Ecohydrological metrics for vegetation communities in turloughs (ephemeral karstic wetlands)

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Abstract
A 28-year hydrological record on four intermittent wetlands (turloughs) in a hydraulically linked karst area in the west of Ireland was used to assess eco-hydrological metrics for different vegetation communities. A methodology using a combination of continuous water level monitoring and high resolution topographic surveying was used to develop a detailed hydrological model of the karst network, from which water levels at any point within the turloughs can be defined at any time during the 28-year period (1989 to 2017). The flood conditions experienced across the spatial distributions for different vegetation communities (as mapped by a field survey) have then been collated and presented as statistical distributions for flood duration, flood depth, flood frequency and mean temperature/global radiation at the time of year in spring when the flood waters start to recede. Analysis of these four turloughs has revealed distinct differences between vegetation communities, from Eleocharis acicularis communities at the turlough base typically experiencing 6 to 7 months of inundation per year compared to the limestone pavement community at the top fringes of the turloughs only flooded from 1 to 2 months per year. An approach that used Sentinel-2 satellite data to provide an assessment of whether there have been changes in the spatial distribution of the communities is also presented. Such metrics can be evaluated alongside other variables such as water quality (particularly nutrients), soil type and land-use, in order to understand the habitat requirements for such plant communities and their associated ecological systems.

KEYWORDS
ecohydrology, ephemeral lake, groundwater-dependent terrestrial ecosystems, karst, remote sensing, turlough, vegetation, wetlands

1 | INTRODUCTION

Wetlands can be recognised as transitional (both in space and time) ecosystems, or ecotones, between terrestrial and aquatic ecosystems (Mitsch & Gosselink, 2015). Although covering only 6% to 8% of the global land space, wetlands account for a much higher share of the estimated total value of the ecosystem services of all biomes (possibly around 36% according to De Groot et al., 2012). The services

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normally associated with wetlands are water supply and purification, flood and erosion control, carbon storage and sequestration (and links to climate), and habitat preservation (Barbier, 2011; Millennium Ecosystem Assessment, 2005). However, about half of global wetland areas have been lost, and much of the remaining wetlands are degraded (Zedler & Kercher, 2005). It is therefore crucial that accurate methods of assessing the ecological health of such wetlands are developed in order to feed into appropriate management decisions for their preservation and protection with respect to past, current and future anthropogenic impacts, including the potential impacts of a changing global climate. The presence or absence of a plant species or community can be used as a bioindicator and can provide information on the environmental conditions in the habitat in which it is found. For example, one commonly used method in Europe of quantifying the relationship between indicator plant species and various environmental variables is the Ellenberg index (Ellenberg, 1988; Hill et al., 1999). The type of vegetation/ecology in wetlands and its location is intimately entwined with the hydrological conditions, and hence, these ecohydrological relationships need to be properly understood and evaluated, from which healthy envelopes/metrics can then be defined for the different key wetland habitats. Such metrics can be used in order to assess the ecohydrological status of wetlands and monitor and manage their current and future existence. Regan et al. (2020), for example, have recently evaluated the ecohydrological envelope of different ecotopes on raised bogs in Ireland, using water table duration curves to show that active areas of Sphagnum growth occur where water tables are within 0.1 m of the ground surface for approximately 90% of a given year.

Up to now, the mapping and monitoring of wetlands has largely been carried out manually by field visits to collect data, a process/activity that is both time and resource intensive. In recent years remote sensing has been increasingly used to map and assess the ecological status of different wetlands: for example, Bourgeau-Chavez et al. (2015) have used the seasonal Landsat-8 and microwave satellite data to map wetlands in north America; Dutta et al. (2015) have used satellite data to find ecological disturbances in mangrove forests in India; Bhatnagar et al. (2018, 2020) have used satellite-RS along with spectral indices to map different vegetation communities on wetlands in Ireland. Equally, studies such as Diaz-Delgado et al. (2019) have used both drones and satellite imagery for monitoring the ecological status of a marsh-ecosystem in Romania. A detailed account of wetland remote sensing can be found in review papers by Gou et al. (2015) and Mahdavi et al. (2018).

This research focuses on ephemeral lakes that form in shallow depressions in karst areas, mainly in the west of Ireland, known as turloughs. Such intermittent wetlands usually flood in winter and drain in summer; they exhibit similar dynamics to the ephemeral polje lakes (Dolinar et al., 2010) found in the Dinaric karst region (and elsewhere). Turloughs generally flood (and drain) from their lowest topographic point, often via estavelles linked to the main karst conduit networks (Naughton et al., 2012). This intermittent flooding of the basin produces a distinct hydrological gradient which produces a linked vegetation gradient. Such wetlands are designated priority habitats under the EU Habitat’s Directive and are considered as groundwater-dependent terrestrial ecosystems (GWDTEs) under the EU Water Framework Directive (2000/60/EC). The continually changing environment of turloughs means that they are more accurately considered as ecotones rather than ecosystems; that is, transitional zones between aquatic and terrestrial systems (Reynolds et al., 1998).

There have been many previous studies on turlough geomorphology, hydrology, ecology, and conservation importance (e.g., Coxon, 1987; Sheehy Skeffington et al., 2006) with summaries of turlough wetland plant and freshwater habitats and communities described in Goodwillie and Reynolds (2003). These were followed by a more integrated project (Waldren et al., 2015) studying the conservation status (vegetation habitats, invertebrates, water quality, hydrology, etc.) of 22 turloughs. These were selected to provide a representative range across the different types of turlough found in Ireland, primarily from the perspective of hydrogeological variation (conduit driven or shallow epikarst), depth and duration of flooding, and so on, but were further characterised later according to water quality, soil type and land-use.

Seasonally or intermittently flooded wetlands present a unique environment for plants that are able to live under such conditions, being affected by hydrology, disturbance, spatial heterogeneity and productivity (Pollock et al., 1998). For example, fluctuating inundations change the physical and chemical properties of soils (Ponnamperuma, 1984), which can affect the competitive abilities of different plant species, and therefore the species composition of vegetation communities (Kennedy et al., 2003; Pollock et al., 1998). From a hydrological perspective, duration (hydroperiod), depth and frequency of flooding have been shown to have the greatest effect on the ecology of wetlands (Casanova & Brock, 2000; De Becker et al. (1999); Thompson & Finlayson, 2001). More specifically to turloughs, the hydroperiod (and other linked metrics such as depth of flooding and the timing of the flood recessions) has major effects on both terrestrial and aquatic communities, strongly influencing the distribution of vascular plant species and the zonation of vegetation communities within turloughs (Irvine et al., 2018; Waldren et al., 2015). Hence, this research has evaluated the spatial distribution of different vegetation communities on four turloughs within the same karst network in south Galway (Ireland) in relation to their different hydrological conditions experienced over a 28-year period. These analyses have then been used to define broad ecohydrological metrics for the different vegetation community habitats.

2 | MATERIALS AND METHODS

2.1 | Study sites (geology, hydrology, water quality and land-use)

The four turloughs selected for study (Blackrock, Coy, Coole, Garryland and Caherglassaun) are located in an extensive conduit karst network catchment in south Galway in the west of Ireland (see Figures 1 and S1). This karst has formed in carbonate rock from
the Carboniferous, which has experienced many episodes of karstification, most significantly during the most recent Cenozoic Era (Drew, 2018), and is situated less than 100 m above current sea level.

The total catchment size is ~500 km² of which about one third of the catchment area is formed by the Old Red Sandstone Slieve Aughty mountains (covered in peat and forestry) which is drained by three main rivers. These rivers feed rapid allochthonous run-off down into the lowland karst network. The rest of the catchment receives autogenic rainfall recharge onto the karst which is covered by shallow limestone till. Several studies have shown the interconnected nature of this south Galway karst conduit network (Figure S1) which discharges out into the Atlantic Ocean at an intertidal spring at Kinvara (Gill et al., 2018; Morrissey et al., 2020; Naughton et al., 2018).

All four turloughs in this interconnected lowland karst network contain waters that are a mixture of soft water from rivers draining the Slieve Aughty mountains and hard water from the lowland calcareous parts of their catchments, yielding waters of relatively low alkalinity and also relatively high colour due to the presence of humic and fulvic materials linked to drainage from peats. All four turloughs were deemed to be eutrophic-mesotrophic from total phosphorus measurements but mesotrophic–oligotrophic from chlorophyll A measurements taken during the Walden et al. (2015) study (Cunha Pereira et al., 2010). The development of algal biomass in these turloughs appears not to have been limited by phosphorus in these turloughs (compared to others studied), which was attributed to the more highly coloured waters of these turloughs causing more
predominant light-limitations through the winter instead (Havens & Nurnberg, 2004). More frequent sampling of these turloughs across the 2011/2012 hydrological year showed fairly consistent concentrations of nutrients both in time and between the four turloughs: total phosphorus (TP) ranging from 20 to 50 μg/L, total dissolved phosphorus ranging from 5 to 30 μg/P and total nitrogen ranging from 0.25 to 1.2 mg/L (McCormack et al., 2016), although the nutrient concentrations were found to reduce over the flooded period with such nutrient loss processes thought to be occurring within the turlough systems themselves. Finally, the soil types are very similar between the four turloughs comprised of mineral soils associated with till subsoils. Equally, the land-use is very similar between the four turloughs, with grazing mainly by cattle (with some sheep and horses) at relatively low intensity during the summer.

2.2 | Vegetation surveys

2.2.1 | Field surveys

Vegetation field surveys were conducted over three field seasons, 2006, 2007 and 2008 across 22 turloughs (Sharkey, 2012; Waldren et al., 2015) using 1 × 1-m quadrats—the majority of vegetation being grassland or short herbaceous vegetation. A minimum of five relevés were recorded in each vegetation type. Within each relevé, the vascular plant species present and their cover-abundance were recorded using the Domin score. Twenty-eight vegetation communities were then described from multivariate analyses of the relevés taken across the 22 turloughs. These turlough plant community species and community identification keys were used in the field in 2008 for identifying and mapping the vegetation types in the four turloughs focussed on in this study (Blackrock, Coy, Caherglassaun and Garryland), which found 15 vegetation communities in at least one of these four turloughs. Trimble handheld GPS devices (Nomad and GeoExplorer models) were used for field recording and loaded with georeferenced images of all available aerial photos and Ordnance Survey (OS) maps. Boundaries between vegetation types were recorded along the estimated centre of the observed zone of transition between two types of community. They were recorded along the putative boundary at intervals of 5, 10, 20 or 30 m, depending on particular local topography and spatial configuration of vegetation. Digital photographs were also taken at ground level in various locations to record the general topography, vegetation, water levels and various other features of the turlough as surveyed on the day; these were subsequently used to help improve the confidence of digital spatial representations of vegetation.

2.2.2 | Vegetation community maps

ArcGIS® software was used to generate digital vegetation maps using GPS data recorded in the field. Differentially-corrected data files were exported as ESRI shapefiles using GPS Pathfinder Office software. Point shapefiles were generated during export and loaded into ArcMap® and a map file for the turlough was saved. Polyline shapefiles were created and added to the map to represent vegetation boundaries. OS vector maps were loaded to the map and the OS vector map lines within the turlough boundary which were needed to create vegetation polygons were copied to the previously created land parcel polyline dataset. Vegetation boundary polylines were then drawn to link all boundary points. Separate polygons were then each attributed to a vegetation type, using the information recorded in vegetation identification points or via a deductive process using field data and information from aerial and ground-level photographs.

2.2.3 | Remote sensing

A more recent assessment of the vegetation distribution across the turloughs was made using remote sensing methods from Sentinel-2 (S2) imagery captured on 30th June 2018. All the S2 bands were resampled to 10 m, and additional normalised difference vegetation index (NDVI), enhanced vegetation index (EVI), and normalised difference water index (NDWI) were calculated. The initial pixel-based classification was done using an ensemble Bagged Tree classifier. The pixel-based classification only takes into account the spectral information, and not the spatial relation that exists between the vegetation communities. Therefore, using the mapping vegetation community (MVC) algorithm described in Bhatnagar et al. (2020), the classification was extended to area-based segmentation. A final classification accuracy of approximately 85% was achieved (see Bhatnagar et al., 2020).

2.3 | Hydrology

2.3.1 | Field data (2007 to 2019)

A series of linked research projects have been carried out on the lowland karst network to investigate the hydrology of the wetlands (Gill et al., 2013a; Naughton et al., 2012), flood alleviation (Morrisey et al., 2020; Naughton et al., 2017), nutrient and other chemical fluxes through the turloughs (Gill et al., 2018; McCormack et al., 2016) and as well as freshwater discharges into the Atlantic Ocean at Kinvara bay (McCormack et al., 2014). For this, the following hydrometeorological data have been collected with more detail given in the above references.

- Meteorology

Rainfall data were collected from two tipping bucket rain gauges positioned at 70 mAOD and 150 m AOD in the catchment to assess the spatial distribution of rainfall. These data were then related to the Gort Derrybrien gauge operated by the national Irish meteorological service, Met Eireann, which provided a longer dataset, in order to fill missing gaps.

- Water level
Continuous water level data were collected using pressure transducers with in-built dataloggers at the base of the four turloughs (in the lowland karst catchment between 2007 to 2018). These were recovered in the dry period each summer to be downloaded, with the data compensated against the variations in atmospheric pressure over the flooded period.

- **Topography**

  Topographical data were derived from LiDAR mapping data for the catchment with a grid spacing of 2 m and a vertical accuracy of ±0.15 m. Where such data were not available, further topographical survey data were obtained from manual surveys carried out using a Trimble 4700 GPS system with a minimum accuracy of 0.01 m (horizontal and vertical direction). These topographical data were combined with the available LiDAR data in ArcGIS and a new integrated DEM was constructed using the Kriging method with a 2-m grid spacing. Depth-area-volume relationships for the turloughs and linked floodplains were derived from these DEMs, as required for the hydraulic model (see Section 2.3.2).

### 2.3.2 Hydraulic model

A detailed semi-distributed hydraulic model of the karst network has been developed over many years for various different applications (ecohydrology, flood alleviation, etc.) and was used to fill in any gaps in the turlough water level data, but also to extend the turlough water time series back to 1989 when local rain monitoring started in the region. The model is built using the InfoWorks CS drainage software due to its ability to model the hydraulics of the karst conduit network in both open channel and pressurised pipe flow. The model is described in detail in Gill et al. (2013a, 2013b) with the most recent update in Morrissey et al. (2020). The groundwater-surface water turlough dynamics were modelled as storage ponds in the software which were configured with the same depth-volume characteristics as the surface topography, as derived from the detailed DEM, thereby giving an accurate profile of the exact depth across each turlough at any point in time on an hourly basis. Examples of the correlation of the model for the four turloughs against the water level field data for the four turloughs are shown in Supplemental Information, Figure S2 (as well as in the above references), all showing excellent model performance with Nash Sutcliffe and Kling Gupta efficiencies (NSE and KGEs) of >0.9. This model has been used to extend the time series of the hydrological monitoring back to 1988 to yield 28 years of continuous water fluctuation data in the four turloughs.

### 2.4 Ecohydrology metrics

The key hydrological variables evaluated are depth, duration, frequency and timing of flooding. The model was used to define the hydrological conditions at all times across a 28-year period on the turloughs. Whilst the model produced data at an hourly time step, this was changed to an average daily time step (due to the slow movement of the water levels) in order to make the data processing quicker. From these water level time series, the following metrics were derived for any point across each turlough over a hydrological year (which is taken from the beginning of October to the end of September in the Irish northern maritime temperate climate).

- **Flood duration**—the number of days in a hydrological year that any pixel area was covered in water, averaged out spatially across each community vegetation type.
- **Flood depth**—the average depth of water per day that any pixel area was covered in water. This was then averaged out spatially across the same vegetation type and expressed as an average value over a hydrological year.
- **Flood frequency**—the number of flood events experienced per vegetation community per hydrological year. A flood event was considered for each pixel if the area went from being dry to flooded and then remained flooded for at least 20 days. All the values for the pixels belonging to the same community were then averaged to form the flood frequency metric.
- **Flood timing (in relation to the start of growing season)**—instead of using a date, or Julian day of the year, proxy variables of global radiation and air temperature at the time when the flooding stops and the vegetation were revealed for the first time in the hydrological year, were determined as more causative key vegetation variables. These were derived as the average values over a 30-day period (10 days before and 20 days after the pixels were first revealed).

The vegetation communities from the turlough maps (Section 2.2.2) were defined at a spatial resolution depending on the turlough size: Caherglassaun being the biggest was surveyed at 4 × 4 m, Blackrock at 3.5 × 3.5 m, Coy and Garryland being smaller at 2.5 × 2.5 m. For each of the defined communities, a binary image (2-D) was extracted from the vegetation survey map such that the spatial relationship between the points belonging to the same community was not lost. This spatial relationship is vital to understand the amount and duration of flooding that occurs in the region. For example, a community such as Lolium grassland can stretch across wide areas of the turlough, with some part of the community in flood, and some part which is seldom flooded. The averaged value for the whole community was then measured using ‘mean2’ function, that is, 2-D mean in MATLAB v.2019b (2019). This was repeated for all the communities across all the turloughs. Then, the metrics were combined and compared for common communities. The field vegetation surveyed map created in 2008 was used as the reference throughout the study. The satellite-based study carried out subsequently by Bhatnagar et al. (2020) in 2018 was then used to assess whether there has been any significant change or shift in the turlough communities over the past 10 years (2008 to 2018).
3 | RESULTS

3.1 | Vegetation distribution

The vegetation maps derived in 2008 for the four turloughs are shown in Figure 1, showing the spatial distribution of the 15 different vegetation communities. For comparison, the vegetation maps determined using remote sensing S2 imagery from June 2018 are shown in Figure 2, revealing a close match to the field survey maps.

The topography of the four turloughs is shown in Figure 3 (and Figure S3 in relation to their relative altitudes), showing the steeper sides to Blackrock turlough, compared to the much flatter topography of Garryland turlough. Visually, some correlation can be seen between the topography (Figure 3) and vegetation communities (Figure 1), indicating the influence of flooding on vegetation location in these basins.

3.2 | Hydrological characteristics

Figure 4 shows a sample 3-year period to show the type of flood dynamics of the four turloughs over time. There was a particularly high level of flooding in November 2009, which caused much disruption in the area. In general, the turloughs exhibit one main flood event across the winter with small fluctuations. However, the 2008/2009 hydrological year showed three moderate inundations that winter as well as some minor flood events in the summer 2009 period.

The average depth-duration plots for the four turloughs over the last 28 years is shown in Figure 5. This shows the much more flashy flooding dynamics of Blackrock turlough, which is the steepest in topography as well as being located at the start of the hydraulic network and therefore receiving a less damped hydraulic signal from the allogenic river inputs compared to the turloughs lower down; in comparison, Garryland and Caherglassaun at the lower end of the system show very similar curves. A comparison between the depth-duration profiles for 1997 to 2007 versus 2008 to 2018 is also shown in Figure 5b which is discussed later in Section 3.8 to assess whether any changes in vegetation over the period between the field survey and satellite survey can be linked to changes in hydrological regime between those two periods.

The annual flood duration and flood depth spatial profile across the 28 years is shown for Blackrock turlough in Figure 6 (and in

![Figure 2](null) Vegetation communities for the four turloughs mapped using remote sensing from Sentinel-2 in 2018: (a) Blackrock, (b) coy, (c) Caherglassaun and (d) Garryland
FIGURE 3  Turlough topographies in relation to their lowest point (a) Blackrock (b) coy, (c) Caherglassaun and (d) Garryland (see figure S3 for actual topographies referenced to m AOD)

FIGURE 4  Time series of simulated water levels for the four turloughs between 2007 to 2010
Figures S4–S6 for the other three turloughs) revealing the difference between hydrological years. In particular years 1989/1990, 1991, 1994, 1995, 2009 and 2015/2016 can be seen to have much longer flood durations than many of the other years (and these were years when there was widely reported significant flood disruption in the winters in this area) compared to much drier years of 1997, 2006, etc.

3.3 | Ecohydrology—Flood duration

The flood duration statistics across the 28 years averaged for some of the key communities which were present in all or at least three out of four turloughs are shown in Figure 7 (with the rest in Figure S7). This shows wide fluctuations between different years but also reveals differences between communities. The variations in durations amongst and between the communities are shown more clearly in the boxplots in Figure 10a. A Kruskal-Wallis one-way ANOVA test was carried out to test for differences between the durations for the different community groups which reveals significant differences between several of the communities (see Table S1a) as well as showing that the different communities can be summarised into three broad groupings each exhibiting similar flood duration characteristics: with Eleocharis acicularis, different from a group of Agrostis stolonifera–Ranunculus repens, Poa annua–Plantago major and Potentilla anserina–Potentilla reptans communities, which were then significantly different all the other communities located higher up the turlough basin sides.

3.4 | Ecohydrology—Flood depth

The flood depth statistics across the 28 years averaged for each of the key communities which were present in all or at least three out of four turloughs are shown in Figure 8 (with the rest in Figure S8). This again shows wide fluctuations between different years: Flood depth is very variable for the ‘deeper’ communities, whilst much less so for those communities at the upper end of elevation gradient. The variations in flood depths amongst and between the communities are shown in the boxplots in Figure 10b also. Statistical analysis to test for differences between the flood depths for the different community groups reveals less significant differences between many of the communities compared to the flood duration (see Table S1b), apparently separating the communities into two main groupings.

3.5 | Ecohydrology—Flood timing

As described in Section 2.4, the timing of when the flood receded (and potential start of the growing season for each vegetation community) was determined by looking at the mean global radiation and mean temperature taking an average of the 30 days (10 days before it was first revealed and 20 days after). The global radiation statistics across the 28 years averaged for each of the key communities when coming out of flood, generally in the springtime, are shown in Figure 9 (with Figure S9 showing the equivalent air temperature relationships). Figure S10 also shows the air temperature and global radiation spatial
plots for this turlough. This shows the higher elevation communities are exposed earlier in the year when the mean solar radiation (and average air temperature) is lower, as expected. The variations in average global radiation when first exposed from the flood waters amongst and between the communities are shown in the boxplots in Figure 10c also. This can be compared to the average annual profile of global radiation and air temperature for the area as shown in Figure S11 to enable comparison with the timing during the year of flood recession. The Kruskal–Wallis one-way ANOVA test applied to the global radiation distributions for the different community groups which again reveals less significant differences between many of the communities compared to the flood duration (see Table S1c), apparently separating the communities into two main groupings. The global radiation and the temperature have a direct relationship showing a correlation of 99% from regression analysis (see Section 3.7), as expected.

3.6 | Flood frequency

Table 1 shows the average flood frequency experienced between the different vegetation communities across all turloughs, that is, how many times a vegetation community went from being dry to flooded and stayed flooded for at least 20 days. The variations in flood
frequency per year amongst and between the communities are shown in the boxplots in Figure 10d also. As can been seen most communities only experience a maximum of two flood inundations per year. Statistical tests showed very few significant differences between flood frequency for the different communities (see Table S1d), indicating that flood frequency seems to be much less important than flood duration with respect to differentiating between the different vegetation communities in these turloughs.
Ecohydrological metrics

A summary of the flood duration, depth, global radiation when coming out of flood and flood frequency metrics across all four turloughs for the different vegetation communities is presented in Table 1 (see Table S2 for a full breakdown per turlough), and in Figure 10. The communities have been ordered according to the flood duration on the graphs and tables ranging from *E. acicularis* (experiencing the most flooded conditions in a year) to the flooded pavement community (experiencing the least amount of flooding).

As the location of the communities stays more or less consistent throughout the years (see Section 3.8), regression analysis has been used to determine the relationship between the four key variables depth, duration, temperature and global radiation with respect to the vegetation type (see Table S3). A variable regression technique was used such that the best fit between the variables can be found. From Table S3 it can be seen that most variables are related linearly. However, variables such as duration-global radiation, and duration-temperature exhibit exponential relationships. The strength of the regression analysis is given by the $R^2$ value. The analysis shows that all four variables are highly correlated, with the highest correlation between depth-duration, and global radiation-temperature. This therefore suggests that just one or two variables can be used to form metrics to frame the required ecohydrological condition of the turloughs.

A hierarchical spatial clustering analysis was then used on the 28-year dataset on the four turloughs on the key ecohydrological variables discussed heretofore (i.e., flooding depth, duration, temperature and global radiation) to identify clusters of similar hydrological years. The aim was to refine the ecohydrological metrics to what the different vegetation communities experienced in what might be considered to be more ‘normal’ years. The hierarchical clustering enables the clusters to be formed based on relative distance. For this study, the hierarchical clustering was done using the formation of ‘Dendrogram’ using Matlab v.2019b (MATLAB, 2019b). The $y$ axis of the dendrogram represents the distance (in this case, Euclidean distance) or dissimilarity between the variables and the $x$-axis displays all the years. The variables here are the depth, duration, global radiation and frequency for all 28 years. The clusters are formed using agglomerative hierarchical cluster tree (Day & Edelsbrunner, 1984). This was carried out using centroid linkage in Matlab v.2019b (MATLAB 2019), which is the distance (Euclidean) between the clusters (two or more). It is represented by Equation 1.

\[
\text{Dissimilarity} = \left| \frac{\text{cluster}_1 - \text{cluster}_2}{\text{cluster}_1} \right|
\]

where dissimilarity is the Euclidean distance between the clusters, and $\text{cluster}$ represents the mean of the cluster.

In order to extract clusters from the linkage tree diagram, a threshold-cut-off line was drawn equal to the median of the dissimilarity (see red line on Figure 11). A total of 8 clusters were formed consisting of all 28 years: cluster 1 (4, 13, 15, 5, 7, 9, 17, 3, 19, 26 and 28); cluster 2 (1, 25, 6, 14, 10, 21 and 20); cluster 3 (11, 12 and 27); cluster 4 (2, 16, 23 and 24); cluster 5 (6); cluster 7 (18 and 22). Cluster 1, with the highest number of years associated, appeared to pick up the years with least amount of extreme fluctuations and so was used to refine the ecohydrological metrics (flood depth, duration, etc.) for all the vegetation communities across all four turloughs again (Table 2). In general, the refined statistics reveal slightly tighter range envelopes (for flood duration, temperature and global radiation) as might be expected, although the flood depth ranges for the different vegetation have extended somewhat, possibly indicating a more unimodal consistent flood peaks in the winter for these hydrological years.

Change in communities over 10-year period

As detailed in Section 2.4, the field surveys were carried out in 2008 and then 10 years later another map of the turloughs was produced using the Sentinel-2 satellite approach (Figure 2) using the mapping vegetation communities (MVC) algorithm presented in Bhatnagar...
et al. (2020). A comparison between these two maps (and the spatial coverage of the different vegetation therein) was made to assess if there have been any shifts in vegetation community spatial distribution which could then be linked to possible changes in hydrological regimes over that 10-year period. The depth-duration plots comparing the 10 year period up to the 2008 vegetation survey (1998 to 2008) and from then up to the satellite survey in 2018 (2008 to 2018) are shown in Figure 5b which shows that there does appear to have been more severe/longer lasting flooding over the past 10 years on all turloughs except Coy turlough that is known to have a higher level overflow in the karst system which thereby maintains fairly uniform peak flood depths, which are unresponsive to additional rainfall. The 2008 to 2018 period has been marked by more extreme rainfall and weather extremities, particularly 2009 and 2015/2016 with two exceptional periods of flooding.

A visual comparison of Figures 1 and 2, set out in Table 3, shows that the majority of the communities appear to stay intact, such as Lolium grassland, P. anserina–P. reptans, etc.; even the smaller spatial extent communities like E. acicularis have been identified well in all four turloughs. Hence, this confirms the assumption that has been made in order to derive the ecohydrological metrics, that the vegetation communities essentially stay intact, with the 2008 field derived vegetation map used as the reference throughout.

Table S4 shows all the different hydrological metric data plotted for the two different time periods whilst Table 3 shows the Jaccard similarity and percentage change in the area of all the communities across turloughs between the vegetation survey (2008) and Sentinel-2 survey (2018). It should be noted that the S2 bands were resampled to 10 m; therefore, the spatial resolution of the satellite-derived maps is 10 m. Given the challenge with more coarse spatial resolution for the satellite images, 15+ communities have been identified. Classification accuracies of 85% for Blackrock, 89.5% for Caherglassaun, 90.2% for Coy and 91.8% for Garryland were achieved, making an average accuracy for classification of turloughs to be 89%.

As can be seen from Table 3, the main trend seems to be a reduction in areas of the communities located higher up the turlough slopes with some increases in the wetter communities in Caherglassaun, Coy and to a lesser extent Garryland. This perhaps corroborates the increase in more flooded conditions over the past 10 years. However, communities like A. stolonifera–Glyceria fluitans were not well identified using S2 imagery due to their small size, which is thought to be the reason for such low similarity and high percentage areal change. Other communities like limestone grassland and flooded pavement were also not identified well. This mainly depends on the condition of the community at the time when the satellite images were captured. Other than that, most of the key vegetation communities show 20% of the change in the area. The main question is whether this is an actual change in the spatial coverage of the communities or whether it is more a function of the satellite resolution and pixel mixing.

4 | DISCUSSION

Turlough vegetation generally exhibits a readily observed zonation, from the unaffected terrestrial communities outside the turlough
| Vegetation community                  | Depth (m) | Duration (months) | Frequency (per year) | Global radiation (J/m²) |
|--------------------------------------|-----------|-------------------|----------------------|-------------------------|
|                                      | MIN  | MEAN  | MAX  | RANGE    | MIN  | MEAN  | MAX  | RANGE    | MIN  | MEAN  | MAX  | RANGE    |
| Open water                           | 0.23 | 1.31  | 2.41 | [0.65–1.24] | 5    | 6.62  | 7.99 | [6.20–7.91] | 1.00 | 1.55  | 3.5  | [1.00–1.95] |
| Eleocharis acicularis                 | 0.56 | 1.7   | 3.1  | [0.49–1.26] | 5.33 | 6.63  | 7.25 | [6.26–7.38] | 1.00 | 1.39  | 2.32 | [1.25–1.70] |
| Agrostis stolonifera-Ranunculus repens | 0.06 | 0.95  | 1.86 | [0.40–0.95] | 2.84 | 4.58  | 5.61 | [4.36–5.31] | 1.00 | 1.4   | 2.81 | [1.10–1.60] |
| Poa annua–Pantago major               | 0.01 | 0.65  | 1.7  | [0.26–0.80] | 2.48 | 4.25  | 5.48 | [4.36–5.31] | 1.00 | 1.18  | 2.81 | [1.10–1.60] |
| Potentilla anserina–Potentilla erecta | 0.5  | 1.07  | 2.01 | [0.78–1.70] | 2.79 | 4.14  | 5.29 | [3.90–5.00] | 1.04 | 1.4   | 3.19 | [1.18–1.60] |
| Filipendula ulmaria–Potentilla erecta–Viola sp | 0.16 | 0.48  | 0.91 | [0.42–0.71] | 1.98 | 3.2   | 4.45 | [2.80–4.05] | 1.00 | 1.2   | 2.66 | [1.15–1.50] |
| Carex nigra–Carex panicea            | 0.04 | 0.2   | 0.42 | [0.04–0.42] | 1.13 | 2.85  | 4.44 | [1.76–3.85] | 1.00 | 1.14  | 1.97 | [1.05–1.45] |
| Woodland                             | 0.28 | 0.57  | 1.14 | [0.47–0.77] | 1.5  | 2.61  | 3.79 | [2.30–3.20] | 1.00 | 1.2   | 2.16 | [1.09–1.45] |
| Scrub                                | 0.32 | 0.51  | 0.76 | [0.39–0.62] | 1.51 | 2.57  | 3.74 | [2.25–3.18] | 1.00 | 1.14  | 1.9  | [1.09–1.42] |
| Agrostis stolonifera–Pontetilla anserina–Festuca | 0.21 | 0.71  | 1.24 | [0.65–1.24] | 1.42 | 2.27  | 3.74 | [1.83–3.00] | 1.00 | 1.29  | 2.05 | [1.10–1.41] |
| Limestone grassland                  | 0.34 | 0.52  | 0.73 | [0.40–0.73] | 1.59 | 2.28  | 3.05 | [1.75–2.91] | 1.00 | 1.06  | 1.6  | [1.00–1.25] |
| Carex nigra–Ranunculus flammula      | 0.16 | 0.78  | 1.6  | [0.26–1.40] | 1    | 2.07  | 3.99 | [1.70–3.25] | 1.00 | 1.23  | 2.15 | [1.00–1.20] |
| Lolium grassland                     | 0.2  | 0.54  | 1    | [0.31–0.59] | 1.18 | 1.97  | 3.31 | [1.95–2.59] | 1.00 | 1.2   | 1.98 | [1.02–1.24] |
| Agrostis stolonifera–Glyceria fluitans | 0.13 | 0.76  | 1.46 | [0.55–1.20] | 1    | 1.91  | 3.78 | [1.44–2.80] | 1.00 | 1.22  | 2.89 | [1.00–1.35] |
| Flooded pavement                     | 0.43 | 0.43  | 0.43 | [0.43–0.43] | 1    | 1.78  | 3.14 | [1.31–2.30] | 1.00 | 1.78  | 3.14 | [1.00–1.01] |

**TABLE 1** Summary table of ecohydrological metrics for different communities
boundary to the communities at the bottom of the flooding gradient which experience the longest and deepest inundation. Four turloughs in a linked conduit-dominated karst system have been evaluated in terms of the spatial distribution of different vegetation communities with respect to their hydrological conditions experienced in such fluctuating wetland systems. The four ephemeral wetlands were chosen as they had similar water quality, soils and land use, and so the changes in hydrology parameters should provide the key variables differentiating between the different communities.

It is known that some species can tolerate a range of soil moisture/flooding, and are usually found almost throughout such turlough basins, for example *A. stolonifera*, *P. anserina* and *R. repens*. Whilst others have a more restricted range due to stricter habitat requirements, such as aquatic species which occur only in permanent water bodies.

Located in the lower parts of the turloughs is the *E. acicularis* community. This is not a common community across Ireland, with restricted distribution in a limited number of turloughs making it of very high conservation value. It forms on relatively small patches on drying mud near water, usually at the very base of the turlough. The results here show that it typically experiences (and therefore requires) 6 to 7 months of inundation per year at depths of 0.75 to 1.85 m.

The next set of communities all experiencing similar average flood durations between 3 to 5 months per year on average are the *A. stolonifera–R. repens*, *P. annua–P. major* and *P. anserina–P. reptans* communities. The *A. stolonifera–R. repens* community is found widespread across different turloughs. The community is relatively short (~25 cm) forb-dominated sward. This community was found in the upper to middle zones of turlough basins in the Waldren et al. (2015) project across a wide set of turloughs, with a mean Ellenberg Wetness value 6.7, indicative of damp but not wet soils.

The *P. anserina–P. reptans* community is a herb-dominated community, with a mean sward height of ~10 cm and is usually located in the middle to the bottom of the flooding gradient with mean Ellenberg Wetness value of 6.1, indicative of damp sites. The metrics in these four turloughs show that both communities appear to exist in locations with very similar ranges of flood duration and depth to the *A. stolonifera–R. repens* community. The *P. annua–P. major* community was generally found in areas where the integrity of the soil had been damaged through poaching, allowing the large proportion of ruderal species found in this type to colonise. The species list consists of perennials that can rapidly colonise from the surrounding grassland. This community was found on trampled ground in the upper reaches of the turlough basins as suggested by the mean Ellenberg Wetness value of 5.9.

Next in order of flood duration is the *Filipendula ulmaria–Potentilla erecta–Viola* community which is a herb-rich community occurring in the middle of the flooding gradient. This was found to have a mean Ellenberg Wetness score of 6.2 across all turloughs, again indicating that it occurs in damp sites. It is an important community is as it contains *Viola persicaria* and hybrids, an International Union for the Conservation of Nature and Natural Resources (IUCN) Red List species.

There are then several different communities found in the middle to upper zones of the turloughs which are hard to separate using the ecohydrological variables: These include the *Carex nigra–Carex panicera*, *C. nigra–Ranunculus flammula*, *Woodland*, *Scrub*, *A. stolonifera–P. anserina–Festuca rubra*, *Limestone grassland*, *Lolium grassland* and *A. stolonifera–G. fluitans* communities. Across a wider set of turloughs these were all found to exist in areas with lower Ellenberg Wetness indices from 5 to 6 indicating damp but not constantly wet, substate, with the exception of the *A. stolonifera–G.*

![FIGURE 11 Dendrogram using four parameters (depth, duration, global radiation and frequency) to form clusters of 28 years. Horizontal line is the median dissimilarity amongst samples](image-url)
| Vegetation community                                      | Depth (m) | Duration (months) | Frequency (per year) | Global radiation (J/m²) |
|-----------------------------------------------------------|-----------|------------------|----------------------|-------------------------|
|                                                           | MIN | MEAN | MAX | RANGE | MIN | MEAN | MAX | RANGE | MIN | MEAN | MAX | RANGE |
| Open water                                                | 0.23 | 1.97 | 3.31 | [0.77–2.50] | 5.11 | 6.92 | 9.67 | [6.40–7.19] | 1.00 | 1.57 | 3.49 | [1.00–1.87] |
| Eleocharis acicularis                                     | 0.56 | 1.52 | 2.70 | [0.75–1.85] | 5.00 | 7.01 | 9.46 | [6.12–6.99] | 1.00 | 1.54 | 3.35 | [1.02–1.70] |
| Agrostis stolonifera-Ranunculus repens                   | 0.06 | 1.24 | 2.19 | [0.55–1.62] | 2.84 | 4.57 | 5.83 | [4.10–4.87] | 1.00 | 1.40 | 2.42 | [1.00–1.58] |
| Poa annua–Pantago major                                   | 0.01 | 0.71 | 1.70 | [0.50–1.22] | 2.84 | 4.57 | 5.83 | [4.10–4.87] | 1.00 | 1.40 | 2.42 | [1.00–1.58] |
| Potentilla anserina–Potentilla reptans                    | 0.45 | 1.21 | 2.11 | [0.71–1.50] | 2.79 | 4.48 | 5.71 | [3.25–4.88] | 1.04 | 1.39 | 2.16 | [1.02–1.52] |
| Filipendula ulmaria–Potentilla erecta–Viola sp           | 0.16 | 0.52 | 0.98 | [0.44–0.80] | 1.98 | 3.36 | 4.90 | [2.18–4.04] | 1.00 | 1.29 | 1.95 | [1.00–1.21] |
| Carex nigra–Carex panicea                                 | 0.04 | 0.18 | 0.42 | [0.22–0.47] | 1.00 | 2.82 | 5.00 | [1.55–4.03] | 1.00 | 1.27 | 2.31 | [1.00–1.21] |
| Woodland                                                  | 0.26 | 0.59 | 1.13 | [0.47–0.81] | 1.50 | 2.74 | 4.40 | [1.97–3.10] | 1.00 | 1.20 | 1.76 | [1.00–1.10] |
| Scrub                                                     | 0.32 | 0.60 | 0.96 | [0.49–0.65] | 1.51 | 2.68 | 4.37 | [1.91–3.15] | 1.00 | 1.20 | 1.97 | [1.01–1.12] |
| Agrostis stolonifera-Potentilla anserina–Festuca         | 0.21 | 0.86 | 1.46 | [0.54–0.85] | 1.42 | 2.50 | 4.58 | [1.60–2.72] | 1.00 | 1.18 | 1.59 | [1.04–1.10] |
| Limestone grassland                                       | 0.06 | 0.42 | 0.73 | [0.50–0.63] | 1.20 | 2.37 | 3.95 | [1.76–2.80] | 1.00 | 1.14 | 1.90 | [1.00–1.14] |
| Carex nigra–Ranunculus flammula                          | 0.16 | 0.84 | 1.68 | [0.48–1.36] | 1.00 | 2.40 | 4.36 | [1.03–2.36] | 1.00 | 1.22 | 2.89 | [1.00–1.40] |
| Lolium grassland                                          | 0.18 | 0.61 | 1.00 | [0.20–0.75] | 1.18 | 2.17 | 4.05 | [1.34–2.33] | 1.00 | 1.14 | 1.63 | [1.03–1.10] |
| Agrostis stolonifera–Glyceria fluitans                   | 0.13 | 0.78 | 1.46 | [0.36–1.13] | 1.00 | 2.20 | 4.54 | [1.00–2.00] | 1.00 | 1.21 | 2.66 | [1.00–1.50] |
| Flooded pavement                                          | 0.05 | 0.24 | 0.43 | [0.43–0.43] | 1.00 | 1.88 | 3.88 | [1.15–2.46] | 1.00 | 1.06 | 1.60 | [1.00–1.20] |
TABLE 3  Jaccard similarity and change in the area (m²) of all the communities across turloughs from vegetation survey (2008) to Sentinel-2 survey (2017)

| Vegetation community                      | BL  | CH  | CY  | GL  |
|------------------------------------------|-----|-----|-----|-----|
|                                          | Jaccard | △ area | Jaccard | △ area | Jaccard | △ area | Jaccard | △ area |
| Open water                               | NaN | NaN | 0.86 | 173  | 0.94  | 134    | NaN     | NaN    |
| Eleocharis acicularis                    | 0.04 | 0.64 | 24   | 0.7   | 8     | 0.52   | NaN     | NaN    |
| Potentilla anserina—Potentilla reptans   | 0.74 | 0.69 | 331  | 0.25  | 130   | 0.74   | 0.32    | 0.8    |
| Agrostis stolonifera—Ranunculus repens  | 0.12 | NaN | NaN  | 0.74  | NaN   | 0.4    | NaN     | NaN    |
| Poa annua—Pantano major                  | 0.23 | 0.55 | 7    | NaN   | NaN   | NaN    | NaN     | NaN    |
| Agrostis stolonifera—Potentilla anserina—Festuca | 0.32 | 0.49 | 223  | 0.51  | 138   | NaN    | NaN     | NaN    |
| Filipendula ulmaria—Potentilla erecta—Viola sp | 0.18 | 0.51 | 473  | 0.7   | 204   | 0.4    | NaN     | NaN    |
| Agrostis stolonifera—Glyceria fluitans  | 0.014 | NaN  | NaN  | NaN   | NaN   | NaN    | NaN     | NaN    |
| Carex nigra—Ranunculus flammula         | 0.41 | NaN | NaN  | NaN   | NaN   | NaN    | NaN     | NaN    |
| Carex nigra—Carex panicea               | NaN  | NaN  | NaN  | NaN   | NaN   | NaN    | NaN     | 0.49   |
| Lolium grassland                         | 0.39 | 0.58 | 322  | 0.55  | 138   | NaN    | NaN     | NaN    |
| Woodland                                 | 0.44 | 0.44 | 1011 | 0.56  | 341   | 0.28   | NaN     | NaN    |
| Scrub                                    | 0.25 | 0.44 | 366  | 0.54  | 155   | NaN    | NaN     | NaN    |
| Limestone grassland                      | NaN  | NaN  | 0.37 | NaN   | NaN   | NaN    | NaN     | NaN    |
| Flooded pavement                         | NaN  | NaN  | 0.28 | NaN   | NaN   | NaN    | NaN     | NaN    |

Note: Apparent increases in area are shaded green, decreases in area shaded red. NaN = not applicable to this site.

"fluitans and C. nigra—R. flammula communities which more broadly seem to be located at the base or near the bottom of the turloughs in areas that are likely to retain some standing water throughout the season, with mean Ellenberg value for Wetness is ~8+. This did not seem to be the case in this selection of turloughs, although the communities were only found in very localised areas on the upper slopes of turloughs which perhaps could be near springs, creating locally wet conditions in the soils. Most of these communities are typically found in grazed areas.

Finally, there is the flooded pavement community that occurs on exposed limestone pavement at the upper fringes of turloughs where open limestone pavement abuts the flood zone. The mean Ellenberg Wetness value of 5.7 is indicative of slightly damp soils. This community is of high conservation value, especially as habitat for Potentilla fruticosa, a species which is rare throughout the Britain and Ireland and largely restricted to the fringes of some turloughs in Ireland. The results from these four turloughs suggest that they survive in areas that are only flooded from 1 to 2 months per year.

The findings presented here concur with other studies on turloughs which have found that one of the main factors affecting turlough plant community composition is hydrological regime (e.g., Moran et al., 2008; Praeger, 1932; Regan et al., 2007). The Waldren et al. (2015) project on a wider range of turloughs concluded that duration of flooding and TP in the flood water of turloughs were the environmental variables most closely associated with the distribution of vegetation communities and vascular plant species and are therefore likely to be the most important ecological drivers of turlough vegetation. Given that these four linked turloughs analysed in this paper all had similar levels of phosphorus (as well as soil type and land use/grazing), this analysis does indeed show that flood duration appears to be the key differentiating hydrological variable. There appears to be little to separate the communities with respect to flood frequency—all usually experiencing a regime of one to two floods per year. Equally the global radiation (and air temperature) of the time of year when the floods start to abate does not reveal such large differences between the communities on average, indicating that perhaps this is not such a key parameter to the vegetation within the constraints of the hydrological regime in these four turloughs. It should be noted that Blackrock, Coy, Caherglassaun and Garryland turloughs had been shown to have similar residence times, termed aggregation periods, during the Waldren et al. (2015) study (with monitoring carried out over 2007/2008) of 38 to 67 days, which were much shorter than those times calculated for many of the other turloughs monitored across Ireland during that study. The findings also concur with studies on other wetlands. For example, Wheeler and Proctor (2000), in a study of ecological gradients and floristic variation of north-west European mires, found that most of the variation was accounted for by just three ecological gradients: pH, nutrient availability and the hydrological gradient. Similar findings were reported by De Becker et al. (1999), who concluded that hydrological regime and soil type/management were the main drivers of vegetation community change in a floodplain mire.

Finally, this research has attempted to demonstrate how this overall methodology could be augmented with the use of remote sensing data in order to provide a more regular way of surveying the vegetation communities, thereby allowing any changes to be picked up and attributed to changes in hydrological regime (whether anthropogenic or not). This could be used as an effective way of
monitoring such groundwater dependent terrestrial ecosystems (GWDTEs). Whilst the satellite images did seem to have picked up some changes in the spatial coverage of the communities which could possibly be linked to slightly wetter conditions over the past 10 years, it is difficult to tell whether this is more an artefact of the accuracy of the relatively low resolution of the Sentinel-2 satellite for this type of image analysis, or whether it is actually linked to real changes in spatial distribution on the ground. However, in the future ever more high-resolution satellite data and/or with the use of drone/satellite combination to improve accuracies (Bhatnagar et al., 2021), such an approach should yield further insights into the hydrological metrics affecting different vegetation communities.

This research has demonstrated the sensitivity of the habitats to the influence of hydrology. The variations observed in the flood regime exert a strong selection pressure on biota inhabiting the turlough basins, influencing the distribution of species and the development and succession of communities. Hence, if the natural frequency-duration of flooding is significantly changed, impacts to the distribution of such vegetation communities are likely to result. The main pressures affecting the hydrological function of turloughs are likely to be drainage and water abstraction. Drainage control within the turloughs will have the most direct and potentially severe impact if not carefully considered. For example, in this area of south Galway, serious flooding has occurred in recent years which has led to the development of a flood alleviation scheme formulated on a series of overflow channels connecting the network of turloughs in order to move water to the sea more quickly (Morrissey et al., 2020). The impacts of drainage such as digging drainage channels through the turloughs or, conversely the blocking of karst features (estavelles, swallow holes) in attempts to increase the availability of agricultural land, will have proportionally greater effects towards the deeper part of the turlough basin. Hence, care needs to be taken with any action that reduces the connectivity of a turlough with the karst groundwater system. Which can lead to reduced rate of turlough filling but will also slow down its emptying. Drainage within the wider zone of groundwater contribution for turloughs (i.e., the supporting karst catchment area) is likely to have a more limited effect, but still needs to be evaluated. Such alterations to the flows in/out of a turlough will change the flooding duration frequency relationship and, therefore, in the long term, the distribution of habitats. Equally, the long-term impact of climate change (to rainfall intensities and frequency as well as evapotranspiration rates) on the hydrology of such karst catchments and these linked GWDTEs needs to be established.

5 | CONCLUSIONS

This paper has evaluated the ecohydrology of intermittent wetlands, using the turloughs found in karst areas (and mainly found in Ireland) being extreme exemplars of such ephemeral flooding environments. A methodology of how to derive different ecohydrological variables associated with the spatial distribution of different vegetation communities has been outlined. Such metrics can then be evaluated alongside a wider mix of variables such as water quality (particularly nutrients), soil type and land-use in order to understand the habitat requirements for such plant communities and their associated ecological systems. Such insights into ecosystem functioning, particularly the sensitivity of vegetation to the flood regime, can be used for the development of ecological status assessment strategies and provide metrics against which potential changes to the hydrological regime due to, for example, proposed drainage schemes or future climate changes can be evaluated.

The analysis on these four turloughs on the same karst network has revealed distinct differences between vegetation communities, from E. acicularis found at the base of the turloughs typically experiencing 6 to 7 months of inundation per year compared to the limestone pavement community at the top fringes of the turloughs only flooded from 1 to 2 months per year.

Finally, an approach that uses remotely sensed data to provide an assessment of whether there have been changes in the spatial distribution of the communities has been presented. A change in the spatial coverage of the communities with a slight decrease in ‘drier’ communities and increase in ‘wetter’ communities seems to correlate with statistically wetter conditions in the turloughs measured over the past 10 years, compared to 10 years prior to that. However, more research is needed to assess whether such changes are skewed as a result of the relatively low resolution of the Sentinel-2 satellite compared to the field survey used for this comparison over a 10-year period.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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