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ABSTRACT

Plants and animals influence biomass production and nutrient cycling in terrestrial ecosystems; however their relative importance remains unclear. We assessed the extent to which mega-herbivore species controlled plant community composition and nutrient cycling, relative to other factors during and after the Late Quaternary extinction event in Britain and Ireland, when two-thirds of the region's mega-herbivore species went extinct. Warmer temperatures, plant-soil and plant-plant interactions, and reduced burning contributed to the expansion of woody plants and declining nitrogen availability in our five study ecosystems. Shrub biomass in particular was consistently one of the strongest predictors of ecosystem change, equaling or exceeding the effects of other biotic and abiotic factors. In contrast, there was relatively little evidence for mega-herbivore control on plant community composition and nitrogen availability. The ability of plants to determine the fate of terrestrial ecosystems during periods of global environmental change may therefore be greater than previously thought.

INTRODUCTION

Which biotic factors are most important for determining the structure and function of terrestrial ecosystems? If animal consumers are a primary driver of ecosystem change, then the extinction of large-bodied herbivores could result in major upheaval in ecosystems worldwide (Dirzo *et al.* 2014). Alternatively, if primary producers control the fates of ecosystems, then ongoing climate change will likely be a more urgent threat, possibly via acceleration of the current extinction crisis (Cahill *et al.* 2013). Documented declines in consumer populations – including extinctions – provide an opportunity to test these alternatives. The fossil record provides key information about the structure and functioning of past ecological communities (Jeffers *et al.* 2015b) that can be used to infer the relative strength of these top-down versus bottom-up drivers of ecosystem change (Jeffers *et al.* 2012) during an extinction event.

The most recent major extinction event occurred toward the end of the last glacial period. Over half (55%) of the 42 mega-herbivore genera (defined here as herbivorous mammals weighing on

average ≥44 kg) extant in the northern hemisphere during the Late Pleistocene period are now globally extinct (est. from Koch & Barnosky 2006). A further seven of these (16%) are extinct on at least one continent. This extinction event offers an opportunity to study the impacts of concurrent changes in climate and ecological communities on ecosystem structure and function. The loss of mega-herbivore species was largely coincident with a reduction in plant species diversity across the Arctic (Willerslev *et al.* 2014), the encroachment of woody plants in northern ecosystems (Lister & Stuart 2008) and global declines in terrestrial nitrogen availability (McLauchlan *et al.* 2013). Early Holocene terrestrial ecosystems were therefore less open, less species diverse and less fertile than in the preceding glacial period. Were these changes a response to the Late Quaternary extinctions? In North America and Australia, palaeoecological research suggests that the mega-herbivore extinctions immediately preceded changes in plant communities and fire activity (Gill *et al.* 2009; Rule *et al.* 2012; Johnson *et al.* 2015). This has been interpreted as evidence that the Late Quaternary mega-herbivore extinctions caused the observed changes in ecosystem structure and function (Gill 2014; Bakker *et al.* 2016; Doughty *et al.* 2016). To understand the impacts of this extinction event, and – importantly – to be able to predict the consequences of future losses of large herbivores on ecosystem structure and function, it is essential to systematically disentangle the biotic and abiotic factors contributing to Late Quaternary ecosystem change.

Here we report palaeoecological proxy data that reflect concurrent changes in mega-herbivore biomass (Gill *et al.* 2013), nitrogen availability (McLauchlan *et al.* 2007), growing season temperatures (Brooks & Birks 2001; Heiri *et al.* 2011), fire activity (Tinner *et al.* 1998) and above-ground plant biomass of herbs, shrubs and trees (Seppä *et al.* 2009) spanning the full extinction event from the Late Pleistocene to the middle Holocene (ca. 16,000 to 4,800 years ago) in Britain and Ireland. Since trends in Late Quaternary ecosystem dynamics are often time-transgressive and tend to follow unique, context-specific successional trajectories, it is essential for any investigation of the ecological consequences of mega-herbivore declines to represent this temporal and ecological breadth. The British Isles provide an excellent model system with which to investigate the ecological context of Eurasian mega-herbivore extinctions in steppe-tundra ecosystems because

they offer many well-stratified localities that exemplify the variety of floral and faunal assemblages, and range of climatic conditions found across the Palearctic steppe-tundra biome over this period (Lowe & Walker 1997).

Our palaeoecological dataset contains 179 published radiocarbon dates from the nine mega-herbivore species extant in Britain and Ireland during the extinction event and 241 observations of fossil pollen, dung fungal spore and charcoal influx; 199 of these records also have corresponding stable nitrogen isotope and chironomid-based mean July air temperature data. This is – to our knowledge – the first such study to utilize independent lines of evidence of ecosystem *and* climate change measured concurrently through time at a number of study sites in order to assess the relative importance of plants, animals, burning and climate in determining the fate of terrestrial ecosystems at the Pleistocene – Holocene transition. Importantly, unlike related studies, we applied statistical modelling to these data in order to assess the evidence for each of the proposed drivers of ecosystem change during this extinction event.

MATERIALS AND METHODS

Study sites. Britain and Ireland represent the western front of the Eurasian Late Quaternary mega-fauna extinctions, many of which proceeded from west to east (Stuart & Lister 2012), tracking the retreat of continental conditions and open habitats (Allen *et al.* 2010). Our five sites (Fig. S1) span mire and heathland communities dominated by oceanic conditions in the northwest, to species-diverse grasslands with continental climates in the southeast (Appendix 1). The palaeoecological time-series data were derived from new and existing sedimentary sequences in England (Quidenham Mere 14,527 to 8,309 cal. yr. BP; Jeffers *et al.* 2011a), Scotland (Dubh-Lochan, 10,888 to 4,863 cal. yr. BP; Froyd 2005), and Ireland (Lough Nadourcan, 15,712 to 10,481 cal. yr. BP; Turney *et al.* 2006; Watson *et al.* 2010; Ballynahatty, 10,328 to 8,232 cal. yr. BP; Plunkett *et al.* 2008; and Long Lough, 13,939 to 9,837 cal. yr. BP; unpublished data collected by E.S. and G.P.). These sites contain plant (TableS2) and animal assemblages (Movie S3) that are representative of the Eurasian steppe-tundra.

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Fossil pollen, spores and charcoal. We used fossil pollen influx as a proxy for above-ground plant biomass (Seppä *et al.* 2009). Influx data account for variation in sedimentation rates through time and provide independent measures of change. Fossil pollen grains were extracted from lake and bog sediments following standard chemical treatments (Bennett & Willis 2001) and identified to plant family or genus level. Pollen concentrations of each plant taxon were calculated relative to the quantities of exotic pollen of a known concentration, which were introduced during sediment preparation (Bennett & Willis 2001). Pollen influx was calculated by multiplying the concentration values by sediment accumulation rates estimated for each core (FigS4). Influx rates for each plant taxon were combined for all herbs, shrubs and trees (TableS2) in order to determine the factors affecting plant community structure across sites (see Appendix 1 for information on dynamics within each plant functional group).

Sporormiella is a dung-associated fungus and the influx of its spores into lake and bog sediments is indicative of mega-herbivore density (Gill *et al.* 2013) and biomass (Baker *et al.* 2016) in the landscape surrounding the basin. We counted individual spores of *Sporormiella* from pollen slides and calculated concentrations and influx in the same way as for pollen (Baker *et al.* 2013). The influx of micro-charcoal fragments reflects patterns of fire activity at the landscape to regional scale. Micro-charcoal fragments were counted from pollen slides (Tinner & Hu 2003) and converted into charcoal concentrations and then influx as above. Pollen, *Sporormiella* and charcoal influx data were obtained for all five of our study sites, resulting in a database of 241 concurrent observations of plant and mega-herbivore biomass and landscape burning spanning ca. 16,000 to 4,800 cal. yr BP (i.e. over the full extinction event), at an average temporal resolution of c. 90 years.

Stable isotopes of nitrogen. The stable nitrogen isotope composition of bulk lake sediments (δ^{15}N) reflects broad trends in nitrogen availability to plants within the lake catchment (McLauchlan *et al.* 2007; see Appendix 2). Sedimentary δ^{15}N was measured from bulk lake sediments at the Godwin Laboratory for Palaeoclimate Research, University of Cambridge. One to two cubic centimetres of sediment were dried at 50°C and homogenized with a mortar and pestle then approximately 10 mg of sediment was placed in a tin capsule and sealed. Samples were analysed for δ^{15}N using a

Costech Elemental Analyzer attached to a Thermo MAT 253 mass spectrometer in continuous flow mode. Known reference standards from IAEA (USGS 40, $\bar{x} = -4.61‰$, $\sigma = 0.04$ and caffeine, $\bar{x} = 1.11‰$, $\sigma = 0.04$) were analyzed at various points throughout the run; these values were used to calibrate the sample results to the international standards. Results are reported relative to air (atmospheric N_2) with a value of 0‰. Standard deviations were determined from replicate analyses to be 0.08‰. Sedimentary $\delta^{15}N$ data were available for three of these sites: Lough Nadourcan, Ireland (Jeffers *et al.* 2012); Quidenham Mere, England (Jeffers *et al.* 2011a); and Dubh-Lochan, Scotland (Jeffers *et al.* 2015a).

Mean July air temperature. We reconstructed mean July air temperatures (°C) with a chironomid-based transfer function (Brooks & Birks 2001; Heiri *et al.* 2011). Head capsules of larval chironomids were extracted from 0.5-3 g of bulk sediment and mounted on microscope slides for identification with a light microscope following Brooks *et al.* 2007. Chironomid assemblages were translated into mean July air temperatures with a three-component WA-PLS transfer function based on a 153-lake modern Norwegian chironomid training set (Brooks & Birks 2001; Heiri *et al.* 2011). Temperature reconstructions were available for Lough Nadourcan (Jeffers *et al.* 2012), Quidenham Mere (Jeffers *et al.* 2011a) and Dubh-Lochan (Jeffers *et al.* 2015a). Prediction errors for each site ranged between 1.05-1.55°C. In total, we had 199 records of sedimentary $\delta^{15}N$ and mean July air temperature that were concurrent with our pollen, charcoal and *Sporormiella* influx.

Sedimentary sequence chronology. Chronologies for each of the five sedimentary sequences were developed in OxCal (version 4.2.3) (Ramsey 1995) using existing radiocarbon dates as reported in the original publications for each site (TableS5). New dates were obtained for the Long Lough sequence at the [14]CHRONO Laboratory at Queen's University Belfast. All chronologies were built using a P-sequence model (Ramsey 2008), where depositional events are modelled using a Poisson process and deposit increments are controlled by a parameter, *k*, set by the user. A total of 52 accelerator mass spectroscopy (AMS) radiocarbon dates were used to build the chronologies for these five sites (OxCal model specifications available in FileS6).

Fossil bone chronology. Since large herbivore species vary in terms of their impact on ecosystem structure and function (Pastor *et al.* 2006), it is important to know the species assemblage dynamics across the study period. The dung fungus *Sporormiella* is able to sporulate on a variety of dung substrates (Baker *et al.* 2013); therefore the influx of these spores in lake sediments cannot be used to infer the ecological impacts of particular herbivore species. The only unequivocal evidence of herbivore species presence comes from AMS ^{14}C-dated fossil bone assemblages obtained from deposition sites within the region. We reconstructed mega-herbivore species assemblage dynamics through time by collating published records of directly-dated fossil bone specimens from each of the nine species that were extant in Britain and Ireland between 16,000 and 4,800 cal. yr BP: elk (*Alces alces*), aurochs (*Bos primigenius*), domestic cattle (*Bos taurus*), red deer (*Cervus elaphus*), wild horse (*Equus ferus*), woolly mammoth (*Mammuthus primigenius*), giant deer (*Megaloceros giganteus*), reindeer (*Rangifer tarandus*) and saiga antelope (*Saiga tatarica*). This resulted in a dataset of 205 AMS radiocarbon dates for these nine mega-herbivore species. Only directly-dated specimens with adequate taxonomic identification to species level for which AMS dates were obtained on purified collagen after 1980 (i.e. when sample pretreatment became widely used) were retained, following the methodology used in Stuart & Lister 2012 and Lister & Stuart 2013. After auditing, 179 samples from published records remained in our fossil bone database. The taxonomy of two *Bos taurus* specimens from Sutton Shell Midden in Ireland has recently come into question (Milner 2010); however we chose to retain the dates of these specimens in our database since their identification as cattle has not been convincingly discounted.

The fossil bone dates were combined for all species and modelled in OxCal (Ramsey 1995) using the Sum function to generate cumulative probability densities that represent the best estimate for the chronological distribution for each mega-herbivore species (OxCal model code available in FileS7). Specifically, all dates for each species were grouped by region and then modelled as independent, single Phases (i.e. groups of ^{14}C-dated bones that are assumed to co-occur within a relatively short period of time), delimited by start and end Boundaries that help constrain the width of the resulting probability density functions. A Sum command was placed inside each Phase to

calculate a cumulative probability density across all modelled dates. The cumulative probability densities reflect the best possible estimate of the true range of AMS ^{14}C dates collected per species.

Statistical modelling. The four response variables were sedimentary δ^{15}N (our proxy for nitrogen availability) and the influx of herb, shrub and tree pollen (all proxies for above-ground plant biomass). All influx data were log-transformed (FigS8) using the formula $\log_{10}(x+1)$ prior to analysis in order to minimize the impacts of extreme values (i.e. from periods of increased sediment accumulation, FigS4) on the model results and to correct for non-normal distributions in the response and explanatory variables (Birks *et al.* 2012). While log-transformation may not remove all effects of varying sedimentation from the influx variables, we found no consistent pattern of correlation between the influx variables or between these variables and time after log-transformation (TableS9).

Scatter plots of the log-transformed data indicated non-linear relationships between predictor and response variables (data not shown). We therefore chose to use generalized additive modelling (GAM) to interrogate our palaeoecological data. This local, non-parametric regression method provides a useful tool for modelling non-linear relationships between variables in time-series data (Birks *et al.* 2012). GAM smoothers are used to estimate the dependence of the mean response variable on one or more of the predictor terms (Hastie & Tibshirani 1990). Model-fitting was conducted in R version 3.4.3 (R Core Team, 2017), using the *mgcv* package version 1.8-22 (Wood 2006). The GAMs were estimated with cubic regression splines using generalised cross-validation to automatically determine the optimal level of smoothing for each term in the model and automatic term selection (see Appendix 3 for further details). The significance of each term in the model was determined using the summary function in *mgcv*, which applies a Wald test to approximate p-values for each smoother term (Wood 2006). The approximate p-values for individual smoother terms are known to underestimate the true p-value (Zuur 2009); therefore we focused primarily on the individual model terms with the highest level of significance (p≤0.001).

We applied a series of GAMs to the time series data and recorded model selection metrics for each iteration. We calculated Akaike Information Criterion (AIC) weights to identify the models that were best able to predict the observed changes in each of our four response variables. AIC weights are a normalized indicator of support for each model given the evidence within each dataset while penalizing more complex models (Burnham & Anderson 1998). We obtained AIC scores using the AIC function in R and calculated AIC weights relative to the model with the lowest AIC score. AIC values can only be compared across a common dataset, so we fitted the GAMs and calculated AIC weights separately for the three-site (n=199, i.e. including all available sedimentary $\delta^{15}N$ and temperature records) and five-site (n=241, i.e. containing all influx data without climate and nitrogen isotope records) datasets.

RESULTS

Ecosystem dynamics. Six of the mega-herbivore species that were extant in Britain, and three in Ireland, between 16,000 and 12,000 years ago (Fig. 1 and MovieS3) became regionally extinct by 10,000 calendar years before present (cal. yr BP): woolly mammoth (*Mammuthus primigenius*), wild horse (*Equus ferus*), saiga (*Saiga tatarica*), giant deer (*Megaloceros giganteus*), moose or elk (*Alces alces*) and reindeer (*Rangifer tarandus*). This represents a loss of more than two-thirds of the extant mega-herbivore species in the region. The only mega-herbivore species remaining in Britain after 10,000 cal. yr BP were red deer (*Cervus elaphus*) and aurochs (*Bos primigenius*), before the arrival of domestic cattle (*Bos taurus*).

There are far more AMS radiocarbon-dated mega-herbivore bone specimens (and greater species diversity) in England (n=152) than Ireland (n=22) or Scotland (n=5). The modelled dates are concentrated in time between 15,000 and 11,000 years ago (Fig. 1) and show good correspondence in time with our *Sporormiella* influx records of mega-herbivore biomass in England and Ireland (Fig. 2). Unfortunately, there were no modelled mega-herbivore dates in Scotland that overlapped with our sedimentary record from Dubh-Lochan; however the *Sporormiella* values suggest increasing large herbivore biomass from the start of the Holocene (Fig. 3).

Shrub and herb biomass increased during the Bølling warm period (ca. 14,500 to 14,000 cal. yr BP) and tree biomass was minimal when mega-herbivore species were relatively abundant (Fig. 2). Both plant and mega-herbivore biomass declined during the Younger Dryas cold period until 11,000 cal. yr BP, when shrub and then tree biomass increased coincident with rising mean July air temperature (Fig. 2). While mega-herbivore biomass increased slightly around this time, it did not recover to peak (late-glacial) levels and the timing of these changes varied by site.

Sedimentary δ^{15}N, our proxy for terrestrial nitrogen availability, declined from ca 15,000 to 13,500 cal. yr BP coincident with rising plant and mega-herbivore biomass, recovered during the Younger Dryas cold period and declined again around the start of the Holocene epoch (ca 11,000 cal. yr BP) concurrently with increasing shrub and tree biomass and growing season temperatures (Fig. 2). These trends are consistent with broad global patterns of declining nitrogen availability from 15,000 to 7,500 cal yr. BP, as terrestrial ecosystems accumulated carbon (McLauchlan *et al.* 2013).

Generalised Additive Modelling. Did the loss of mega-herbivore species cause the expansion of woody plants and declines in nitrogen availability? In order to answer that question, we applied a series of GAMs to our palaeoecological time-series data and used AIC weights to identify the best-fitting model(s) for predicting observed changes in nitrogen availability and herb, shrub and tree biomass.

To explain changes in sedimentary δ^{15}N we began with a model whereby only *Sporormiella* influx (our proxy for mega-herbivore biomass) was used as a predictor variable. We compared the goodness of fit of this model to an alternative where the effects of *Sporormiella* influx on sedimentary δ^{15}N were site-specific. The AIC scores for these models show that the site-specific model (AIC = 499.0207) offered a better prediction of nitrogen dynamics than the model specified by a constant effect of mega-herbivores across all sites (AIC = 513.918, Table 1). Then, to investigate the influence of mega-herbivore biomass on nitrogen availability relative to changes in plant biomass, growing season temperature and fire activity, we added the following terms to the basic model: pollen influx for each of the three plant growth types at each site, mean July air temperature

and charcoal influx. Each term was added incrementally to the original model and model selection metrics were recorded at each step (Table 1). The AIC weights (w_i) show that the best-fitting model (AIC = 407.8037, w_i = 100%) for explaining the dynamics of sedimentary δ^{15}N included site-specific effects of *Sporormiella* and pollen influx combined with changes in mean July air temperature and charcoal influx (Table 1). Model validation analyses show that the results do not violate the model assumptions of homogeneity or normally distributed errors (Fig. 3; see Appendix 3 for more model validation analysis and details of alternative model configurations and results).

Model statistics for individual smoother terms indicate the strength and form of impacts on the dependent variables by each predictor term. Fire, mean July air temperature and woody plants (shrubs at Dubh Lochan, and trees at Lough Nadourcan) had the highest significance levels for explaining changes in nitrogen availability (Fig. 4A, see TableS10 for additional parameter statistics). In general, higher woody plant biomass, increasing growing season temperatures and declining fire activity were predicted to result in reduced nitrogen availability (Fig. 4A). Mega-herbivore biomass was a moderately significant factor for determining changes in nitrogen availability at Quidenham Mere, although it was less significant than burning and growing season temperatures (Fig. 4A). At the other sites, mega-herbivore biomass had only a low significance (Dubh-Lochan) or was excluded from the model due to lack of support (Lough Nadourcan).

We then applied the same series of models to explain changes in the pollen influx from herbs, shrubs and trees. As with sedimentary δ^{15}N, the full model including mean July air temperature, charcoal influx and site-specific effects of pollen and *Sporormiella* influx provided the best explanation for changes in the pollen influx of all three plant growth types (Table 1) when applied to the three-site dataset (*n*=199). Since there were no mean July air temperature records for Ballynahatty or Long Lough, we re-applied the same set of models to the full dataset (*n*=241) and obtained independent model-selection metrics using the summary function as above (Table 2). Again, the full model with charcoal influx and site-specific effects of the biotic factors (*Sporormiella* and herb, shrub and/or tree pollen influx) provided the best fit to this five-site dataset (Table 2).

Shrub biomass was the most significant biotic factor for predicting changes in herb biomass at the majority of sites. At Long Lough shrubs were only moderately significant (Fig. 4B and see FigS11 for results using 3-site dataset only; additional parameter statistics are available in TableS12 and TableS13). At Quidenham Mere, mega-herbivore biomass was slightly more significant than shrubs. The smoothers for Dubh-Lochan had very wide confidence intervals, particularly at lower values. This is likely due to the relatively few observations for this site (n=19). At all sites, burning was the most significant term (p=1.62e^{-33}) overall for predicting changes in herb biomass. Increased burning was predicted to result in higher levels of herbaceous plant biomass (Fig. 4B).

At all but one site, herbs or trees were the most significant factors for predicting changes in shrub biomass, exceeding the effect of mega-herbivores, growing season temperature and – to a lesser extent – burning (Fig. 4C). Dubh-Lochan was the exception in that no biotic factors were predicted to have a significant effect on shrub biomass. Contrary to expectations, mega-herbivore biomass was not a highly significant factor for predicting shrub biomass at any study site (Fig. 4C).

Shrub biomass was the most significant term for predicting changes in tree biomass at all but one site and the shrub smoother terms show a predominantly positive effect of shrubs on tree biomass (Fig. 4D). Again Dubh-Lochan was the exception; trees dominated the landscape at that site from the beginning of the sequence (Fig. 2) and herb biomass was the most significant term (p=1.22e^{-20}) for predicting changes in tree biomass. Mega-herbivore biomass was a highly significant term for predicting tree dynamics at Lough Nadourcan (p=1.44e^{-06}) only, although shrubs were slightly more significant at that site (p=1.91e^{-14}). Growing season temperature was found to be highly significant for predicting tree biomass and rising temperatures were predicted to have a positive but saturating effect on tree biomass (Fig. 4D); however, biotic factors were more significant for predicting tree dynamics at all sites.

DISCUSSION

We presented palaeoecological evidence from ecosystems within Britain and Ireland spanning the full period of Late Quaternary megafauna change and extinction in northern Europe when two-thirds

of the extant mega-herbivores went extinct. This is, to our knowledge, the highest density of sites, number of palaeoecological proxies and observations used to assess the ecological consequences of the Late Quaternary extinctions. Extinction chronologies built on fossil bone records and modelled vegetation dynamics have previously demonstrated that several Eurasian mega-herbivores persisted during the productive Bølling warm period when herbaceous biomass increased relative to the preceding glacial period but then went extinct with the subsequent expansion of trees (Huntley *et al.* 2013). High temporal correspondence between the abundance of large herbivores and the extent of open landscapes in Great Britain during the Holocene has also been suggested by fossil beetle data (Sandom *et al.*, 2014). In the ecosystems we studied, we see a similar pattern where the loss of mega-herbivore species (Fig. 1) and declines in herbivore biomass (Fig. 2), were coincident with or preceded an increase in both shrub and tree biomass, although the timing of these changes varied by site. Yet, chronologies alone are not adequate for investigating the relative importance of contributing factors to long-term ecosystem changes.

Here, we used statistical analysis to infer the relative importance of biotic and abiotic factors in driving changes in plant community composition and nitrogen availability across the Late Quaternary extinction event from our palaeoecological data. Our results indicate support for a more holistic model of ecosystem change than has previously been proposed from qualitative interpretation of comparable datasets from other regions (Rule *et al.* 2012; Gill 2014; Johnson *et al.* 2016).

Firstly, our model-selection results show that mega-herbivores alone cannot explain the observed changes in nitrogen availability and plant community composition as the *Sporormiella*-only model had the least evidence for explaining changes in these variables (Tables 1, 2). Model fit, as indicated by lower AIC scores which penalizes against models with more terms, improved when we allowed for site-specific effects of mega-herbivores. Including the effects of fire and mean July air temperatures further improved the model fit; however, the inclusion of plant growth forms as a predictor variable provided the greatest improvement in model fit (i.e. largest reduction in AIC scores). All of the evidence in the data (as indicated by AIC weights) points to the model with site-

specific effects of mega-herbivores and plant growth forms operating alongside changes in the abiotic environment from fire and mean July air temperatures to drive shifts in nitrogen availability and plant community composition across the extinction event.

Secondly, the significance level for each predictor term within the model provides insights into the relative strength of each factor in determining long-term changes in nitrogen availability and plant biomass. Multiple factors were often found to be highly significant (i.e. p-value ≤ 0.001) and the significance of each biotic term tended to vary between sites. Yet despite this complexity, a pattern emerged that indicates an important role for woody plants in determining long-term changes in nitrogen availability and plant community composition.

The expansion of shrubs and trees had an equally significant effect on nitrogen availability as landscape burning and changing growing season temperatures (Fig. 4A). Woody plants sequester nitrogen in long-term pools; therefore increasing woody biomass results in less nitrogen available for recycling, leading to long-term declines in nitrogen availability (McLauchlan *et al.* 2013), as predicted by the progressive nitrogen limitation hypothesis (Luo *et al.* 2004).

Fire and growing season temperature alter nitrogen availability directly by driving gaseous losses of nitrogen from soils (Morris *et al.* 2015) and indirectly through gains and losses in plant biomass (Craine *et al.* 2015). Declining fire activity in the early Holocene (Fig. 2) would have further limited the release of nutrients from long-term plant and soil pools (Dunnette *et al.* 2014), thus accelerating reductions in fertility at the ecosystem-scale.

Shrubs were the most significant biotic factor in determining changes in both herb and tree biomass at most sites; their impact was often comparable to changes in landscape burning and exceeded the effects of rising growing season temperatures (Figs. 4B and 4D). There was only one site, Dubh-Lochan, where trees were dominant in the landscape for the entire Holocene series (Fig. 2), where this pattern did not hold; yet even there the most significant factor for predicting changes in tree biomass was herb biomass, not burning or changing temperatures (Fig. 4D). These results

support the important role of plant-plant interactions in determining changes in plant community composition (Jeffers *et al.* 2015a).

Could shrubs have facilitated tree expansion in the early Holocene period? We see here that shrub expansion typically preceded the establishment and expansion of trees (Fig. 2) and our modelling results indicate a strongly positive effect of shrubs on tree biomass (Fig. 4D). Shrubs act as nurse plants for tree seedlings in stress-prone environments and thus have a net positive effect on tree recruitment, particularly in water-limited conditions (Gómez-Aparicio *et al.* 2004; Gómez-Aparicio *et al.* 2008) and grazed ecosystems (Vera 2006). Taken together, we argue that this is good evidence that shrubs played a key role in the postglacial expansion of trees in northwestern Europe. As described above, increasing woody plant biomass in turn had a significantly negative effect on nitrogen availability, contributing directly and indirectly to declining nitrogen availability in these terrestrial ecosystems. Our results implicate shrub encroachment as a key trigger for long-term changes in terrestrial ecosystem structure and function in this region during the Late Quaternary, just as it is in modern Arctic tundra (Myers-Smith *et al.* 2011) and grassland ecosystems worldwide (Bond 2016).

In conclusion, our results do not support the prevailing notion that the loss of mega-herbivore species caused the expansion of woody plants (Zimov *et al.* 2012; Bakker *et al.* 2016) and declining ecosystem fertility (Doughty *et al.* 2016) in terrestrial ecosystems at the end of the last glacial period. Instead, our findings indicate a relatively high level of plant control on ecosystem structure and function, an effect which has heretofore been largely overlooked as a direct driver of Late Quaternary ecosystem change (Willis *et al.* 1997; Jeffers *et al.* 2011b; Jeffers *et al.* 2015a). We suggest that interactions among plant growth forms and plant-soil feedbacks may have been more important than trophic interactions in determining changes in terrestrial nitrogen availability and above-ground plant biomass in northern European ecosystems as the Earth transitioned out of the last glacial period.

Overall, our results indicate that the mega-herbivores remaining in these ecosystems were not able to stem the expansion of woody plants at the onset of postglacial warming. Instead, it appears likely that plants strongly influenced the fate of the once extensive steppe-tundra biome in northwestern Europe. Whether or not these ecological changes contributed to the extinction of the Late Quaternary mega-herbivores in this region remains to be determined; however, our results are consistent with suggestions (Guthrie 1984; Stuart & Lister 2012; Willerslev *et al.* 2014) that ecological mechanisms may have played an important role in the extinction process. In a bottom-up world, where plants engineer the structure and function of terrestrial ecosystems, survival of even the largest consumers could chiefly depend on the fate of primary producers, and how they respond to changing climate and interactions with other plant growth forms.

DATA ACCESSIBILITY STATEMENT Data used in the analysis are available from Dryad: DOI: doi:10.5061/dryad.845n3f6

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FIGURE LEGENDS

Fig. 1. Mega- herbivore species assemblage dynamics. Cumulative probability densities for mega-herbivore species extant in Ireland, England and Scotland during the study period. The outputs shown were obtained by summing the selected radiocarbon dates for each species within bounded phases in OxCal, which defines periods of expected co-occurrence of individuals of the same species in time. The area circumscribed by the probability densities is proportional to the number of dated specimens. Note that the y-axes on the plots are shown on different scales due to large differences in the number of records available across the regions.

Fig. 2. Time-series of ecosystem dynamics. Pollen influx of herbs, shrubs and trees is used as a proxy for above-ground plant biomass at each of our five study sites (n=241). The influx of *Sporormiella*, a dung fungus, provides a proxy for mega-herbivore biomass in the surrounding landscape. Charcoal influx, shown with y-axes along the right hand side in the combined plots, reflects fire activity in the landscape and wider region. Nitrogen availability within the catchment is inferred from sedimentary $\delta^{15}N$ (n=199). Mean July air temperatures were reconstructed from chironomid assemblage data using a WA-PLS transfer function (n=199). All data were measured at concurrent levels across each sediment core.

Fig. 3. Model validation. Scatter plots of the response variables used in generalized additive models over time by site (A). Histogram plots of model residuals and scatter plots of residuals versus fitted values (B) for generalized additive models built using the 3-site data set (for predicting sedimentary $\delta^{15}N$) and the full 5-site data set (for predicting the three pollen influx variables).

Fig. 4. Modelled effects of biotic and abiotic factors on nitrogen availability and plant biomass. Plots of GAM smooth functions from the best-fitting model for describing changes in sedimentary $\delta^{15}N$ (A) at Dubh Lochan, Quidenham Mere and Lough Nadourcan, and pollen influx of herbs (B), shrubs (C) and trees (D) at all five sites. Nitrogen availability was modelled using data from the three sites for which these data are available (n=199) whereas plant biomass was modelled using data from all five sites (n=241) except for the climate term, which was modelled using only the 3-site dataset (see

FigS11 for all GAM smoother plots for the 3-site dataset). Asterisks indicate significance levels: * = p-value ≤ 0.05; ** = p-value ≤ 0.01; *** = p-value ≤ 0.001. Gray areas represent two standard errors around the estimated effect. Tick marks along the x-axis indicate data points. The model included site-specific effects of the biotic factors on each response variable. Models were fit using the *mgcv* package in R with generalized cross-validation and automatic term selection. Flat lines indicate where a term has been dropped from the model. Note that some y-axes are shown on a different scale due to wide confidence intervals.

Supplementary Information

FigS1. Map of the five sedimentary sequences and fossil bone assemblages used in this study

TableS2. Plant taxa within each functional group present at each study site

MovieS3. Spatio-temporal probability densities for large herbivore species in Britain and Ireland

FigS4. Sediment accumulation rates by site

Appendix 1. Further information on study sites

Appendix 2. Sedimentary $\delta^{15}N$ as a proxy for nitrogen availability

TableS5. Age determinations of sedimentary sequences

FileS6. OxCal model specification for sedimentary sequence chronologies

FileS7. OxCal model specification for fossil bone chronologies

FigS8. Log-transformed time series data used in statistical modelling

TableS9. Correlation coefficients for the 3-site and 5-site datasets

Appendix 3. Statistical modeling

TableS10. Model statistics for individual smooth terms describing changes in sedimentary $\delta^{15}N$ at Dubh-Lochan, Quidenham Mere and Lough Nadourcan

FigS11. Modelled effects of biotic and abiotic factors on plant biomass using 3-site dataset

TableS12. Model statistics for individual smooth terms describing changes in pollen influx using 3-site dataset

TableS13. Model statistics for individual smooth terms describing changes in pollen influx using 5-site dataset

Table 1. Goodness of fit of each generalised additive model to the sedimentary δ¹⁵N and pollen influx data from Dubh-Lochan, Quidenham Mere and Lough Nadourcan (3-site dataset). Model selection metrics were obtained using the *summary* function in the *mgcv* package in R. AIC weights (w_i) indicate the relative support for each model given the evidence in the data.

Models predicting Sedimentary δ^{15}N				
Model	**Dev. Exp.**	**Adj. R^2**	**AIC**	**w_i**
δ^{15}N ~ log(Dung spores)	9.42%	0.0699	513.918	0.00%
δ^{15}N ~ log(Dung spores$_i$, for all sites i)	22.90%	0.171	499.0207	0.00%
δ^{15}N ~ log(Dung spores) + Temp.	20.8	0.182	489.614	0.00%
δ^{15}N ~ log(Dung spores$_i$, for all sites i) + Temp.	32.50%	0.282	468.513	0.00%
δ^{15}N ~ log(Dung spores) + log(Charcoal)	26.80%	0.228	481.5945	0.00%
δ^{15}N ~ log(Dung spores$_i$, for all sites i) + log(Charcoal)	36.80%	0.309	465.6523	0.00%
δ^{15}N ~ log(Dung spores) + Temp. + log(Charcoal)	37.50%	0.336	453.0914	0.00%
δ^{15}N ~ log(Dung spores$_i$, for all sites i) + Temp. + log(Charcoal)	45.20%	0.398	439.3393	0.00%
δ^{15}N ~ log(Dung spores) + Temp. + log(Charcoal) + log(Herb pollen) + log(Shrub pollen) + log(Tree pollen)	43.90%	0.39	439.9324	0.00%
δ^{15}N ~ log(Dung spores$_i$, for all sites i) + Temp. + log(Charcoal) + log(Herb pollen$_i$ for all sites i) + log(Shrub pollen$_i$ for all sites i) + log(Tree pollen$_i$, for all sites i)	57.20%	0.505	407.8037	100.00%
Models predicting Herb Pollen Influx				
Model	**Dev. Exp.**	**Adj. R^2**	**AIC**	**w_i**
log(Herb pollen) ~ log(Dung spores)	42.10%	0.406	320.6112	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i)	69.10%	0.679	201.0699	0.00%
log(Herb pollen) ~ log(Dung spores) + Temp.	52.20%	0.486	300.0072	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i) + Temp.	71.80%	0.702	188.3001	0.00%
log(Herb pollen) ~ log(Dung spores) + log(Charcoal)	65.40%	0.644	219.2119	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal)	78.40%	0.775	129.4189	0.00%
log(Herb pollen) ~ log(Dung spores) + Temp. + log(Charcoal)	70.30%	0.676	210.8094	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i) + Temp. + log(Charcoal)	81%	0.798	111.8856	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal) + log(Shrub pollen$_i$ for all sites i) + log(Tree pollen$_i$ for all sites i)	91.40%	0.891	15.98281	100.00%
Models Predicting Shrub Pollen Influx				
Model	**Dev. Exp.**	**Adj. R^2**	**AIC**	**w_i**
log(Shrub pollen) ~ log(Dung spores)	26.10%	0.244	288.6781	0.00%
log(Shrub pollen) ~ log(Dung spores$_i$, for all sites i)	39.40%	0.364	259.004	0.00%
log(Shrub pollen) ~ log(Dung spores) + Temp.	26.10%	0.241	290.6055	0.00%
log(Shrub pollen) ~ log(Dung spores$_i$, for all sites i) + Temp.	43.10%	0.400	248.5863	0.00%
log(Shrub pollen) ~ log(Dung spores) + log(Charcoal)	36.70%	0.338	266.794	0.00%
log(Shrub pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal)	48.80%	0.461	227.0712	0.00%
log(Shrub pollen)~ log(Dung spores) + Temp. + log(Charcoal)	36.70%	0.338	266.794	0.00%
log(Shrub pollen)~ log(Dung spores$_i$, for all sites i) + Temp. + log(Charcoal)	51.80%	0.485	220.1156	0.00%
log(Shrub pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal) + log(Herb pollen$_i$ for all sites i) + log(Tree pollen$_i$ for all sites i)	85.10%	0.818	33.31709	100.00%
Models Predicting Tree Pollen Influx				
Model	**Dev. Exp.**	**Adj. R^2**	**AIC**	**w_i**
log(Tree pollen) ~ log(Dung spores)	24.60%	0.234	333.4624	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i)	53.50%	0.51	251.1887	0.00%
log(Tree pollen) ~ log(Dung spores) + Temp.	39%	0.363	302.1733	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i) + Temp.	56.20%	0.524	251.1237	0.00%
log(Tree pollen) ~ log(Dung spores) + log(Charcoal)	48.10%	0.446	278.13	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal)	58.10%	0.546	241.1491	0.00%
log(Tree pollen) ~ log(Dung spores) + temp. + log(Charcoal)	54.20%	0.497	263.4908	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i) + Temp. + log(Charcoal)	61.50%	0.572	233.38	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal) + log(Herb pollen$_i$ for all sites i) + log(Shrub pollen$_i$ for all sites i)	78.60%	0.757	124.1384	100.00%

Note: 'Pollen' refers to the pollen accumulation rate or influx, 'dung spores' are the influx of *Sporormiella* (the dung fungus), 'charcoal' refers to charcoal influx and the subscript i indicates terms with site-specific effects on the response variable, where i represents Dubh-Lochan, Quidenham Mere and Lough Nadourcan.

Table 2. Goodness of fit of each generalised additive model to the pollen influx data from all five study sites (5-site dataset). Model selection metrics were obtained using the *summary* function in the *mgcv* package in R. AIC weights (w_i) indicate the relative support for each model given the evidence in the data.

Models predicting Herb Pollen Influx				
Model	**Dev. Exp.**	**Adj. R^2**	**AIC**	***w_i***
log(Herb pollen) ~ log(Dung spores)	37%	0.354	397.6484	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i)	67.10%	0.651	257.6245	0.00%
log(Herb pollen) ~ log(Dung spores) + log(Charcoal)	53.80%	0.524	325.6721	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal)	76.90%	0.75	181.599	0.00%
log(Herb pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal) + log(Shrub pollen$_i$, for all sites i) + log(Tree pollen$_i$, for all sites i)	91.70%	0.886	28.8873	100.00%
Models Predicting Shrub Pollen Influx				
Model	**Dev. Exp.**	**Adj. R^2**	**AIC**	***w_i***
log(Shrub pollen) ~ log(Dung spores)	17.50%	0.159	456.269	0.00%
log(Shrub pollen) ~ log(Dung spores$_i$, for all sites i)	56.40%	0.524	334.299	0.00%
log(Shrub pollen) ~ log(Dung spores) + log(Charcoal)	33.30%	0.308	413.4638	0.00%
log(Shrub pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal)	61.40%	0.58	303.6918	0.00%
log(Shrub pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal) + log(Herb pollen$_i$, for all sites i) + log(Tree pollen$_i$, for all sites i)	89.60%	0.871	44.73457	100.00%
Models Predicting Tree Pollen Influx				
Model	**Dev. Exp.**	**Adj. R^2**	**AIC**	***w_i***
log(Tree pollen) ~ log(Dung spores)	21.10%	0.198	456.6042	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i)	61.60%	0.591	305.3566	0.00%
log(Tree pollen) ~ log(Dung spores) + log(Charcoal)	39.70%	0.376	400.7345	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal)	65.90%	0.626	289.6825	0.00%
log(Tree pollen) ~ log(Dung spores$_i$, for all sites i) + log(Charcoal) + log(Herb pollen$_i$, for all sites i) + log(Shrub pollen$_i$, for all sites i)	82.40%	0.802	143.24	100.00%

Note: 'Pollen' refers to the pollen accumulation rate or influx, 'dung spores' are the influx of *Sporormiella* (the dung fungus), 'charcoal' refers to charcoal influx and the subscript *i* indicates terms with site-specific effects on the response variable, where *i* represents Dubh-Lochan, Quidenham Mere, Lough Nadourcan, Ballynahatty and Long Lough.