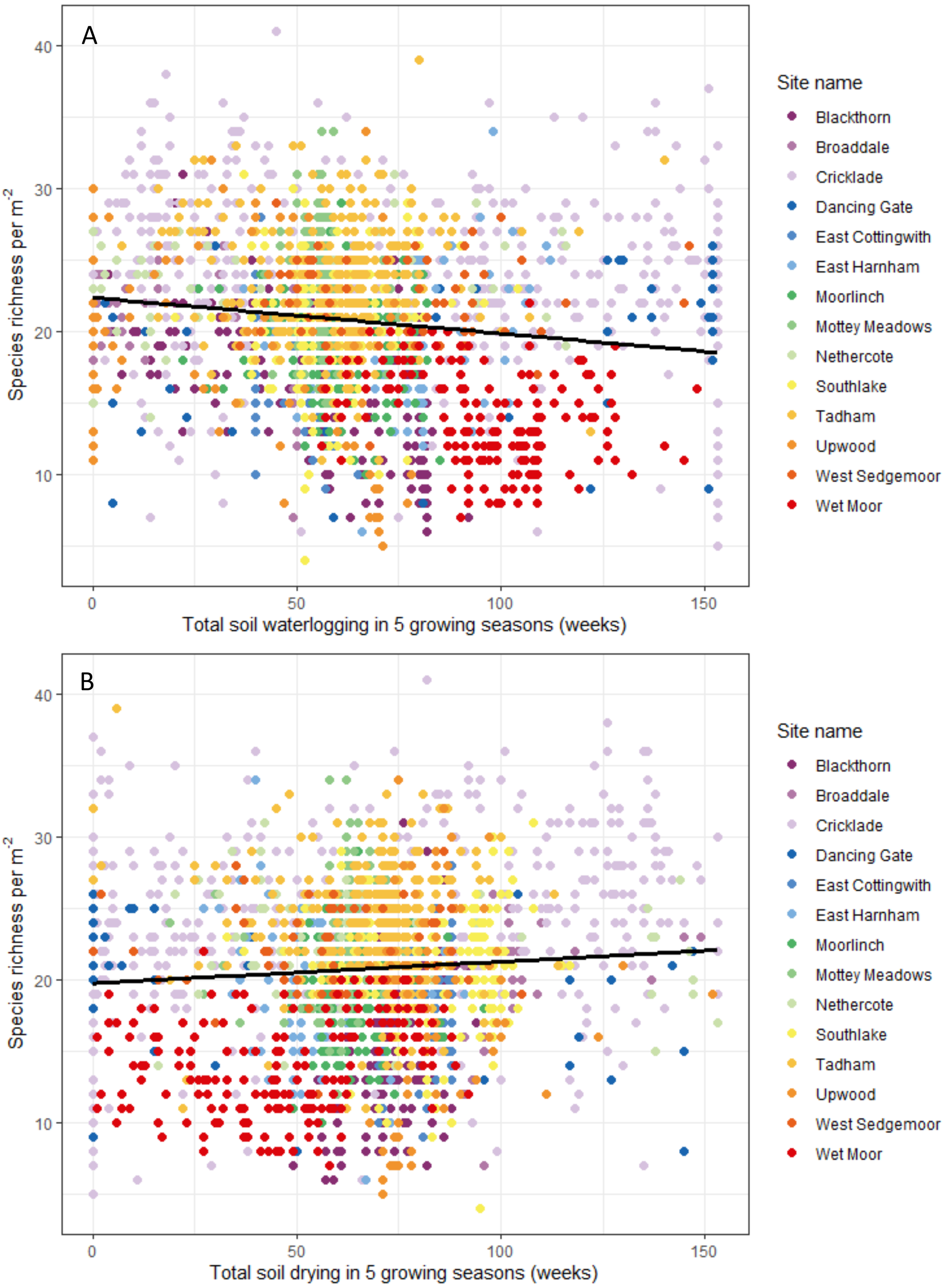


Figure 3.3: Relationship between the total number of weeks the soil (a) waterlogging (b) drying threshold is exceeded across five growing seasons and species richness per 1m^2 quadrat (n=2218).

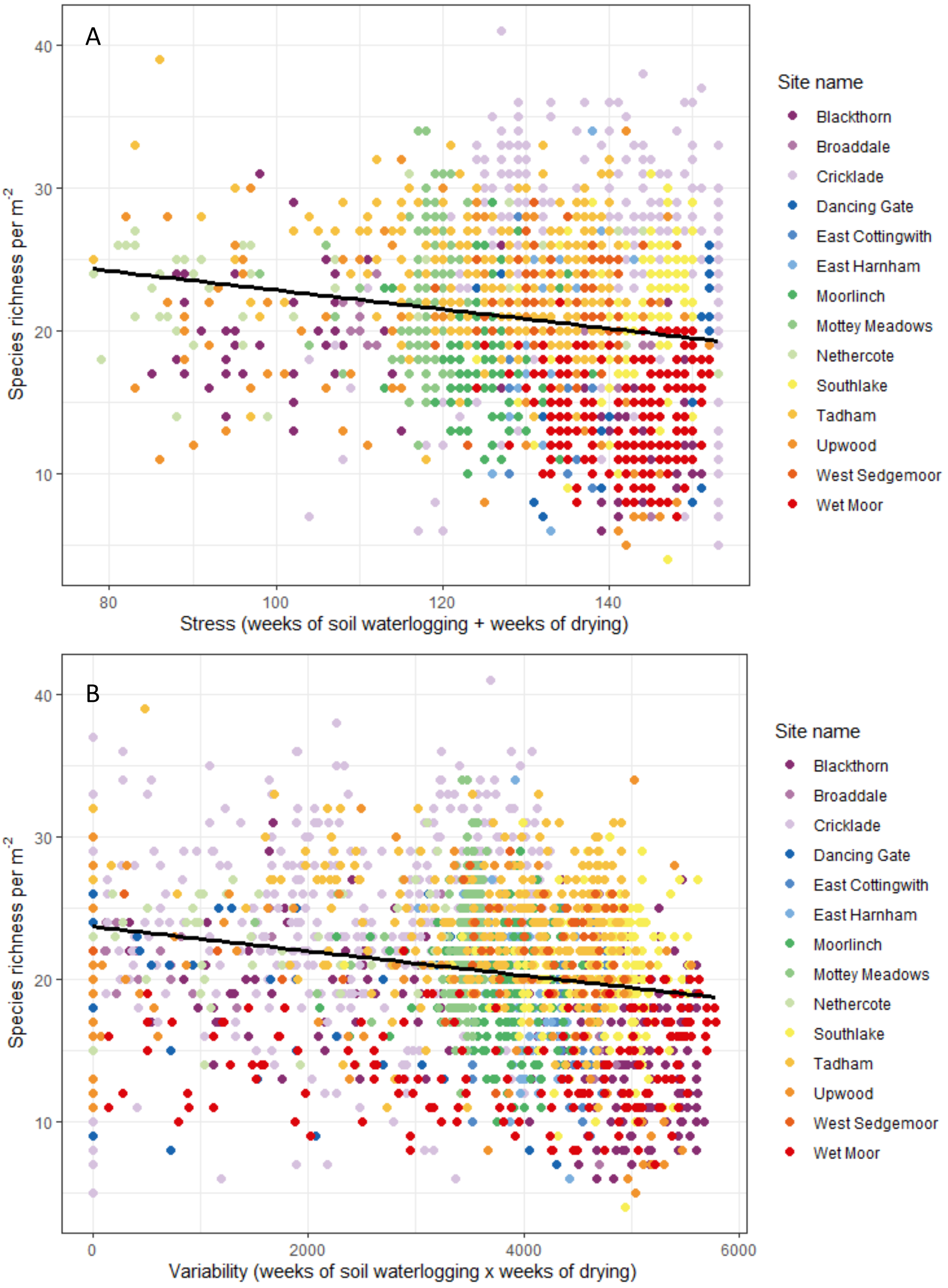


Figure 3.4: Relationship between (a) stress (b) variability and species richness per 1m^2 quadrat (n=2218); stress was calculated by adding the total number of soil waterlogging and drying weeks across five growing seasons (data presented in figures 3.3a and 3.3b), and variability was calculated by multiplying the total number of soil waterlogged weeks by those of soil drying.

There is a decline in species richness as total stress increases (p-value <0.01, R^2 0.03). The majority of quadrats experience more than 110 weeks of stress over five growing seasons (figure 3.4a). There is also a significant decline in species richness as hydrological variability increases and the correlation is stronger (p-value <0.01, R^2 0.05; figure 3.4b). When inherent site differences are accounted for using a lme model, the relationships remain significant (p-values <0.01).

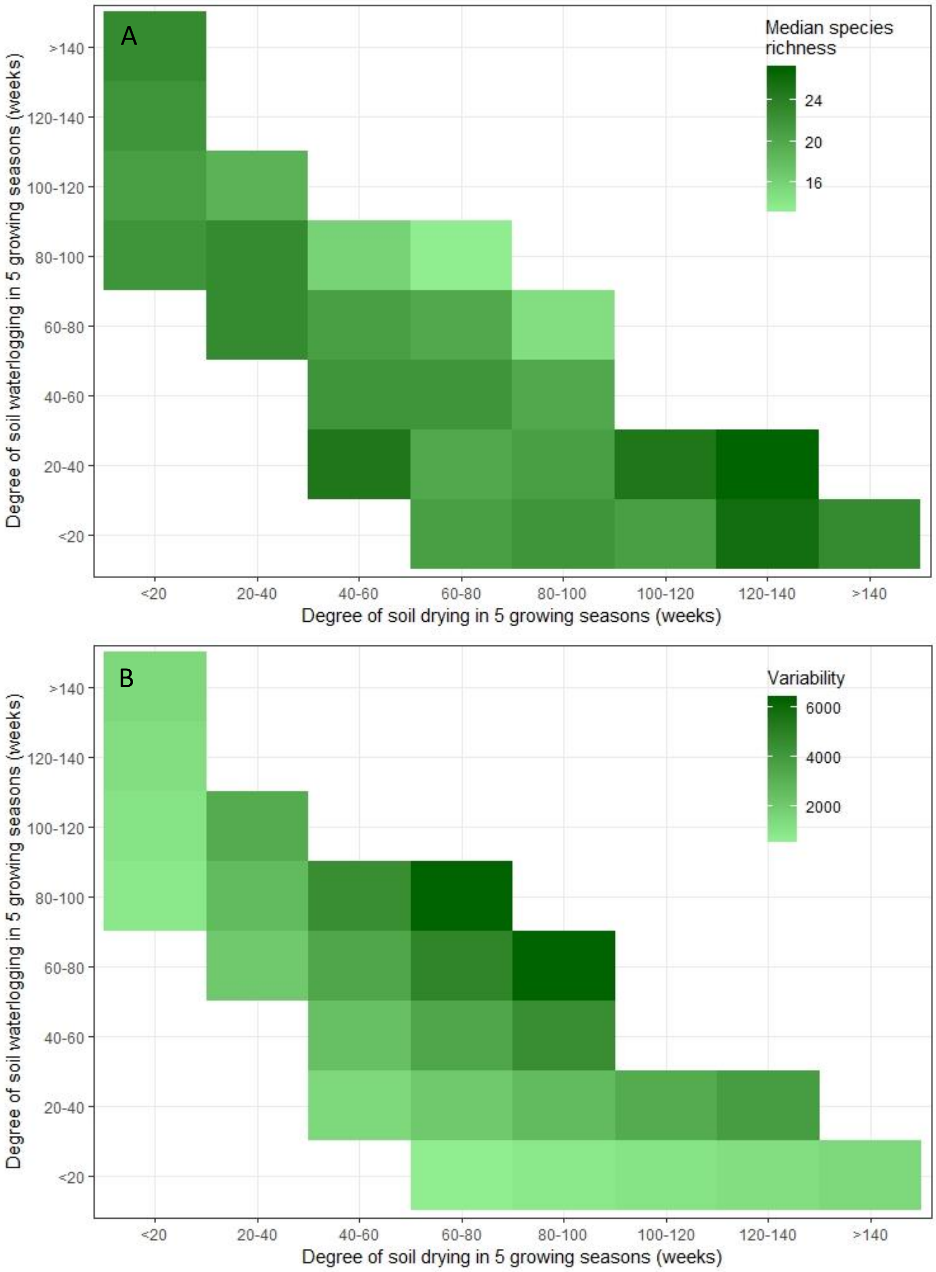


Figure 3.5: Botanical quadrats (n=2218) plotted in categories based on the number of soil waterlogging and drying weeks at that location across five growing seasons; green colour scale refers to (a) median species richness (calculated for all quadrats in each category, minimum n=5) and (b) variability (mean number of soil drying and waterlogging weeks in each category multiplied); blank cells have insufficient data.

The highest median species richness occurs where the degree of soil drying is greater and soil waterlogging is rare or non-existent, and those quadrats which are constantly waterlogged are less species rich (figure 3.5a). The lowest species richness (light green; figure 3.5a) occurs in the most variable quadrats, which experience both a high number of weeks' soil drying and waterlogging (dark green; figure 3.5b). This indicates a possible link between species richness and hydrological variability at these sites, and that high levels of waterlogging may be more detrimental to species richness than soil drying (figure 3.5a and 3.5b).

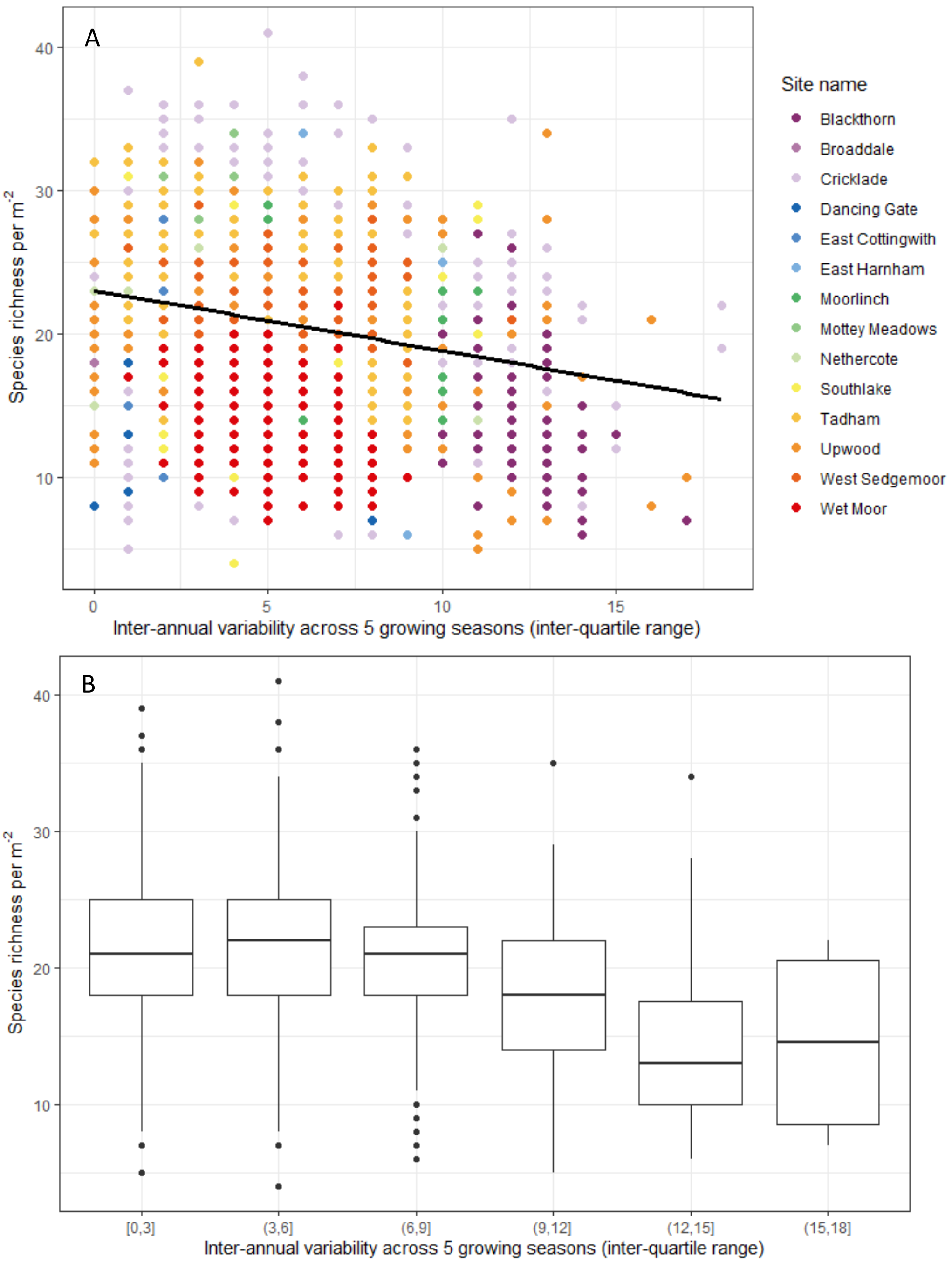


Figure 3.6: Inter-annual variability versus species richness for (a) each quadrat (n=2218) and (b) six groupings of quadrats (boxplot shows median, first and third quartiles, minimum, maximum, and outliers); see section 3.2.3 and appendix B for details on how inter-annual variability was calculated.

There is clear negative correlation between the degree of inter-annual hydrological variability and species richness (p-value <0.01, R^2 0.06). Taking into account the effect of site differences in the lme model, the trend is still highly significant (p-value <0.01; figure 3.6a). Figure 3.6b shows the decline in median species richness in each group of quadrats as the variability increases; the greatest range of richness occurs in the first three categories of low to medium variability. There are no distinct patterns between the different sites (see appendix D).

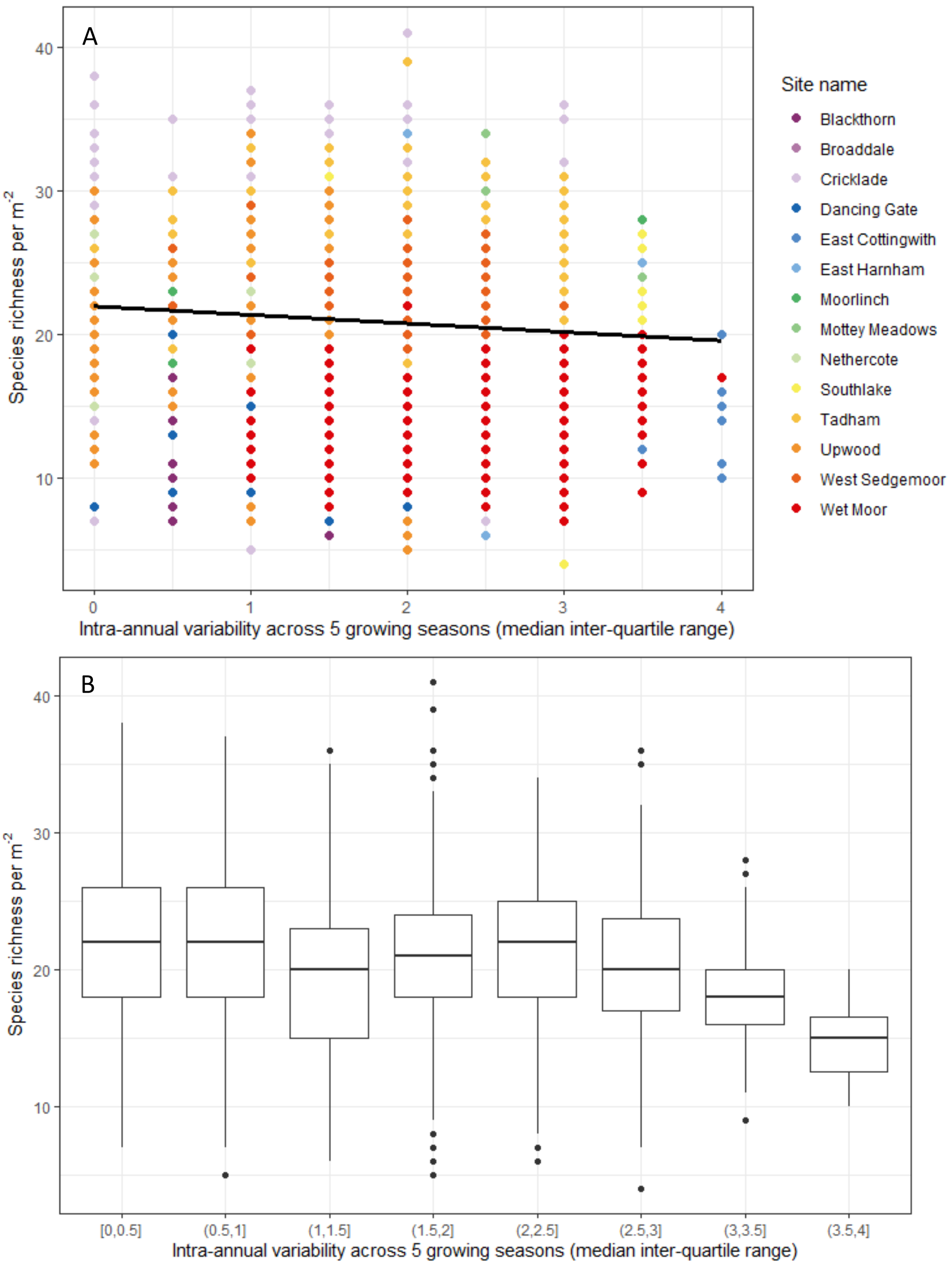


Figure 3.7: Intra-annual variability versus species richness for (a) each quadrat (n=2218) and (b) six groupings of quadrats (boxplot shows median, first and third quartiles, minimum, maximum, and outliers); see section 3.2.3 and appendix B for details on how intra-annual variability was calculated.

There is also a negative relationship between the degree of intra-annual hydrological variability and species richness, but the correlation is weaker (p-value <0.01, R^2 0.01). Taking into account the effect of site differences in the lme model, the trend is still significant (p-value 0.01; figure 3.7a). Figure 3.7b shows the gradual decline in median species richness in each group of quadrats as the variability increases; the decline is more noticeable where intra-annual variability is greatest (the last two categories). There are no distinct patterns between the different sites (see appendix D).

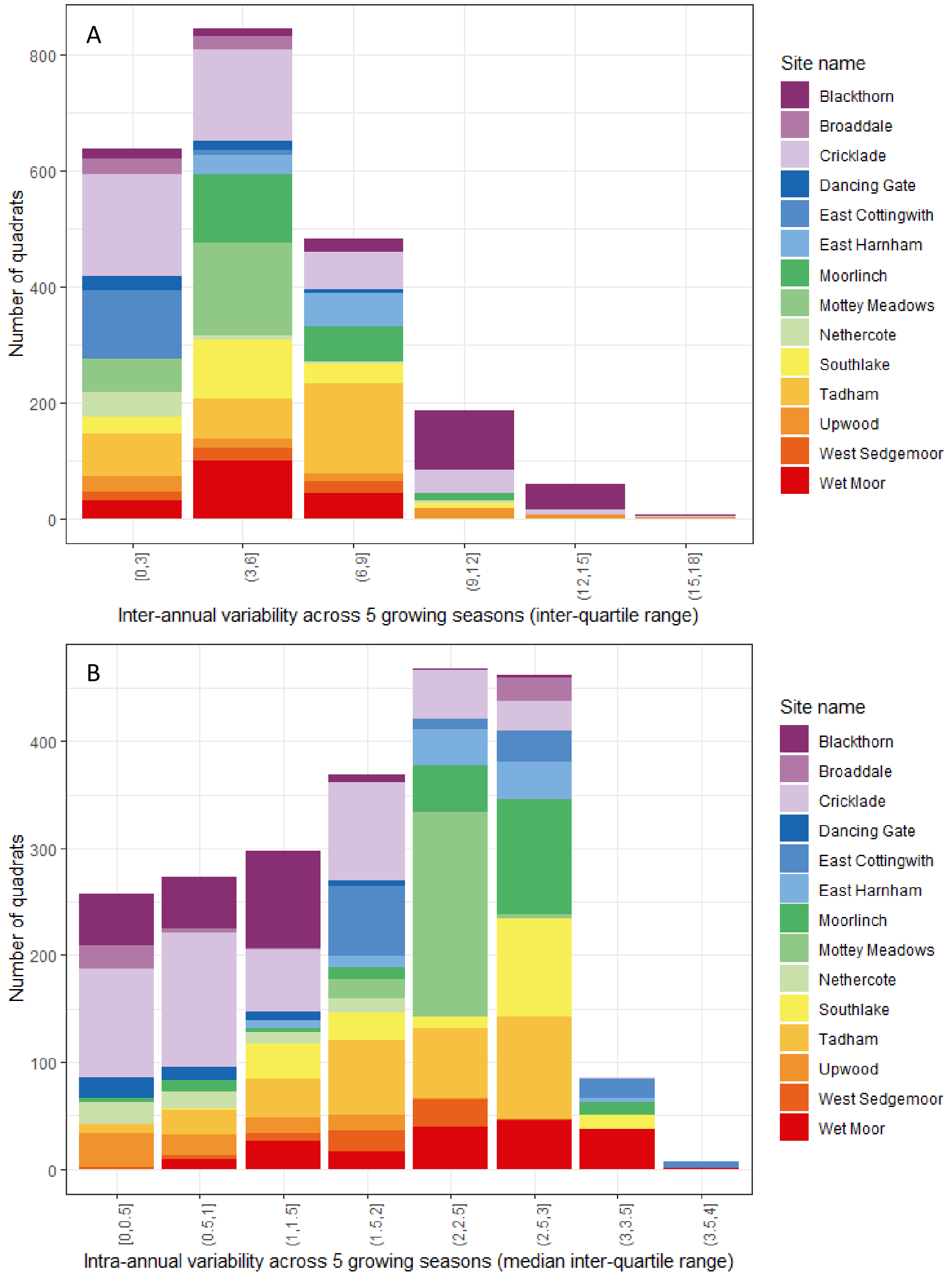


Figure 3.8: The distribution of quadrats (n=2218) from each site per category of (a) inter- and (b) intra-annual variability (as shown in figures 3.6b and 3.7b respectively); see section 3.2.3 and appendix B for details on how variability measures were calculated.

The majority of quadrats occur in the low to medium categories of inter-annual variability and all sites are represented here; whereas, fewer quadrats and only 6 sites are represented in the latter three categories of greater variability (figure 3.8a). Fewer quadrats and sites are also represented in the categories with greatest intra-annual varaibility (five sites in the last two categories), whereas the majority of quadrats and all sites are represented in the first six categories (figure 3.8b).

3.4 Discussion

3.4.1 Impact of soil drying and waterlogging on species richness

The results of this study show that an increase in soil waterlogging duration correlates with a decline in species richness at a selection of English floodplain meadows; in contrast, an increase in the duration of soil drying relates to an increase in species richness, although this (positive) correlation is less strong than with waterlogging. These trends have been found across several other floodplain habitat studies, which found that increased flooding affects species richness negatively (Mathar *et al.*, 2015; Garssen *et al.*, 2017) and drier conditions result in higher species richness (Casanova and Brock, 2000; Toogood *et al.*, 2008).

There are numerous effects of flooding and/or waterlogging which can be detrimental to plants. A saturated soil can cause oxygen deficiency for the roots, while complete submergence removes the link to the atmosphere, limiting gas exchange further and photosynthesis as a result. A plant can 'escape' the flooding stress using strategies such as shoot elongation, aerenchyma formation, adventitious roots and anatomical leaf changes; or they will adopt a 'quiescence' strategy, such as lowering growth rates and avoiding high metabolic activity (Garssen *et al.*, 2015). Plants similarly have strategies either to avoid or tolerate soil-drying stress, and have a particular reliance on dormancy in order to tolerate drought (Crawford, 1989). Drought-avoiding species include those which can survive drought as a seed, or in dormancy as a rhizome or bulb for example. Tolerance strategies include leaf desiccation, the maintenance of water uptake and retention by either lengthening roots to reach the water table or stomatal closure and osmoregulation (Crawford, 1989).

Therefore, it is likely that at the floodplain meadows in this study, the level of waterlogging stress at certain locations is reducing the number of species present (to the few species that are able to grow under those stressful conditions), whereas at drier locations more species are able to thrive (under the relatively less stressful conditions). However, it is not only hydrology influencing the species richness of these sites, there will also be other abiotic factors as well as competitive dynamics. It has been found elsewhere that species richness is controlled by abiotic factors (related to flooding) in the frequently flooded zone and by plant interactions at drier higher elevation along river shorelines in the Netherlands (Lenssen *et al.,* 1999).

Nutrients dynamics can also influence species richness. For instance, although species richness was found to decline with increased flooding stress in a European stream study, higher levels of extractable soil phosphorus were also strongly correlated with increased flooding (Garssen *et al.*, 2017). The availability of nutrients is highly inter-correlated with flooding dynamics, and therefore it is difficult to distinguish the impact of each variable separately on species richness. As mentioned in the methods, all the sites studied here have received no inputs of artificial nutrient or pesticide in recent decades, but differences in nutrient levels between sites will be still be a factor; for instance, some study sites will flood regularly, adding nutrients into the meadow system, whereas other sites are disconnected from the nearby river and do not receive these additional nutrient inputs. This compounding factor has not been addressed in the current study but does require further investigation.

3.4.2 Impact of overall stress and variability on species richness

Although changes in mean and/or total values are important, there is evidence that species diversity responds to extreme rather than to average conditions (Knapp *et al.*, 2002). Flooding is irregular in its nature and there is an intrinsic amount of variability in the water regime of any wetland; it is the amount of hydrological variability which can change temporally and spatially that affects plant survival. Depending on the amount of deviation from previous conditions, annual variations in flood depth, duration and timing can cause distinct changes in the functional structure and species composition of floodplain meadows (Mathar *et al.*, 2015). For instance, summer flooding can affect plant survival much more than regular winter flooding in temperate lowland floodplains (Eck *et al.*, 2006). The floodplain meadow sites in this study show a decline is species richness when there is an increase in either overall stress or variability (both of which include a measure of soil drying and waterlogging). These trends are in agreement with the findings of other studies, which also found that species respond to hydrological fluctuations (Casanova and Brock, 2000; Leyer, 2005; Magee and Kentula, 2005).

3.4.3 Impact of inter- and intra-annual variability on species richness

The results of this study show a downward trend in species richness as a result of an increase in both inter- and intra-annual variability, with the correlation being stronger in the former than the latter (R^2 0.06 and 0.01 respectively). These findings are not in agreement with much of the modern literature on this topic, which found that an increase in both between and within year variability can have a positive effect on species richness (e.g. Knapp *et al.*, 2002; Riis and Hawes, 2002; Capon, 2005; Capon and Brock, 2006). Other studies however, have found similar trends, where an increase in hydrological variability was detrimental to plant diversity (e.g. Garssen *et al.*, 2015), or highest species richness was found at intermediate levels of disturbance (e.g. Connell, 1978; Pollock *et al.*, 1998; Magee and Kentula, 2005).

It is not appropriate to analyse the trends in intra-annual variability from the present study further, because of the weakness of the correlation and given the small amount of published literature from dissimilar habitats. There is, however, far more literature on the positive effects of inter-annual variability, and associated storage effects, on plant species richness. The storage effect has become a core concept in community ecology, explaining how environmental fluctuations can promote coexistence and maintain biodiversity, and there is now a large amount of empirical evidence supporting the theory in general (Angert *et al.*, 2009; Ellner *et al.*, 2016; Tredennick *et al.*, 2017) and specifically in floodplain habitats (Hölzel and Otte, 2004; Capon, 2005; Mathar *et al.*, 2015). Therefore, it is surprising that the findings of the present study contrast with this prevalent literature and it is clear that further investigation is required to account for the unexpected downward trend in species richness as a result of a greater degree of inter-annual hydrological variability.

Our results also show that highest species richness occurs where hydrological conditions are most stable (i.e. low levels of inter- and intra-annual hydrological variability). It is important to note that those quadrats experiencing no hydrological fluctuations in this study are either constantly waterlogged (i.e. highly stressed) or constantly non-waterlogged (i.e. not stressed); and that there was a large range of species richness values at the non-fluctuating end of the variability spectrums. These patterns are probably the result of constantly waterlogged locations with lower species richness and the constantly non-waterlogged locations will have higher species richness. This is the most likely scenario given the findings of this study, and those of others (e.g. Casanova and Brock, 2000), on how increases in soil drying and wetting positively and negatively affect species richness respectively.

3.4.4 Implications of climate change for species richness in floodplain meadows

Alterations in water regime due to climate change events such as floods and drought are a significant threat to internationally important wetlands, including floodplain wet grasslands (Brotherton *et al.*, 2019a). Under future climate change, soil moisture content dynamics will increase with greater temperature and rainfall variability; and in combination with increased plant physiological demands for both oxygen and water, this will lead to an increased occurrence of wet and dry extremes of plant stresses. Using downscaled IPCC scenarios to simulate waterlogging and drought stress, it is predicted that both waterlogging and drought stress will increase, on average by ∼20% at sites where both stresses occur, in a warmer and more variable future (2050) climate. This is likely to have a greater negative impact on currently endangered plant species (causing a reduction of ∼16%) than on common species (Bartholomeus *et al.*, 2011).

A field study was carried out to assess the immediate and longer-term response of English floodplain grassland to hydrological alteration as a result of climate change (Brotherton *et al.*, 2019a). Plants were transplanted between wetter and drier sites, and the results demonstrated that some functionally important species may succumb within weeks to a hydrological alteration, unless they are able to tolerate the challenging conditions. Additionally, Garssen *et al.* (2014) looked at the effects of increased summer drought under future climate change and found that an increase in intense events lasting more than 30 days would rapidly lead to a decline of riparian species richness. The effects of extreme flooding may also result in a subsequent reduction in plant species diversity, with species possessing traits for tolerating submersion and soil saturation most likely to survive; however, it was found that plants in higher-diversity assemblages were less negatively affected by flooding (Wright *et al.*, 2017).

Given the climate change predictions listed above and the results of other studies on the impacts of hydrological alterations, it is likely that climate change will have a significant impact on plant species richness at the current study sites. A greater occurrence of soil waterlogging will probably result in a continuation of the current trend of reducing floodplain meadow species richness, and an increase in soil drying could also begin to have a negative effect on richness. Additionally, given the importance of hydrological variability in driving the plant species richness of sites studied here, and the apparent negative effects of both greater inter- and intra-annual variability on richness, it is highly likely that an increase in climate variability and extreme weather events under future climate change is going to have a significant and potentially negative impact on these highly valued species-rich habitats in the UK and elsewhere.

Reyer *et al.* (2013) conducted a large-scale literature review on how plants respond to extreme climatic conditions induced by changing climatic variability. It was concluded that plant water relations are very vulnerable to extremes driven by changes in temperature and precipitation, and that extreme drought and flooding have stronger impacts on physiological processes than changing mean climate. Similarly using a meta-analysis approach, Garssen *et al.* (2015) found a reduction in species richness at sites where a relatively stable regime became more variable. Therefore, given the predictions of our future climate, it is vital that we continue to research the impacts of greater hydrological variability on all aspects of ecological functioning.

Climate change has already affected plant species by disrupting phenology and physiology, and altering community dynamics (Anderson, 2016). Those species which are not able to adapt to conditions or disperse fast enough to keep pace with rapidly changing climates and environmental change risk extinction (Willis *et al.*, 2008; Corlett and Westcott, 2013). Some plants are capable of rapid adaptation to novel climates, for example Thompson *et al.* (2013) demonstrated an adaptive reduction in winter freezing tolerance of wild thyme (*Thymus vulgaris*) populations in response to warming from the 1970s to 2010, but this is not common; additionally, some populations can adjust plastically (Anderson and Gezon, 2015) which could aid population persistence under climate change.

3.4.5 Impact of this study for management and further research

It is clear from the results presented here that the guidance provided for the management of lowland wet grassland in the UK, and potentially elsewhere, needs to take into account the variability of water regimes and not simply the total or mean values of drying and waterlogging required to maintain target plant species, communities and overall richness. It is of great concern that not only an increase in overall soil waterlogging, but also inter- and intra-annual hydrological variability, currently relate to a decline in species richness, as these stresses are only set to increase in the near future.

More research is required on the mechanisms relating to hydrological variability that affect species richness, as the present study found a lack of research relating to intra-annual variability and found a contradictory result of greater inter-annual variability reducing species richness than that found in the wider literature. Floodplain meadows are a vital biodiversity resource in the UK and elsewhere, not only for their species-rich plant communities but also the fauna they support, the flood mitigation properties they provide and the social and cultural heritage they represent. The conservation of species-rich plant communities relies on an adequate understanding of how diversity is regulated, and it is vital that these habitats are protected going forward into a highly unpredictable future under climate change.

Chapter 4

Hydrological characterisation of plant communities in Ireland’s groundwater-fed, temporary wetlands (turloughs)

4.1 Introduction

Turloughs are temporary wetland ecosystems which occur on karst limestone and are so far known to be almost exclusive to the west of Ireland. They occur in regions with a seasonally high groundwater supply and typically fill in the autumn via springs and fissures, and dry out in the summer when they are used for low-intensity grazing, although heavy rainfall at other times of the year can also result in flooding (Sheehy Skeffington *et al.*, 2006). As a result of these large inputs from groundwater sources, turloughs are classified as Groundwater Dependent Terrestrial Ecosystems under the Water Framework Directive (European Community, 2000) along with fens, marl lakes and dune slacks (Tynan *et al.*, 2007). Similar seasonal lakes do occur in Slovenia (both turloughs and poljes) (Sheehy Skeffington and Scott, 2008), England (Breckland Meres) and the karst regions of North America (Goodwillie and Reynolds, 2003), but in Britain, only one site has been granted ‘turlough’ status at Pant-y-llyn in Wales (Campbell *et al.*, 1992; Farr *et al.*, 2012). Despite the broad similarities between turloughs and other temporary wetlands, the former are distinct and have therefore been classified as a priority habitat in Annex 1 of the EU Habitats Directive (European Community, 1992).

The characteristic hydrological dynamics of turloughs are the key mechanism driving the ecological functioning of this habitat (Bonacci *et al.*, 2009). The hydrological regime, driven by patterns in groundwater flooding, results in a distinctive ecology with unique vegetation and fauna (Naughton *et al.*, 2012; Tynan *et al.*, 2007). Basins are grass- or sedge-dominated, often with a wetter community at their base such as fen, marsh or a permanent pond (Sheehy Skeffington *et al.*, 2006), and there is a distinct absence of trees and shrubs (Praeger, 1932). Turloughs have been described as ecotones acting as a shore or floodplain for underground rivers, a transitional habitat between aquatic and terrestrial ecosystems (Goodwillie, 2003; Reynolds, 1998; Naughton *et al.*, 2017a). Turloughs superficially appear to be grassy hollows, but they actually contain a regular zonation of plant communities (Goodwillie, 2003). This zonation was first noted by Praeger (1932), who also stated the difficulty in establishing a correlation between the flora and fluctuation of water levels because of the variability between sites and years. The majority of subsequent research into the mechanisms driving plant community composition and distribution has had to rely upon qualitative proxy measures, in lieu of measured hydrological information, to characterise the hydrological regimes of turloughs. Some studies related the presence of particular woody scrub or moss species to differing flood height, duration or frequency (Praeger, 1932; Coxon, 1987a; Proctor, 2010), and others equated basin depth with susceptibility to inundation (Goodwillie, 1992; Lynn and Waldren, 2003; Moran *et al.*, 2008a; Moran *et al.*, 2008b).

One investigation into the relationship between flood duration and plant community distribution compared the topographic height occurrence of plant communities identified in a historical report (Goodwillie, 1992) with flood duration calculated using contemporary hydrological data collected across 1-3 years (Tynan *et al.*, 2007). It was concluded that the plant communities present were largely constrained by their ability to withstand either a maximum or minimum flood-duration, but some communities could withstand a very wide range of flood duration conditions. The conclusions drawn by that study were limited because it was not possible to ascertain what hydrological conditions had driven the formation of the plant communities studied, since the botanical and hydrological data were not contemporaneous.

The duration of flooding was thought to be the main driver of plant community zonation in turloughs (Sheehy Skeffington *et al.*, 2006), but it has been suggested that flood depth, linked with basin elevation, also plays a significant role in affecting plant community composition and soil properties (Moran *et al.*, 2008a; Moran *et al.*, 2008b). Additionally, in a large-scale analysis of 30 turloughs, overall site 'wetness' (soil moisture and drainage) was found to be the primary controlling environmental factor determining turlough plant communities, and fertility was the second; although hydrological data were only collected for one year (Regan *et al.*, 2007). In a single turlough in County Mayo (Skealoghan), hydrological regime and grazing, which combine to affect soil properties such as organic content, were the most important factors determining species richness (Moran *et al.*, 2008a). Plant and beetle communities have also been found to differ within the same flood zone due to differences in farming practice (Ní Bhriain *et al.*, 2002, 2003), whereas mollusc communities were seen to correlate with emergent vegetation height as well as water pH within one turlough flood zone (Williams and Gormally, 2009).

A lack of hydrological data in turlough research, looking to relate their ecological and hydrological functioning, is no longer the constraint it once was. In recent years there has been considerable advancement in the monitoring, modelling, and understanding of turlough hydrology and hydrogeology. In particular, there has been much work on quantifying the hydrological regimes of a particular group of twenty-one turloughs (Naughton *et al.*, 2009, 2012) and developing models for predicting water levels using meteorological records (Gill *et al.*, 2013; Naughton, 2011).

Flooding can take many forms, varying in terms of its seasonality, duration and extent, and thus it imposes different types of stress on plants (Crawford, 2008). The characteristic unpredictable flooding in turloughs disrupts the physical environment of the ecosystem, thus changing what resources are available to plants. The key mechanism by which flooding controls vegetation is the depletion of oxygen in the soil which can result in the death or inhibition of root growth (Goodwillie *et al.*, 1997). Additional impacts include the build-up of toxic substances in the soil that are caused by anaerobic metabolism of plants or bacteria and changes in soil structure (Tynan *et al.*, 2007). Plants have different strategies for coping with the periodic inundations and oxygen deficiencies, they can survive *in situ* using specialist adaptations to flooding stress or they can quickly colonise bare ground (Goodwillie, 2003).

The highly variable environment shaped by unpredictable flooding creates a disturbance regime in turloughs that some species are able to exploit. Some notable species associated with turloughs include *Limosella aquatica*, which requires bare mud to be exposed early and remain wet throughout the growing season, and *Rorippa islandica*, which is a species characteristic of damp muddy sites that are kept bare by late flooding or trampling (Goodwillie, 1992); both species are listed as rare in Ireland's Red Data Book (Curtis and McGough, 1988). Turloughs are home to other rare species including *Potentilla fruticosa* and *Viola persicifolia*, which are classified as vulnerable and near threatened in the Ireland Red List respectively (Wyse Jackson *et al.*, 2016). The unpredictable fluctuations in water depth are also believed to have caused an evolutionary adaptation in one species of buttercup, *Ranunculus repens*, where those plants growing in turloughs are characterised by more highly-dissected leaves than the typical broad-leaved form found in neighbouring terrestrial environments (Lynn and Waldren, 2001, 2002).

Seasonal inundation is a key mechanism supporting turlough ecology, with flooding strongly influencing species composition and ecosystem physicochemical properties (Sheehy Skeffington *et al.*, 2006; Kimberley *et al.*, 2012). Therefore, the delicate balance that exists between the hydrological and ecological functioning of these ecosystems is likely to be disrupted as a result of climate change, with the intensification of the seasonal hydrological cycle and increased winter rainfall predicted for the west of Ireland in the coming decades (Sweeney *et al.*, 2003; Steele-Dunne *et al.*, 2008; Sweeney *et al.*, 2013). Climate change will also result in flood mitigation works, which could then have indirect impacts on turlough ecosystem functioning. Internationally, the flood mitigation benefits provided by functioning wetlands has gained popularity (Naughton *et al.*, 2017b) and works have been proposed in Ireland to mitigate groundwater flooding, which would involve the re-direction of floodwaters towards turlough basins (Naughton *et al.*, 2017a) thus changing their hydrological regime. Therefore, it is critical to understand the relationship between hydrology and plant communities in this unique habitat to inform future management initiatives. In addition, turloughs provide a useful analogue when looking at the impact of increased flood risk under climate change on plant communities that are currently under a more stable hydrological regime in Ireland and beyond.

Several studies have examined the hydrological complexities and plant-community assemblages of turloughs, but the link between the water levels and flora has not yet been clearly quantified. Therefore, the specific objectives of this chapter are to:

1. Describe the plant communities along a flooding gradient in three turlough basins;
2. Describe the hydrological regimes and soil characteristics of the study sites;
3. Establish which aspect of the hydrological regime is driving the plant-community composition at the study sites;
4. Summarise the management implications for plant communities in turloughs and other wetlands vulnerable to altered hydrological regimes under future climate change.

4.2 Methods

4.2.1 Study area and sites

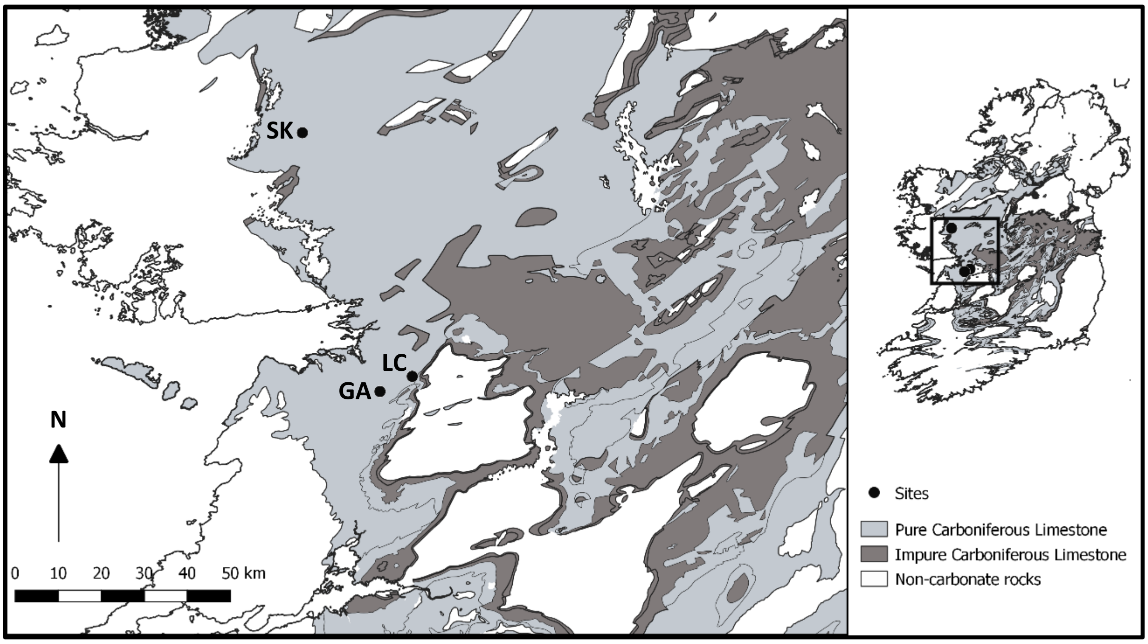


Figure 4.1: Map of the turlough study sites in Western Ireland in relation to limestone geology; map drawn using QGIS software (v 3.0.3), geological data from Geological Survey of Ireland.

Table 4.1: Turlough study site information table; basin areas and Irish Grid references from Goodwillie (1992).

Site name	Site code	Area (ha)	Irish Grid reference	Latitude / longitude
Garryland	GA	25	M 4104	53.083583, -8.8813253
Lough Coy	LC	36	M 4907	53.111364, -8.7624117
Skealoghan	SK	28	M 2563	53.611653, -9.1340674

The study area lies in a limestone region of the west of Ireland (figure 4.1). The turloughs occur on near-surface pure, well-bedded Carboniferous limestone because, unlike other limestones, it is very susceptible to karstification (Coxon, 1987b; Sheehy Skeffington *et al.*, 2006). Three turloughs (table 4.1) were selected for study based on the following criteria: (i) presence of distinct plant-community zonation; (ii) the availability of hydrological data for a minimum of five continuous years; and (iii) contrasting hydrological regimes between the sites.

Skealoghan (SK) turlough, situated in south-east County Mayo (Moran *et al.*, 2008b), is a shallow basin surrounded by gentle slopes with a relatively steady hydrological regime and maximum recorded flood depth of 3.2 m (Naughton, 2011). Garryland (GA) and Lough Coy (LC) are in south-west County Galway (Goodwillie *et al.*, 1997) and form part of a network of five turlough basins which are linked hydrologically via underground channels (Gill *et al.*, 2013). GA and LC are steep-sided basins with more variable hydrological regimes and maximum recorded flood depths of 10.9 and 10.6 m respectively, with LC having the flashiest hydrograph (Naughton, 2011). All study sites have been designated as Special Areas of Conservation (SAC) under EU and national legislation. Garryland is also part of the Coole-Garryland National Nature reserve and is a designated Ramsar site under the Ramsar Convention (https://rsis.ramsar.org/ris/473).

4.2.2 Vegetation and soils

Initial site visits were made in August 2016 and at each site an area of study was selected and divided into four distinct sampling zones based on a visual identification of the plant communities present. A preliminary botanical survey was conducted with four relevés (1 m x 1 m quadrats) randomly distributed in each sampling zone. The sampling quadrat size of 1 m^2 was selected in order best to correlate with any fine-scale spatial variability in the hydrology (Gowing *et al.*, 1997). In each quadrat all plant species present were given a percentage cover value and identified to species level where possible, following the nomenclatures of (Stace, 1991) for vascular plants and Smith (2004) for mosses. A second botanical survey was conducted in August 2018 to repeat the original permanently marked quadrats and to add four new quadrats in each zone; an additional zone was identified at LC (zone 0) that had previously been inundated, and eight quadrats were sampled here.

In 2016, one bulk soil sample (approximately 250 g) was collected per sampling zone; a soil corer was used to collect a sample from the upper 10 cm of the soil column. The samples were air-dried in Ireland and subsequently tested in the laboratory for pH and extractable phosphorus concentration (Olsen *et al.*, 1954). In May 2017, Plant Root Simulator (PRS®) probes were installed to measure plant-available nutrients in the rooting zone (ca 10 cm depth); four locations per vegetation zone were selected for installing the probes at LC and SK (GA was inaccessible due to high water levels). The probes were in the ground for 53 days and recovered in June 2017; they were cleaned in the laboratory using distilled water and shipped to Western Ag Innovations in Canada for analysis (visit www.westernag.ca for methodology). All sampling locations were recorded to an accuracy of 3 ± 1.5 cm using a high precision Leica Zeno 20 GPS.

4.2.3 Hydrology

In 2016, five dipwells were installed at each site in an 'L' shape to monitor ground water levels and establish the direction of flow (see chapter 2 for details). Pressure-transducer water-level loggers (LevelSCOUT, Seametrics, Seattle) were installed in the lowest dipwell at each site, and a barometer (BaroSCOUT, Seametrics, Seattle) was installed at SK for measuring atmospheric pressure for offsetting the overall pressure recorded by the LevelSCOUTs. An additional two water level loggers were installed at LC and SK, and an additional one at GA, in the adjacent dipwell/s in 2017. A minimum of two manual dipwell readings were taken to calibrate each LevelSCOUT.

A waterlogging threshold depth was calculated for the soil in each sampling zone at the three sites (table 4.2). Three undisturbed soil cores (5 cm depth x 5 cm diameter) were collected per zone, saturated and then placed on a sand table to determine the soil-moisture-release curve. The tension at which 10% air-filled porosity was reached was calculated for each soil core (Gowing *et al.*, 2002), and the median value of the three replicates was used to assign a waterlogging threshold to each sampling zone (appendix E), plus an additional 10 cm to account for the relationship between rooting depth and aeration (Dumortier, 1991).

The total number of waterlogged days (growing season only; i.e. 1st March to 30th September) was then calculated for the five years preceding the botanical survey for each quadrat sampled in 2016 and 2018 (i.e. August 2011 to July 2016, and May 2013 to April 2018); data were not available for May to July 2018. The daily water levels were provided by collaborators at Trinity College Dublin, and these data were a combination of measured and modelled elevations (see Naughton, 2011 for model details). The five-year mean duration of summer inundation (daily values) has been deemed the best hydrological parameter to explain a contemporary plant community (Noest, 1994).

Table 4.2: The threshold depths for soil waterlogging for each vegetation sampling zone (depth below surface in cm) at each turlough study site; the thresholds in the table have been adjusted (10 cm has been added) to account for the relationship between rooting depth and aeration (Dumortier, 1991), i.e. for GA sampling zone 1 the threshold of 42 cm increased to 52 cm below the surface.

Site	Sampling zone	Wet threshold
GA	1	52
	2	17
	3	22
	4	30
LC	0	50
	1	17
	2	20
	3	20
	4	25
SK	1	50
	2	24
	3	31
	4	31

4.2.4 Data analysis

To identify those plant communities present across the three study sites objectively, a TWINSPAN analysis was conducted using the WinTWINS program on all the botanical data collected in 2016 and 2018. A total of 152 samples and 113 species were inputted, together with a percentage cover for bare ground and bare rock. The default cut levels of 0, 2, 5, 10 and 20 were used and no rare species were omitted; the total number of species and pseudo-species was 355. Six plant community groups were defined and drawn onto site maps using QGIS software (version 3.0.3). All remaining data manipulation and analysis were carried out using the software packages R (R Core Team, 2013) and Excel (Microsoft, 2013).

The Shapiro-Wilk test was used to test for normality in the soil data. The data were then transformed if necessary and the appropriate tests were selected. Olsen-P and pH values were normally distributed and thus a one-way ANOVA was used to test for differences in samples between the four sampling zones and the three sites. No methods successfully transformed the PRS probe data into a normal distribution; therefore Kruskal-Wallis was used to test for differences in the samples between the four sampling zones and two sites (SK and LC only); and Spearman Rank was used to test for correlations between the different elements.

A non-metric multidimensional scaling (NMDS) ordination was used to examine relationships within the plant-community data (presence-absence); quadrats were grouped in polygons to represent the six end groups identified by TWINSPAN. Four variables were then overlaid onto the NMDS plot to see whether the principal dimensions aligned with the hydrological parameter of waterlogging. In addition to waterlogging, the mean Ellenberg values per quadrat for moisture (F), pH (R) and fertility (N) were overlaid onto the ordination (calculated using original Ellenberg scores; Ellenberg *et al.*, 1991).

4.3 Results

4.3.1 Site characteristics

Soils

Mean values of plant-available phosphorus (Olsen-P) are highest in the wetter zones 1 and 2, and there is a significant difference between values recorded in the four sampling zones across all three sites (p-value 0.01; figure 4.2); the range is as expected for species-rich grassland communities (Gilbert *et al.*, 2009). The difference in the mean soil pH between the three sites is significant (p-value 0.01; figure 4.3) with GA (5.72 ± 0.11) being the most acidic and SK the closest to neutral (6.75 ± 0.39). The differences in Olsen-P between sites and pH between sampling zones are not significant.

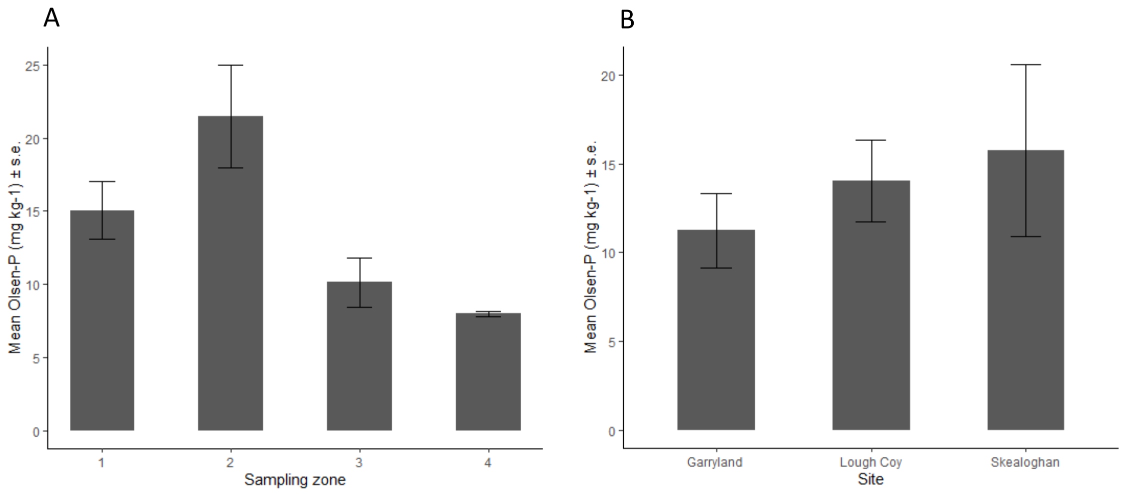


Figure 4.2: Mean values with standard error bars of plant-available phosphorus (Olsen-P) (a) across all sampling zones (significant differences at 95% confidence interval); and (b) at each site.

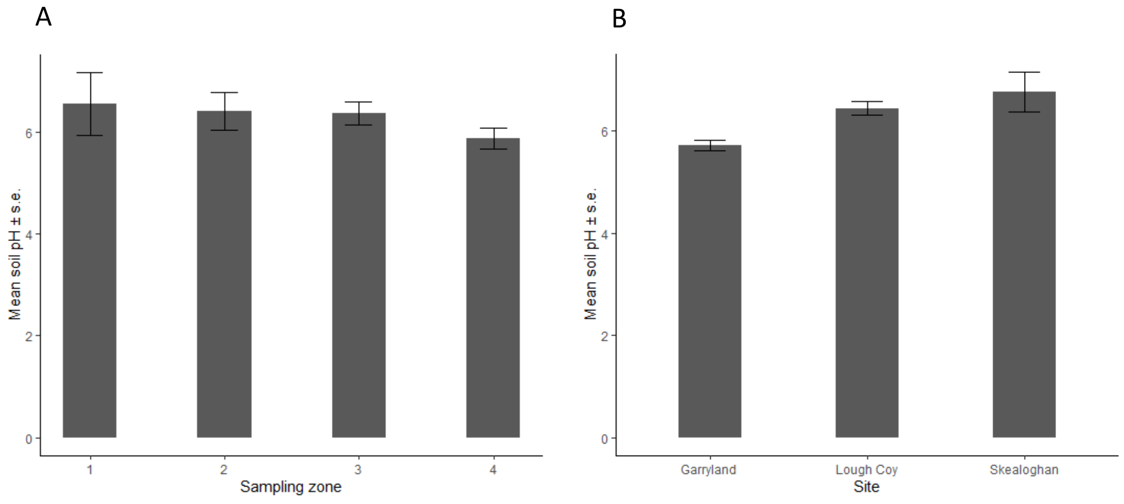


Figure 4.3: Mean values with standard error bars of soil pH (a) across all sampling zones and (b) at each site (significant differences at 95% confidence interval).

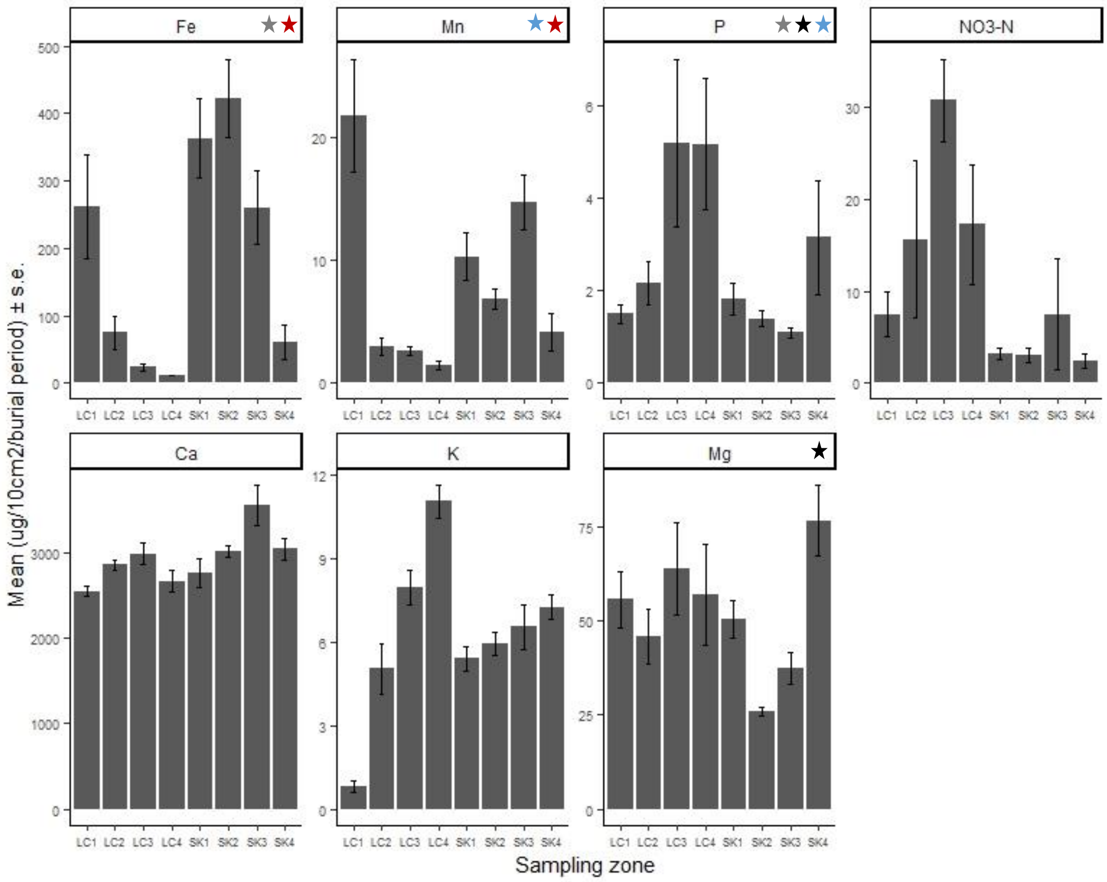


Figure 4.4: Mean values of Plant Root Simulator (PRS) probe measurements (with standard error bars) for the four sampling zones (1-4) at LC and SK; stars of the same colour denote significant correlations between pairs of nutrients at 95% confidence interval.

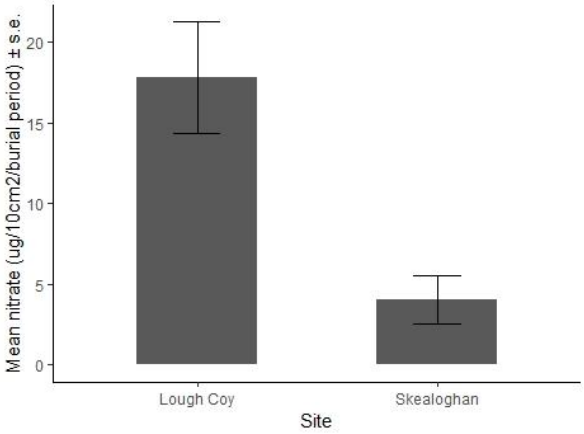


Figure 4.5: Mean values with standard error bars of Plant Root Simulator (PRS) probe measurements of NO3-N for two sites (LC and SK); significant difference between sites at 95% confidence interval.

Figure 4.4 shows the nutrients that are most relevant to plant growth measured by the PRS probes at LC and SK; ammonium is excluded because values in 80% of samples were below detection limits. Phosphorus is significantly associated with magnesium, iron and manganese (p-values 0.02); iron and manganese are also significantly correlated (p-value 0.04). There are no significant differences in PRS-derived elements between sampling zones. Only nitrate levels are significantly different between two sites (LC and SK; p-value 0.04; figure 4.5).

Hydrology

During a two-year period, from July 2016 to August 2018, the maximum flooding depths recorded at each site were 1.6 m at SK (figure 4.6), 10.4 m at GA and 9.1 m at LC (figure 4.7); these depths were all recorded during the winter months. There is much variation in water elevation during the summer months at LC and GA, whereas SK remains relatively constant with the water tables remaining close to the ground surface at lower elevations throughout summer. There were significant summer floods at both GA and LC from July 2017 onwards, reaching peaks of 3.2 m and 4.2 m respectively in early August. In contrast, in 2018 the water levels were largely below the surface from early June to late August.

The hydrographs for LC and GA are highly similar because these sites are linked hydrologically via underground channels, and LC is one of a number of turloughs which drains into GA. LC has a flashier hydrograph than GA, with a greater number of peaks and troughs which are more pronounced as the basin fills and empties more rapidly. However, during the high amplitude flood events the water is slower to recede at LC than at GA, and therefore the water elevation at LC remains higher for longer during peak flood events.

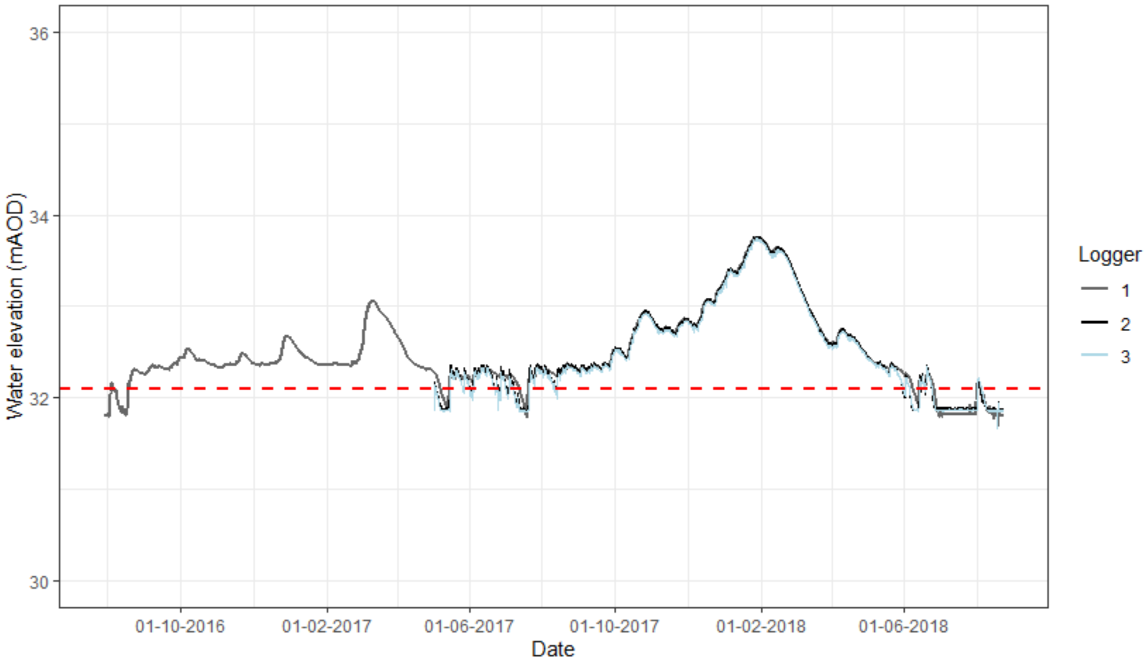


Figure 4.6: Water elevation recorded at 3-hour intervals by data-loggers at SK; horizontal dashed line marks the ground surface; logger 1 was installed in July 2016 in the central and lowest dipwell (D3); loggers 2 and 3 were installed in adjacent dipwells in June 2017 (D2 and D4); see figures 2.5 and 2.6 in Chapter 2 for details of dipwell locations.

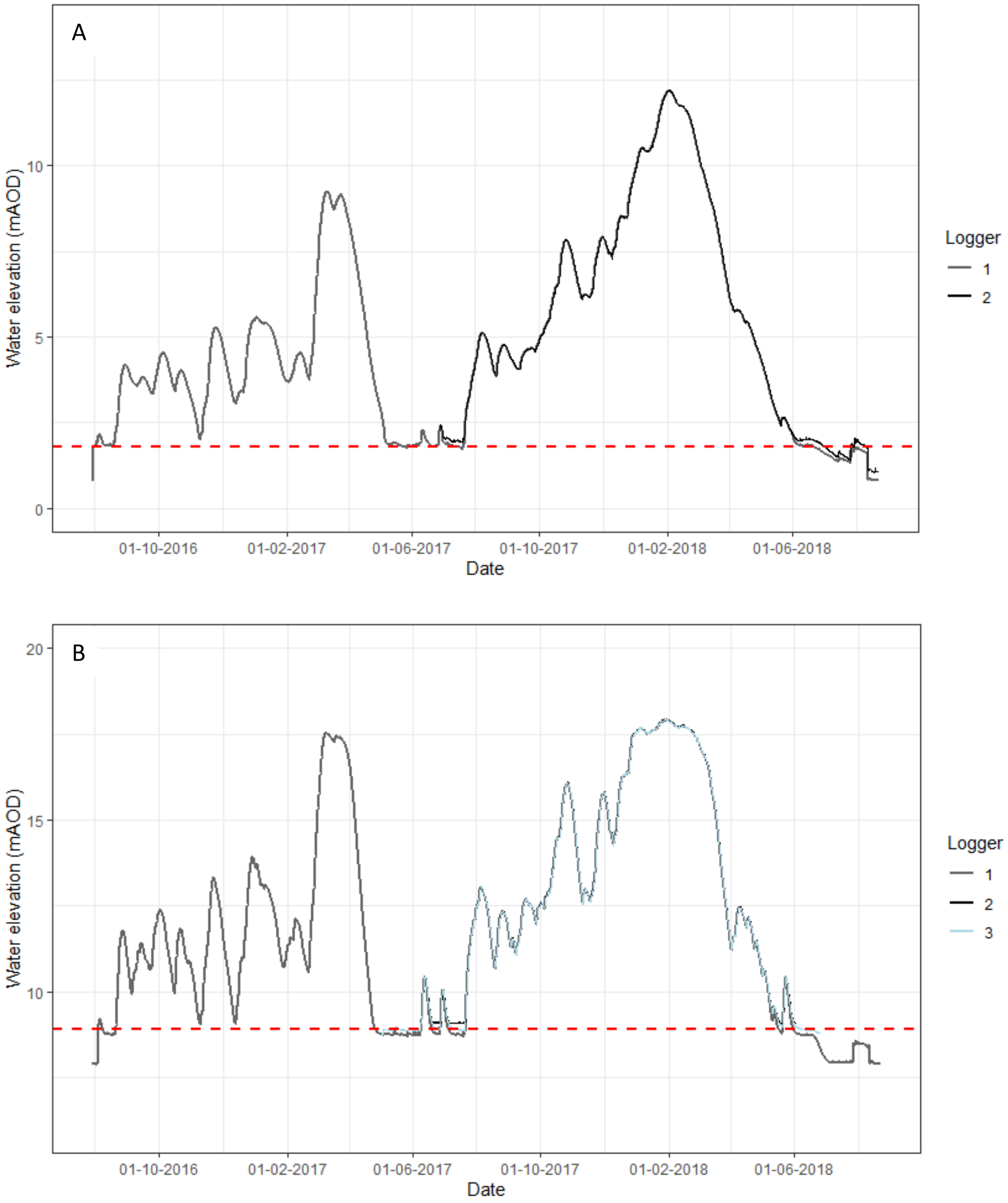


Figure 4.7: Water elevation recorded at 3-hour intervals by data-loggers at (a) GA and (b) LC; horizontal dashed line marks the ground surface; logger 1 was installed in July 2016 in the central and lowest dipwells (D3); logger 2 at LC and GA, and logger 3 at LC, were installed in the adjacent dipwell/s in June 2017 (D2 and/or D4); see figures 2.5 and 2.6 in Chapter 2 for details of dipwell locations.

3.3.2 Plant communities and hydrology

A total of 110 plant species were recorded in 152 quadrats surveyed across the three turloughs in both 2016 and 2018. The TWINSPAN analysis grouped the quadrats from the original thirteen sampling zones into six distinct plant communities (table 4.3). Group 5 contains the greatest number of species overall at 59, whereas plant communities 3 and 4 have the lowest species richness with only 28 and 25 respectively; this pattern is also reflected in the average number of species recorded per 1 m x 1 m quadrat at 19 in group 5, and 6-7 in groups 3 and 4.

Carex nigra and *Potentilla anserina* were almost ubiquitous across the study areas, occurring in all six plant communities (table 4.3). An additional four species were recorded in all the plant communities: *Mentha aquatica*, *Leontodon autumnalis*, *Agrostis stolonifera* and *Galium palustre*; whereas 65 species (excluding mosses) were labelled as infrequent, only occurring in 1 or 2 plant communities at no more than 5% cover (table 4.3; appendix F).

Plant communities 1, 3, 4 and 6 were found at both LC and GA, whereas communities 2 and 5 occur at SK only (figure 4.8). The plant communities coloured in shades of blue (1, 2, 3) and green (4, 5, 6) were found at lower and higher elevations in the turlough basins, respectively. The boundaries drawn between the six plant communities on the site maps are based on the locations of the quadrats and/or the presence of noticeable changes in topography found during the field mapping exercise.

Plant community 1 is characterised by a greater amount of bare ground and presence of the indicator species *Eleocharis acicularis*, *E. palustris* and *Rorippa palustris*; it is also home to *E. uniglumis,* three species of *Persicaria*, and two notably rare species, *Limosella aquatica* and *Rorippa islandica* (table 4.3). The indicator species for community 2 is *M. aquatica*, and it also contains the aquatic species *Glyceria fluitans*, *Potamogeton natans* and *Sparganium erectum*. The species in plant community 3 are very similar to those in community 1, but the former is characterised by a greater cover of *C. nigra* and *P. anserina*.

The indicator species for communities 4 and 6 are *P. anserina* and *C. nigra* respectively; these species are present in both communities, and although 6 is characterised by a lesser cover of *C. nigra* than in other communities, it contains few other potential indicator or associated species (table 4.3). Community 4 also contains a high cover of *Phalaris arundinacea*, whereas 6 has a greater amount of *Filipendula ulmaria*. Community 5 is home to a number of species which do not occur elsewhere in the study areas, for example *Plantago lanceolata* (indicator species), and the grasses *Festuca rubra*, *F. arundinacea* and *Poa pratensis*.

Table 4.3: List of plant species recorded at the turlough study sites, excluding infrequent species (see appendix F); indicator species for the six TWINPSAN groupings are highlighted, along with associated key species for each grouping; constancy values I to V denote: <2, 2-5, 5-10, 10-20 and >20 % (following method by: Dring, 2000); values represent mean cover across all quadrats in each plant community.

Plant community		**1**	**2**	**3**	**4**	**5**	**6**
No. of quadrats		**26**	**24**	**31**	**21**	**24**	**26**
No. of species per community		**36**	**38**	**28**	**25**	**59**	**39**
Average no. of species per quadrat		**9**	**15**	**6**	**7**	**19**	**12**
CODE	**FULL NAME**						
Indicator species							
BareG	*Bare ground*	V	V	III	II	III	IV
EleAci	*Eleocharis acicularis*	V		IV			
ElePal	*Eleocharis palustris*	IV	II	III		I	
RorPal	*Rorippa palustris*	II		I			
MenAqu	*Mentha aquatica*	III	III	II	II	I	IV
CrxNig	*Carex nigra*	IV	V	V	V	III	IV
PotAns	*Potentilla anserina*	II	III	V	V	IV	V
LeoAut	*Leontodon autumnalis*	I	I	I	I	III	II
PlaLan	*Plantago lanceolata*					IV	
Associated species							
EleUni	*Eleocharis uniglumis*	V		III			
PhaAru	*Phalaris arundinacea*		I	V	V		II
PersAmp	*Persicaria amphibia*	IV	I	IV		I	
BidTri	*Bidens tripartita*	III		IV			
PersHyd	*Persicaria hydropiper*	IV		II	I		I
PersMac	*Persicaria maculosa*	IV		II	I		
PolAvi	*Polygonum aviculare*	III		I			
AlisPla	*Alisma plantago-aquatica*	III					
TarOff	*Taraxacum officinale*	II		I	I	I	
CrxDis	*Carex disticha*		IV			I	
GlyFlu	*Glyceria fluitans*		III				
HydVul	*Hydrocotyle vulgaris*		III			III	
JunArt	*Juncus articulatus*		III			II	
PotaNat	*Potamogeton natans*		III				
SpaEre	*Sparganium erectum*		III				
PotRep	*Potentilla reptans*			II	III		II
MenArv	*Mentha arvensis*				III		III
PotEre	*Potentilla erecta*				II	II	II
CrxFla	*Carex flacca*		II			IV	
JunEff	*Juncus effusus*					IV	
LolPer	*Lolium perenne*					IV	
LotCor	*Lotus corniculatus*				I	IV	III
FilUlm	*Filipendula ulmaria*		II		I	III	V
TriRep	*Trifolium repens*		II		I	III	III
AloPra	*Alopecurus pratensis*					III	
BareR	*Bare rock*					III	
CrxHir	*Carex hirta*				I	III	II
CrxPan	*Carex panicea*					III	
FesRub	*Festuca rubra*					III	
PhlPra	*Phleum pratense*		I			II	I
RumAce	*Rumex acetosa*	I				II	II
TriFra	*Trifolium fragiferum*						IV
AgrCap	*Agrostis capillaris*			I	II	I	III
SteMed	*Stellaria media*	I		I	I		I
CerFon	*Cerastium fontanum*			I		I	I

Plant community		**1**	**2**	**3**	**4**	**5**	**6**
CODE	**FULL NAME**						
Common species							
AgrSto	*Agrostis stolonifera*	II	IV	II	III	IV	V
GalPal	*Galium palustre*	I	II	I	II	I	I
PlaMaj	*Plantago major*	II		I	I	II	II
PoaAnn	*Poa annua*	III		I	I	I	I
RanRep	*Ranunculus repens*	I	II		II	II	II
Rare species							
ApiInu	*Apium inundatum*		II				
BelPer	*Bellis perennis*					II	
BracRiv	*Brachythecium rivulare*				I		I
BracRut	*Brachythecium rutabulum*					I	
CallCus	*Calliergonella cuspidata*		IV			III	I
CallGia	*Calliergon giganteum*		II				
CalSp	*Callitriche seedling/sp*	II		I			
CarPra	*Cardamine pratensis*		I			I	
ClimDen	*Climacium dendroides*				I		IV
CirArv	*Cirsium arvense*					II	
CrxOva	*Carex ovalis*					II	
DrepAdu	*Drepanocladus aduncus*		II	I			
ElyRep	*Elytrigia repens*						II
EquFlu	*Equisetum fluviatile*	I	II				
FesAru	*Festuca arundinacea*					II	
GalVer	*Galium verum*						II
GnaUli	*Gnaphalium uliginosum*	II					I
LimAqu	*Limosella aquatica*	I					
LytPor	*Lythrum portula*	II		I			
LytSal	*Lythrum salicaria*			II			
Myolax	*Myosotis laxa*		I			I	
PoaPra	*Poa pratensis*					II	
PruVul	*Prunella vulgaris*					II	
RanAcr	*Ranunculus acris*					II	
RanFla	*Ranunculus flammula*	I	I				
RhytSqu	*Rhytidiadelphus squarrosus*					I	I
RorIsl	*Rorippa islandica*	I					
RubFru	*Rubus fruticosus*	I					I
RumCri	*Rumex crispus*					I	II
Salix	*Salix seedling/sp*	I					I
ScorRev	*Scorpidium revolvens*		II			II	
SteGra	*Stellaria graminea*		I			I	
SucPra	*Succisa pratensis*					II	
TripIno	*Tripleurospermum inodorum*	II					
VioCan	*Viola canina*				II		I
VioRiv	*Viola riviniana*					I	I

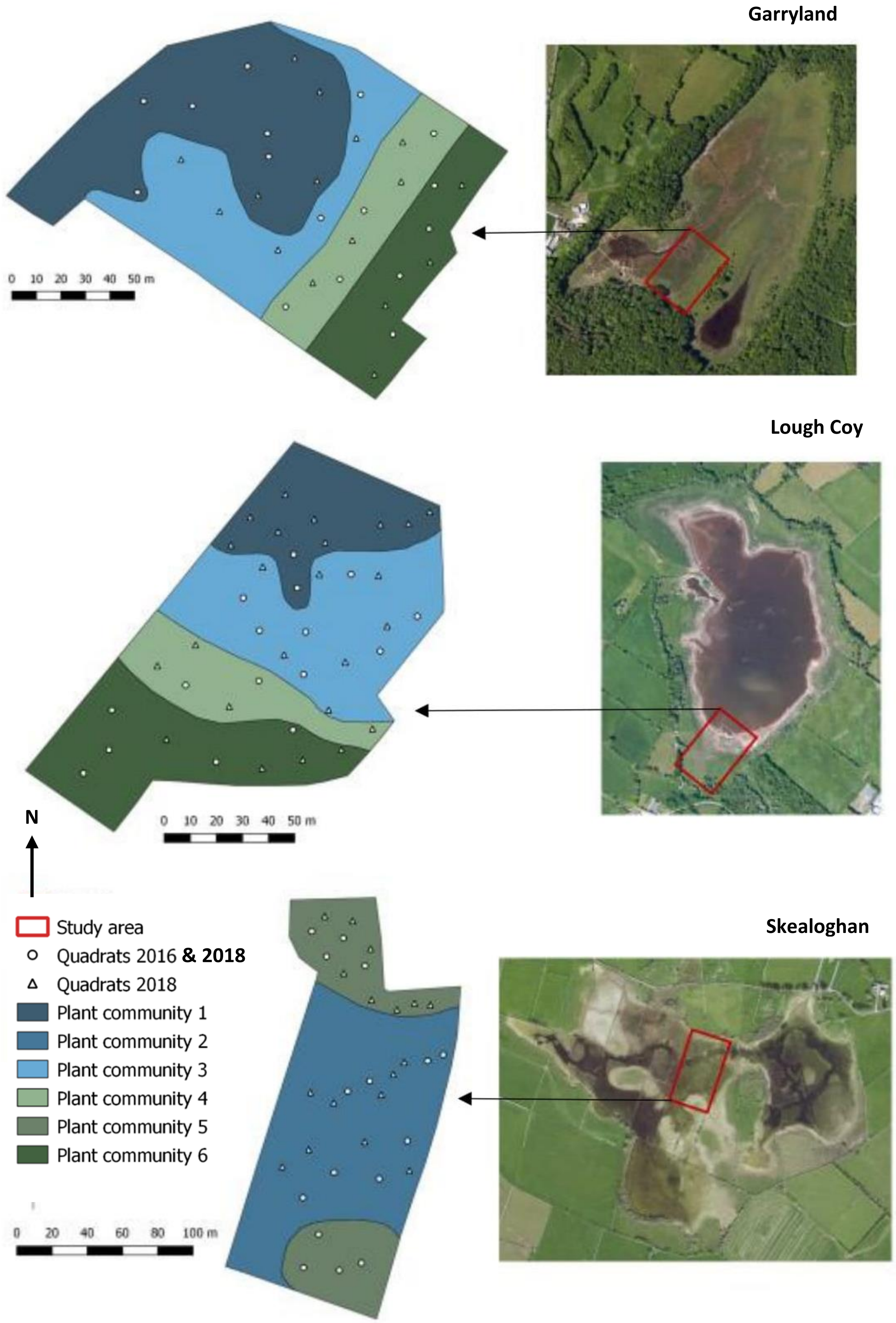
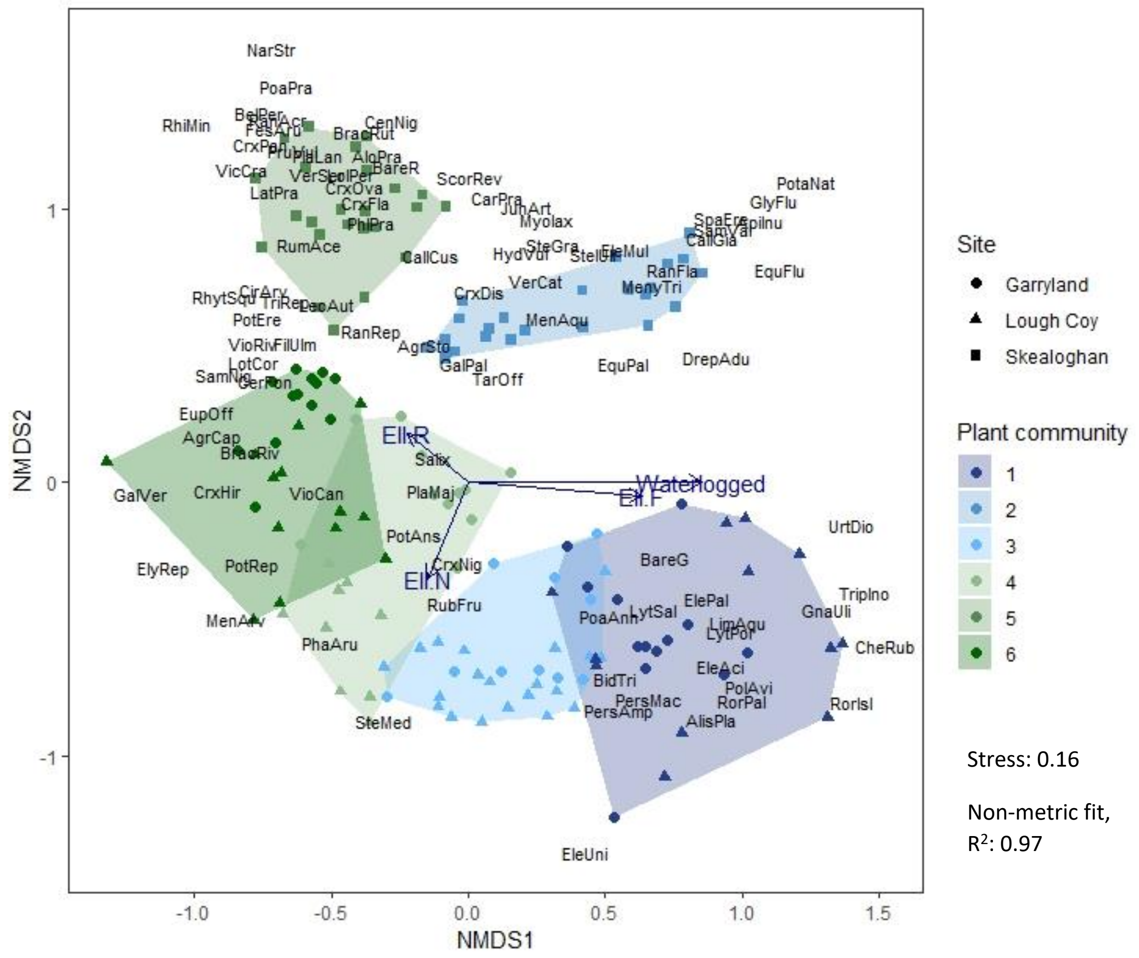


Figure 4.8: Maps of the six TWINSPAN plant communities at each site with quadrat positions marked (communities 2 and 5 at SK only; 1, 3, 4 and 6 at LC and GA; plant communities in shades of blue and green were found at lower and higher elevations in the basins, respectively); the boundaries between the plant communities displayed relate to 2018 data (see chapter 2 for maps showing the boundaries in relation to 2016 data). Aerial photographs of the turloughs (partially full) on the right, with study area outlined; maps drawn using QGIS software (v 3.0.3), aerial photography source: Google Maps.

The six plant communities which were grouped in the TWINSPAN analysis are also distinct in the NMDS ordination space (figure 4.9). The Ellenberg value for moisture (F) is closely correlated with waterlogging along the first NMDS axis, whereas fertility (Ellenberg N; based on nitrogen) is closely aligned with the second axis and is likely to be the secondary driver of community composition after hydrology; pH (R) is not correlated with either axis. The length of the arrows, representing the derived variables, denote the degree of goodness of linear fit in the ordination; the waterlogging and Ellenberg F vectors present the best fit, whereas Ellenberg N and R are the most skewed resulting in shorter arrow lengths (figure 4.9). The plant data in the NMDS appears to be strongly aligned with the hydrological gradient along axis 1 and there is a clear difference between the mean duration of waterlogging amongst the quadrats in the different plant communities, with group 1 being almost constantly waterlogged and group 6 being rarely inundated during the growing season (figure 4.10).

The plant communities 4 to 6 occur on the left of the NMDS plot (figure 4.9) and are rarely inundated during the growing season, with community 6 experiencing the lowest numbers of days of waterlogging (figure 4.10). Some species associated with these drier grassland communities include *Rumex acetosa*, *L. autumnalis* and *C. hirta*. Plant community 1 contains those species that can withstand the constantly waterlogged conditions and/or are able to colonise bare ground rapidly once water levels have receded for a long enough period of time, for example *Chenopodium rubrum* and *Rorippa islandica* (figure 4.9). Communities 2 and 3 contain plant species which can thrive during long, but not constant, periods of waterlogging in the growing season, such as *C. nigra*, *A. stolonifera* and *Juncus articulatus* (figure 4.9-10).

The relevés from SK, which make up plant communities 2 and 5, are completely separate in the ordination space (figure 4.9); this shows that SK is botanically distinct from the other two sites, and it contains a number species which were not recorded at LC and GA. SK lies at the lower end of the fertility (Ellenberg N) gradient, and LC and GA are at the higher end. The presence of the species *Elytrigia repens* and *Stellaria media* at LC and GA also indicate a possible higher nutrient status of these sites (figure 4.9). It is also clear from figure 4.10 that plant communities 2 and 3, and 4 and 5, have almost identical waterlogging durations and therefore the difference between these communities may be driven by other environmental factors, such as nutrient status.



Stress: 0.16

Non-metric fit, R^2: 0.97

Figure 4.9: NMDS ordination of all relevé data (2016 and 2018 quadrats; n=152); quadrats are grouped into 6 polygons representing the TWINSPAN communities (Table 3.1); the ordination plot is overlain with vectors representing duration of waterlogging (days per quadrat, 5 years, growing season only), and mean Ellenberg values for moisture (F), pH (R) and fertility (N) per quadrat (see appendix G for trend surfaces); 20 species labels were omitted for legibility.

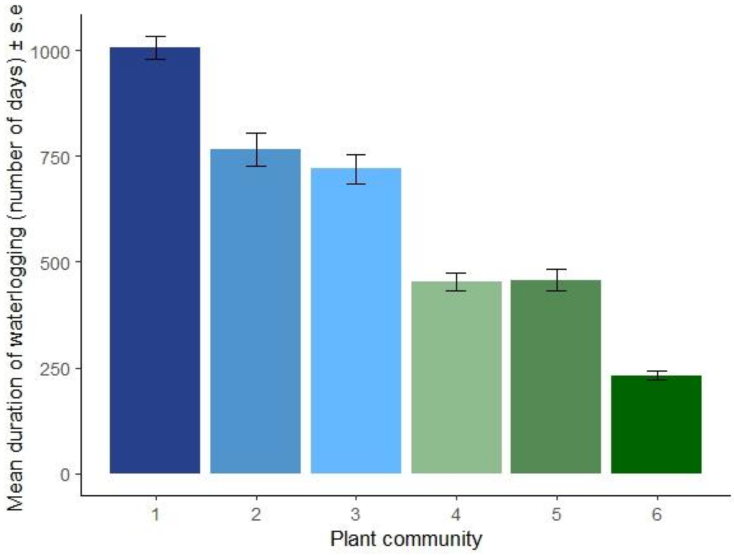


Figure 4.10: Mean duration of waterlogging across the quadrats which belong to the six TWINSPAN plant communities, with standard error bars (number of days across 5 years, growing season only).

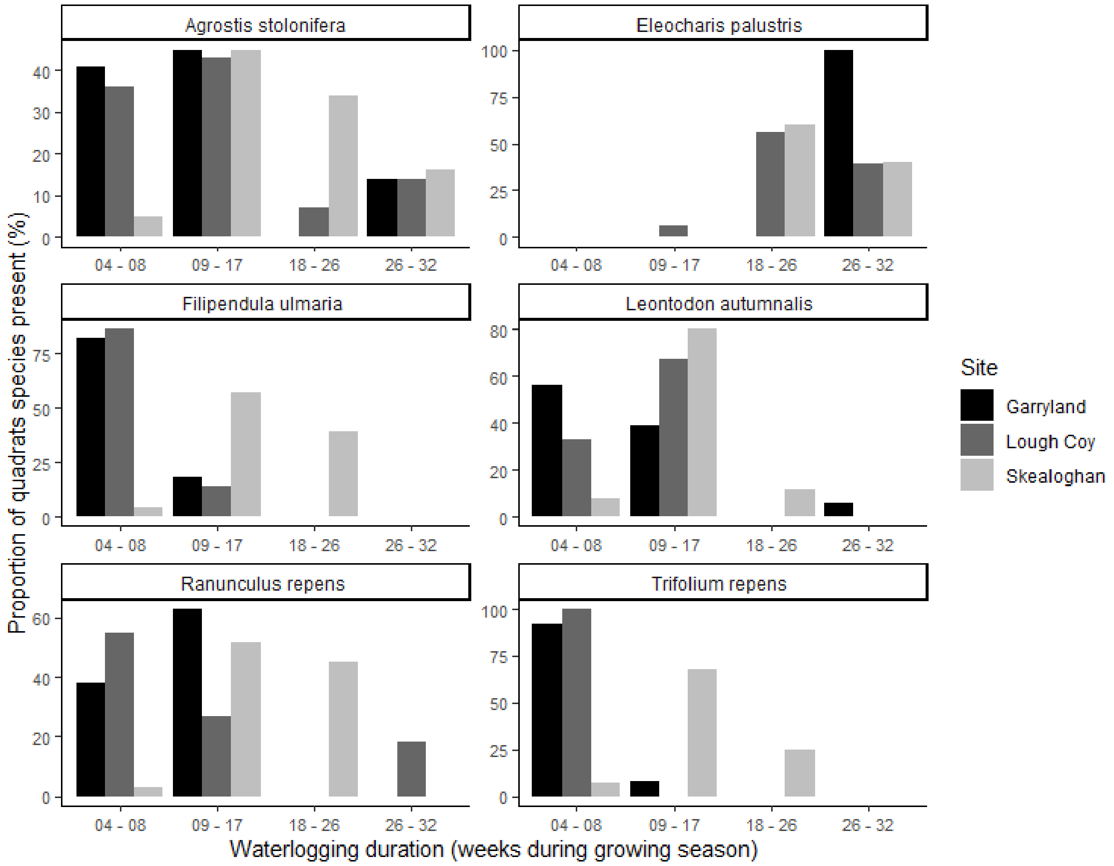


Figure 4.11: Six vascular plant species (selected for their common occurrence at the study sites) and the hydrological conditions in which they were found to grow in this study; each bar represents the percent of quadrats in which the species was present in a given waterlogging band per site (number of weeks per growing season across 5 years).

It is clear that individual species are responding to hydrology in a similar way across the different sites. Figure 4.11 shows the distribution of five vascular plant species in relation to hydrology across the three study sites. The species were chosen because they occur at each site, and in 30-53 % of the 152 quadrats overall. Each bar represents the percent of quadrats with the species present in a given waterlogging band. The absolute number of weeks of waterlogging in each category is consistent across the sites. The six species show different hydrological preferences: *E. palustris* occurs in quadrats with a high degree of waterlogging; *A. stolonifera* and *Ranunculus repens* occur across a wide range of hydrological conditions but are generally absent from quadrats which are constantly waterlogged; *F. ulmaria*, *L. autumnalis* and *Trifolium repens* are found in drier quadrats only.

3.4 Discussion

3.4.1 Plant communities

Ivimey-Cook and Proctor (1966) classified the vegetation of Burren turloughs as part of a phytosociological study and relevés from this, and from 17 turloughs in County Clare and Galway (MacGowran, 1985), were included in a phytosociological overview of Irish wetland vegetation (O'Connell *et al.*, 1984). A comprehensive typology of turlough plant communities was later produced by Goodwillie (1992), who mapped 32 communities across 60 undrained turloughs (greater than 10 ha). Goodwillie *et al.* (1997) extended this work as part of the Gort lowlands flooding study (Southern Water Global, 1998) to include turloughs of a smaller size, but only in a single catchment, and described 36 plant communities. These two typologies were then consolidated into 24 principal plant communities by Goodwillie (2003).

A total of nine plant communities were described across 30 turloughs in south-east Co. Galway by Regan *et al.* (2007), but details of the site-specific communities were not given and therefore cannot be compared to the present study sites. A more recent vegetation mapping project was undertaken as part of a National Parks & Wildlife Services report (Waldren, 2015) and covered 22 turloughs (Sharkey *et al.*, 2015), 17 of which had also been mapped by Goodwillie (1992).

As mentioned previously, turloughs are ecotones, which lie in a transitional zone between fully aquatic and terrestrial systems. This inherent variability, combined with the use of different typologies (described above), makes it difficult to: i) create a comprehensive typology of plant communities applicable to all turloughs; ii) ascertain whether the differences in plant communities recorded reflect variation over time and/or in methodologies (table 4.4). Thus, a single classification system is required, to allow the direct comparison of results between studies undertaken at different points in time and at contrasting sites.

To aid the interpretation of the plant communities described in this study, and allow them to be compared to future work, they have been matched with the vegetation types defined by Goodwillie (2003) (table 4.5), which represent the two most comprehensive turlough mapping projects to date (Goodwillie, 1992; Goodwillie *et al.*, 1997). Both the presence and abundance of Goodwillie's (2003) diagnostic species were used to relate the communities, as well as the more complete community descriptions provided by Goodwillie (1992).

Table 4.4: The TWINSPAN end groups from this study, alongside the comparable plant communities previously recorded at the study sites (references given in the table); the position of each community is related in each case to elevation within the site; TWINSPAN end group 3 is excluded because it could not be matched with any of the plant communities previously recorded.

Site	Elevation within each site	TWINSPAN end groups	Goodwillie (1992)	Goodwillie et al. (1997)	Sharkey et al. (2015)
SK	Low ↓ High	2	11B Peaty pond		13 *P. anserina - C. nigra*
			7A *Polygonum amphibia* (grassy)		
			6A Dry *Carex nigra*		8 *C. nigra - C. panicea*
			5D Sedge fen		
		5	3B Sedge heath		5 Limestone grassland
			2B Poor grassland		
LC	Low ↓ High	1		28 *Eleocharis acicularis - Limosella*	26 *E. acicularis*
		4		12 Dry *C. nigra*	3 *Agrostis stolonifera - Ranunculus repens*
		6		10 *P. reptans - Viola canina*	20 *Filipendula ulmaria - P. erecta - Viola sp.*
GA	Low ↓ High	1	9B *Eleocharis acicularis*	28 *Eleocharis acicularis - Limosella*	26 *E. acicularis*
		4	6A Dry *C. nigra*	12 Dry *C. nigra*	3 *A. stolonifera - R. repens*
		6	5B *Potentilla reptans* (species poor)	11 *P. reptans - C. nigra*	20 *F. ulmaria - P. erecta - Viola sp.*

Table 4.5: The TWINSPAN end groups from this study with the comparable plant communities described by Goodwillie (2003) for each of the study sites.

Site/s	Elevation within site	TWINSPAN end groups	Goodwillie (2003)
SK	Low ↓ High	2	4D *Wet Carex nigra* 7A *Peaty C. nigra*
		5	2D *Lolium grassland*
GA and LC	Low ↓ High	1	6B *Eleocharis acicularis - Limosella*
		3	N/A
		4	4B *Potentilla reptans - C. nigra* 4C *Dry C. nigra*
		6	4A *P. reptans - Viola canina* 4B *P. reptans - C. nigra*

At SK, the lower plant community 2 is most similar to '4D Wet *C. nigra* and '7A Peaty *C. nigra*', which are both associated with peaty substrates that are slow to dry out or remain close to the summer water table, respectively (Goodwillie, 2003); and the higher elevation community 5 best matches with '2D *Lolium* grassland'. This only represents half the number of communities recognised by Goodwillie (1992) in the same study area. Whereas, in another study at the same site, three main plant communities were found in the same study area: 'Sedge Fen', 'Dry *Carex nigra*', and 'Sedge Heath' (Moran *et al.*, 2008a). This again highlights the need to follow the same turlough plant community typology for all sites and studies going forward.

Plant community 1, which lies at the deepest part of both GA and LC basins, is comparable to the '6B *Eleocharis acicularis – Limosella*' community (table 4.4); described by Goodwillie (2003) as a short-lived vegetation type on exposed mud. The next plant community (3) did not fit well with any vegetation types listed and appears to be a transitional band between the adjacent communities 1 and 4, rather than a distinct community in itself. Plant community 4 is comparable to both '4C Dry *Carex nigra*' and '4B *Potentilla reptans – C. nigra*'; and the latter is also well matched with community 6 at higher elevations, along with '4A *P. reptans – Viola canina*'.

The difficulties in matching some of the plant communities found in this study to a single Goodwillie (2003) vegetation type may be because four of those plant communities (1, 3, 4, 6) occur across two sites (GA and LC), but it also highlights the difficulty in drawing distinct boundaries between plant communities of turloughs which lie along a continuum of wet to dry conditions. Regan *et al.* (2007) similarly found that their relevés did not fit neatly with a previous typology, as they identified nine communities which had corresponded to eighteen Goodwillie (1992) categories.

3.4.2 Hydrology as the main driver of plant community zonation

Within sites

It is clear from the findings of this study that the zonation of plant communities within these particular turloughs is driven by their hydrology, and more specifically, the duration of waterlogging during the growing season. Those ruderal species which are able to colonise the bare mud exposed during the growing season are found at lower parts of the basins, for example *E. acicularis* and *Rorippa islandica*. Plant communities found at mid-basin elevations are subjected to alternating conditions of wetting and drying; hence species found here such as *C. nigra* and *P. reptans* can cope with rapidly changing conditions (Goodwillie *et al.*, 1997). Growing conditions are more stable at higher elevations where plants are less likely to experience waterlogging stress during the growing season, which leads to formation of grass-dominated communities.

Flooding changes the soil environment in which the plants are growing and therefore drives community composition. The wetter sampling zones (1 and 2) in this study had higher levels of Olsen-P than the drier zones; additionally, in the PRS-derived nutrient dynamics measured here, phosphorus was significantly associated with magnesium, iron and manganese. Waterlogging creates reducing conditions in soils leading to the production of reduced ions, many of which are potentially toxic to plants (Crawford, 1989). During flooding, oxygen in the soil is quickly depleted and this releases free manganese, sulphide and iron, which adds to root stress and intensifies with time (Goodwillie *et al.*, 1997). Phosphorus dynamics are also closely related to iron cycling in wet sediments; in anoxic soils, phosphorus is released, and can either be seized by biota or amorphous iron particles (Baldwin and Mitchell, 2000). Higher plants that are flood-tolerant and are in possession of aerenchyma have a greater ability to withstand iron poisoning toxicity than non-flood-tolerant plants; where the diffusion of oxygen through the root escapes into the rhizosphere protecting the plant against the uptake of potentially harmful concentrations of iron by oxidizing the ferrous ions to the ferric form (Crawford, 1989).

The findings of this study (see figure 4.10) support the consensus in the literature that it is flood duration, rather than depth, which is the main driver of plant community zonation in turloughs (Sheehy Skeffington *et al.*, 2006; Tynan *et al.*, 2007). Despite the lack of hydrological information in earlier research, there was an assumption that basin depth correlated to the frequency and/or duration of flooding and that this was driving vegetation zonation. Although some more recent studies have used hydrological data, they have been limited by the amount of data available (e.g. Regan *et al.*, 2007; Moran *et al.*, 2008a; Moran *et al.*, 2008b). The present study has examined turlough plant communities in relation to fine scale variation in the hydrology, using botanical and hydrological data which are contemporaneous. In addition, any previous hydrological data used were based on the inundation of vegetation alone, as opposed to the methods employed here of looking at the waterlogging thresholds of the different soils across the sites; this is an important distinction to make, because plants can experience the impacts of flooding stress, i.e. oxygen depletion, before water reaches the ground surface.

Previous studies have found that different grazing strategies within the same turlough can result in differences in faunal and flora diversity and composition within the same vegetation type and flood zone (Ní Bhriain *et al.*, 2002, 2003). Grazing impacts are not within the scope of this study; and as such, sites were selected on the basis that they were being actively grazed and the study areas within each site lie in a single management type. However, grazing affects the sward directly by livestock targeting more palatable species (Ní Bhriain *et al.*, 2003), and it has been suggested that herbage palatability in turloughs is affected by their inherent hydrological variability (Ryder *et al.*, 2005). For instance, livestock could graze the first flush of soft vegetation during a relatively dry Spring but, a subsequent flood event would prevent further grazing and result in the presence of coarser, less palatable vegetation when stock are returned to the site (Ryder *et al.*, 2005). Therefore, it is likely that grazing pressure will be affecting some degree of the within-site variation in plant communities in this study. This is in agreement with another turlough study where the main factors controlling plant community composition were hydrological regime and grazing (Moran *et al.*, 2008a).

Between site differences

The factors controlling plant-community composition between the sites may differ to those driving variation within individual turloughs, predominantly the differences between plant communities at SK and those at GA/LC. There are two sets of two communities which experience almost identical waterlogging durations, but they vary considerably in their plant community compositions; i.e. plant community 2 at SK versus 3 at LC/GA, and community 4 at LC/GA versus 5 at SK. It has been demonstrated that individual species are responding to waterlogging stress in a similar way between all three sites and that species are exhibiting preferences for different hydrological niches (figure 4.11). The theory of hydrological niche segregation (HNS) in plant communities was developed in relation to floodplain meadow research in the UK (Silvertown *et al.*, 1999) but subsequent studies have also found HNS to be driving plant community composition in other habitats (e.g. Araya *et al.*, 2011; García-Baquero *et al.*, 2016).

Firstly, it is possible that the hydrological parameter used in this study does not sufficiently capture all the complexities of the hydrological regimes required to explain the plant community composition and distribution fully. For instance, it is likely that plant communities 3 and 4 experience more variable hydrology than communities 2 and 5. GA and LC are steep-sided basins, which experience sizable summer flood events, whereas SK is relatively flat topographically with a steady hydrological regime and only one major flood per year, which is largely confined to the winter months. Therefore, communities 3 and 4 can experience multiple short-term waterlogging events within a single growing season, whereas community 2 remains constantly damp throughout the summer and any waterlogging events experienced by community 5 are more likely to be at peak flood times before floodwaters recede or after they have nearly fully risen.

Further evidence that plant communities 3 and 4 experience greater hydrological variability is the observation that the highest abundance of *Phalaris arundinacea* occurs within these communities whereas it is absent from community 5. This species flourishes in environments with fluctuating water levels (Figiel *et al.*, 1995; Magee and Kentula, 2005), as does the fen violet, *Viola persicifolia* (Pullin and Woodell, 1987) which grows at the same elevation as *P. arundinacea* at LC. These patterns could be linked to grazing pressure as *P. arundinacea* is highly palatable which could explain its absence from community 5 (Goodwillie *et al.*, 1997).

There are additional environmental factors which differ between the study sites that could also be influencing species composition; for example, the nutrient status of a site is mediated through both the soil and inflowing water. SK soils are circumneutral and peaty, with an extensive area of fen peat in the basin floor making up 65% of the total area; whereas GA and LC have moderately acidic and inorganic soils (Kimberley *et al.*, 2015) and contain significant marl deposits (Kimberley & Waldren, 2012). As mentioned previously, LC and GA are hydrologically linked and in fact they receive non-limestone recharge from the Slieve Aughty mountains (with sandstone geology), in contrast to SK which receives its recharge from the limestone area only (Naughton *et al.*, 2009).

It is likely that nutrient status is the secondary influence on vegetation in turloughs, and this could explain some of the differences between plant communities at SK and GA/LC. The nitrate concentrations recorded in this study are significantly higher in soils at LC and lower at SK, and it is likely the GA is also nutrient-rich given its similarities with LC (listed above). Goodwillie (1992) stated that GA is eutrophic and SK oligotrophic, and using the Ellenberg N index Goodwillie *et al.* (1997) surmised that GA and LC are mesotrophic sites. Although results from another study indicated that the Ellenberg F index is a good model for hydrological variables, more work is needed before the Ellenberg N and R indices can be accepted as dependable surrogates for relevant environmental variables on turloughs (Williams *et al.*, 2011).

4.4.3 Impact of this study for management and further research

To measure any changes in turlough ecology in the future, it is vital that we gather as much baseline data as possible before these sites are (further) affected by a changing climate and/or drainage schemes. There are novel methods of data collection being developed and applied to turlough plant communities, where Satellite imagery is being used to map wetland communities (Bhatnagar *et al.*, 2020). Although this will accelerate the gathering of information, it will lack the detail and precision of data collected in the field, and it employs yet another plant community classification system (which is only comparable to studies employing similar methods).

As stated by Goodwillie *et al.* (1997), ‘Any flood measures which take ecological conservation as their flood level control will have to respect this essential feature [fluctuating hydrology] of the habitat and allow for year to year differences in water height’. Turloughs represent a stronghold for some nationally and internationally rare species (e.g. *Rorippa islandica*, *Viola persicifolia)*, which rely upon the inherent variability of this ecosystem to survive, and therefore any changes in their hydrological regimes are likely to be detrimental to the highly specialised flora and fauna of Ireland’s turloughs. It is therefore vital that any studies which examine the impact of turlough hydrology on their ecology, use some measure of hydrological variability, but this is not the focus of current research. One component of a study by

Morrissey *et al.* (2020) was to assess the possible ecological impact/s of proposed flood alleviation schemes, but this impact was only assessed at one turlough and with regard to the reduction of winter peak floods which largely affect non-wetland vegetation.

It is important to study these ecosystems, which experience highly variable hydrology, to provide us with information on what might happen to wetlands that have a more stable hydrological regime under present climate conditions. Climate change will force difficult decisions upon us. For example, in Ireland, there is a need to protect homes, businesses and agricultural land, but also the ecological functioning of our wetland ecosystems. The increased variability brought on by climate change will bring a whole new challenge to habitat and wildlife conservation, where ecosystems will not only become wetter or drier, but more variable and unpredictable. Thus, we may no longer be able to manage sites in the same way we do now, and site managers will need to be flexible and adapt their strategies as quickly as the climate is changing.

Chapter 5

Effect of inter- and intra-annual hydrological variability on the species richness of vegetation in Irish turloughs: a comparison with English floodplain meadows

5.1 Introduction

There is a general consensus within the published literature, including those studies which use hydrological data, that the development of plant communities in turloughs is largely driven by flood duration, rather than mean flood depth (Goodwillie, 2003; Sheehy Skeffington *et al.*, 2006; Tynan *et al.*, 2007). In addition to this, the current study (research summarised in chapter 4) suggests that the duration of soil waterlogging is an important mechanism driving the composition of turlough plant communities. There are, however, no empirical studies which investigate role of hydrological variability as a potential driving mechanism of species richness in this habitat.

The results of chapter 3 indicate that inter- and intra-annual hydrological variability are important drivers of species richness in English floodplain meadows. Praeger (1932), who first noted the plant-community zonation of turlough basins, commented on the difficulty of establishing a connection between the flora and fluctuation of water levels in turloughs because of the variability between years. It is possible that inter- and intra-annual hydrological variability may also be important drivers of plant species richness in turloughs.

Turloughs have been described as ecotones acting as a floodplain for underground rivers (Goodwillie, 2003; Reynolds, 1998; Naughton et al., 2017a), and they often contain similar species assemblages to English floodplain meadows. However, turloughs are more variable hydrologically and therefore provide a useful comparison for what might occur in English floodplain meadows under a more variable hydrological regime such as that predicted under future climate change (IPCC, 2014).

The literature published thus far (summarised in chapter 3) on the impacts of hydrological variability upon plant-community dynamics and species richness are highly contradictory. The research findings of chapter 3 showed that an increase in soil waterlogging, and inter- and intra-annual hydrological variability, all correlate with a decline in species richness at a selection of English floodplain meadows. It is assumed that these patterns will be similar in Irish turloughs.

Therefore, to address the research gaps identified here, the aims of this chapter are to (a) investigate the effect of soil waterlogging, and inter- and intra-annual hydrological variability, on turlough plant species richness, (b) explore whether the effect of soil waterlogging, inter- and intra-annual hydrological variability on the plant species richness, is similar for both English floodplain meadows and Irish turloughs, and (c) examine whether turloughs experience greater levels of hydrological variability than English floodplain meadows. Following the results of chapter 3 and the evidence given here, the following hypotheses are suggested:

1. Increases in soil waterlogging, inter- and intra-annual variability, will all lead to a decline in turlough species richness;
2. Both meadow and turlough species richness will respond in a similar way to increases in soil waterlogging, inter- and intra-annual variability;
3. The hydrological regimes observed in turloughs will extend the known range of both the inter- and intra-annual variability observed in floodplain meadows.

5.2 Methods

The criteria employed to select field sites and the data collection methods used for English floodplain meadows and Irish turloughs are detailed in chapter 2.

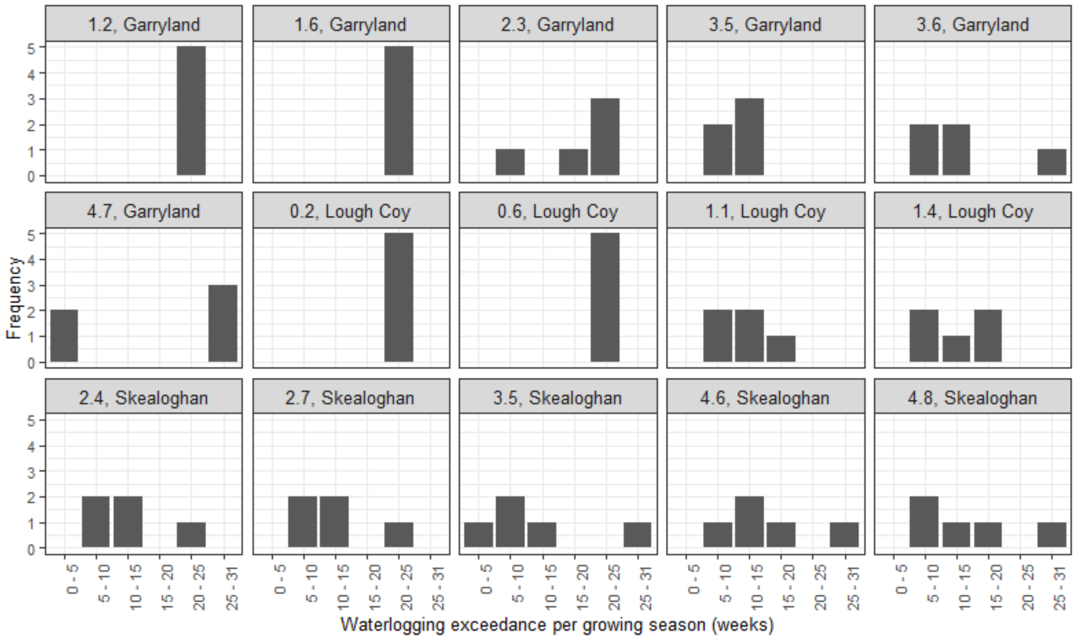
See chapter 3 (and appendix B) for details on how the measures of inter- and intra-annual hydrological variability were calculated. This chapter only addresses the impact of soil waterlogging, not drying, on plant species richness for the following reasons:

- Analysis of English floodplain meadow data indicated that high levels of waterlogging are more detrimental to species richness than soil drying (chapter 3; figure 3.5a and 3.5b).
- Inter- and intra-annual variability in soil waterlogging drives species richness in English floodplain meadows (chapter 3);
- Duration of waterlogging drives turlough plant-community zonation (chapter 4);

Additionally, it was found that a large proportion of the study area within each turlough basin experienced either no (at Lough Coy and Skealoghan), or very little (Garryland), measurable drying stress during the two-year measuring period (Jul-16 to Aug-18). The drying SEVs were calculated using data from the instrumented dipwells which had the longest data-series available (i.e. lowest lying dipwells). The small amount of variation in values at Garryland are likely to be too small to affect the plant communities present at the lower parts of the basins, but it is possible that soil drying could be a factor influencing vegetation in the upper parts of the basin slopes. In order to apply the SEV drying approach in the present study, would involve further data collection on groundwater levels by drilling into the bedrock and this was beyond the scope and logistical capabilities of the current study (see section 7.2 for suggestions on further work in this research area).

The data analyses were carried out as per the methods described in chapter 3 (section 3.2.3). The daily water-level values for turloughs were transformed into weekly means to allow their comparison with meadows. The distributions of raw data were checked visually for normality (figure 5.1); most deviated from normality, therefore the summary statistics of inter-quartile range and median were selected. Linear mixed effect (lme) models were fitted as per the methods described in chapter 3 (see appendix C.2 for model outputs).

Inter-annual hydrological variability



Intra-annual hydrological variability

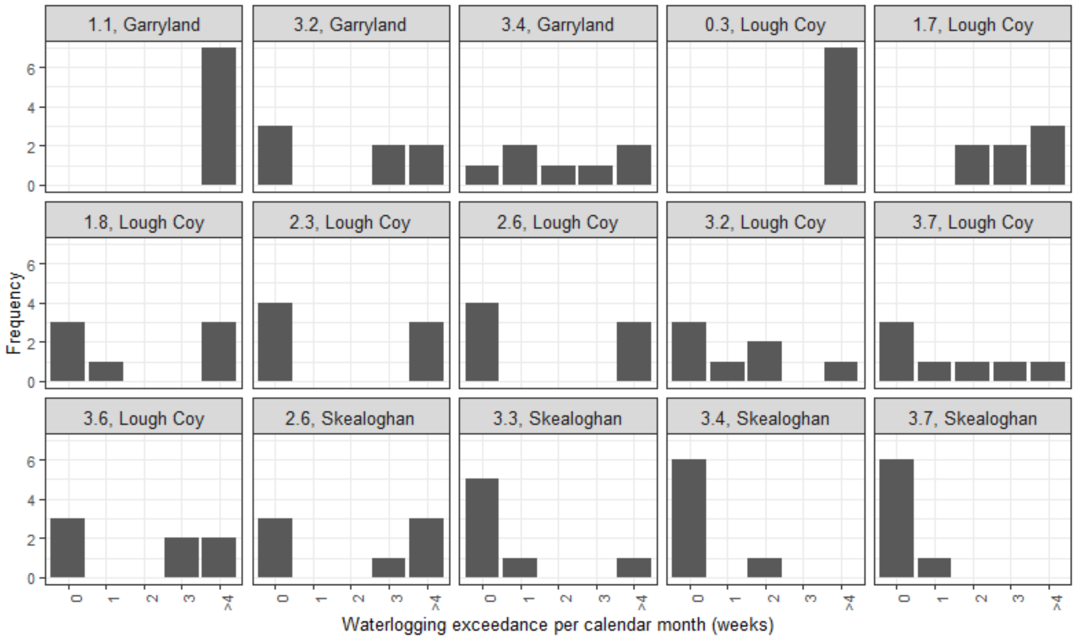


Figure 5.1: Random samples selected to illustrate the distribution of the data used to calculate (a) inter- (n=5) and (b) intra-annual (n=7) variability; the quadrat (i.e. sample) number and the site it belongs to are given; see chapter 3 text (and appendix B) for details of how measures are calculated.

5.3 Results

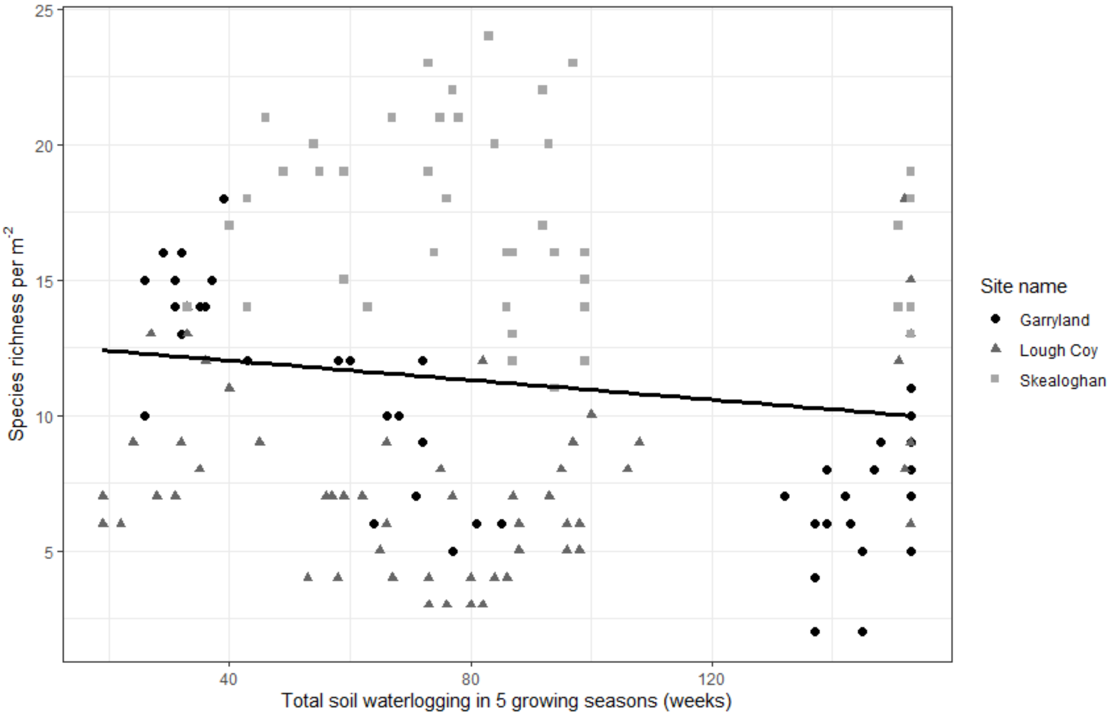


Figure 5.2: Relationship between the total number of weeks the soil waterlogging threshold is exceeded across five growing seasons and species richness per quadrat; each symbol represents one quadrat (n=152).

The relationship between the total number of weeks a quadrat is waterlogged across five growing seasons and the species richness of that quadrat is not significant ($p = 0.09$, $R^2 = 0.02$; figure 5.2). However, when inherent site differences are accounted for using a lme model, this relationship becomes highly significant ($p < 0.01$). The quadrats from Garryland and Lough Coy, which are mixed together on the graph, represent the lower end of the species richness gradient and are spread along the entire gradient of total soil waterlogging. The Skealoghan quadrats are almost entirely separate and they have greater species richness but do similarly follow the trend of declining species richness with increasing waterlogging.

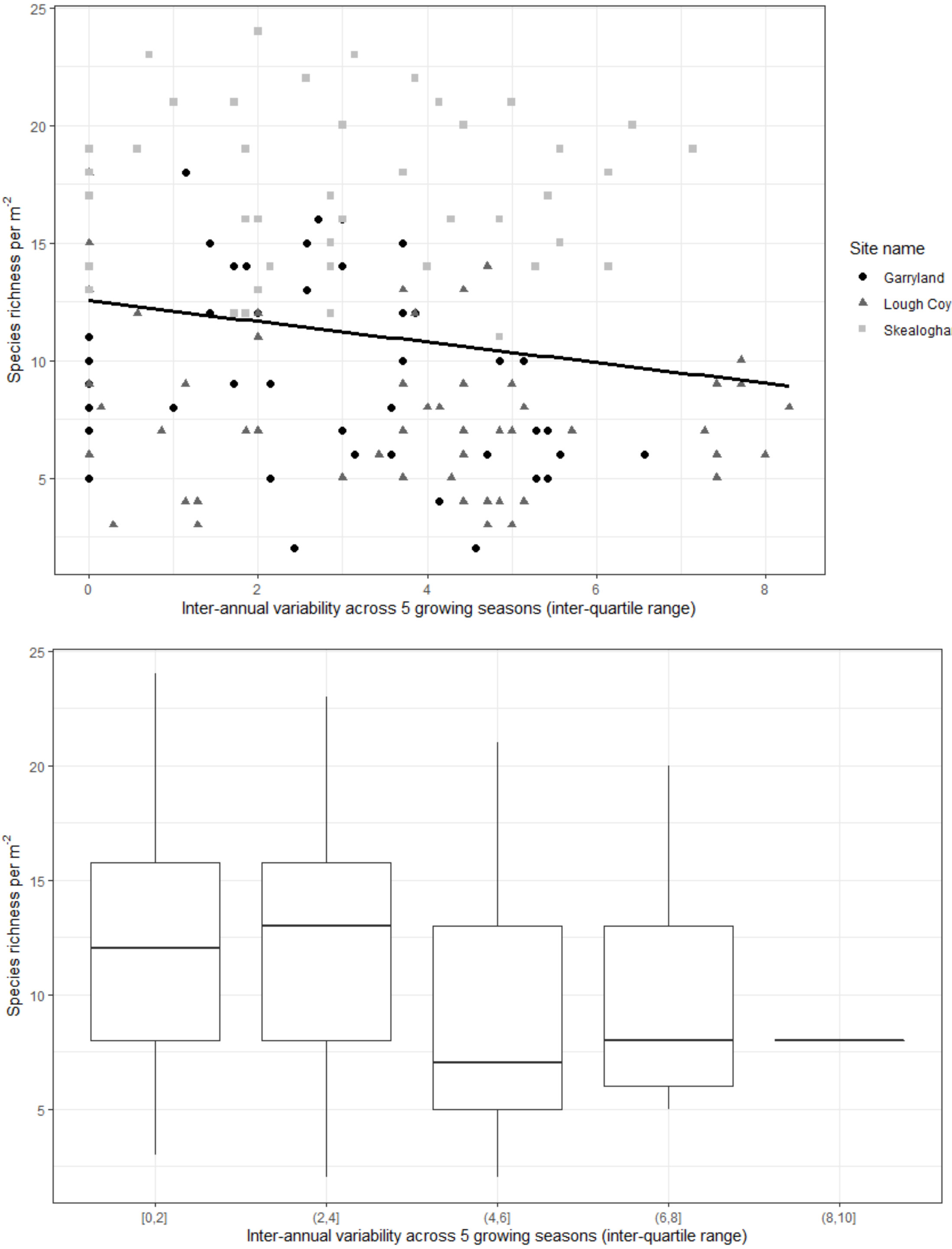


Figure 5.3: Inter-annual variability versus species richness for (a) each quadrat (n=152) and (b) five groupings of quadrats (boxplot shows median, first and third quartiles, minimum, maximum, and outliers).

There is a negative correlation between the degree of inter-annual hydrological variability and species richness ($p = 0.03$, $R^2 = 0.03$; figure 5.3a). Taking into account the effect of site differences in the lme model, the trend remains significant ($p < 0.05$). Figure 5.3b shows the decline in median species richness in each group of quadrats as the variability increases.

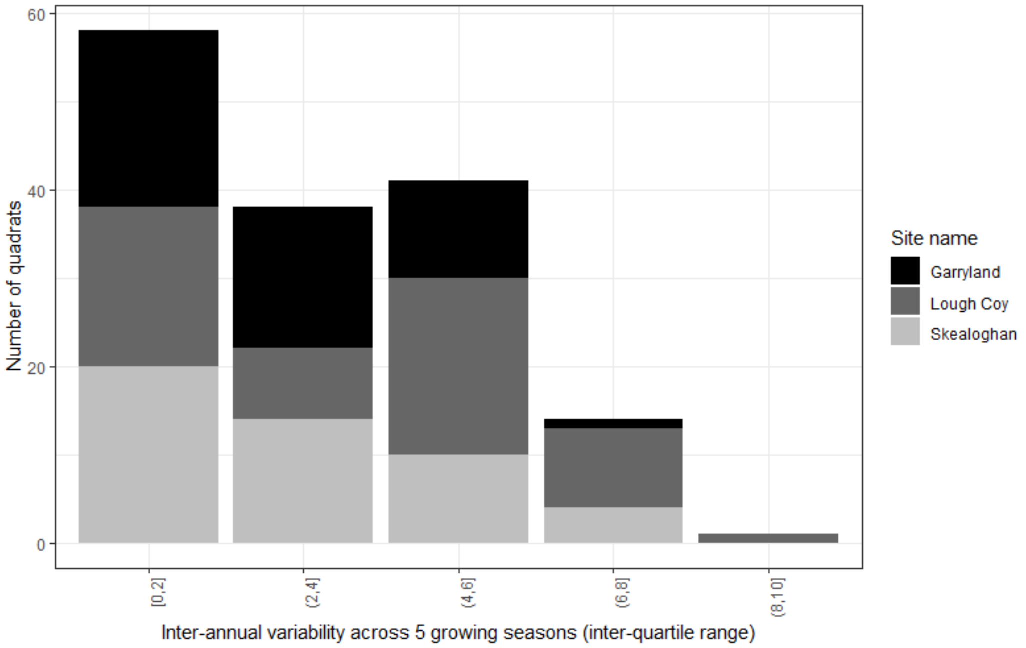


Figure 5.4: The distribution of quadrats from each site (GA n=48, LC n=56, SK n=48) per grouping of inter-annual variability.

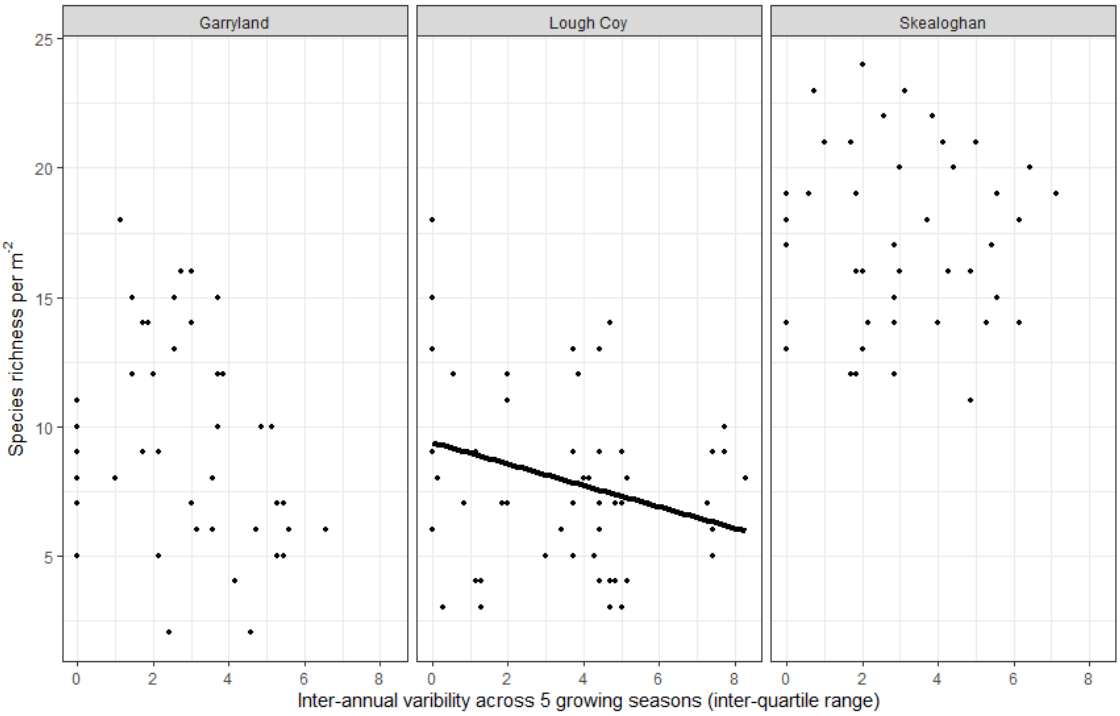


Figure 5.5: Inter-annual variability versus species richness for each quadrat, with each site plotted separately (GA n=48, LC n=56, SK n=48); line of best fit displayed only for statistically significant results.

The majority of quadrats occur in the low to medium categories of inter-annual variability, and all sites are represented in four out of the five groupings (figure 5.4). When the relationship between inter-annual variability and species richness is examined at individual sites, there is only a significant negative correlation at Lough Coy ($p = 0.03$, $R^2 = 0.08$; figure 5.5).

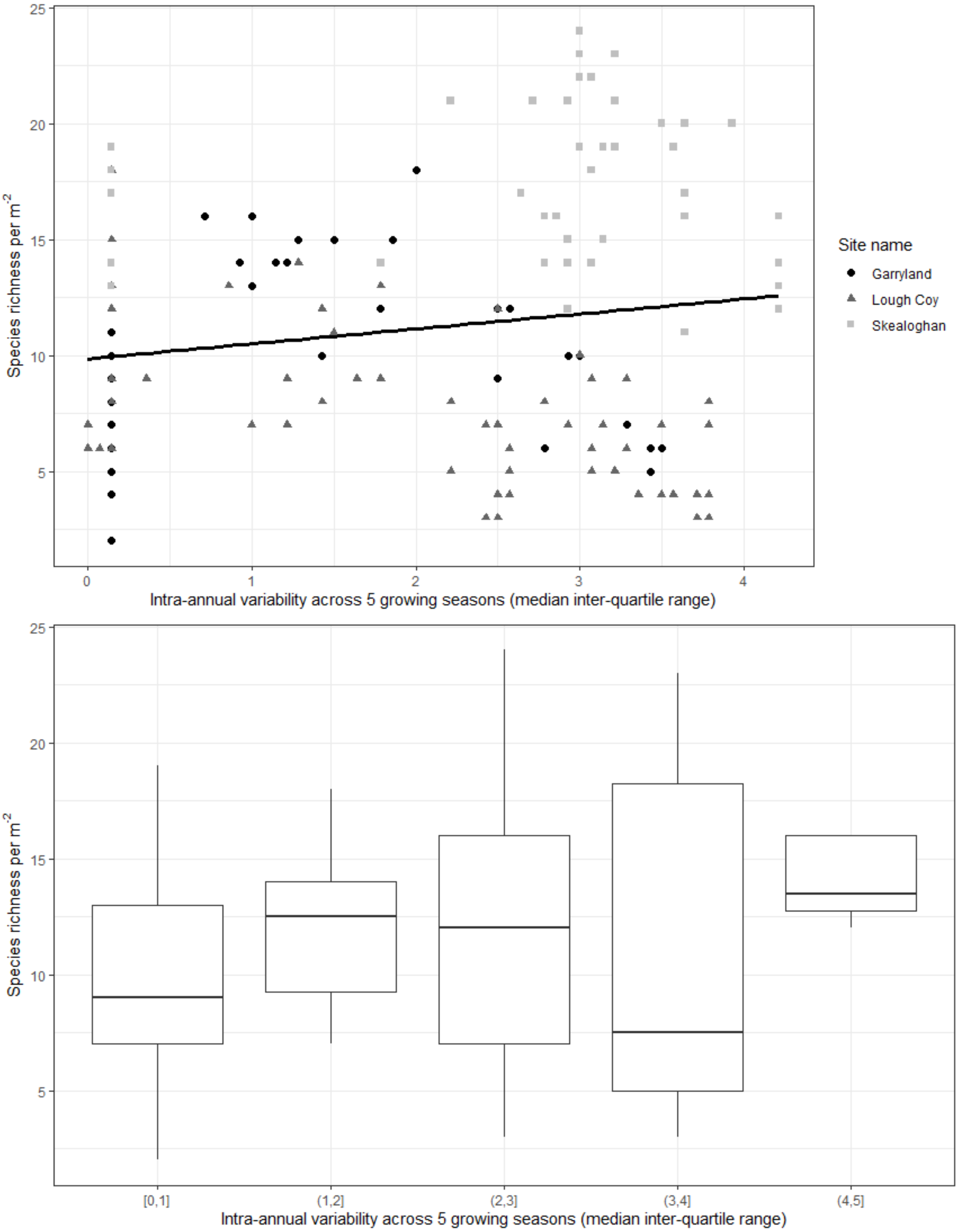


Figure 5.6: Intra-annual variability versus species richness for (a) each quadrat (n=152) and (b) five groupings of quadrats (boxplot shows median, first and third quartiles, minimum, maximum, and outliers).

There is a positive correlation between the degree of intra-annual hydrological variability and species richness (p-value 0.04, R^2 0.03; figure 5.6a). The trend is no longer significant however when the effects of inherent site differences are taken into account using the lme model (p = 0.06). Figure 5.6b shows the increase in median species richness in each group of quadrats except the fourth, as the variability increases.

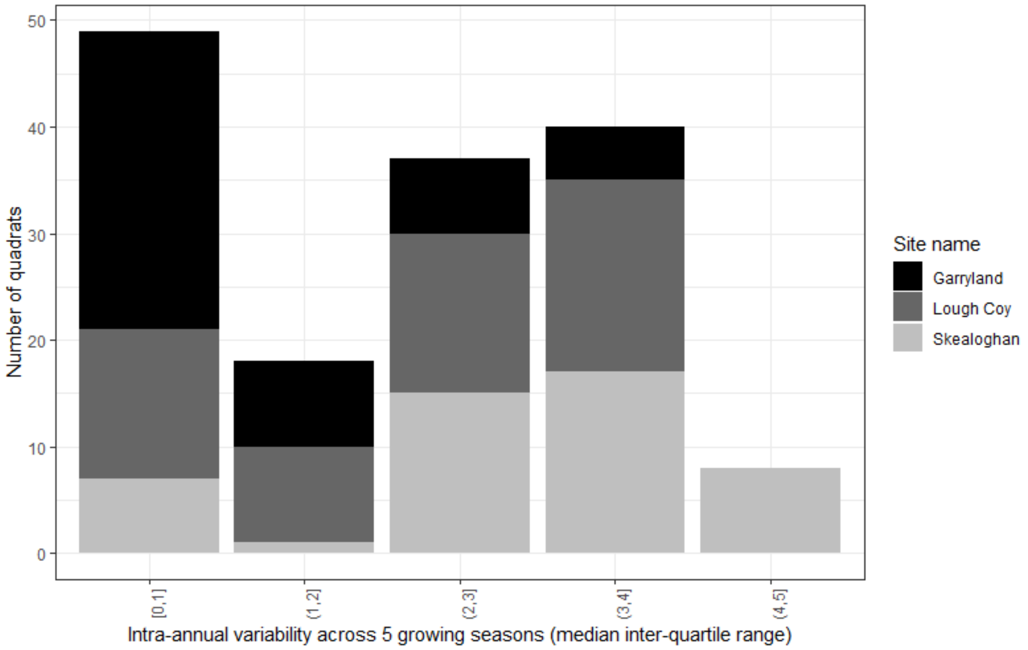


Figure 5.7: The distribution of quadrats from each site (GA n=48, LC n=56, SK n=48) per grouping of intra-annual variability.

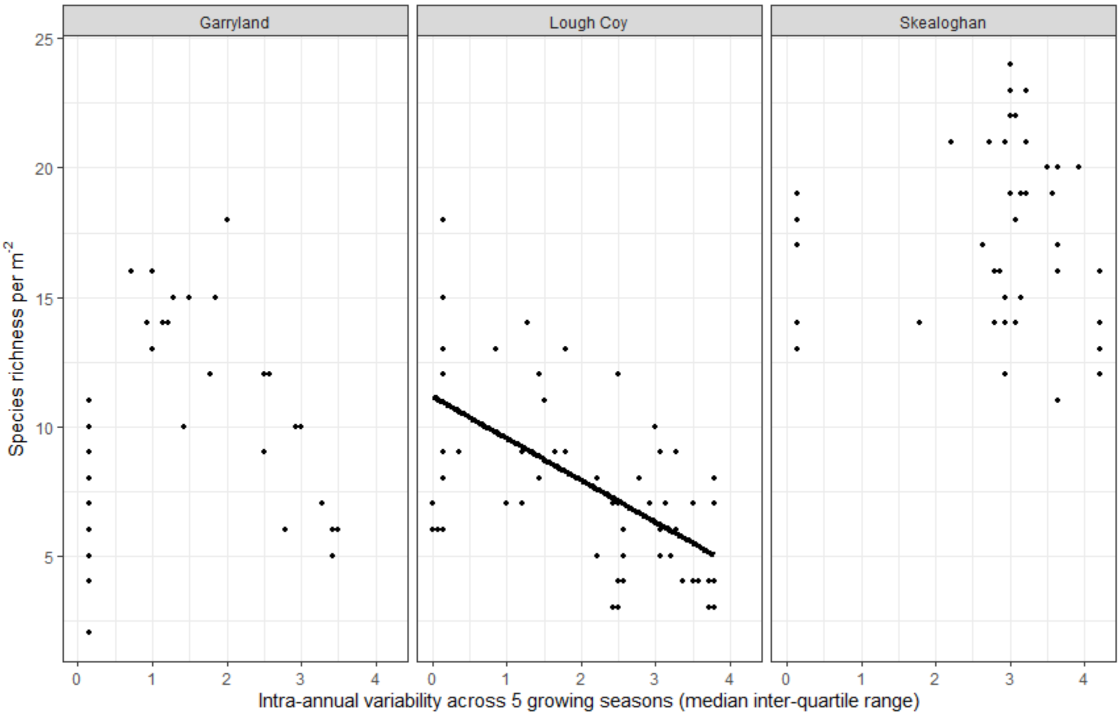


Figure 5.8: Intra-annual variability versus species richness for each quadrat, with each site plotted separately (GA n=48, LC n=56, SK n=48); line of best fit displayed only for statistically significant results.

The majority of quadrats occur in the low to medium categories of intra-annual variability, and all sites are represented in four out of the five groupings (figure 5.7). When the relationship between intra-annual variability and species richness is examined at individual sites, there is a significant negative correlation only at Lough Coy ($p < 0.001$, $R^2 = 0.33$; figure 5.8).

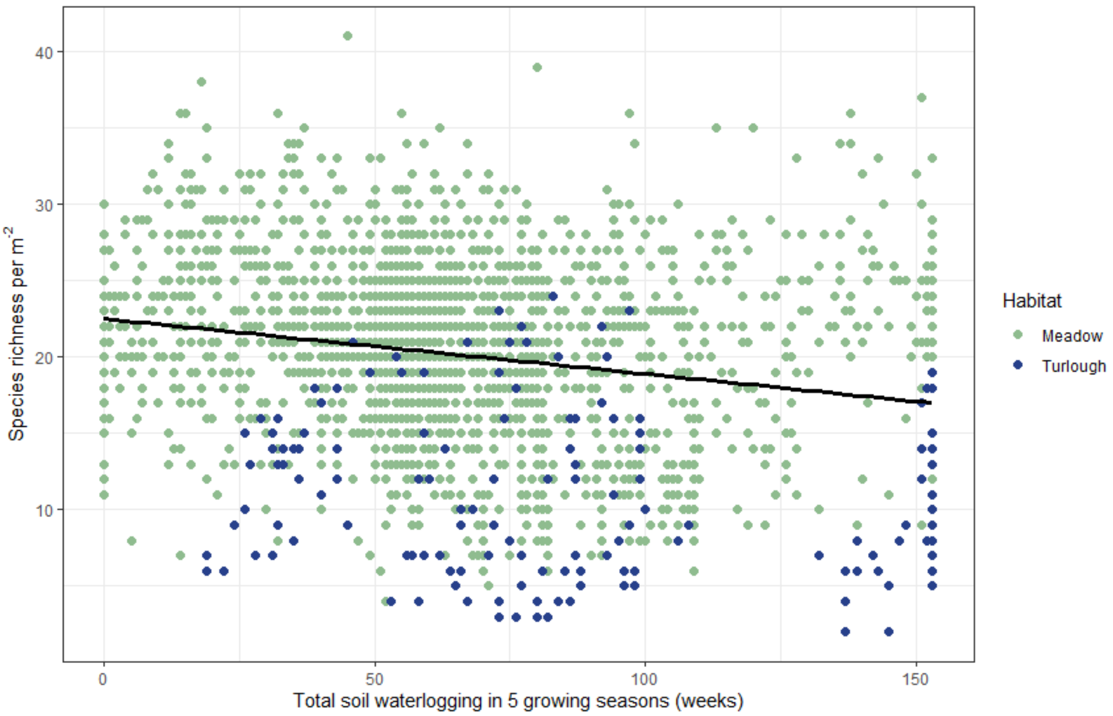


Figure 5.9: Relationship between the total number of weeks the soil waterlogging threshold is exceeded across five growing seasons and species richness for each quadrat belonging to meadows (n=2218) and turloughs (n=152).

There is a negative relationship between the total number of weeks a quadrat is waterlogged across five growing seasons and the species richness of that quadrat when both English floodplain-meadow and Irish turlough data are combined ($p < 0.01$, $R^2 = 0.05$; figure 5.9). Taking into account the effect of site differences in the lme model, the trend remains significant (p-values <0.01). Whilst the turlough quadrats represent the lower end of the species richness spectrum, they are spread along almost the entire gradient of total soil waterlogging, representing a similar range to the meadow quadrats and are only absent from the driest end of the spectrum (i.e. where quadrats experience no or very little soil waterlogging stress).

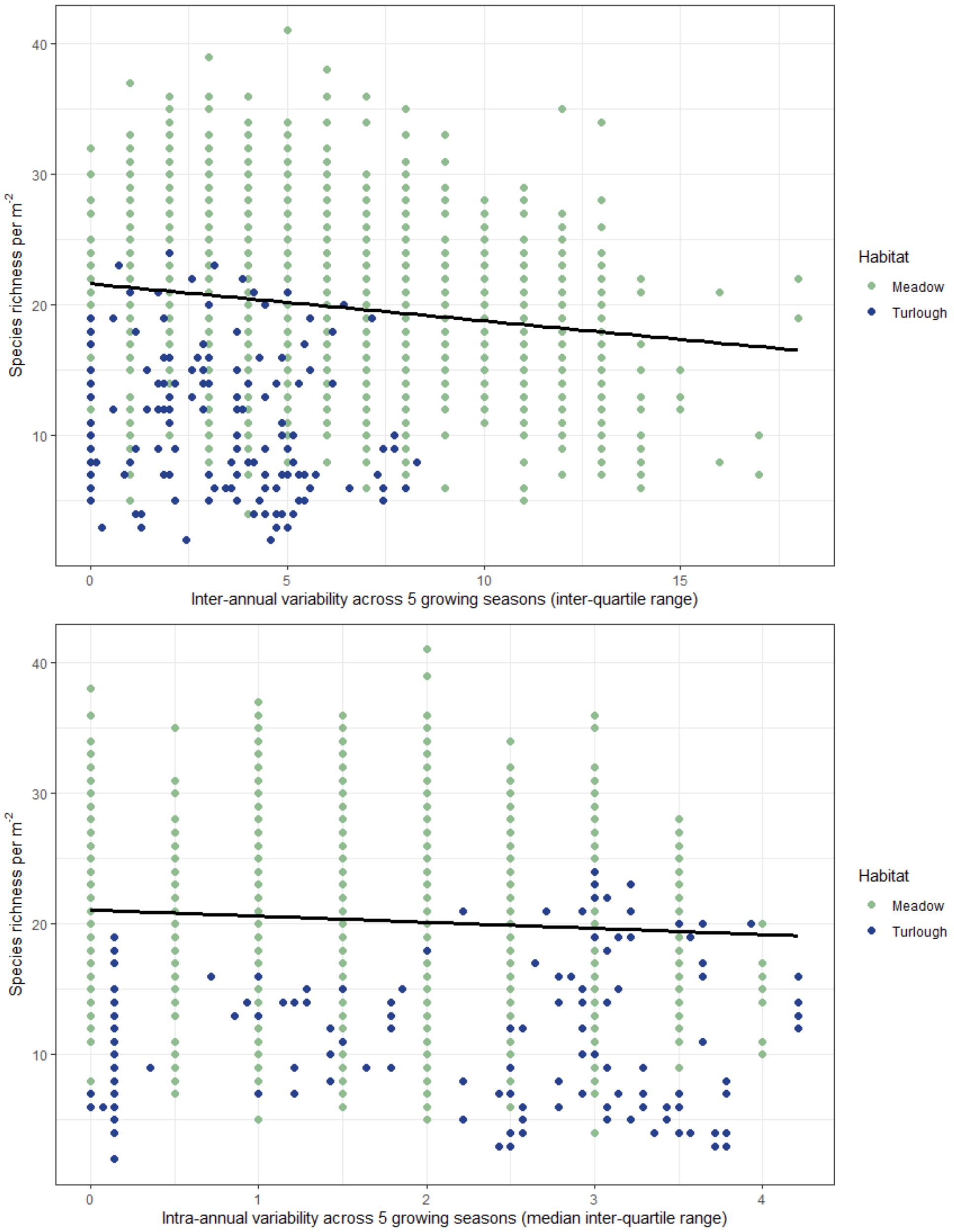


Figure 5.10: (a) Inter- and (b) intra-annual variability versus species richness for each quadrat belonging to meadows (n=2218) and turloughs (n=152).

There is a negative relationship between the degree of inter- and intra-annual hydrological variability across five growing seasons, and the species richness of that quadrat, when data from both English meadows and Irish turloughs are combined (figure 5.10). Both trends are highly significant ($p < 0.01$), however the correlation for intra-annual variability is weaker ($R^2 < 0.01$) than for inter-annual variability ($R^2 = 0.02$). Both relationships remain significant when inherent site differences are accounted for using the lme model ($p < 0.01$). The turlough quadrats only occur at the lower end of the inter-annual variability spectrum, whereas they are spread all along the intra-annual variability spectrum with a greater density of points at the upper end.

5.4 Discussion

5.4.1 Impact of soil waterlogging on turlough species richness

The results of this study show that an increase in soil waterlogging correlates with a decline in species richness at the three Irish turloughs studied here. These trends are similar to those found in floodplain habitat studies, where an increase in flooding negatively affects species richness (chapter 3 findings; also Maher *et al.*, 2015; Mathar *et al.*, 2015; Garssen *et al.*, 2017). It is interesting that this relationship is only significant once inherent site differences has been accounted for; this could be explained by the fact that quadrats at SK have notably higher species richness compared to those at GA and LC, but importantly the overall trend is the same across all three sites. Therefore, soil waterlogging is an important component of turlough ecosystem functioning at the selected sites, as a potential driver of both the species richness and composition (chapter 4 findings) of plant communities.

5.4.2 Impact of inter- and intra-annual variability on turlough species richness

The results of this study show a downward trend in species richness as a result of an increase in inter-annual variability across all three sites; which provides further evidence that greater levels of hydrological variability can be detrimental to plant species richness. This finding agrees with the research conducted on English floodplain meadows (chapter 3) and elsewhere (e.g. Garssen *et al.*, 2015). It is however, not in agreement with literature on the storage effect, which is thought to be a key mechanism driving plant-community dynamics in other floodplain environments (Hölzel and Otte, 2004; Capon, 2005; Capon and Brock, 2006; Mathar *et al.*, 2015). It is not possible to analyse the positive correlation between species richness and intra-annual variability found in this study, because the correlation does not remain significant once the lme model is applied.

When the data from each turlough were analysed individually, a significant result was only found at LC. An increase in both inter- and intra-annual variability correlates with a decline in species richness at LC, but the correlation is noticeably stronger with the latter (R^2 = 0.08, R^2 = 0.33 respectively). This site was selected for study because of its ‘flashy’ hydrograph and to represent the more extreme end of the hydrological variability spectrum observed in turloughs (see chapter 2 for details). It is surmised that the high levels of disturbance at LC, caused by the variable hydrology, is having a detrimental impact on species richness; this follows the logic of the Intermediate Disturbance hypothesis (Connell, 1978) and evidence given elsewhere (e.g. Pollock *et al.*, 1998). The question remains, why was this relationship not significant at the other two sites? One possible explanation is that the relative extreme nature of the variability at LC has surpassed a threshold, whereby the disturbance caused by the variable hydrology has become the overriding mechanism driving the establishment and survival of plant species. Whereas at other sites, that are less disrupted by fluctuating water levels, other biotic and abiotic factors are driving the development and diversity of the plant communities present.

5.4.3 Impact of soil waterlogging, intra- and inter-annual variability on turlough and meadow species richness

Meadow and turlough species richness similarly respond to increases in soil waterlogging, inter- and intra-annual hydrological variability, when analysed simultaneously; and these findings agree with the author's hypothesis. The species richness of both habitats is negatively correlated with increasing intra- and inter-annual hydrological variability; but the correlation is stronger with inter-annual variability (intra-annual R^2 <0.01, inter-annual $R^2 = 0.02$), as in chapter 3 when the meadows data only were analysed. A stronger correlation was not expected given the inherent noise generally found in ecological data, and particularly in such a large dataset. Additionally, in both habitats, there will be other controlling factors of species richness which will account for the unexplained variation in the data; for instance, soil type, grazing regime, nutrient status and/or fertiliser use, have been found to be important drivers of diversity in both floodplains (Mountford *et al.*, 1993; Maher *et al.*, 2015) and turloughs (Sheehy Skeffington *et al.*, 2006; Regan *et al.*, 2007).

5.4.4 Extending the range of hydrological variability observed with meadows

One outcome that was expected in this analysis was for the range of inter- and intra-annual hydrological variability, observed with English floodplain meadows alone, to be extended once the Irish turlough data were added. This was expected because of the contrasting hydrological regimes of the meadow and turlough habitats, where the former is notably less variable than the latter. This research, however, found that the range of inter-annual variability did not increase, and the spectrum of intra-annual variability was only lengthened slightly once the turlough data were added to the analysis. There are several factors contributing to these surprising findings.

First, the hydrological parameter used in the analysis could mask the extent to which turlough hydrology is more variable than that of meadows. To summarise the hydrological regimes of these two habitats in an ecologically meaningful way, a measure of inter- and intra-annual hydrological variability was calculated, which used the data on how often soil waterlogging thresholds were exceeded (see section 3.2.3, and appendix B). Therefore, this analysis does not measure the full extent of hydrological variability at turlough sites, where much of that variability results from large fluctuations in water level. Second, it is important to stress that the three turlough sites were selected to represent different levels of variability characteristic of turloughs. However, these three sites are only a small fraction of the turloughs found in Ireland, as well as other turloughs and similar habitats which occur elsewhere (Sheehy Skeffington *et al.*, 2006), and therefore they are unlikely to be fully representative of the total variability shown across this habitat.

Notwithstanding the points discussed above, some interesting trends have emerged from this analysis. The findings show that the meadow study sites experience greater inter-annual hydrological variability in waterlogging stress than the three turlough sites studied, and that the total amount of waterlogging stress occurring at turlough sites is relatively consistent between years. Conversely, the turlough study sites experience greater intra-annual hydrological variability than the meadow sites; with fewer turlough quadrats plotting at the lower end of the variability spectrum, and fewer meadow quadrats at the upper end. Therefore, to summarise, in terms of the total waterlogging stress experienced at the study sites – meadows are more variable between years, and turloughs are slightly more variable within years (and it is likely that this would be heightened if the hydrological parameter used also measured water-depth variance).

5.4.5 Implications of this study for management and further research

It is of great concern that not only an observed increase in overall soil waterlogging across a gradient, but also inter- and intra-annual hydrological variability, currently relate to a decline in species richness at both Irish turloughs and English floodplain meadows, as these stresses are only set to increase in the near future. As discussed in chapter 3, there is a lack of research relating to intra-annual variability, and the current study on meadows and turloughs found a contradictory result of greater inter-annual variability reducing species richness than that found in other studies. Therefore, more research is required on the mechanisms relating to hydrological variability that affect species richness at these sites and other grassland habitats, to inform their management under future climate change.

There is much to learn from the turlough habitat, particularly with regards to the relationship between hydrological variability and species richness – and its potential for enlightening future impacts of climate change on grassland communities elsewhere, which currently experience less variable hydrological conditions but are predicted to become more variable in the near future. It is possible that under climate change, with more variable weather conditions, more turloughs than currently known could become dominated by flashy hydrological regimes and this may cause a decline in species richness, like the situation in LC; furthermore, intra-annual hydrological fluctuations could become more pronounced in English floodplain meadows, which could then begin to impact on diversity. Lastly, as the variability measures used in this study do not fully encapsulate the contrasting hydrological regimes of these two habitats, more work is needed to find appropriate hydrological measures to investigate these important habitats further.

Chapter 6

Effects of fluctuating water levels on four species of *Ranunculus* in a mesocosm experiment

6.1 Introduction

There is now a large body of evidence to support the idea that plant species, and communities as a whole, respond to fine-scale shifts in hydrology across time and space (see chapters 3 and 4). Historically, research has centred around the impact of water-table depths on plant communities. Water-regime descriptors commonly used in the ecological analysis of vegetation patterns include: mean (Jones *et al.*, 2017), median (Hájek *et al.*, 2013) and maximum (Aldous and Bach, 2014) water table depth, amplitude (Kotowski *et al.*, 1998), and thresholds (Noest, 1994; Gowing *et al.*, 1997). However, there has been relatively little focus on the role of hydrological variability, beyond amplitude, in shaping plant communities. For example, the Sum Exceedance Value (SEV) method focuses on the total number of weeks of waterlogging and drought per growing season that individual plants and communities can tolerate (Silvertown *et al.*, 1999), but does not consider the distribution (timing, frequency and duration) of those weeks.

There is evidence from field studies which suggests that hydrological variability affects plant communities in English floodplain meadows and Irish turloughs (see chapters 3 and 5), as well as in other wetland habitats. For instance, it has been suggested that plant species can exhibit unique responses in occurrence and/or abundance in relation to water-level variability in North American urban wetlands (Magee and Kentula, 2005) and river floodplain habitats in Germany (Leyer, 2005). Similarly, Pollock *et al.* (1998) found a linear relationship between species richness and the spatial variation of flood frequencies (SVFF) at multiple wetland sites in Alaska; sites with intermediate flood frequencies and high SVFF were species-rich, whereas sites that are frequently, rarely, or permanently flooded and with low SVFF were species-poor. It has also been suggested that it is the extreme, rather than average, flood events which are greater determinants of riparian plant species distributions (Vervuren *et al.*, 2003). In contrast, Kotowski *et al.* (1998) examined the behaviour of wetland plant species along a moisture gradient in two European lowland river valleys, using mean water level and water-level amplitude, and found that most species were consistent in their response to mean water level rather than fluctuation and suggested that overall wetness is more important than fluctuation.

Controlled experiments are a useful method for testing theories in plant ecology, which can be used independently or in conjunction with gathering field evidence. A number of experimental studies support the idea that changing water levels affect plant biomass and community composition (e.g. Potvin *et al.*, 2015; Weiher and Keddy, 1995). There is also general agreement on the impacts of flood timing and duration on plants communities, which can affect plant reproductive traits and biomass (e.g. Eck *et al.*, 2004; Mony *et al.*, 2010; Greet *et al.*, 2013), and growth form (e.g. Herr-Turoff and Zedler, 2007; Brotherton *et al.*, 2019b). Conversely, those studies which address the effects of hydrological variability on plants are not all in agreement. Nygaard and Ejrnaes (2009) manipulated nutrients, water-table depth and the degree of water-level variance in mesocosms and found that the effect of fluctuation regimes on species composition and species richness was not statistically significant.

The evidence from experimental research focussing on how plant physiological and morphological adaptations in response to changing water levels can be detrimental to growth rates, when compared to more stable conditions, is not clear-cut. Casanova and Brock (2000) conducted an experiment based on Australian temporary wetlands, where seed bank samples were exposed to 17 different water-level treatments with different depths, durations and frequencies of flooding. The greatest biomass was recorded in a low-fluctuation (i.e. never flooded) and high-fluctuation (i.e. short frequent floods) treatment, and the lowest biomass occurred in another low-fluctuation treatment (i.e. continuously flooded). Other studies have found some similar patterns in riparian and wet dune slack plant communities, where highly fluctuating water levels led to lower total biomass production than lower fluctuating or permanent water levels (Bakker *et al.*, 2007; Luo *et al.*, 2018). In contrast, another study examined the impact of different flood lengths and fluctuations on a *Carex* species and found that it was the duration of flooding which limited biomass accumulation (Gao *et al.*, 2016).

Some experimental studies have found that the growth form and reproductive traits of plants, including some species of *Ranunculus*, can shift in response to fluctuating hydrology. The unpredictable fluctuations in water depth characteristic of a type of temporary wetland in the West of Ireland (turloughs) are believed to have caused an evolutionary adaptation in one species of buttercup, *Ranunculus repens*; where those plants growing in turloughs are characterised by more highly-dissected leaves, resulting in enhanced gas exchange, than the typical broad-leaved form found in neighbouring terrestrial environments (Lynn and Waldren, 2002). Another species of *Ranunculus*, the aquatic *R. peltatus*, also exhibits plasticity in growth form, and is able to survive and reproduce under alternating wet and dry conditions when it was transferred to three different hydrological conditions at either a vegetative state or at the onset of flowering; these findings may explain why *R. peltatus* is successful in temporary marshes (Volder *et al.*, 1997). Additionally, when *Ranunculus acris* was subjected to different flood-length treatments (2- and 7-day), the leaf lengths produced were significantly longer under the more extreme flooding conditions compared to the 2-day treatment and control, and the onset of flowering was also delayed in the 7-day treatment (Brotherton *et al.*, 2019b). Blom *et al.* (1990) investigated the morphological and physiological adaptations of particular riverine plant species to fluctuating water levels; for instance, species were able to elongate stems to protrude above the water surface and/or change their timing of reproduction. Similarly, Ayi *et al.* (2016) found that terrestrial plants experiencing oxygen deficiency due to flooding can form aquatic adventitious roots, which are capable of absorbing oxygen to alleviate some stress and delay death.

Competition is another important factor which can affect the growth of plants in experimental studies. For instance, Byun *et al.* (2017) investigated the response of three emergent wetland plants, in terms of their biomass, to five flooding regimes and found that only one of the three species responded positively to increased water levels and flooding frequency, and it out-competed the other two species irrespective of hydrological regime. Foxx and Fort (2019) conducted a meta-analysis on the competitive aspect of root and shoot interactions under water stress and found that competition was most intense between roots at low water availability (i.e. drought stress). Repeating the design of a well-known experiment by Ellenberg, where six plant species grown in both mixtures and monocultures were subjected to different water-table depths (WTD) and soil thicknesses, Bartelheimer and Poschlod (2016) demonstrate that hydrology is more important than competition along a WTD gradient; this finding differed from the original study, which has been attributed to the exclusion of the different soil thicknesses in the experiment. Regarding the effects of water fluctuation and competition on the growth of plants, Murillo *et al.* (2019) found that the biomass of two emergent macrophytes did not differ between the species when grown in monocultures, but that one species showed a competitive edge when grown in mixtures under intermittent flooding conditions.

The cosmopolitan genus *Ranunculus* has more than 400 species worldwide and is a common feature of the European flora, where the yellow buttercups form a prominent part of many meadows, pastures, or waste places during spring and summer (Steinbach and Gottsbergek, 1994). The four species studied here are the perennial herbs *Ranunculus acris*, *R. bulbosus*, *R. flammula* and *R. repens*, which are thought to have distinct hydrological preferences in the field. In an early ridge and furrow grassland study, it was found that *R. acris* occupies the narrow zone on the sides of the ridges, *R. repens* is found in the waterlogged furrows and *R. bulbosus* on the well-drained tops of the ridges (Harper, 1957). *R. flammula* is a wetland species found in mires and at the edges of waterbodies such as lakes and streams, and can also occur in grasslands subjected to winter flooding; it is not strictly an aquatic species, but can be temporarily submerged (Grime *et al.*, 1988). More recent research suggests that *R. repens* can adapt its growth form to cope with a constantly fluctuating environment (Lynn and Waldren, 2002) and that *R. acris* is a plastic species, which can cope with a wide range of environmental conditions (Oaten, 2005). However, it is not known how *R. flammula* and *R. bulbosus* will cope with differing levels of water fluctuation and how this could affect the competitive dynamics of these species when grown together in the same environment.

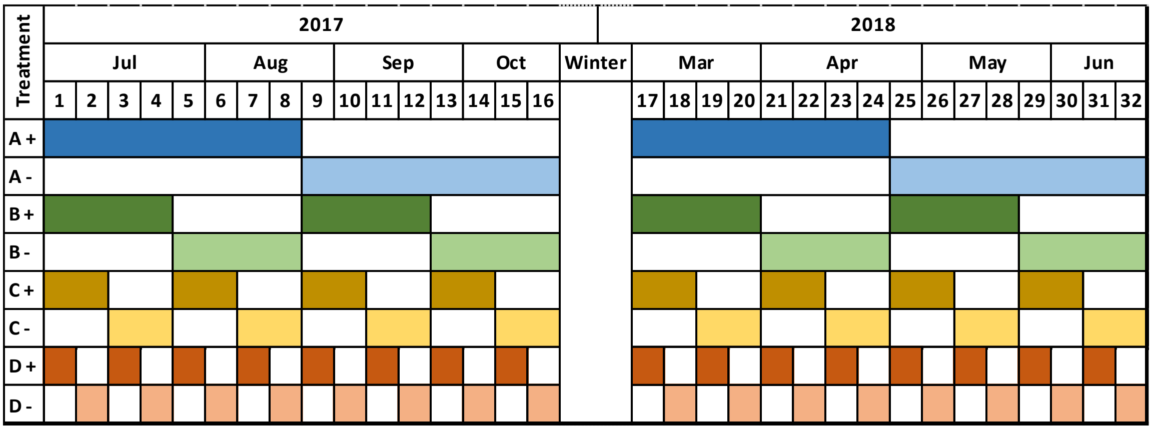
With the onset of climate change, which is set to increase hydrological variability in the UK further (IPCC, 2014), it is vital that we further our understanding of the links between plant growth and hydrological variability. It has been argued that experiments are best suited to the simulation of the extreme events which are predicted to occur under climate change (Reyer *et al.*, 2013). Therefore, this chapter will examine how an identical waterlogging duration being distributed in four different flooding treatments (i.e. different frequency and duration of individual events) affects four species of *Ranunculus*. There will also be two different flood timings within each of the four flooding treatments, to ensure that all periods of the growing season are represented. The following hypotheses are proposed:

1. There will be a difference in the flowering effort and percentage cover of each *Ranunculus* species between the four flooding treatments;
2. There will be a difference in the above- and below-ground biomass of the whole community between the four flooding treatments;
3. The timing of flooding within treatments of longer flood duration (treatments A and B) will affect the growth of the plants (i.e. flowering, cover, biomass);
4. The timing of flooding within treatments of shorter flood duration (treatments C and D) will not affect the growth of the plants (i.e. flowering, cover, biomass).

6.2 Methods

6.2.1 Experiment setup and design

The experiment was constructed on a purpose-built levelled gravel area at the Open University campus located in Buckinghamshire, UK. The level of individual mesocosms was checked using a high precision Leica Zeno 20 Global Positioning System (GPS) and adjusted accordingly to sit within a tolerance range of 3 cm. The experiment ran for one year, between July 2017 and June 2018, with treatments imposed for 32 weeks largely during the growing season (figure 6.1). The treatments were paused when the first frost occurred in October 2017 (i.e. the pots were disconnected and allowed to freely drain), in order to protect the experimental infrastructure from frost damage, and then re-instated at the start of March 2018 (figure 6.1).



Treatment	2017																	2018															
	Jul					Aug				Sep				Oct			Winter	Mar				Apr					May				Jun		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
A +	■	■	■	■	■	■	■	■										■	■	■	■	■	■	■	■								
A -									■	■	■	■	■	■	■	■										■	■	■	■	■	■	■	■
B +	■	■	■	■					■	■	■	■						■	■	■	■					■	■	■	■				
B -					■	■	■	■					■	■	■	■						■	■	■	■					■	■	■	■
C +	■	■			■	■			■	■			■	■				■	■			■	■			■	■			■	■		
C -			■	■			■	■			■	■			■	■				■	■			■	■			■	■			■	■
D +	■		■		■		■		■		■		■		■			■		■		■		■		■		■		■		■	
D -		■		■		■		■		■		■		■		■			■		■		■		■		■		■		■		■

Figure 6.1: Timetable showing the flooding regimes of the four main treatments (A to D) and sub-treatments (+/-) for the length of the experiment; no treatments were imposed in the winter months.

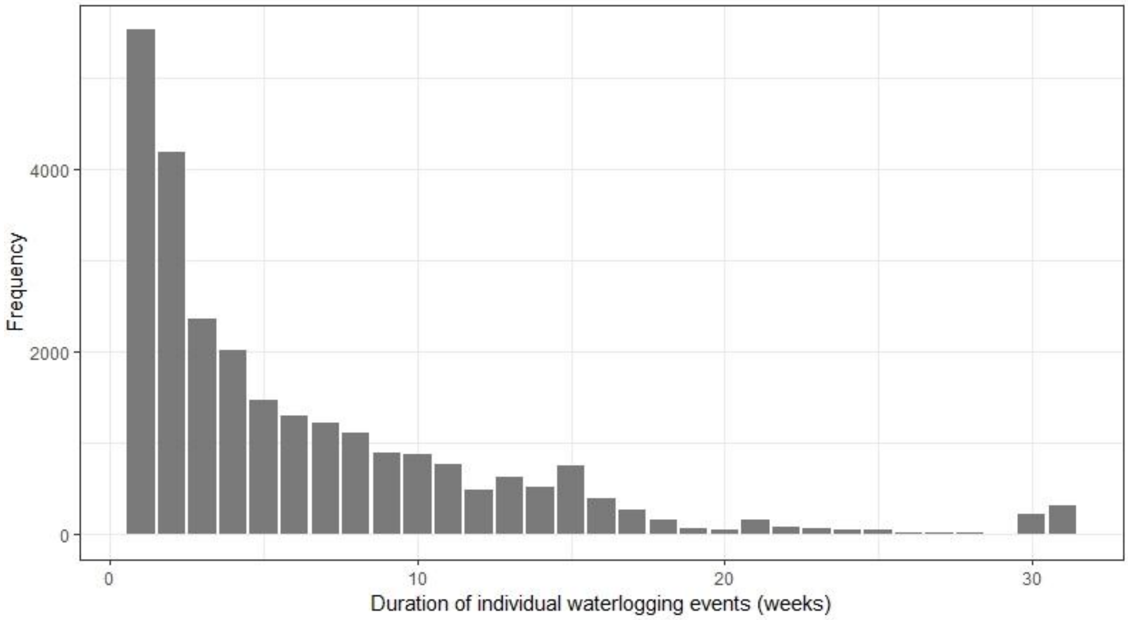


Figure 6.2: Hydrological data from 14 English floodplain meadows showing the frequency of individual flood events of differing durations; data were collected between 1981 – 2001 (growing season only); see section 3.2.2 for further details on the individual sites and hydrological years used for those sites.

The lengths of the flooding events used in the four treatments A, B, C and D (8, 4, 2 and 1-week durations; figure 6.1), were selected following the analysis of a large dataset collected from 14 English floodplain meadows (see chapter 3 for site details). Flood events of between 1- and 8-week duration occur most frequently (more than 1000 occurrences; figure 6.2). Sub-treatments (+/-) were used to ensure that all periods of the growing season were represented in each overall treatment in order to remove timing effects (figure 6.1).

To select an appropriate growing medium, the following mixes of Somerset sedge peat and coarse sand were placed on a sand table and their soil moisture release curves derived: 100% and, 75% sand, 50:50 sand and peat, 75% peat and 100% peat (see appendix H for results). Subsequently, the 50:50 mix was selected because it is a highly conductive growing medium which ensures a speedy alteration between the high (stressed) and low (non-stressed) water table depths (WTDs); this is particularly important in the more variable treatments C and D with fortnightly and weekly changes in WTD.

The high WTD represents the waterlogging threshold (i.e. depth at which 10% air-filled porosity is reached; Gowing *et al.*, 1997) derived from the soil moisture release curve for the 50:50 mix and 10 cm is added to account for the relationship between rooting depth and aeration (Dumortier, 1991). The low WTD represents free-draining (non-stressed) growing conditions. The sand table procedure was subsequently repeated on soil samples which had been left to weather and settle over time. This settlement resulted in waterlogged conditions being reached at a different depth and therefore the low WTD was altered in 2018. In 2017 the high and low WTDs were set at 5 cm and 25 cm; whereas in 2018 the low WTD was altered to 37 cm. The low WTD should have ideally been lowered to 39 cm, but this would have placed the water level in the gravel layer at the bottom of the mesocosm and potentially resulted in a loss of hydrological connection with the root zone, therefore the WTD was set at a level just within the fine sand layer (figure 6.3e).

The sedge peat used in the growing medium is pH 4.3 and contains 12.6 mg kg^{-1} (+/- 1.3) extractable phosphate. An inoculate soil was collected from a wet and dry meadow and then added to the growing medium (100 ml per mesocosm) to introduce microbes to an otherwise sterile growth environment. The water tank (capacity 5000 l) was filled from a local mains tap and sugar beet pulp was added to remove this supplementary source of nitrogen. This was done because the purpose of the experiment is to look at the effect of hydrology rather than nutrition, and therefore it was necessary to exclude nutrient inputs, which could give some species an advantage. The original Ellenberg scores for nitrogen vary between the *Ranunculus* species; *R. bulbosus* and *R. flammula* are scored 3 and 2 respectively, and *R. repens* is 7

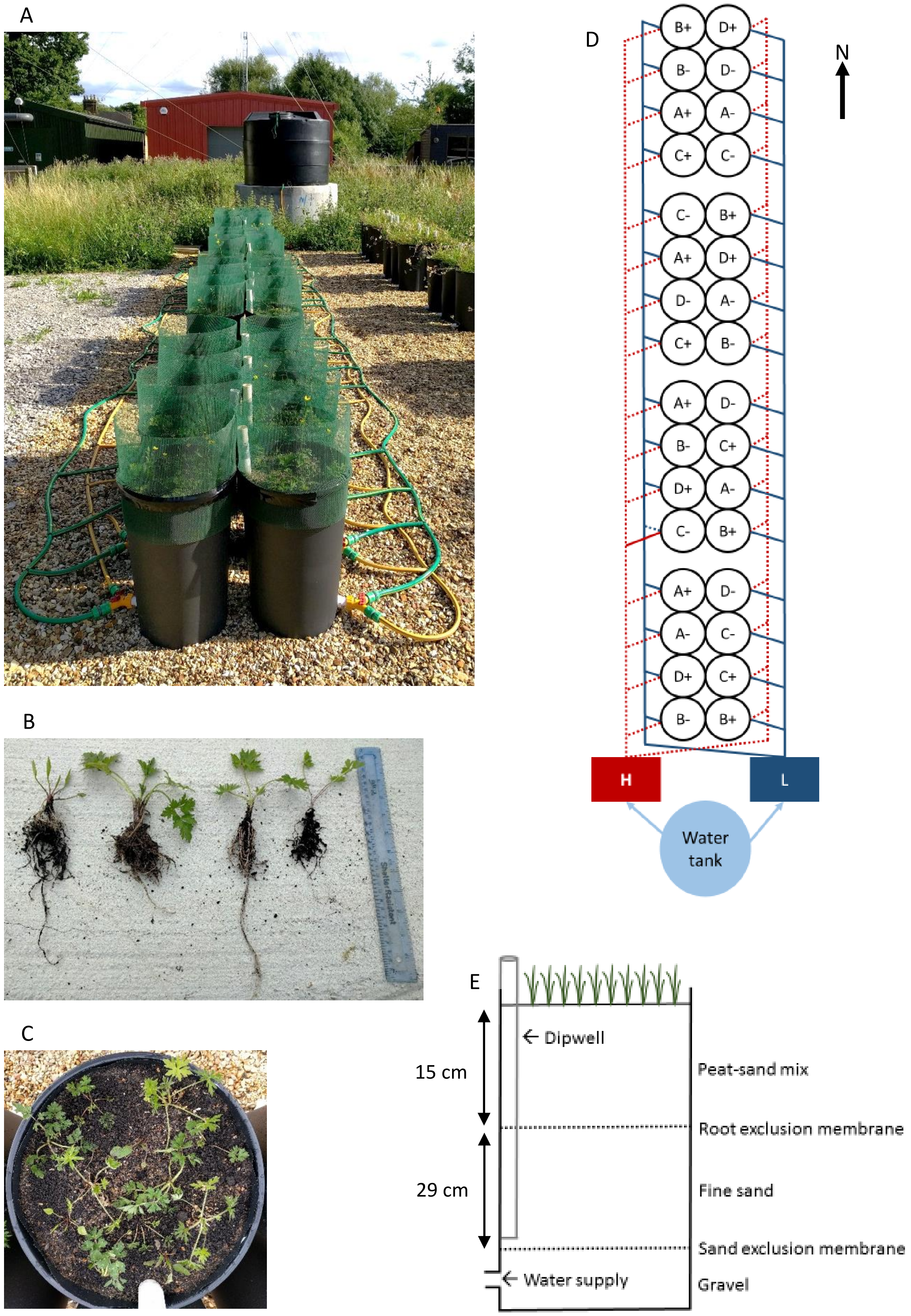


Figure 6.3: Photographs on the left: a) complete array of mesocosms at the experimental site, b) size of specimens planted in April 2017 (left to right: *R. flammula*, *R. repens*, *R. acris* and *R. bulbosus*), c) planting layout in a mesocosm. The layout of the experiment is shown (d) with the position of the main water and two header tanks (H for high and L for low) and the construction of the mesocosms (e).

(Ellenberg *et al.*, 1991). Sugar beet pulp is rich in free carbohydrate and therefore stimulates microbial activity, thus deoxygenating and denitrifying the water (Araya *et al*., 2010). Initially, 10 kg of dried molasses sugar beet shreds were added to the water tank monthly, following the method employed by Araya *et al.*, (2010). Further tests revealed that 500 g were sufficient to reduce the level of nitrate (20.5 ppm mg/L in tap water) to undetectable within 24 hours, and therefore this amount was used subsequently.

A protective circle of green plastic shading (mesh size 3 mm) was attached to each mesocosm at 20 cm above the rim (figure 6.3a) to create a micro-habitat and mimic the effect of taller surrounding vegetation characteristic of the meadow habitat. Multiple attempts to germinate the *Ranunculus* species from seed were unsuccessful and therefore plants were gathered from three sources: *R. flammula* and *R. acris* from a commercial grower (Cumbria Wildflowers), *R. bulbosus* from a meadow in Bedfordshire, and *R. repens* from a meadow on the OU campus in Buckinghamshire. Three individuals of a similar size (figure 6.3b) of each *Ranunculus* species were planted per pot equidistant to each other (12 plants in total; pot diameter 33 cm; figure 6.3c) on 29th April 2017 and left to adjust to the growth environment before starting treatments on 6th July 2017. The treatments were distributed randomly within a block design (figure 6.3d) to reduce the impact of any environmental gradients present e.g. shade. The construction of the mesocosms (figure 6.3e), and the controlled WTD system used to set the high and low WTDs, follow the design detailed in Araya *et al.*, (2010).

6.2.2 Measurements and sampling

During the experiment, the growth environment was monitored using a variety of instruments (appendix I). Pressure-transducer water-level loggers (LevelSCOUT, Seametrics, Seattle) were installed in two mesocosms to check that the high and low WTDs were being maintained; the loggers were placed in treatments D+/D- in 2017 and A+/A- in 2018. The loggers were calibrated using manual readings taken using a dipstick, and the readings were adjusted for atmospheric pressure using barometric data from an onsite weather station (Vantage Pro, Davis, Hayward). Additional measurements were collected in 2018 by instruments connected to a data-logger (GP2, Delta-T, Cambridge): soil oxygen concentration (treatments C+/C-; Soil Oxygen Sensor MIJ-03, Environmental Measurement Japan Co. Ltd., Fukuoka), soil water tension (treatment C-; SWT5 tensiometer, Delta-T, Cambridge), and soil temperature (treatments A+/A- and nearby ground; thermistors).

A number of plant measurements were collected to determine the effects of the treatments on each species of *Ranunculus*. To look at the growth of individual species the following measurements were taken: percentage cover using pin quadrat (October 2017 and June 2018), and flowering effort (mid- and late-June 2018). The number of individual flower structures at particular growth stages (bud, flower, seed-head, empty seed-head) were counted for *Ranunculus acris* plants only, because no other species successfully completed flowering; the first survey was planned to coincide with the peak flowering period (but this was missed by approximately one week) and the second survey was completed at the end of the experiment.

To examine the community as a whole above- and below-ground biomass were sampled in a destructive harvest at the end of the experiment (June 2018; appendix I). All aboveground biomass was collected, dried at 40 °C for 72 hours and weighed. Three soil cores were collected at random from the top of the soil surface to a depth of 5 cm from each mesocosm for the below-ground biomass. Samples were taken to the laboratory, placed in water for 30 minutes and then washed through two sieves (2.00 mm and 710 µm), the roots were then: separated and rinsed, placed into containers, dried at 40 °C for 72 hours, and weighed. It was not possible to distinguish between the roots of the different *Ranunculus* species and therefore, to make the above- and below-ground biomass measurements comparable, both biomasses were recorded for the whole plant community rather than per species.

6.2.3 Statistical analyses

All data manipulation and analysis was carried out in Excel (Microsoft, 2013) and R (R Core Team, 2013). Data were checked for normality using histogram plots and the Shapiro-Wilk test. Above- and below-ground biomass and flowering effort data had a normal distribution, and those data which could not be transformed to a normal distribution were percentage cover. The parametric Student's T-test was applied to normally distributed data (i.e. above- and below-ground biomass, flowering effort) and the non-parametric Mann-Whitney U-test was applied to the percentage cover data. These tests were used to assess whether there were any significant differences in the growth of individual species (represented by flowering effort and percentage cover) and the whole community (above- and below-ground biomass) between the different treatments and sub-treatments. Linear mixed effect (lme) modelling was used to investigate relationships within the data further, with plant measurements as dependent factors, treatment and sub-treatments as fixed effects, and block as a random effect. It was necessary to test the data using lme modelling to see whether the block design was having an impact on the experiment, which could potentially mask significant patterns in the data.

6.3 Results

6.3.1 Abiotic environment

The soil moisture release curves, derived from newly mixed and settled soil cores collected in 2017 and 2018, show the different depths at which the 10% air-filled porosity is reached (blue lines and arrows; figure 6.4). The waterlogging threshold is reached at 16 cm and 29 cm in the newly mixed and settled soil cores, respectively. Figure 6.5 shows the quick response time (<2 hours) of the soil water tension in the root zone to treatment changes.

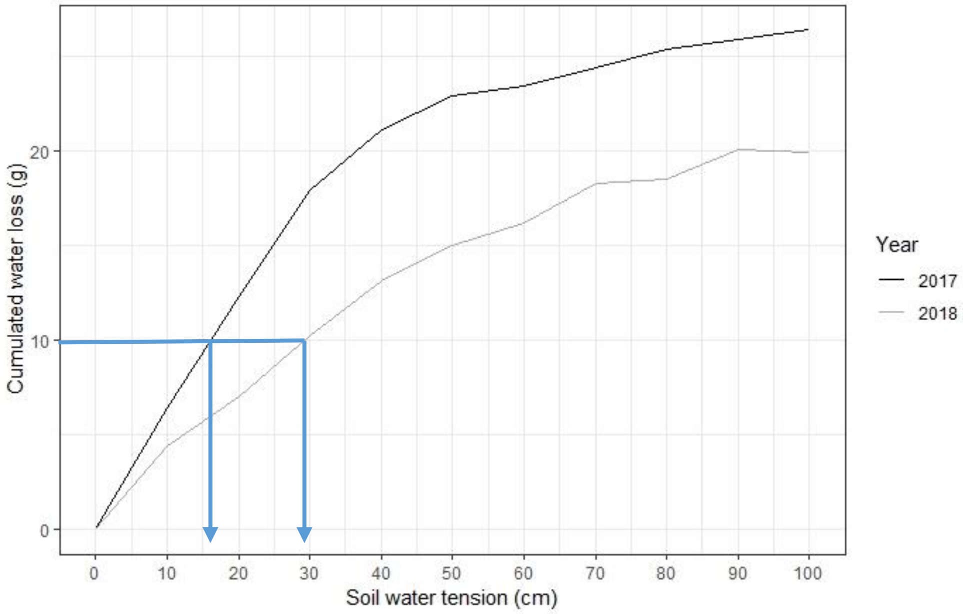


Figure 6.4: Soil moisture release curves derived from newly mixed and settled soil cores collected in 2017 and 2018, and the waterlogging threshold for each soil type (see section 6.2.1 for full details).

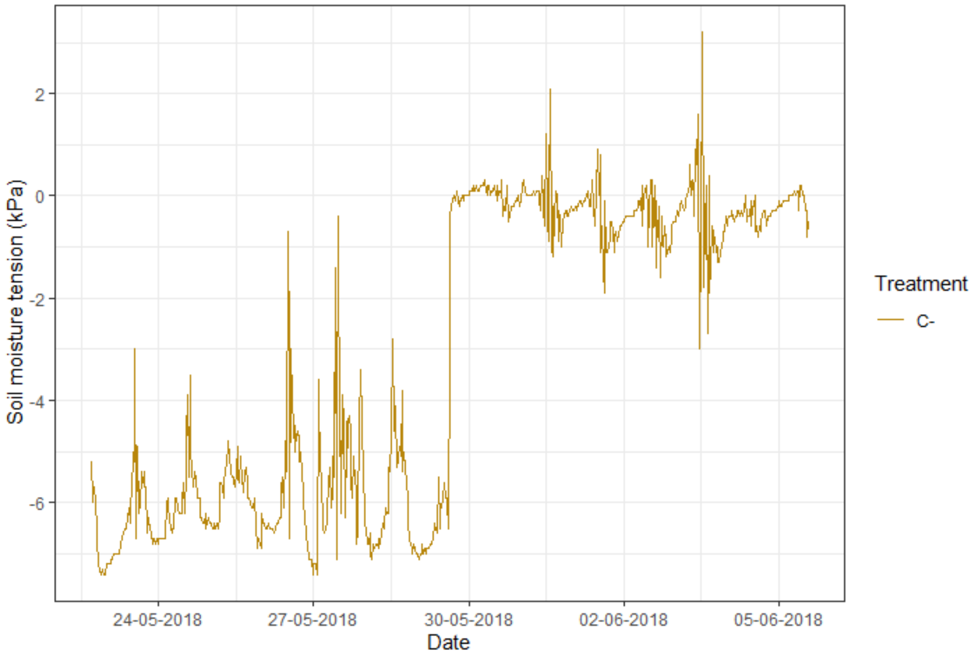


Figure 6.5: Soil water tension measured in the root zone in treatment C- in 2018.

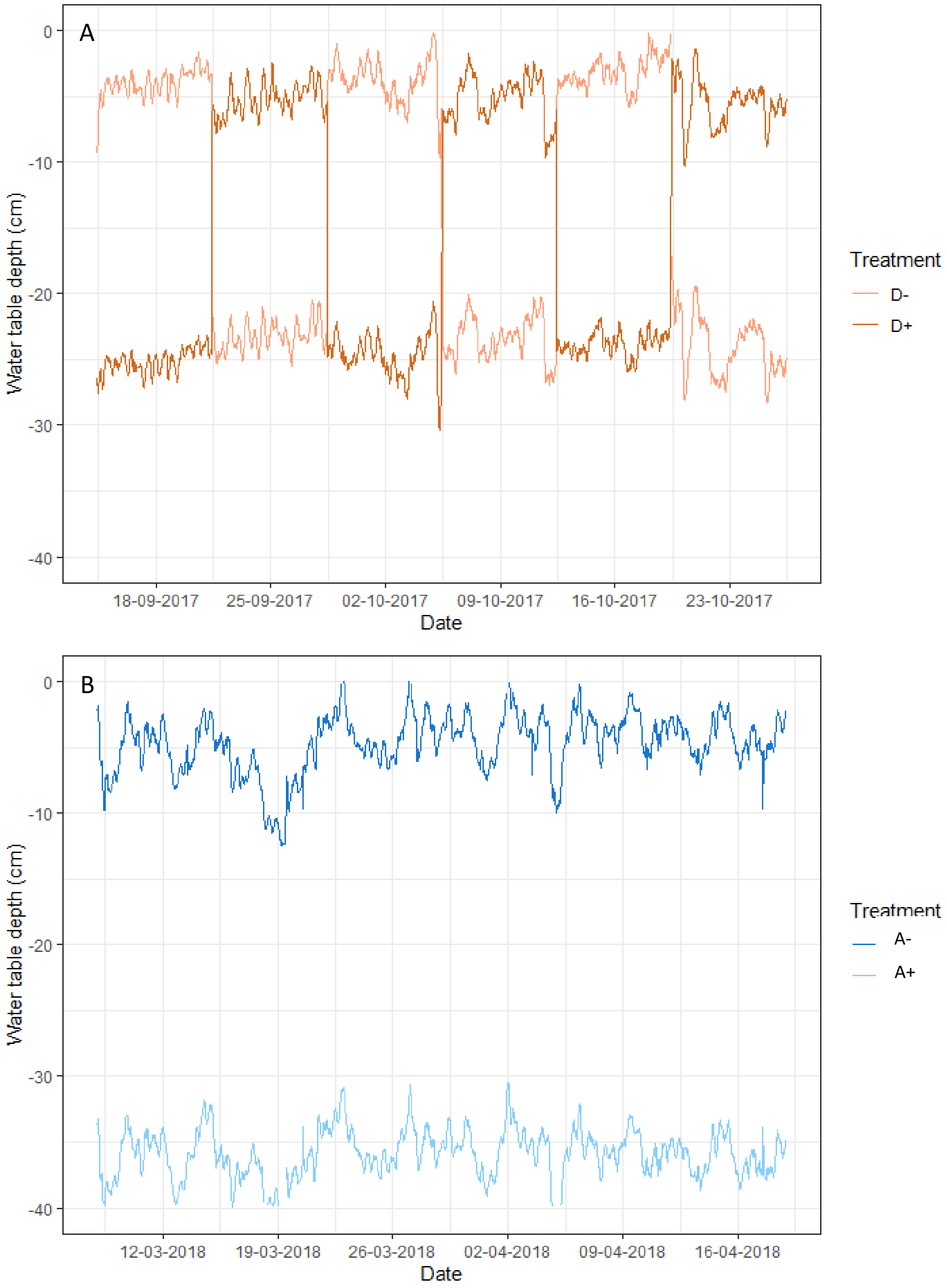


Figure 6.6: Water table depths recorded in (a) treatments D+ and D- in 2017 and (b) treatments A+ and A- in 2018.

The mean high WTD is 5 cm throughout the experiment, whereas the mean low WTD was altered from 25 cm in 2017 to 37 cm in 2018 (figure 6.6a and 6.6b). The swift change in water level (<2 hours) is clearly visible in figure 6.6a where the WTD is adjusted weekly in treatment D.

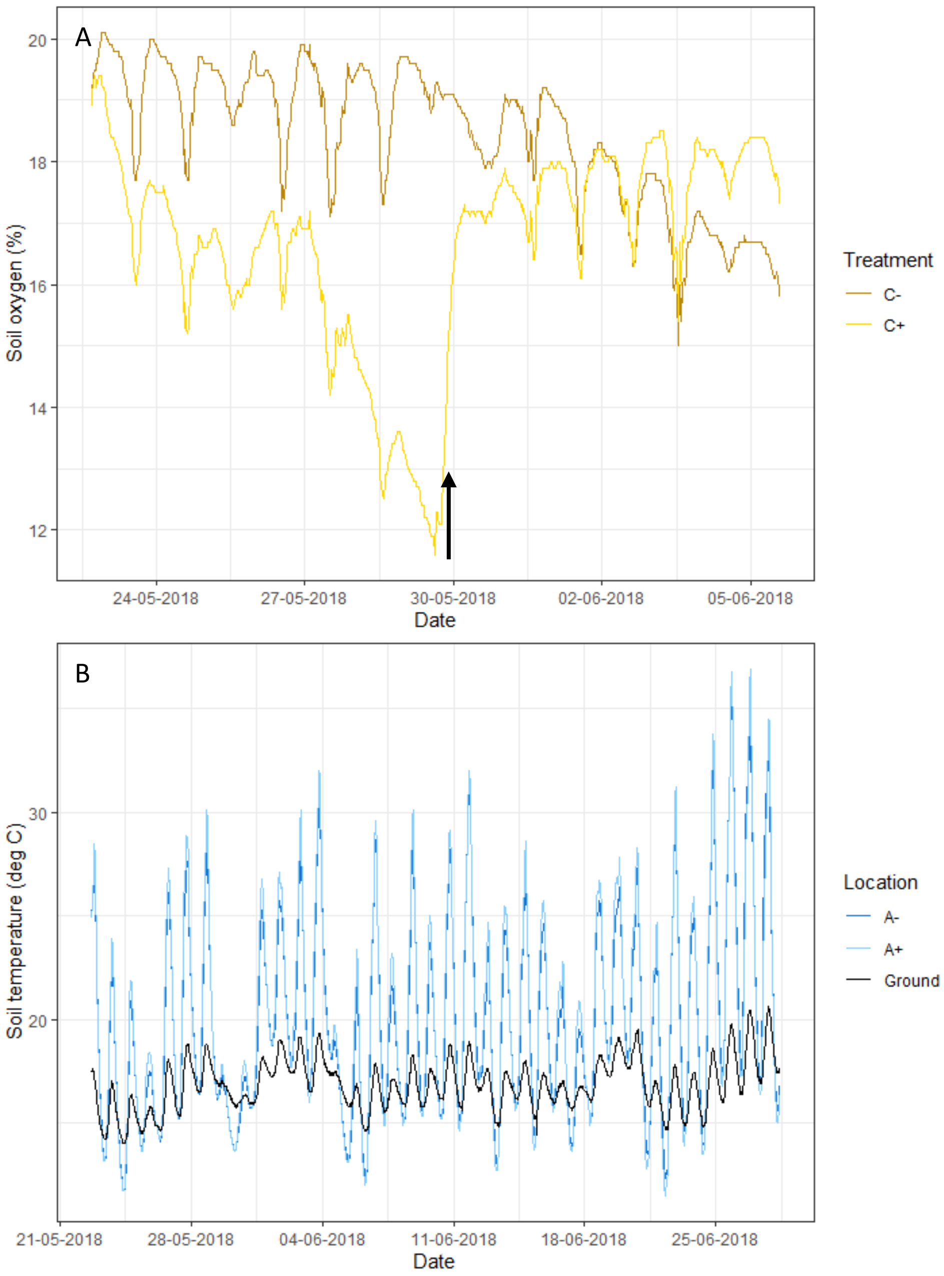


Figure 6.7: (a) Soil oxygen concentration (%) recorded in treatments C- and C+ in 2018 (arrow indicates when the treatments were changed from high to low WTD in C+, and low to high WTD in C-); and (b) soil temperatures recorded in treatments A- and A+ and the nearby ground in 2018.

There is a steep decline in soil oxygen concentration in treatment C+ during its second week of waterlogging, but the concentration recovers quickly in response to the lowering of the water level when the treatment is changed (figure 6.7a). There is a decline in C- soil oxygen once the higher water level is imposed. The process of de-oxygenation takes days whereas oxygenation occurs within hours; therefore, it is likely that treatment D, which has weekly WTD changes, is not becoming as de-oxygenated as other treatments. The daily high and low soil temperatures recorded are substantially greater in both mesocosms in comparison to the nearby ground (figure 6.7b).

6.3.2 Flowering effort of *Ranunculus acris*

In the mid-June 2018 survey, treatments A and C have the greater mean number of buds and flowers, whereas treatments A and B have the most seed-heads (figure 6.8a). In terms of total flowering effort, treatment A has the highest mean count, followed by B, C and D. The mean counts for sub-treatment A- are consistently higher than those for A+, whereas this trend is reversed in the B treatment; there is no noticeable difference between the sub-treatments of C and D (figure 6.8b). However, there are no statistically significant trends in the data when the Student's T-tests is applied, or when block effect is accounted for using a lme model.

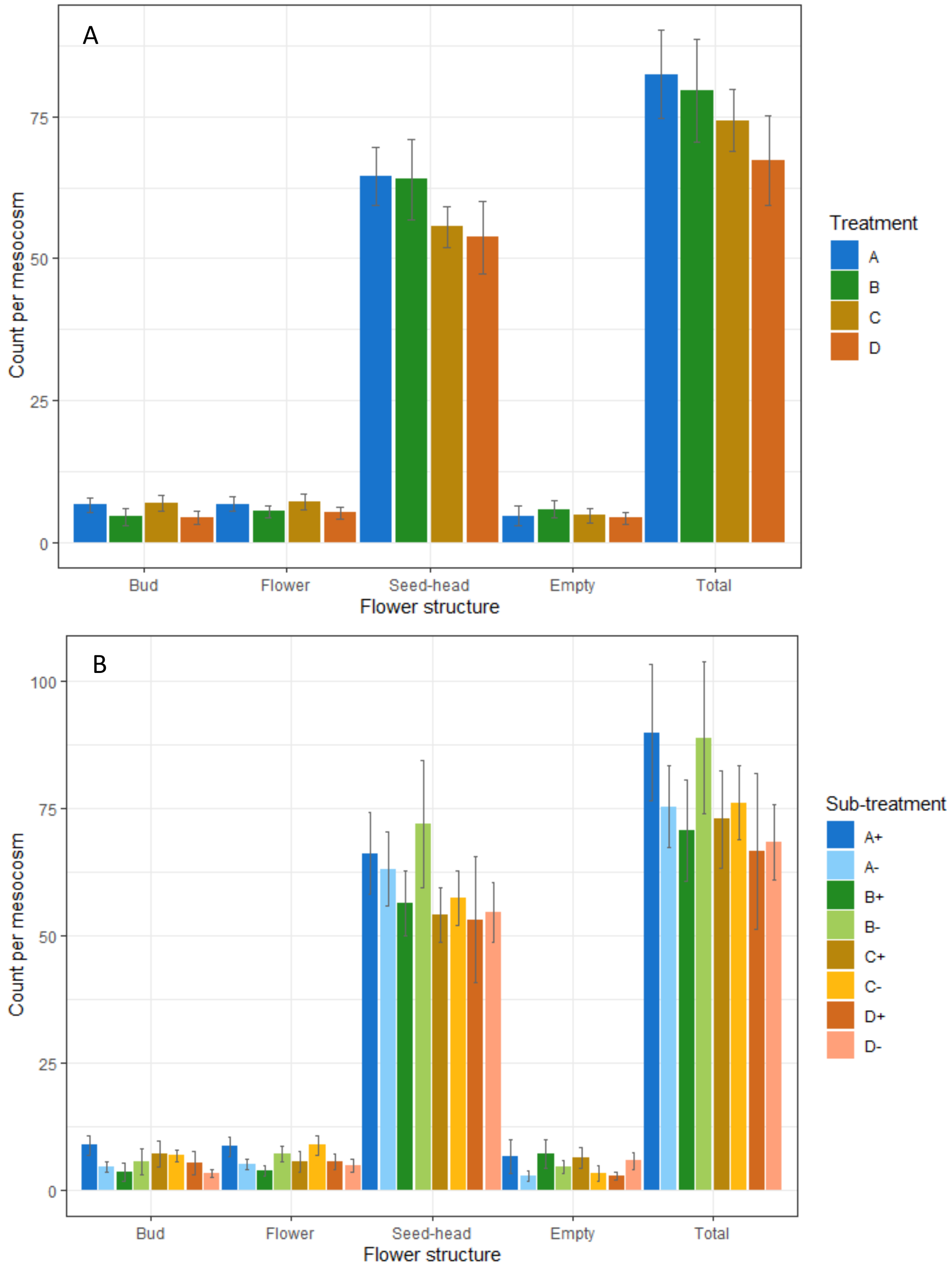


Figure 6.8: Flower structures for *R. acris*; data collected on 14th June 2018 for the (a) four flooding treatments (A to D) and (b) sub-treatments (+/-). Error bars represent standard error of the mean.

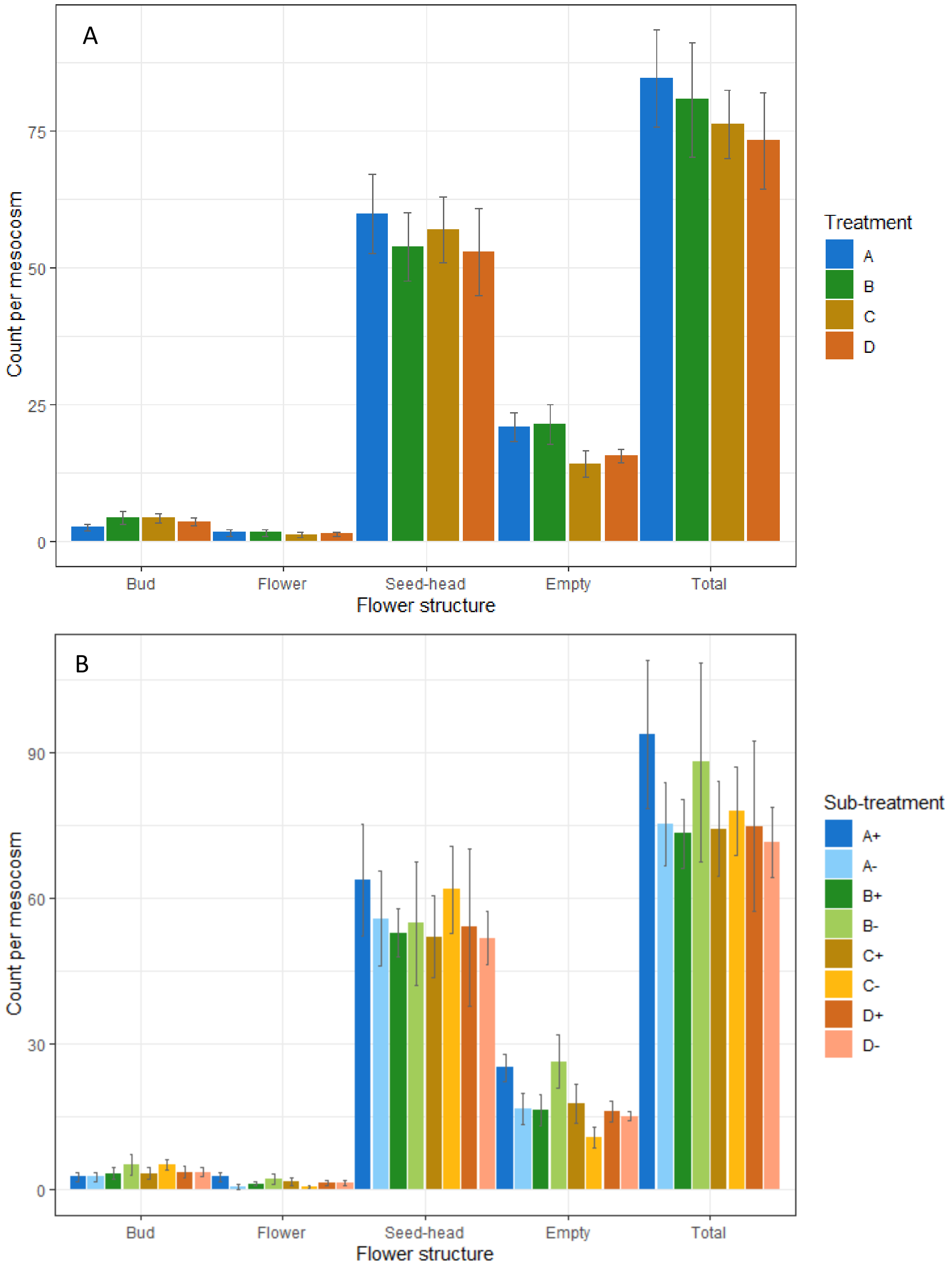


Figure 6.9: Flower structures for *R. acris*; data collected on 28th June 2018 for the (a) four flooding treatments (A to D) and (b) sub-treatments (+/-). Error bars represent standard error of the mean.

In the late-June survey, there are no clear patterns in the mean counts of the different flowering structures across the four flooding treatments; except the mean total count, which is again greatest in treatment A followed by B, C, and D (figure 6.9a). The A+ sub-treatment has higher mean counts than A-, and the B+ sub-treatment has lower counts than B-, in almost all flowering types (figure 6.9b). Again, there are no statistically significant trends in the data when the Student's T-tests is applied, or when block effect is accounted for using a lme model.

6.3.3 Plant cover

In 2017, the percentage cover of both *R. acris* and *R. repens* is slightly higher in treatment B in comparison to the other three treatments (figure 6.10a). The percentage cover of *R. bulbosus* plants is similar across all four flooding treatments, as it is for *R. flammula*. However, these trends are not significant when tested using the Mann-Whitney U-test, or when block effect is accounted for using a lme model. *R. acris* cover is noticeably higher in the B+ sub-treatment (Mann-Whitney U-test p-value 0.03) compared to B- (figure 6.10b). Additionally, *R. bulbosus* is either absent, or much lower in cover, in all the '-' sub-treatments (lme p-value 0.02).

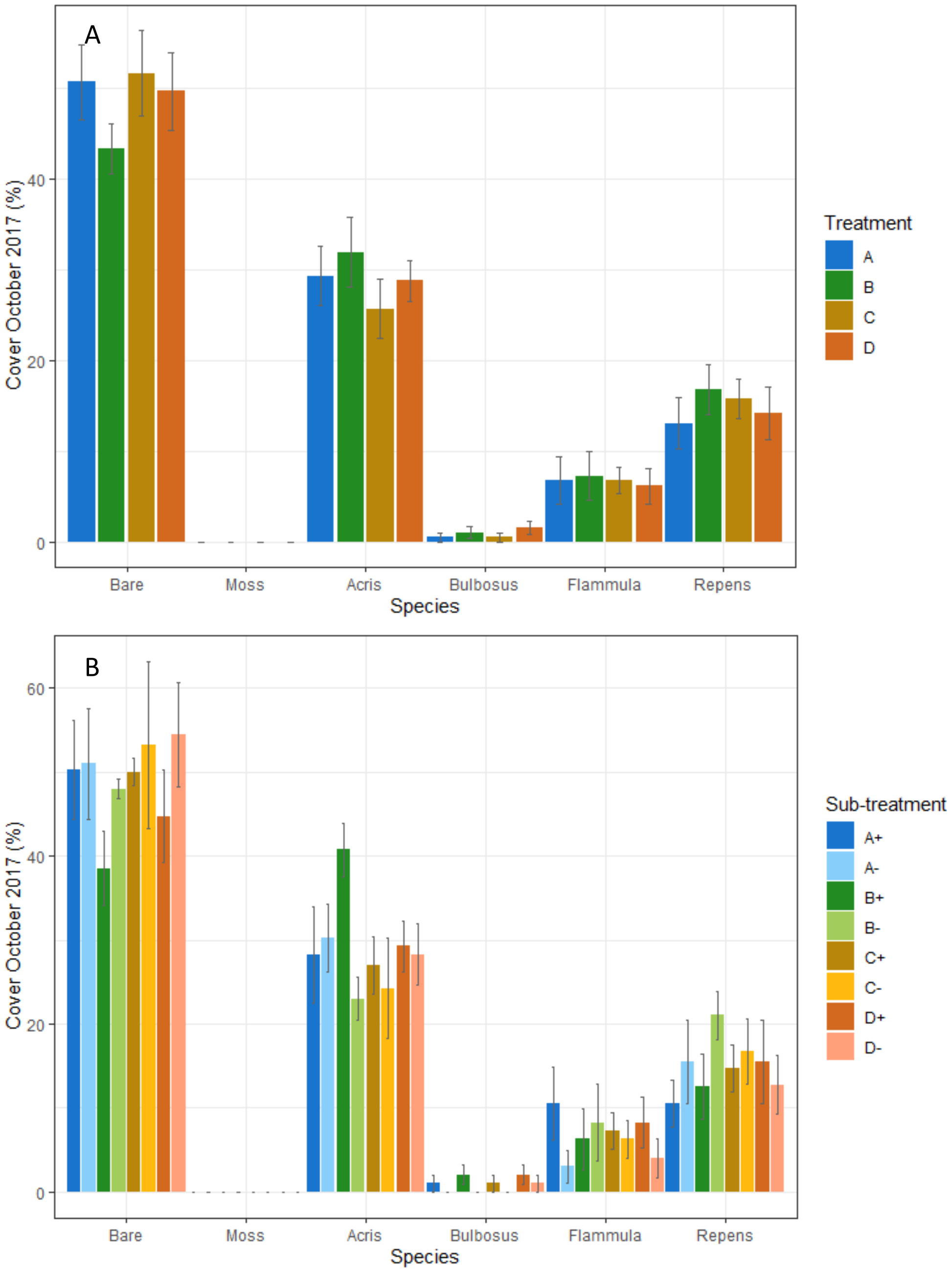


Figure 6.10: Percentage cover of bare-ground, moss, *R. acris*, *R. bulbosus*, *R. flammula* and *R. repens* measured in October 2017 for the (a) four flooding treatments (A to D) and (b) sub-treatments (+/-). Error bars represent standard error of the mean.

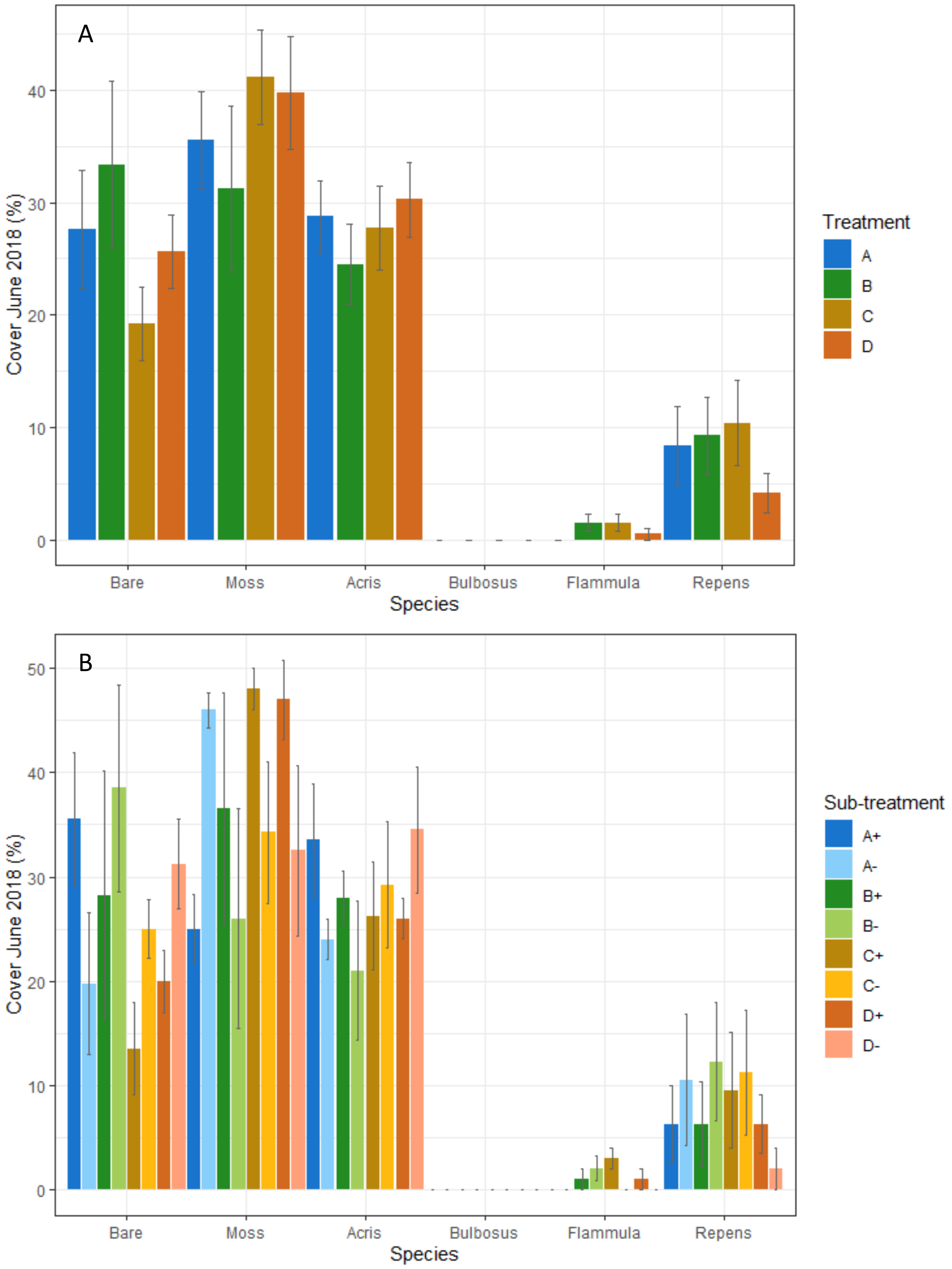


Figure 6.11: Percentage cover of bare-ground, moss, *R. acris*, *R. bulbosus*, *R. flammula* and *R. repens* measured in June 2018 for the (a) four flooding treatments (A to D) and (b) sub-treatments (+/-). Error bars represent standard error of the mean.

In 2018, the percentage cover of *R. acris* is highest in treatment D, at a similar level in A and C, and lowest in B; whereas *R. repens* cover is noticeably much lower in treatment D than the other three treatments (figure 6.11a). *R. flammula* cover is very low in treatments B to D and absent from A, and *R. bulbosus* is absent from all treatments. However, none of these trends is significant when the Mann-Whitney U-test is applied, or when block effect is accounted for using a lme model. Moss cover is significantly lower in the A+ sub-treatment (figure 6.11b; Mann-Whitney U-test p-value 0.03).

6.3.4 Biomass

Treatments A and B have slightly higher mean shoot biomass than treatments C and D (figure 6.12a). There are no clear differences between the mean shoot biomass of the different sub-treatments (figure 6.12b). There are no statistically significant trends in the data when the Student's T-test is applied, or when block effect is accounted for using a lme model.

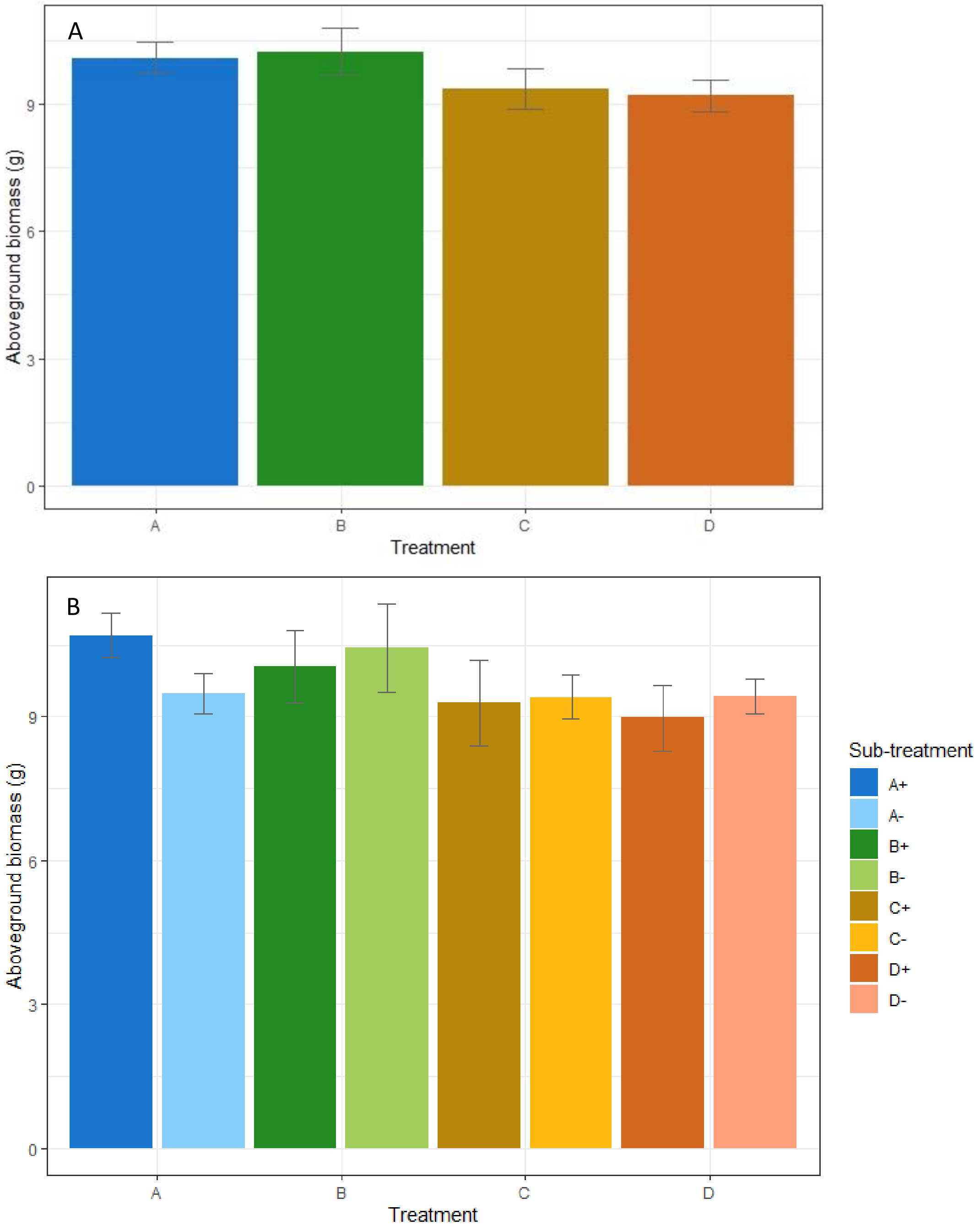


Figure 6.12: Above-ground (shoot) biomass of the whole plant community for the (a) four flooding treatments (A to D) and (b) sub-treatments (+/-). Error bars represent standard error of the mean.

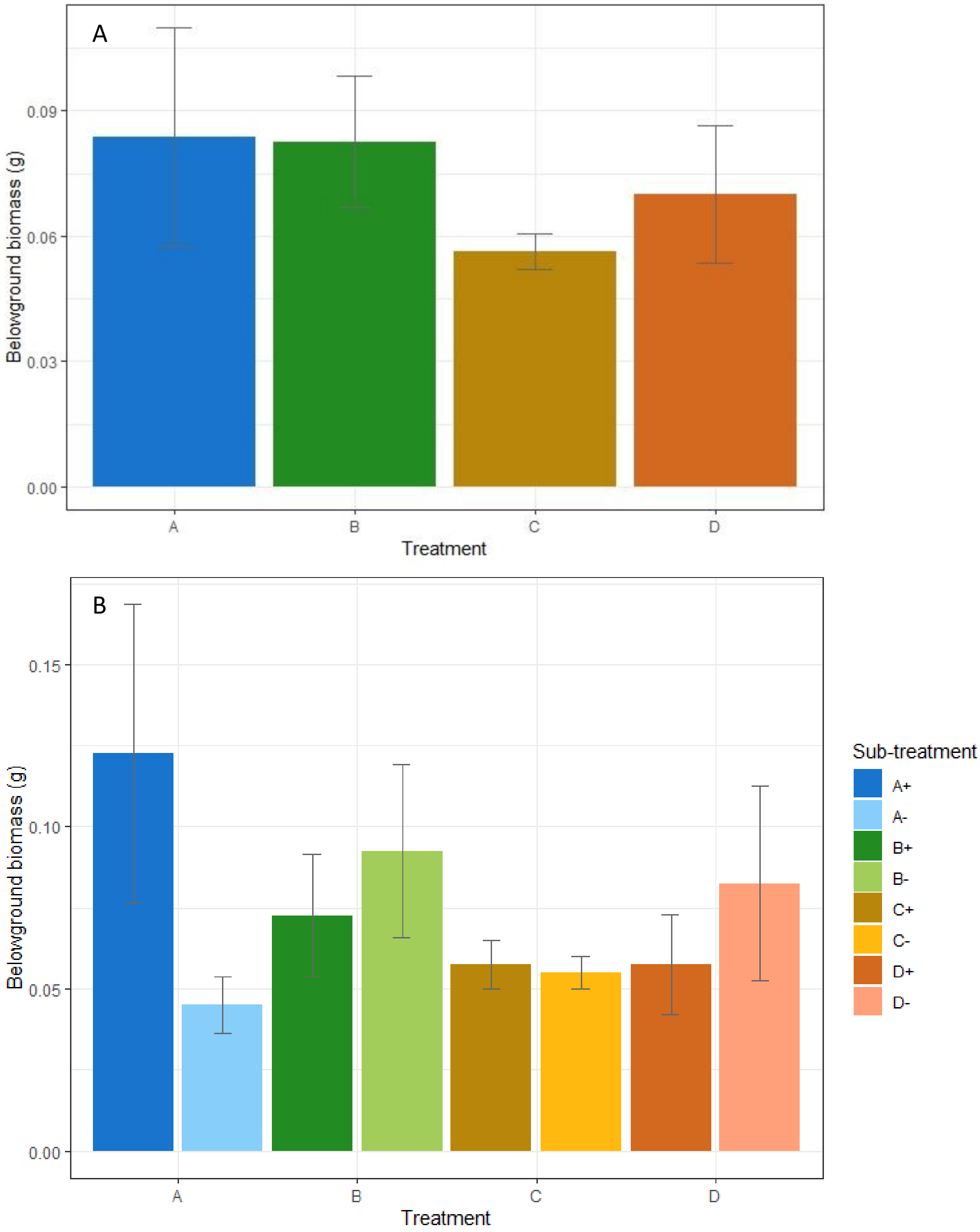


Figure 6.13: Below-ground (root) biomass sample of the whole plant community for the (a) four flooding treatments (A to D) and (b) sub-treatments (+/-). Error bars represent standard error of the mean.

Similarly to shoot biomass, the mean root biomass is highest in treatments A and B, and lower in treatments C and D (figure 6.13a), but these trends are not statistically significant (Student's T-test and lme model applied). The mean root biomass of A+ is noticeably larger than A-, but again, this trend is not statistically significant (Student's T-test p-value 0.06; figure 6.13b).

6.4 Discussion

6.4.1 Effect of hydrological variability on flowering, cover, and biomass (H1 and H2)

In this study there were no significant differences in the flowering effort of *Ranunculus acris* plants between the four main flooding treatments. It is, however, interesting to note that there was a trend of total flowering effort in *R. acris* declining (highest in treatment A to lowest in treatment D) with increasing hydrological variability (i.e. fewer flowering structures were produced under more hydrologically variable conditions) and also with decreasing individual flood duration.

Mony *et al.* (2010) similarly found that two annual species had greater number of flowers with increased duration of inundation, promoting extensive seed production. However, there is a lack of research into the links between fluctuating hydrology and plant reproductive strategies, and it is clear that further investigation is required. With the increases in hydrological variability predicted under future climate change (IPCC, 2014), it is vital that we understand what degree of fluctuation would be detrimental to the ability of plants to set seed and germinate, and to continue their establishment.

There were no significance differences, or noticeable trends, in the percentage cover of the four *Ranunculus* species across the four main flooding treatments. There were also no significant differences in the above- or below-ground biomass of the whole *Ranunculus* community between the four flooding treatments; however, it is interesting that the shoot and root biomass were both marginally higher in the less variable treatments A and B, and lower in the highly fluctuating treatments C and D. Other experimental studies have found that hydrological variability can have a negative impact on the biomass accumulation of wetland plants (Bakker *et al.*, 2007; Luo *et al.*, 2018).

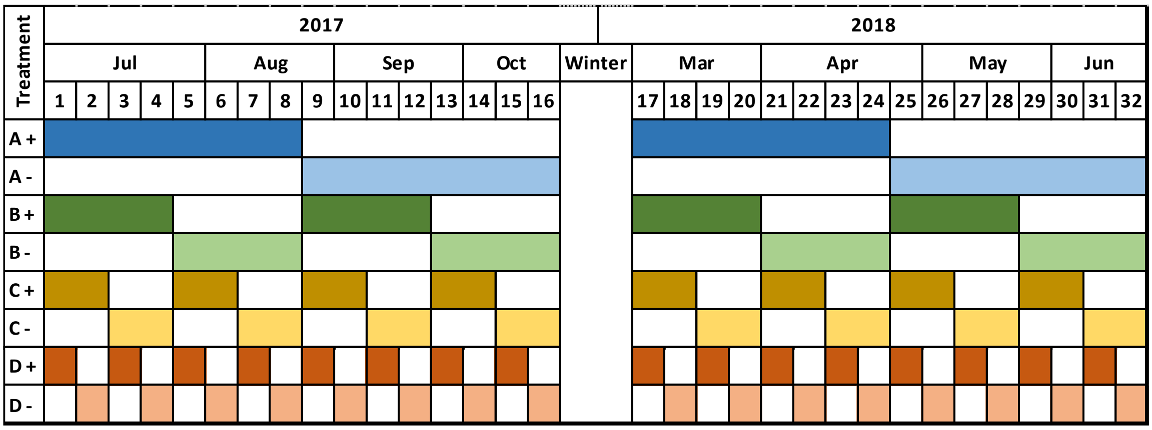
The higher biomass accumulation in this study was found in the treatments with the longest individual flood durations (i.e. treatments A and B), and this finding is contrary to those of other experimental studies where an increase in flood length led to a reduction in plant biomass (Eck *et al.*, 2004; Gao *et al.*, 2016). However, interpretation of these results is limited by the lack of statistical significance, and because the biomass accumulation and percentage cover were extremely low across all treatments and sub-treatments. It is therefore likely that there are factors other than the treatments which are having an overriding effect on the growth of the plants in this experiment, and these are discussed in detail below (section 6.4.3 and 6.4.4).

6.4.2 Effect of flood timing on flowering, cover, and biomass (H3 and H4)

There were no significant differences in flowering effort of the *R. acris* plants between the sub-treatments, but the onset of flowering may have been accelerated across all the experimental treatments. It is estimated that peak flowering occurred in early June across all treatments (buds had begun to form by 22nd May and some seed-heads were visible on 6th June 2018). However, it has been noted that the peak of flowering for *R. acris* in the Midlands and eastern England are mid- and late-June, respectively (Harper, 1957).

There are two key reproductive strategies used to survive flooding, one is to delay flowering and seed production during unfavourable conditions and survive as vegetative plants, and the second is to accelerate flowering during short dry periods in order to produce seeds in the intervals between two successive floods (Blom *et al.*, 1990). Therefore, it is possible that the *R. acris* plants in this study were accelerating flowering due to the stressful growth conditions they were under in all treatments. In contrast, another experiment found that the phenological response of *R. acris* plants differed according to the intensity of the flooding treatments they were exposed to; flowering was delayed in the more extreme treatment, whereas the relatively less stressed plants flowered earlier (Brotherton *et al.*, 2019b). Other studies have also found that flood timing is an important factor affecting plant reproduction in floodplain and riparian habitats (Voesenek and Blom, 1992; Greet *et al.*, 2013).

There were some significant trends in percentage cover for two species of *Ranunculus* between the sub-treatments. In October 2017, *R. acris* percentage cover was significantly higher in the B+ sub-treatment compared to B- and *R. bulbosus* was absent from the A-, B- and C- sub-treatments. Additionally, the above- and below-ground biomass of the whole community was higher in the A+ sub-treatment in comparison to A- (particularly noticeable in the root biomass data), but these trends were not statistically significant.



Treatment	2017																	2018															
	Jul					Aug				Sep				Oct			Winter	Mar				Apr					May				Jun		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
A +	■	■	■	■	■	■	■	■										■	■	■	■	■	■	■	■								
A -									■	■	■	■	■	■	■	■										■	■	■	■	■	■	■	■
B +	■	■	■	■					■	■	■	■						■	■	■	■					■	■	■	■				
B -					■	■	■	■					■	■	■	■						■	■	■	■					■	■	■	■
C +	■	■			■	■			■	■			■	■				■	■			■	■			■	■			■	■		
C -			■	■			■	■			■	■			■	■				■	■			■	■			■	■			■	■
D +	■		■		■		■		■		■		■		■			■		■		■		■		■		■		■		■	
D -		■		■		■		■		■		■		■		■			■		■		■		■		■		■		■		■

Figure 6.14: Timetable showing the flooding regimes of the four main treatments (A to D) and sub-treatments (+/-) for the length of the experiment; no treatments were imposed in the winter months.

The sub-treatments were included to ensure that the plants in each flooding treatment experienced waterlogging during all periods of the growing season (figure 6.14), in case timing was a factor. It is interesting that most of the trends in the percentage cover and biomass accumulation data described above occurred within treatments A and B, which is in agreement with the author's hypotheses. Weather can be highly variable during the growing season and this variability can either enhance or lessen the impacts of the waterlogging treatments. For instance, higher soil temperatures during waterlogging periods can exacerbate the oxygen depletion in the root zone (Drew, 1983). The distribution of the waterlogging weeks in treatments A and B meant that long periods of stressful growth conditions were more likely to occur in the warmer or cooler parts of the growing season (figure 6.14), and therefore the timing of flooding events may have influenced plant growth within these two treatments. However, the interpretation of these results is again limited by the lack of statistical significance and/or the extremely low plant growth across all treatments.

Although flood timing could be *one* factor explaining the higher *R. acris* cover in B+, it is unlikely to be the only cause of the absence of *R. bulbosus* from multiple sub-treatments where the timing and duration of waterlogging events differed greatly. The life-cycle of *R. bulbosus* differs from the other *Ranunculus* species in this experiment. It is active early in the growing season, completes its flowering in June and subsequently the plant dies down into its corm and over-winters as a rosette (Harper, 1957). Therefore, the time at which this species usually dies back coincided with the onset of the treatments in 2017, and no flowering plants were observed before the treatments began. The *R. bulbosus* plants in this study may have employed different survival strategies depending on the timing of the waterlogging they experienced in July to October 2017. Some individuals may have gone dormant at the usual time (i.e. in July) and thus avoided any treatment induced stress; and others may have been killed off by the treatment stress early in the experiment, particularly if periods of waterlogging coincided with high temperatures. Other individuals may have attempted to 'hang on' later into the season than usual (hence they were still visible in October) to build up extra reserves before dying back into the corm phase.

6.4.3 Competitive dynamics

The effects of competition were not tested directly in this study, but it is surmised that there was little or no direct competition occurring between the plants in any of the treatments, because the biomass was very low and therefore the individuals were not interacting. It is more likely, that the extremely low root and shoot biomass, and percentage cover, measured in all treatments is a sign of the stress of the whole community (see section 6.4.4), as the pot size was not constraining growth at the end of the experiment; i.e. there was enough room for plants to expand their roots and shoots, but they were unable to make use of the available space.

One interesting finding is that *R. acris* out-competed the other three species irrespective of hydrological regime. These results are contradictory to those of Bartelheimer and Poschlod (2016), which suggest that hydrology is more important than competition along a water-table depth gradient; but they are in agreement with Byun *et al.* (2017), who similarly found that one emergent wetland species (*Zizania latifolia*), typical of South Korean wetlands, out-competed the other two (*Scirpus tabernaemontani*, *Typha orientalis*) irrespective of hydrological regime.

It was, however, surprising that *R. repens* did not perform better across the flooding treatments in this experiment. *R. repens* is a competitive ruderal species especially abundant where drainage is impeded, and when growing under changing environmental conditions it responds with an increase in stolon development rather than seed production (Harper, 1957). Additionally, this species is known to cope with both waterlogged conditions and fluctuating water levels (Harper, 1957), in Irish turloughs for example (Lynn and Waldren, 2001). It is therefore interesting that *R. acris* was a better competitor than *R. repens* in this experiment. It is possible for competitive dynamics to shift in response to intermittent flooding conditions (Murillo *et al.*, 2019); however, it is more likely that another factor was limiting the growth of *R. repens*, because it grew poorly in all experimental treatments (see section 6.4.4).

6.4.4 Other factors influencing plant measurements

There were substantial differences in both the duration and frequency of individual flood events across the four main experimental treatments in this study, which created a spectrum of high to low hydrological variability. Given the considerable hydrological differences between treatments, it is surprising that so few significant differences were found in the plant measurements between treatments. There are several possible explanations for this outcome.

First, it is possible that overall wetness was more important than hydrological variability in this study, as all four treatments had the same total and mean waterlogging duration across the growing season. This explanation is in agreement with some studies, where overall wetness and flood duration were the most important factors affecting either plant reproduction, growth or biomass (Kotowski *et al.*, 1998; Herr-Turoff and Zedler, 2007; Mony *et al.*, 2010; Gao *et al.*, 2016; Brotherton *et al.*, 2019). Additionally, the four flooding treatments had the same mean water level, which has also been found to influence the occurrence of plant species (Weiher and Keddy, 1995; Potvin *et al.*, 2015).

The second possible explanation is that the growth environment was too stressful across all the experimental pots and therefore no treatment effects could be distinguished. The measurements of the abiotic environment demonstrate the high levels of stress all plants were under as a result of numerous factors (detailed below); and this stress may have inhibited all plant growth, irrespective of the differences in hydrological variability. One of the key additional stresses to the growth environment were the extreme soil temperatures recorded in the pots combined with periods of waterlogging stress, intensifying the oxygen depletion in the root zone (Drew, 1983). However, this does not explain the lack of plant activity in the most variable treatment (D), because these pots were not being flooded long enough to bring on significant oxygen depletion in the root zone, and therefore more plant growth would be expected relative to the other treatments.

Another possible explanation could be due to the lack of nutrients in the growth medium, which may have skewed some of the experimental results. As discussed above (section 6.4.3), *R. repens* was expected to grow well in this experiment, because is known to cope with both waterlogged conditions and fluctuating water levels, but in fact it grew poorly in all the experimental treatments. It is likely that *R. repens* was largely limited by the lack of nutrients in the growth medium, rather than the fluctuating hydrology, because of its preference for high nutrient conditions (original Ellenberg score 7 for nitrogen; Ellenberg *et al.*, 1991). This lack of nutrients might have diminished *R. repens* usual competitive-ruderal growth strategy (Grime *et al.*, 1988).

6.4.5 Improvements to experimental design

There were some weaknesses in the current experimental design and implementation which may have also disrupted the results of this study. First, some of the abiotic conditions imposed on the plants were not representative of growing conditions usually found in the field. For instance, the large temperature range in the pots was much greater than that recorded in the nearby ground, and it could be argued that the speed at which the water levels changed in the pots was much quicker than what occurs in more natural conditions. Therefore, it would be preferable to use pots that are insulated from temperature extremes (e.g. they could be sunken in the ground).

The controlled water depth system used here, designed by Araya *et al.* (2010), has a number of advantages. It is a low-cost, low maintenance and highly reliable system which maintains constant water levels across multiple pots, with greater consistency between repeats than other methods (e.g. topping up levels by hand) used in different studies (e.g. Bartelheimer and Poschlod, 2016). However, it could benefit from having the pace of the water-level alterations slowed to reflect field conditions more closely.

There were some crucial parameters that had to be changed part way through the experiment, which placed unintended stress onto the plant community before they were resolved. These include the lowering of the high (stressed) water level due to the settling of the growth medium, and the addition of too much sugar beet initially. These factors serve as useful lessons for any future experimental work in this area. Additionally, if this experiment were to be repeated it would be preferable to use plant species with similar nutrient requirements in order to remove fertility as a factor.

It would also have been preferable to run the experiment for a number of growing seasons, rather than one, but this was not possible due to logistical constraints, and is not uncommon for experiments documented in the published literature to run for only a few years (Reyer *et al.*, 2013). There are weaknesses in any experimental design and they can never be truly representation of field conditions, but they do remain an important resource in plant ecology research. Experimental research is particularly well suited to the simulation of extreme events, which is vital to our understanding of the impacts of future climate change on plant community dynamics (Reyer *et al.*, 2013).

Chapter 7

Discussion

7.1 Key research outcomes

This section details how the current study has addressed the research gaps identified in chapter 1 (section 1.5). The key findings are discussed below in relation to the research aims laid out in section 1.6 and to the wider literature.

7.1.1 Effects of soil drying and waterlogging on plant species richness and community assembly (objective 1)

The findings of this study demonstrate that within the growing season a greater duration of soil drying relates to an increase in plant species richness (floodplain meadow sites; chapter 3), and a greater duration of soil waterlogging relates to a decline in richness (floodplain meadow and turlough sites; chapter 3 and 5), in seasonally flooded grassland habitats. These findings are in agreement with other European floodplain habitat studies which found that an increase in flood duration negatively affects species richness (Maher *et al.*, 2015; Garssen *et al.*, 2017) and that drier conditions support the development of species-rich communities (Toogood *et al.*, 2008). Similar trends have also been found in other wetland habitat types, where a decrease in flood duration and depth relate to an increase species richness in Irish turloughs (e.g. Moran *et al.*, 2008a) and Alaskan wetlands (Pollock *et al.*, 1998).

Put another way, the preceding hydrological conditions (in this case, drying and waterlogging stress as measured over a five-year period; growing season only) affect the species richness of the plant communities in these two habitats with fluctuating hydrological regimes, English floodplain meadows and Irish turloughs. The role which previous hydrological conditions play in structuring plant communities has been demonstrated in some studies (e.g. Noest, 1994; Gowing *et al.*, 2002; Curreli *et al.*, 2013) but despite their importance, there is still a lack of research on the quantification of ecological memory and its effect on current ecological processes (Ogle *et al.*, 2015).

An awareness of the antecedent conditions in wet grassland habitats can aid the interpretation of results greatly because these fluctuating habitats are constantly shifting in response to changing environmental conditions. For instance, a number of relatively dry years with few flood events can lead to the development of a diverse, but flood-intolerant, floral assemblage

(Toogood and Joyce, 2009) which can then suffer a marked decline in species richness as the result of a drastic summer flood event (Mathar *et al.*, 2015). If one only examines the current or recent history of a site, then any trends in species richness and/or community composition may be attributed to an incorrect driving mechanism. The current research examined the effects of the ecological memory of waterlogging and drying stress on plant species richness at the study sites, and the results show the importance of possessing this previous knowledge when examining the current situation at habitats characterised by fluctuating hydrology.

Another important finding of this study is the evidence to support the Hydrological Niche Segregation (HNS) hypothesis at an additional habitat, where the fluctuating hydrology in Irish turloughs appears to drive the composition of the plant communities. Specifically, it is the duration of soil waterlogging into and during the growing season which influences the zonation of plant communities (chapter 4). The theory of HNS has become a core concept in ecology and it is a phenomenon which has been found to occur at a wide range of ecosystems, from temperate floodplain-meadow grasslands (Silvertown *et al.*, 1999) to tropical forests (Engelbrecht *et al.*, 2007).

7.1.2 Effects of inter- and intra-annual hydrological fluctuations on species richness (objective 2)

This study presents a substantial body of evidence showing that high levels of hydrological fluctuation can result in a decline in plant species richness. Hydrological fluctuation was measured as the degree of between-year (inter-annual) and within-year (intra-annual) variation in soil waterlogging across the preceding five growing seasons. Both increasing inter- and intra-annual variability correlate with a decline in species richness at English floodplain meadows (chapter 3 and 5) and Irish turloughs (chapter 5). However, the relationship was stronger, and most consistent across both habitats, with inter-annual variability.

There is evidence, contrary to the results of this study, that intra-annual hydrological variability can have a positive effect on species richness at mesic grassland sites in USA (Knapp *et al.*, 2002), and in the littoral plant communities of lakes in New Zealand (Riis and Hawes, 2002). Additionally, inter-annual fluctuations play a key role in promoting species richness via the storage effect in arid floodplains in Australia (Capon, 2005; Capon and Brock, 2006) and European floodplain meadow communities (Hölzel and Otte, 2004; Mathar *et al.*, 2015). There are also some studies which suggest that species richness is greatest at intermediate levels of intra-annual variability in Alaskan wetlands (Pollock *et al.*, 1998) and inter-annual variation in urban wetlands in USA (Magee and Kentula, 2005). It is, however, difficult to find studies which provide a direct and meaningful comparison to the present study.

First, the majority of the studies detailed above are based on different types of wetland habitat to those examined in the current study (i.e. seasonally flooded grassland habitats). Second, the methods employed by these studies are not directly comparable because inter- and/or intra-annual hydrological variability were measured as the: amount of fluctuation in flood frequency (ranging from low to high frequency, denoting low to high variability) (Pollock *et al.*, 1998; Hölzel and Otte, 2004; Capon, 2005; Capon and Brock, 2006); difference in the mean number of flooding days per year (Mathar *et al.*, 2015); absolute difference in water level (Magee and Kentula, 2005); and variation in rainfall pattern (Knapp *et al.*, 2002). There are other possible explanations, however, as to why some of the results of the current study differed to those of broadly similar research.

It was surprising that a greater degree of inter-annual variability did not have a positive effect on the species richness of the plant communities studied here, as per the findings of similar research (summarised above); but these trends could be explained by the relative amount of annual and perennial plant species which occur at the locations studied. The systems studied here (English meadows and Irish turloughs) are largely populated by long-lived perennials, as opposed to other floodplain habitats which are dominated by ephemeral annuals (e.g. arid floodplains). In an arid Australian floodplain, for example, it was found that the more species-rich, frequently-flooded areas were largely populated by annual species, and that perennial species were poorly represented in the soil seed bank (Capon, 2005; Capon and Brock, 2006).

In line with the Intermediate Disturbance Hypothesis (Connell, 1978), and research conducted elsewhere (Pollock *et al.*, 1998; Magee and Kentula, 2005), it was expected that the greatest species richness would occur at intermediate levels of inter- and intra-annual variability. Although the overall trend in the present study was a decline in richness with increasing hydrological variability, there is a lot of noise in this large and complex data set. For instance, some of the lowest and highest species-richness values occurred at the lowest variability (i.e. those quadrats that are either never or constantly waterlogged). There is an important interplay between overall waterlogging and variability which is difficult to untangle, and it is possible that there are positive responses of species richness to intermediate levels of disturbance hidden within the large data set of the current study. There are, however, other drivers of species richness at the sites in this study, such as land-use and nutrient levels, which add to the noise in the data set.

Water-level fluctuation is a complex variable, which encompasses the amount, frequency and regularity of changes in hydrology. It is apparent from the literature summarised here, that these different components of hydrological fluctuations can affect plant communities in a variety of different ways. In essence, the term ‘variability’ encompasses a myriad of environmental conditions across sites, countries, regions, and those fluctuations will be performing different roles in the functioning of grassland habitats; thus, it is difficult to relate the findings of studies on variability to one another.

7.1.3 Effects of hydrological fluctuation on the growth of a sample plant community (objective 3)

In order to meet this objective, a controlled mesocosm experiment was undertaken to test the effects of hydrological fluctuation on four species of *Ranunculus*. Each treatment had the same overall flood duration, but this was divided into individual flood events of differing frequency and duration (i.e. variability). The results of this experiment suggest that overall wetness could be more important than variability because there were no significant differences in the survival, biomass accumulation or flowering of the plants between the treatments imposed. There were, however, some additional stresses in the growth conditions which surfaced during the experiment and this is likely to have influenced the results of this experimental study; these issues are addressed fully in chapter 6. Additionally, experimental treatments were only imposed for one year, due to logistical constraints, and therefore other patterns might have emerged had it run over a longer timescale.

There are experimental studies that have similarly found that overall wetness and flood duration are the most important factors affecting either plant reproduction, growth or biomass (Kotowski *et al.*, 1998; Herr-Turoff and Zedler, 2007; Mony *et al.*, 2010; Gao *et al.*, 2016; Brotherton *et al.*, 2019b). Additionally, a meta-analysis of experimental data from 23 published studies (largely conducted during the growing season) was undertaken by Garssen *et al.* (2015), and they found that an increase in flood duration led to a rise in both seedling and adult plant mortality. There are fewer experimental studies which address the role of hydrological variability on plant growth, and the results from these studies are not in agreement. Water-level variance was found to have no significant effect on species richness and composition (Nygaard and Ejrnaes, 2009). In terms of biomass, one study found that the highest levels of biomass occurred in a low-fluctuation (i.e. never flooded) and high-fluctuation (i.e. short frequent floods) treatment, and the lowest biomass occurred in another low-fluctuation treatment (i.e. continuously flooded). Other studies found that highly fluctuating water levels led to lower total biomass production than lower fluctuating or permanent water levels (Bakker et al., 2007; Luo et al., 2018). This highlights the need for further work in this area of research.

The present study has revealed some fascinating information regarding the response of two species of *Ranunculus* to highly stressful and fluctuating growth environments, which could aid the interpretation of their behaviour in the field. It was expected that the treatments imposed would have little effect on the growth of *R. repens* species, because it is known to cope well with fluctuating water levels (Harper, 1957; Lynn and Waldren, 2001). It is, however, *R. acris* which appeared to be the superior species in this experiment, as it was able to survive all treatments and successfully flowered, although the flowering may have been accelerated due to the stressful conditions. These results show the importance of the availability of nutrients to *R. repens*, and how adaptive *R. acris* can be in a highly stressful and changeable growth environment with low nutrient levels. These findings are in agreement with other research conducted on *R. acris*, which found it to be a plastic species with the ability cope with a wide range of environmental conditions (Oaten, 2005).

This study also demonstrated how important it is to monitor the growth environment during the course of an experiment, as conditions can fluctuate beyond the parameters one has set. Without the monitoring equipment used here, the author would have been unaware of the high temperatures and large fluctuations in temperature occurring within the growth environment. This lack of information would have hindered the interpretation of the results because these additional stresses may have interacted with the effects of the hydrological treatments imposed on the *Ranunculus* species.

7.1.4 Implications for management under future climate change (objective 4)

Given the results of this study (chapters 3, 4 and 5), it is surmised that future climate change will have a considerable effect on the structure and functioning of floodplain meadows and turloughs, as well as other similar wet grassland habitats in Europe. Similar predictions have been made elsewhere. For instance, in a meta-analysis study, species richness was found to decline in response to an increase in flooding at sites which previously experienced relatively stable hydrological regimes (Garssen *et al.*, 2015). It is predicted that species richness may recover once new hydrological regimes have been established, but it is likely that this will only occur in low nutrient systems. In western Europe, for example, any losses in the species richness of riparian plant communities are unlikely to be regained because of the high nutrient loading which occurs along with the additional pressure of increased flooding; species-rich communities may also struggle to recover in locations where new flooding regimes may become too variable (Garssen *et al.*, 2015). The complex interplay between hydrological variability and nutrient availability will present a considerable challenge for the management of biodiversity in wet grassland habitats in the near future.

Seasonally flooded grassland habitats in England, Ireland and elsewhere, are also vulnerable to alterations of hydrological regimes as a result of climate change mitigation works; the need to protect homes, businesses and human life will necessitate the use of both these habitats for their natural flood storage capabilities, but more regularly and possibly for longer periods each time. These additional pressures present another challenge for site managers, as there is also a need to regulate hydrology for the benefit of biodiversity. For instance, the hydrological fluctuations which occur at turloughs and meadows are what make these habitats unique and drive the zonation of plant communities (e.g. Gowing *et al.*, 2002; Sheehy Skeffington *et al.*, 2006).

Under future climate change, soil-moisture dynamics will increase with greater temperature and rainfall variability; and in combination with increased plant physiological demands for both oxygen and water, this will lead to an increased occurrence of wet and dry extremes of plant stresses (Brotherton *et al.*, 2019a). Using downscaled IPCC scenarios to simulate waterlogging and drought stress, it is predicted that both waterlogging and drought stress will increase, on average by ~20% at sites where both stresses occur, in a warmer and more variable future (2050) climate (Bartholomeus *et al.*, 2011). These factors combined will have major repercussions for the plant communities at the present study sites and elsewhere. Therefore, as per the findings of the present study, it is predicted that the following changes are likely to occur in wet grassland habitats:

- An increase in species richness where there is a greater duration of soil drying;
- A decline in species richness where there is an increase in the overall duration of soil waterlogging stress and/or an increase in the amount it varies within and between years;
- Shifts in community composition associated with the loss or gain of species described above;
- Assembly of new communities as a result of changes in water regimes via mechanisms of hydrological niche segregation.

This list represents a snapshot of the new potential challenges facing site managers, conservationists, and regulatory organisations in the coming decades. Wet grassland habitats are vital strongholds for biodiversity, and they also provide numerous other ecosystem services and benefits to wider society (Lawson *et al.*, 2018). Therefore, it is important that site managers do their best to protect and conserve sites of high conservation value wherever feasible. However, it is likely that many conservation objectives will need to change in response to the substantial shifts in ecosystem processes and functioning which will occur as a result of climate change.

It therefore advisable that we (i.e. academics, scientists, practitioners, conservationists, government agencies) explore and plan how we can protect biodiversity under a highly variable climate. Adaptative management strategies may be needed, where site managers are not governed by set restrictions on water levels, grazing regime or hay cutting times, or the management of sites for the protection of individual species. It will be more important than ever before to create corridors for nature, as species will need to be able to disperse in order to find new locations to establish if current habitats become unsuitable.

This study has demonstrated the importance of ecological memory in the development of plant species richness at wet grassland habitats. In highly fluctuating, transitional habitats, like those studied here, where plant communities are constantly shifting in response to changing environmental conditions, it will be particularly difficult to track changes associated with climate change. Therefore, it is vital that they are monitored to develop (or to build upon current) long-term datasets using consistent surveying techniques, in order to see changes over time in relation to current and historic conditions. Truly long-term datasets are a rare and highly valued resource in plant ecology.

7.2 Further research

The key research areas which relate to the current study and that require further investigation are as follows:

1. Study the mechanisms related to the inter- and intra-annual variability in the duration of waterlogging that appear to be driving a decline in species richness at both English meadows and Irish turloughs.
2. Examine the temporal aspect of hydrological niche segregation specifically (i.e. the storage effect), and how this links to the current species richness and composition of plant communities, at the study sites.
3. Widen the analysis undertaken in this study to more turlough sites in order to further our understanding of the relationship between hydrological variability (particularly intra-annual fluctuations in water levels) and turlough plant communities.

4. Develop and explore the use of other hydrological parameters to investigate the relationship between hydrological variability and plant communities at English meadows and Irish turloughs further.
5. Explore the role that soil drying and drought stress may be exerting on the plant communities of Irish turloughs.
6. Refine the experimental design used in this study and repeat in order to test the effects of hydrological variability on plant growth.
7. Study and compare the Irish and English populations of the fen violet (*Viola persicifolia*) to aid the conservation of this rare species at sites in the UK.
8. Examine measures, other than species richness, that are of conservation importance which can be used to evaluate the effects of fluctuating hydrology and future climate change on wet grassland habitats (e.g. plant community types, indicator species, other ecosystem services).
9. Study more grassland habitats which are characterised by different levels of hydrological fluctuation, in order to extend our current knowledge on what role variability plays in their ecosystem functioning and help predict the effects of climate change on these important habitats.
10. Study the interaction between hydrological variability and nutrients and/or competitive dynamics, which are likely to shift under climate change.

7.3 Conclusion

The present study has provided a substantial body of evidence that demonstrates the key role hydrological variability can play in the development of plant communities in seasonally flooded grassland habitats. Additional research is required, however, to enhance our understanding of the effects of hydrological variability on plant communities in wet grasslands further, especially given the predictions of how our climate will change in the future. The results of the present study have implications for the management of wet grasslands under a more variable climate. It is likely that a more flexible approach to habitat (and biodiversity) management will be needed because it may no longer be feasible to maintain sites as they have 'always been' or to restore them to what they were previously.

Appendices

Appendix A: Laboratory protocols

A.1 Measurement of Soil pH in Water

EQUIPMENT

- pH meter, temperature compensated
- Combination electrode
- pH standards made within the previous 2 months (if not, make new standards)
- 50mL centrifuge tubes with lids

QC SAMPLES

- Check calibration every 10 samples using pH 7, if it varies by more than ±0.1-0.15 then recalibrate the meter.
- Repeat every 10th sample to check for reproducibility.
- Use an in-house soil pH standard - one at the beginning and one at the end of small batches OR every 10 samples, to check the method.

REAGENTS

- Buffer solutions- pH 4, 7 and 9.2 (if using)
- RO water
- Air dried soil, sieved <2mm

PROCEDURE

1. Weigh out 5g soil into vial.
2. Add 25ml distilled water (stagger your samples in batches of 10 samples, 5 minutes apart, so that samples do not wait long for measurement)
3. Shake horizontally for 1 hour at speed 120 on shaker table.
4. Calibrate pH meter as per instructions.
5. Rinse probe in RO water and shake off drips prior to use.
6. Shake once again by hand and measure immediately – stir sample with probe then hang on probe stand to stabilise. Take care to insert the electrode to the same depth in each sample.
7. Check calibration every 10 samples using pH 7, if it varies by more than ±0.1-0.15 then recalibrate the meter.
8. Remove electrode from the sample and rinse with RO water, then shake off drips, before taking the next sample.

A.2 Olsen P (Available Phosphorus)

A.2.1 Sample preparation

As soon as possible after sampling, soils must be dried at 40 degrees for 24 hours to prevent chemical alterations from microbial activity. The dry soils can be stored in labelled grip lock bags until extraction and analysis.

Before extraction, dry soil samples must be crushed or ground, either by hand, using a pestle and mortar, or with a small electric mill, such as a coffee bean grinder. The ground soil should then be sieved to <2mm.

A.2.2 Extraction

PLANNING

- Check consumables are available, and chemicals are in date and order if necessary
- The large centrifuge in the Chemical Preparation Lab can spin 20 x 50ml tubes at once so bear this in mind when planning your batch size.
- If preparing a large batch, you may wish to extract one day, freeze the samples and analyse another day, as liquid extracts degrade chemically after ~12 hours. To avoid thawing and refreezing the whole extract, pipette 5ml aliquots (required for colour complex stage) into new 50ml tubes and freeze these as well as the remainder. The 5ml aliquots will thaw quicker than 50ml tubes on the day of analysis.
- Check there is space in the freezer before starting extracts.
- Glassware and Nalgene bottles must be CLEAN and phosphate free.
    - Wash Nalgene bottles and lids in the glasswasher (cycle 3: acid/ alkali/ DI rinse) then rinse again once with DI and dry in CLEAN oven.
    - Volumetric glassware and Duran bottles in lab cupboards *should* have been washed in glasswasher but to be certain they are clean, soak them in phosphate free Neutracon overnight then rinse at least 3 times in DI (until there are no more bubbles).
    - Soak dispensette tubes in Neutracon in the duran bottle to be used for bicarb reagent and pump neutracon through the dispensette to clean. Then pump DI through the dispensette to rinse.

QUALITY CONTROL

- 3 blanks per batch
- Sample repeats (3 reps in total) must be included in every batch, at a minimum frequency of 1 per 10 samples
- 1 or 2 internal reference samples per batch where possible (e.g. in-house soil standard or previously run samples). 3 reps of each standard.

EQUIPMENT

- Permanent pen
- Measuring spoon, 5ml
- Clean Nalgene bottles, 250ml (1 per sample including reps, soil standards and blanks)

- Balance, 2 d.p
- MQ washbottle
- Clean volumetric glassware for sodium bicarb reagent
- Clean Duran bottle to fit Dispensette, 2.5L
- Clean Dispensette, 50ml
- pH meter
- Really Useful Box for shaking Nalgene bottles
- Shaker table
- Centrifuge, *Heraeus Multifuge* 3L
- Pipettor, 5ml
- Waste beaker
- Freezer

CONSUMABLES

- Tubes, 50ml, x2 per sample/ blank/ standard
- Weighing boats, large and small
- Chemicals *(see Reagents)*
- pH calibration standards
- Neutracon
- Pipette tips, 5ml

REAGENTS

- Sodium bicarbonate reagent (sodium hydrogen carbonate):
    - Dissolve $NaHCO_3$ (42g/l) + NaOH (0.72g/l) in MQ water. Make up to volume*.
    - Adjust to pH 8.5 with weak solutions of NaOH or H_2SO_4.

  **Volume required: 100ml per sample, including reps and blanks, + enough spare to freeze and use as a matrix for Phosphate standards (at least 7x 50ml).*

METHOD

1. Weigh ~5g of each sample into CLEAN, labelled 250mL nalgene bottles and record weights on a data sheet. Include reps and soil standards.
   Tip: Use a 5ml measuring spoon. It's much quicker than a spatula.
2. Add 100ml of sodium bicarb reagent using a bottle-top dispensette.
   Also dispense 100ml into 2 or 3 empty Nalgene bottles for reagent blanks.
   Tip: Express a few millilitres 2 or 3 times into a waste beaker, to eliminate bubbles in dispensette, before filling Nalgenes.
3. Cap bottles tightly and shake at 120rpm for 30 min at 20°C.
   Lay the bottles on their sides (in a box) to ensure sufficient reaction; shaking with the bottles upright results in poor equilibration and poor P extraction.
4. Pour 50ml of each sample or blank into a new labelled centrifuge tube.
5. Spin at 4000rpm for 5 minutes.
6. While spinning, label new 50ml centrifuge tubes for each sample/ blank.
7. Without disturbing the sediment, pipette 5ml of supernatant from each sample and blank into a new labelled tube. This will be used for the colour complex reaction.
8. Freeze the 5ml aliquots, the remaining extracts and the spare bicarb reagent for later use.

A.2.3 Analysis

PLANNING

- Check consumables are available and chemicals are in date and order if necessary
- Clean glassware
- Prepare 1.5M sulphuric acid and ammonium molybdate stock in advance to save time on day of analysis
- On day of analysis, remember to remove sodium bicarb reagent from freezer first, in order to make up P standards

QUALITY CONTROL

- Sample repeats and reagent blanks, as per instructions for extraction process
- Repeat measurements with spectrophotometer to verify consistency of absorbance measurements

EQUIPMENT

- Clean volumetric glassware for reagents
- Permanent pen
- Spatula
- Balance, 2 d.p
- MQ Washbottle
- Fume cupboard
- Clean beakers and measuring cylinders for adding concentrated acids
- Clean Duran bottle to store 1.5M sulphuric acid
- Pipettors, 1ml, 5ml, 10ml
- Tube/ cuvette racks
- Spectrophotometer (Thermo Spectronic Heλios γ)
- Fume cupboard
- Clean dispensette, 25ml
- Clean Duran bottle to fit Dispensette, 2.5L
- Waste beaker
- Large hazardous waste vessel

CONSUMABLES

- Chemicals *(see Reagents)*
- Tubes, 50ml, to make up and develop Phosphorus standards (x15)
- Cuvettes (new, disposable), 1 per sample, repeat, and blank and 2 per phosphorus standard
- Pipette tips, 1ml, 5ml and 10ml
- Hazard labels
- Grip lock bags and waste vessel for hazardous solids and liquid waste

REAGENTS

- 1.5M Sulphuric acid (80ml/l conc. Sulphuric)
  Volume required: 1ml per sample/blank/standard

To make 100ml, add 8ml conc. sulphuric to 92ml MQ water

Ammonium molybdate stock solution

Dissolve the following in MQ water and make up to volume*:

- Ammonium molybdate (12g/l)
- Potassium antimonyl tartrate (0.300g/l)
- Conc. Sulphuric acid (148ml/l)

**Volume required: 10% of total volume of ascorbic acid reagent required. (ie. 100ml in every litre of ascorbic acid reagent)*

Ascorbic acid (Ascorbic-ammonium molybdate solution) – Make up on the day

Dissolve/ combine the following in MQ water and make up to volume*:

- Ascorbic acid (3g/l)
- Ammonium molybdate stock solution (100ml/l)

**Volume required: 25ml per sample/blank/standard*

Phosphorus (P) standards – Make up on the day

- Prepare a 100mg/l stock solution from 1000ppm standard (5ml P in 50 ml MQ water)
- Prepare working standards as below, using $NaHCO_3$ reagent as a matrix

Desired working standard concentration (mg/L)	0	1	2	3	4	5	6	7
Volume (mL) of 100 mg/L P stock solution required in 50mL centrifuge tube	0	0.5	1	1.5	2	2.5	3	3.5
Approximate expected absorbance (using sipper)	0	0.22	0.45	0.7	0.9	1.1	1.4	1.5
Approximate expected absorbance (using cuvette)	0	0.12	0.24	0.36	0.48	0.60	0.72	0.84

METHOD

Colour complex:

1. Turn on the spectrophotometer approximately 1 hour before use
2. Thaw frozen samples (5ml aliquots, not bulk extract)
3. In the fume cupboard, add 1ml of 1.5M Sulphuric acid to each tube
   Tip: Start with the P standards as these must be analysed first
   Acid **MUST** be added ***before*** ascorbic-ammonium molybdate solution
4. Add 25ml of the ascorbic-ammonium molybdate solution with dispensette
5. Allow to stand for 1 hour (use within 4 hours)

Spectrophotometry:

6. Ensure the instrument has had at least 1 hour to warm up.
7. Load the method:
   Menu → Method → Enter; Use down arrow to select Olsen method (Carly Olsen) → Enter
8. Pour your standards into new CLEAN cuvettes (rinse once with standard first) and stand in a cuvette holder or polystyrene rack

9. Use your 0ppm P standard to zero the machine:
   Load cuvette → Press Zero
   Measure 0 standard to check the spectrophotometer has zeroed correctly.
10. Measure standards and note absorbances. Measure each solution at least twice until a repeatable absorbance is achieved. This may need 3 or 4 attempts. Record all measurements on a data sheet. Check your absorbance measurements for standards against the expected absorbances on the previous page.
11. Measure samples in new cuvettes, recording all measurements as you go. You can clear results from the display if desired: Clear Results → Enter.
    (Note: Samples degrade quickly in cuvettes and absorbance drifts down consistently by ~0.001 with each repeat measurement, so best to select first measurement for data analysis. This drift is not seen in the standards.)
12. Monitor any drift by reanalysing your 0 ppm standard and one other standard every 10-15 samples, and the full set of standards at the completion of all samples.

A.3 Soil Moisture Release

Place the soil cores collected in the field in a tray with water to saturate for 24 hours. Once saturated, record their weight (i.e. 0cm tension).

Make sure no water is lost when weighing the saturated core/s. Use a tray to support them as soon as they are removed from the sand table, and also use the tray for weighing. Make sure to deduct the weight of the tray when recording data. Then place the core firmly on the sand table (see photographs below) at manometer position of 10 cm. Allow this set up to equilibrate for 3 days.

Once equilibrated then remove and weigh on a balance once again. After weighing place on sand table firmly once again and then drop the sand table manometer by another 10 cm to 20 cm.

This process of weighing and changing manometer position is continued until 100 cm. At the end of the experiment, dry the soil at 65°C for 4 days and weigh.

Appendix B: Worked examples of inter- and intra-annual hydrological variability

For example,

Site: Blackthorn

Botanical survey: 1995

Quadrat number: 1

Inter-annual variability: Counted the number of weeks that the waterlogging threshold was exceeded per year for each quadrat (growing season only; max. 31 weeks); calculated the inter-quartile range (IQR) across the 5 years preceding the botanical survey.

1	Waterlogging threshold exceeded
0	Waterlogging threshold *not* exceeded

Growing season week	Year 1 (1990)	Year 2 (1991)	Year 3 (1992)	Year 4 (1993)	Year 5 (1994)
1	1	0	1	1	0
2	1	1	1	1	0
3	1	1	0	1	0
4	1	0	0	1	1
5	1	1	0	1	1
6	0	0	0	1	1
7	0	1	0	1	1
8	0	1	0	1	1
…31	0	0	0	0	0
TOTAL	16	8	5	20	17
Inter-quartile range			9		

Intra-annual variability: Counted the number of weeks that the waterlogging threshold was exceeded in each calendar month for each quadrat (max. 5 weeks); calculated the IQR across each growing season (7 months); calculated the median IQR across the 5 years preceding the botanical survey.

Growing season month	Year 1 (1990)	Year 2 (1991)	Year 3 (1992)	Year 4 (1993)	Year 5 (1994)
1 (Mar)	4	4	2	5	4
2 (Apr)	5	4	4	4	5
3 (May)	3	3	4	3	0
4 (Jun)	1	0	2	0	0
5 (Jul)	0	0	0	0	0
6 (Aug)	0	0	0	1	2
7 (Sep)	3	0	2	2	3
Inter-quartile range	3	3.5	2	3	3.5
Median inter-quartile range			3		

Appendix C: Linear mixed effect model outputs

C.1: Chapter 3 lme model output

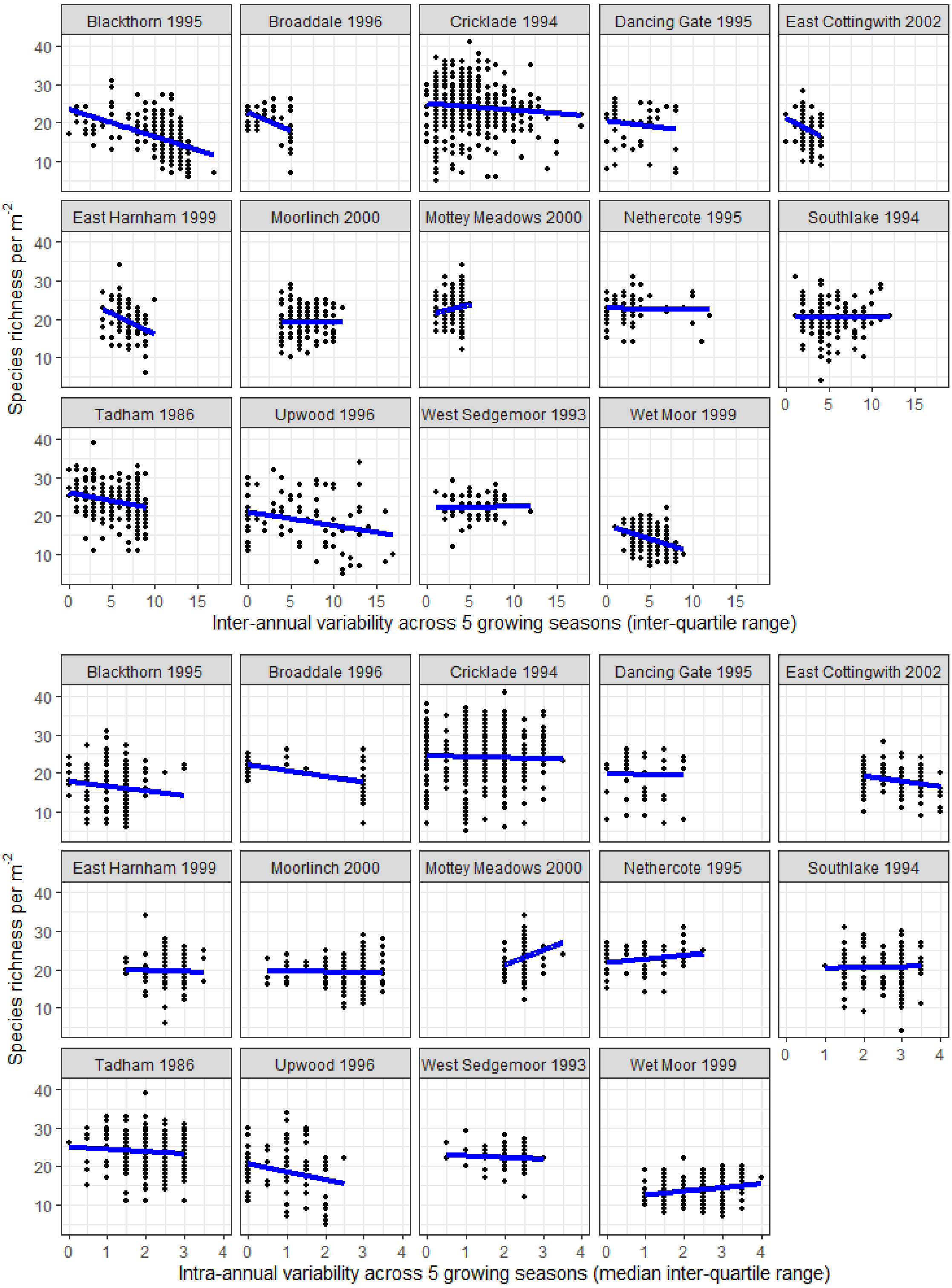
Model	Figure	Estimate	SE	t-value	p-value
species richness ~ total waterlogging	3.3a	-0.7424	0.1101	-6.743	1.97e-11 ***
species richness ~ total drying	3.3b	0.5359	0.1066	5.026	5.40e-07 ***
species richness ~ stress	3.4a	-0.5613	0.1187	-4.728	2.41e-06 ***
species richness ~ variability	3.4b	-0.4699	0.124	-3.791	0.000154 ***
species richness ~ inter-annual variability	3.6a	-1.0338	0.1186	-8.715	< 2e-16 ***
species richness ~ intra-annual variability	3.7a	-0.3574	0.1303	-2.743	0.00613 **

C.2: Chapter 5 lme model output

Model	Figure	Estimate	SE	t-value	p-value
species richness ~ total waterlogging (turloughs)	5.3	-0.021427	0.007021	-3.052	0.0027 **
species richness ~ inter-annual variability (turloughs)	5.4a	-0.5986	0.3014	-1.986	0.0489 *
species richness ~ intra-annual variability (turloughs)	5.7a	-0.6279	0.3323	-1.89	0.0607 .
species richness ~ total waterlogging (turloughs & meadows)	5.10	-2.32E-02	3.17E-03	-7.328	3.19e-13 ***
species richness ~ inter-annual variability (turloughs & meadows)	5.12a	-1.0133	0.1146	-8.839	< 2e-16 ***
species richness ~ intra-annual variability (turloughs & meadows)	5.12b	-0.382	0.122	-3.131	0.00176 **

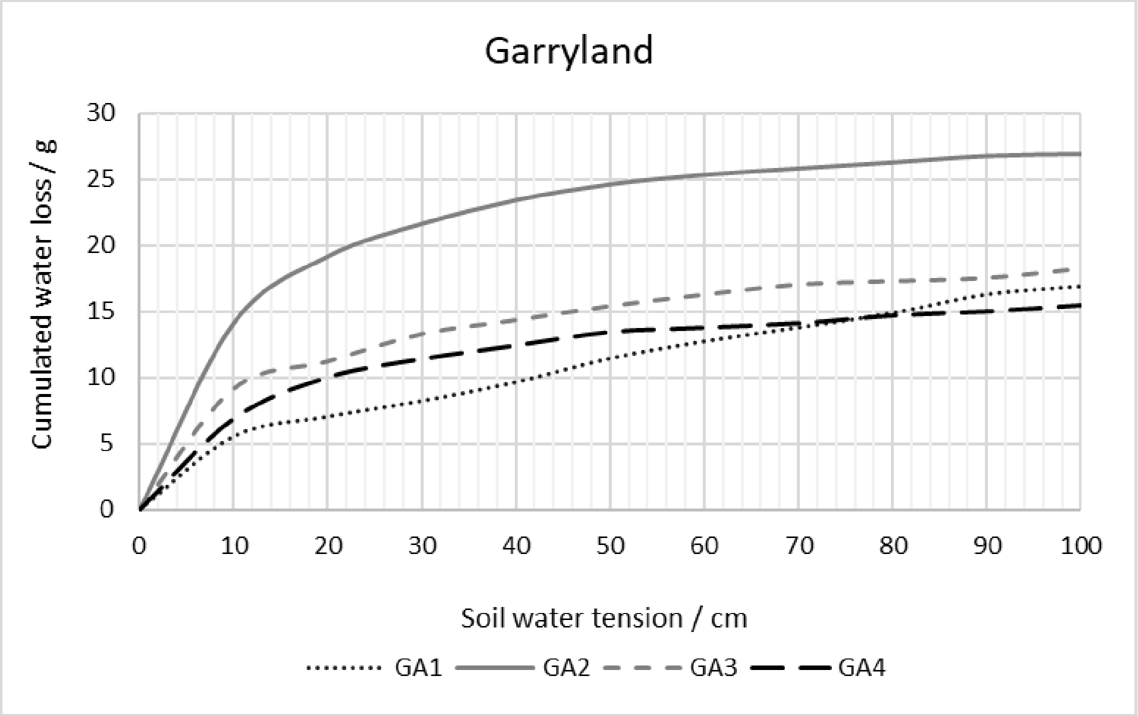
Significance codes:
0 ‘***’
0.001 ‘**’
0.01 ‘*’
0.05 ‘.’
0.1 ‘ ’

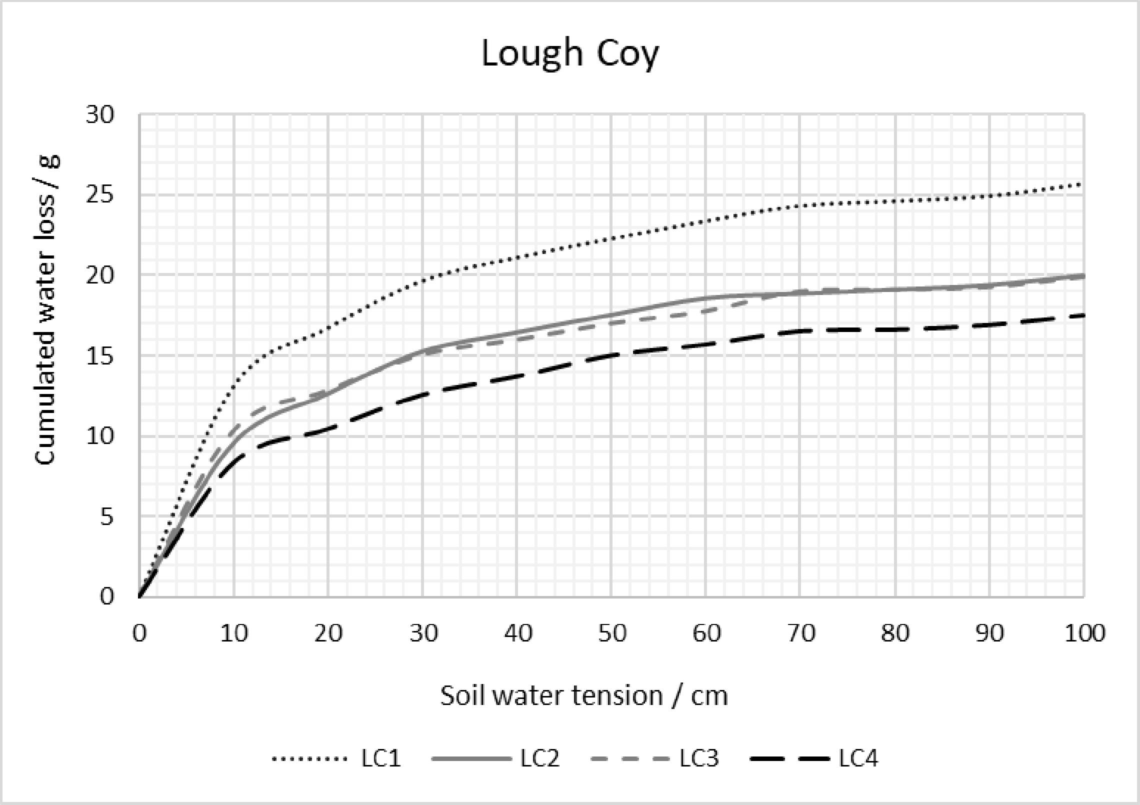
Appendix D: Hydrological variability versus species richness at individual floodplain meadow sites

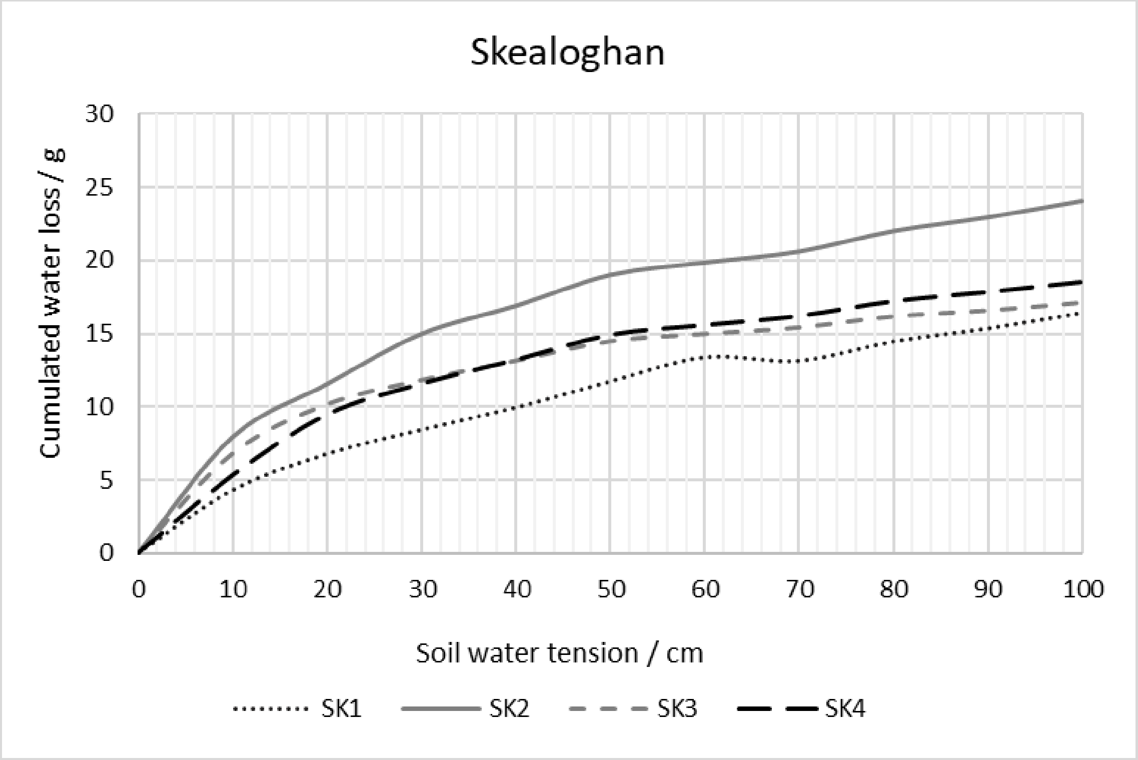


Inter-annual (top) and intra-annual (bottom) hydrological variability versus species richness for each quadrat, with each site plotted separately; the line of best fit displayed (blue lines) do not denote statistically significant results.

Appendix E: Soil moisture release curves for turlough vegetation sampling zones







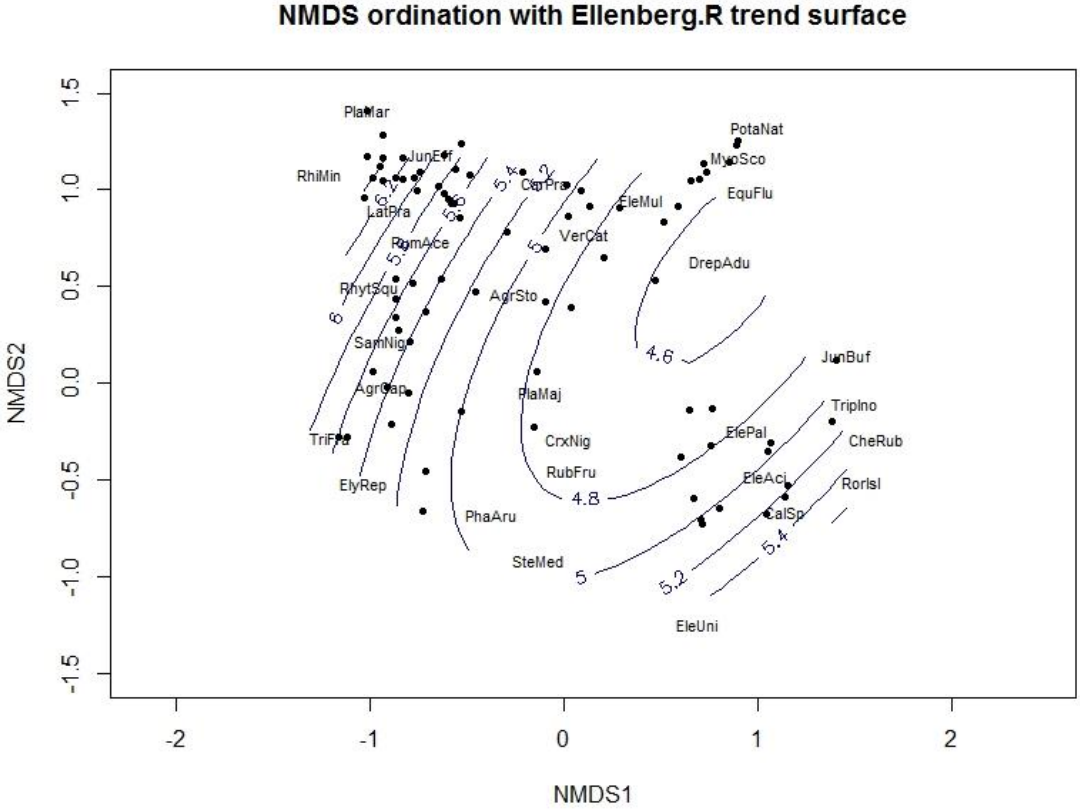
A waterlogging threshold depth was calculated for the soil in each sampling zone (1 to 4) at the three sites (Garryland, Lough Coy, Skealoghan). Three undisturbed soil cores (5 cm depth x 5 cm diameter) were collected per zone, saturated and then placed on a sand table to determine the soil-moisture-release curves displayed above (median values only). The tension at which 10% air-filled porosity was reached was calculated for each soil core (Gowing *et al.*, 2002), and the median value of the three replicates was used to assign a waterlogging threshold to each sampling zone. An additional 10 cm was added to account for the relationship between rooting depth and aeration (Dumortier, 1991); i.e., a 20 cm threshold becomes 30 cm below the surface.

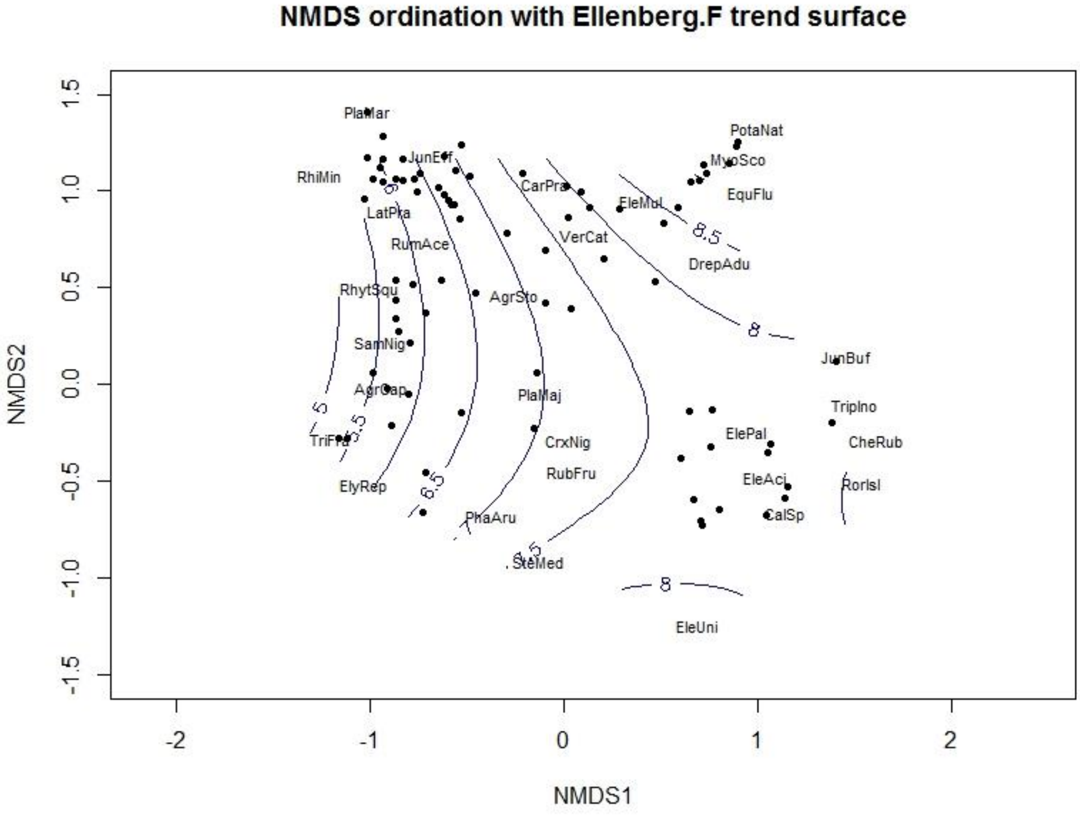
Appendix F: Full list of plant species recorded at turlough study sites

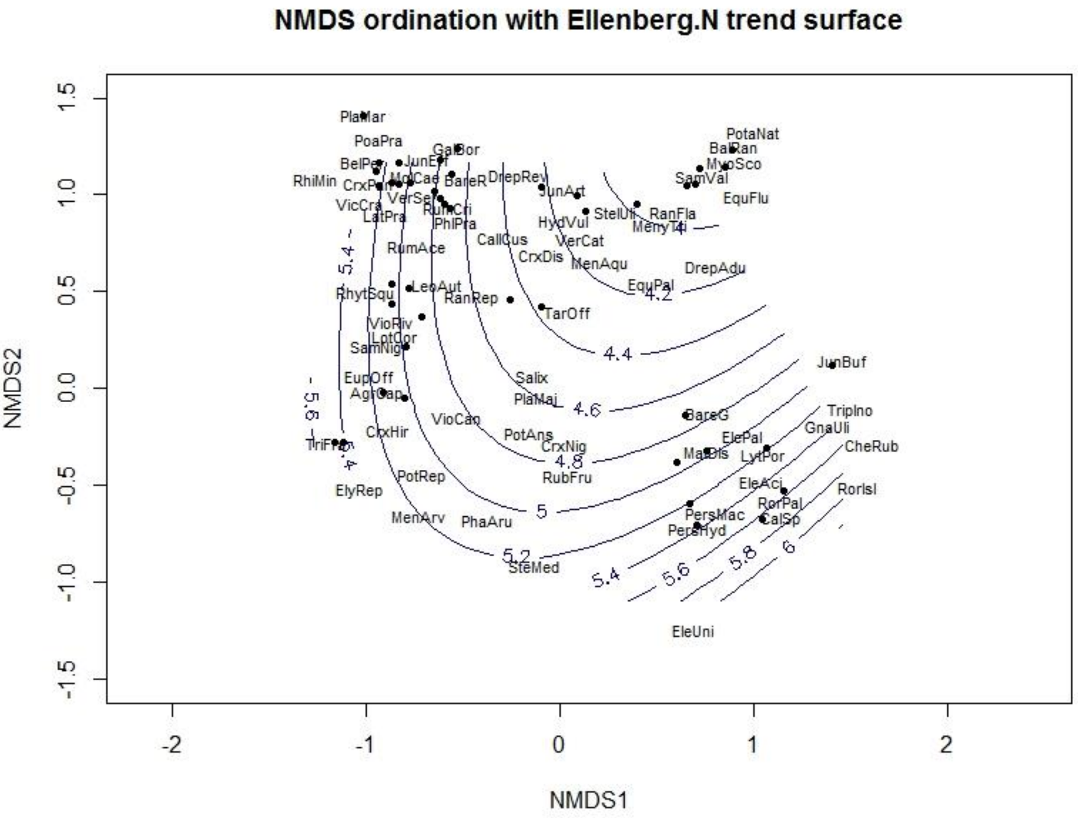
CODE	**FULL NAME**	**CODE**	**FULL NAME**
AgrCap	*Agrostis capillaris*	MenAqu	*Mentha aquatica*
AgrSto	*Agrostis stolonifera*	MenArv	*Mentha arvensis*
AlisPla	*Alisma plantago-aquatica*	MenyTri	*Menyanthes trifoliata*
AloPra	*Alopecurus pratensis*	MolCae	*Molinia caerulea*
ApiInu	*Apium inundatum*	Myolax	*Myosotis laxa*
BalRan	*Baldellia ranunculoides*	MyoSco	*Myosotis scorpioides*
BareG	*Bare ground*	NarStr	*Nardus stricta*
BareR	*Bare rock*	OphVul	*Ophioglossum vulgatum*
BelPer	*Bellis perennis*	PersAmp	*Persicaria amphibia*
BidTri	*Bidens tripartita*	PersHyd	*Persicaria hydropiper*
BracRiv	*Brachythecium rivulare*	PersMac	*Persicaria maculosa*
BracRut	*Brachythecium rutabulum*	PhaAru	*Phalaris arundinacea*
CallGia	*Calliergon giganteum*	PhlPra	*Phleum pratense*
CallCus	*Calliergonella cuspidata*	PlaLan	*Plantago lanceolata*
CalSp	*Callitriche seedling/sp*	PlaMaj	*Plantago major*
CarPra	*Cardamine pratensis*	PlaMar	*Plantago maritima*
CrxDis	*Carex disticha*	PoaAnn	*Poa annua*
CrxFla	*Carex flacca*	PoaPra	*Poa pratensis*
CrxHir	*Carex hirta*	PolAvi	*Polygonum aviculare*
CrxNig	*Carex nigra*	PotaNat	*Potamogeton natans*
CrxOva	*Carex ovalis*	PotAns	*Potentilla anserina*
CrxPan	*Carex panicea*	PotEre	*Potentilla erecta*
CenNig	*Centaurea nigra*	PotRep	*Potentilla reptans*
CerFon	*Cerastium fontanum*	PruVul	*Prunella vulgaris*
CheRub	*Chenopodium rubrum*	RanAcr	*Ranunculus acris*
CirArv	*Cirsium arvense*	RanFla	*Ranunculus flammula*
ClimDen	*Climacium dendroides*	RanRep	*Ranunculus repens*
CynSp	*Cynosurus sp*	RhiMin	*Rhinanthus minor*
DrepAdu	*Drepanocladus aduncus*	RhytSqu	*Rhytidiadelphus squarrosus*
EleAci	*Eleocharis acicularis*	RorIsl	*Rorippa islandica*
EleMul	*Eleocharis multicaulis*	RorPal	*Rorippa palustris*
ElePal	*Eleocharis palustris*	RubFru	*Rubus fruticosus*
EleUni	*Eleocharis uniglumis*	RumAce	*Rumex acetosa*
ElyRep	*Elytrigia repens*	RumCri	*Rumex crispus*
EquFlu	*Equisetum fluviatile*	Salix	*Salix seedling/sp*
EquPal	*Equisetum palustre*	SamNig	*Sambucus nigra*
EupOff	*Euphrasia officinalis*	SamVal	*Samolus valerandi*
FesAru	*Festuca arundinacea*	ScorRev	Scorpidium revolvens
FesRub	*Festuca rubra*	SenAqu	*Senecio aquaticus*
FilUlm	*Filipendula ulmaria*	SpaEre	*Sparganium erectum*
GalBor	*Galium boreale*	SteGra	*Stellaria graminea*
GalPal	*Galium palustre*	SteMed	*Stellaria media*
GalVer	*Galium verum*	StelUli	*Stellaria uliginosa*
GlyFlu	*Glyceria fluitans*	SucPra	*Succisa pratensis*
GnaUli	*Gnaphalium uliginosum*	TarOff	*Taraxacum officinale*
HydVul	*Hydrocotyle vulgaris*	TriFra	*Trifolium fragiferum*
JunArt	*Juncus articulatus*	TriRep	*Trifolium repens*
JunBuf	*Juncus bufonius*	TripIno	*Tripleurospermum inodorum*
JunEff	*Juncus effusus*	Bryo	*Unidentified Bryophyte*
LatPra	*Lathyrus pratensis*	UrtDio	*Urtica dioica*
LeoAut	*Leontodon autumnalis*	VerCat	*Veronica catenata*
LimAqu	*Limosella aquatica*	VerScu	*Veronica scutellata*
LolPer	*Lolium perenne*	VerSer	*Veronica serpyllifolia*
LotCor	*Lotus corniculatus*	VicCra	*Vicia cracca*
LytPor	*Lythrum portula*	VioCan	*Viola canina*
LytSal	*Lythrum salicaria*	VioRiv	*Viola riviniana*
MatDis	*Matricaria discoidea*	Species highlighted in red appear in the appendix only	

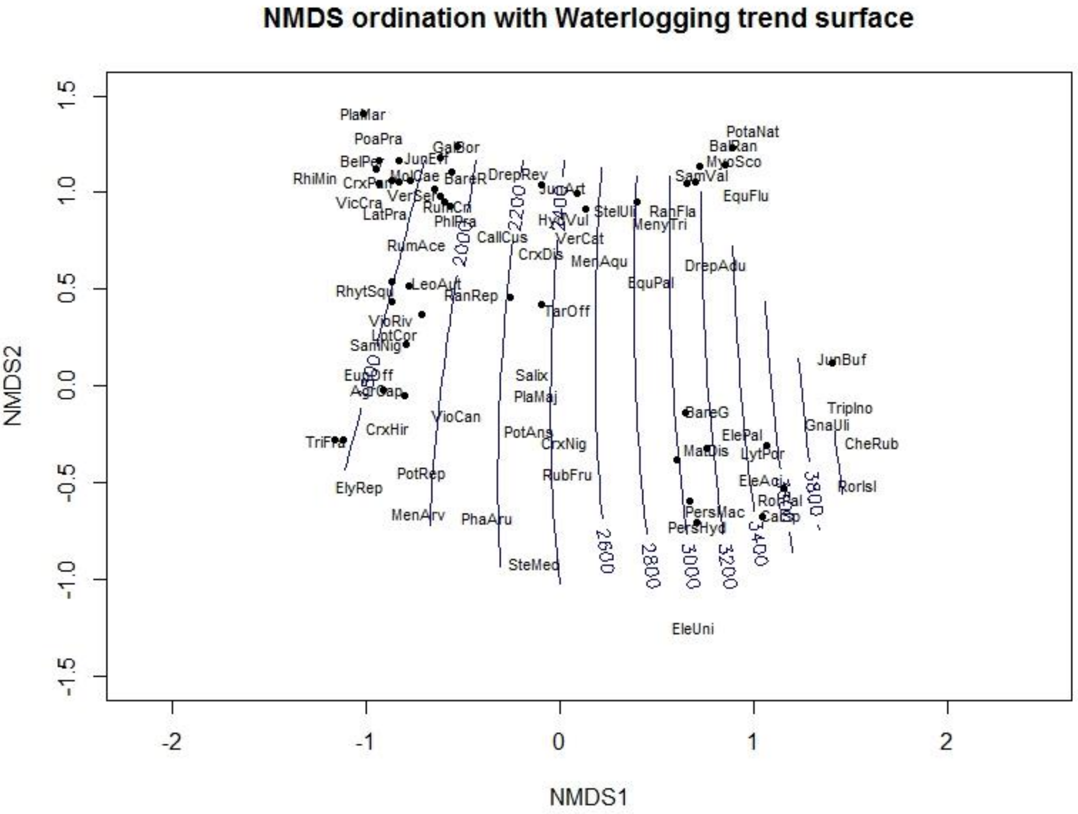
Species names in the table follow the nomenclatures of Stace (1991) for vascular plants and Smith (2004) for mosses.

Appendix G: NDMS ordination trend surfaces

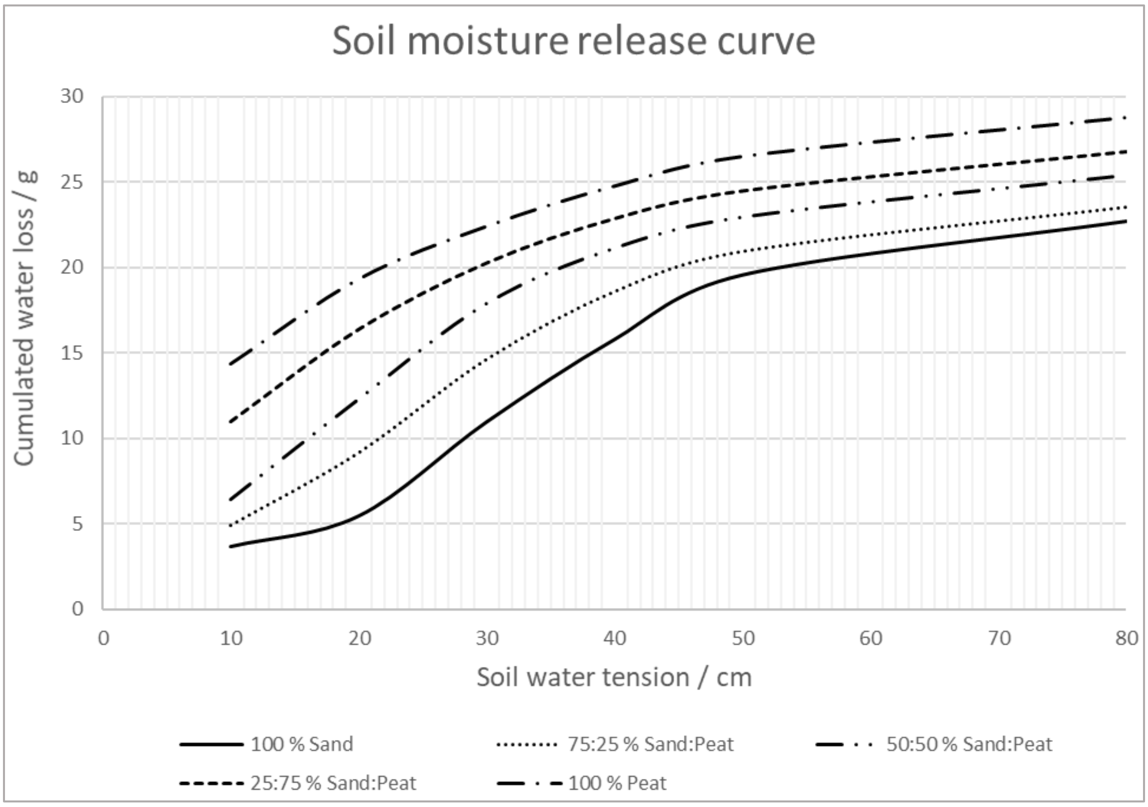

NMDS ordination with Ellenberg.R trend surface


NMDS ordination with Ellenberg.F trend surface


NMDS ordination with Ellenberg.N trend surface
NMDS2
NMDS1
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2
-1
0
1
2
4
4.2
4.4
4.6
4.8
5
5.2
5.4
5.6
5.8
6
PlaMar
PoaPra
GalBor
BelPer
JunEff
RhiMin
CrxPan
MolCae
BareR
DrepRev
VicCra
VerSer
LatPra
RumCri
PhlPra
JunArt
HydVul
StelUli
RanFla
MenyTri
PotaNat
BalRan
MyoSco
SamVal
EquFlu
CallCus
VerCat
CrxDis
MenAqu
RumAce
DrepAdu
EquPal
RhytSqu
LeoAut
RanRep
TarOff
VioRiv
LotCor
SamNig
JunBuf
EupOff
AgrCap
Salix
PlaMaj
TripIno
GnaUli
CheRub
CrxHir
VioCan
PotAns
BareG
CrxNig
ElePal
MatDis
LytPor
TriFra
RubFru
EleAci
PotRep
RorIsl
ElyRep
RorPal
MenArv
PhaAru
PersMac
CalSp
PersHyd
SteMed
EleUni


NMDS ordination with Waterlogging trend surface
NMDS2
NMDS1
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2
-1
0
1
2
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
PlaMar
PoaPra
GalBor
BelPer
JunEff
RhiMin
CrxPan
MolCae
BareR
DrepRev
VicCra
VerSer
LatPra
RumCri
PhlPra
JunArt
HydVul
StelUli
RanFla
MenyTri
PotaNat
BalRan
MyoSco
SamVal
EquFlu
CallCus
VerCat
CrxDis
MenAqu
RumAce
DrepAdu
EquPal
RhytSqu
LeoAut
RanRep
TarOff
VioRiv
LotCor
SamNig
JunBuf
EupOff
AgrCap
Salix
PlaMaj
TripIno
GnaUli
CheRub
CrxHir
VioCan
PotAns
BareG
CrxNig
ElePal
MatDis
LytPor
TriFra
RubFru
EleAci
PotRep
RorIsl
ElyRep
RorPal
MenArv
PhaAru
PersMac
CalSp
PersHyd
SteMed
EleUni

Appendix H: Soil moisture release for trial mixes of experimental growth medium



A waterlogging threshold depth was calculated for five different mixes of fine sand and peat for the mesocosm experiment. Three cores (5 cm depth x 5 cm diameter) of each mix were taken, saturated and then placed on a sand table to determine the soil-moisture-release curves displayed above (median values only). The tension at which 10% air-filled porosity was reached was calculated for each soil core (Gowing *et al.*, 2002), and the median value of the three replicates was used to assign a waterlogging threshold to each mixture. An additional 10 cm was added to account for the relationship between rooting depth and aeration (Dumortier, 1991); i.e., a 20 cm threshold becomes 30 cm below the surface. The 50:50 mix of sand and peat was selected for the experiment, see Chapter 6 for details.

Appendix I: Photographs of the controlled mesocosm experiment

Photographs showing the monitoring equipment installed in block one (top); Theta and oxygen probes, and tensiometer (bottom left); thermistor (bottom right).

Photographs showing: two of the experimental pots in June 2018 immediately before the final detructive harvest (top right and left), with only *R. acris* plant remaining; the complete array after the final harvest (bottom left); and one of the below-ground biomass samples (bottom right).

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