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Carbon storage in phosphorus limited grasslands may decline in response to elevated nitrogen deposition: a long-term field manipulation and modelling study

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Abstract

In many temperate ecosystems, nitrogen (N) limits productivity, meaning anthropogenic N deposition can stimulate plant growth and subsequently carbon (C) sequestration. Phosphorus (P) and N-P co-limited grasslands are widespread, yet there is limited understanding of their responses to N deposition, which may transition more ecosystems toward P-limited or N-P co-limited states. Here, we investigate the consequences of enhanced N addition on the C-N-P pools of grasslands in different states of nutrient limitation. We explored the response of a long-term nutrient-manipulation experiment on two N-P co-limited grasslands; an acidic grassland of stronger N-limitation and a calcareous grassland of stronger P-limitation, by combining data with an integrated C-N-P cycling model (N14CP). To explore the role of P-access mechanisms in determining ecosystem state, we allowed P-access to vary, and compared the outputs to plant-soil C-N-P data. Combinations of organic P access and inorganic P availability most closely representing data were used to simulate the grasslands and quantify their temporal response to nutrient manipulation. The model suggested N additions have increased C stocks in the acidic grassland, but decreased them in the calcareous, where N provision exacerbated P-limitation and reduced biomass input to the soil. Furthermore, plant acquisition of organic P may play an important role in reducing P-limitation, as both simulated grasslands increased organic P uptake to meet P demand. We conclude that grasslands of differing limiting nutrients may respond to N deposition in contrasting ways, and stress that as N deposition shifts ecosystems toward P-limitation, a globally important carbon sink risks degradation.

1. Introduction

Grasslands represent up to a third of terrestrial net primary productivity (NPP) [Hoekstra et al., 2005] and potentially hold over 10% of the total carbon stored within the biosphere [Jones and Donnelly, 2004]. The ecosystem services provided by grasslands, such as carbon storage, are highly sensitive to perturbations in their nutrient cycling, including the perturbation of nitrogen (N) inputs from atmospheric deposition [Phoenix et al., 2012].

Since the onset of the industrial revolution, human activity has doubled the global cycling of N, meaning that anthropogenic sources of fixed N now surpass natural sources by 7 Tg N yr^{-1} [Fowler et al., 2013]. Much of this additional N is deposited on terrestrial ecosystems from atmospheric sources. This magnitude of N deposition results in a range of negative impacts on ecosystems (including grasslands) such as reductions in biodiversity [Bobbink et al., 2010; Southon et al., 2013], acidification of soil, and the accumulation of toxic levels of ammonium [Carroll et al., 2003; Horswill et al., 2008; Phoenix et al., 2012]

Despite large anthropogenic fluxes of N, most terrestrial ecosystems on temperate post-glacial soils are thought to be N-limited (biomass production is most restricted by N availability) [Vitousek and Howarth, 1991; Du et al., 2020], as weatherable sources of phosphorus (P) remain sufficiently large to meet plant P demand [Vitousek and Farrington, 1997; Menge et al., 2012]. Both empirical and modelling studies have shown that pollutant N, when deposited on N-limited ecosystems, can increase productivity [Tipping et al., 2019] and soil organic carbon (SOC) storage [Tipping et al., 2017], largely as a result of stimulated plant growth. This suggests that while there are negative consequences of N deposition, there may also be benefits from enhanced plant productivity and increases in carbon storage.

Whilst most research focuses on N limited ecosystems [LeBauer and Treseder, 2008], a number of studies have highlighted that P limitation and N-P co-limitation are just as prevalent, if not more

https://doi.org/10.5194/bg-2020-392
Preprint. Discussion started: 9 November 2020

widespread, than N limitation [Fay et al., 2015; Du et al., 2020; Hou et al., 2020]. In a meta-analysis of grassland nutrient addition experiments spanning five continents, Fay et al. [2015] found that aboveground annual net primary productivity (ANPP) was limited solely by P in 8 sites and co-limited by N and P in 25, compared to only 10 sites showing N limitation alone [Fay et al., 2015]. Similarly, P additions in 652 field experiments increased aboveground plant productivity by an average of 34.9% [Hou et al., 2020], while it is estimated that co-limitation of N and P could constrain up to 39% of the terrestrial surface's productivity [Du et al., 2020].

Furthermore, P limitation may be exacerbated by N deposition [Johnson et al., 1999; Phoenix et al., 2004], or become increasingly prevalent as previously N-limited ecosystems transition to N-sufficient states [Goll et al., 2012]. For example, in parts of the Peak District National Park, UK, N deposition has exceeded 3 g m^{-2} yr^{-1}, with further experimental additions of 3.5 g m^{-2} yr^{-1} leading to decreases rather than increases in productivity of calcareous grasslands [Carroll et al., 2003], in contrast to previous studies of N deposition enhancement of N-limited productivity [Tipping et al., 2019]. This makes P limitation and N-P co-limitation critical to understand in the context of global carbon and nutrient cycles. By definition, N deposition should impact N-P co-limited and P-limited ecosystems differently to N-limited ones, yet there is little understanding of how N deposition impacts P and N-P co-limited ecosystems.

While N deposition may worsen P limitation in some instances, plant strategies for P acquisition, such as changes in root architecture and increased root exudation [Vance et al., 2003] require substantial investments of N, suggesting that in some areas with P depleted soils, N may facilitate enhanced P uptake [Long et al., 2016; Chen et al., 2020]. It has been shown that N deposition can stimulate additional production of extracellular phosphorus-cleaving enzymes by plants [Johnson et al., 1999; Phoenix et al., 2004], thereby increasing plant availability of organic forms of phosphorus in order to help meet plant P demand. This response could be driven by exacerbated P-limitation resulting from N deposition or extra N availability making elevated enzyme production possible.

These interdependencies of the C, N and P cycles make understanding an ecosystem's response to perturbations in any one nutrient cycle challenging, particularly when ecosystems are not solely limited in N. This highlights the need for integrated understanding of plant-soil nutrient cycling across the C, N and P cycles, and in ecosystems that are not solely N-limited.

Process-based models have a role to play in addressing this, as they allow us to test our mechanistic understanding and decouple the effects of multiple drivers. There has been increasing interest in linking C with N and P cycles in terrestrial ecosystem models [Wang et al., 2010; Achat et al., 2016; Jiang et al., 2019] as the magnitude of the effects that anthropogenic nutrient change can have on biogeochemical cycling are realised [Yuan et al., 2018]. Yet, few modelling studies have explicitly examined the effects of P or N-P co-limitation, likely mirroring the relatively fewer empirical studies of these systems.

Combining process-based models with empirical data from long-term nutrient-manipulation experiments offers a valuable opportunity for understanding ecosystem responses to environmental changes that may only manifest after extended periods of time, such as with changes in soil organic C, N and P pools, which typically occur on decadal timescales [Davies et al., 2016a, Janes-Bassett et al., 2020]. Ecological data from these experiments can be used to drive and calibrate process-based models, which in turn can disentangle multiple interacting processes involved in plant-soil nutrient cycling, that otherwise makes interpretation of empirical experiments complex. This allows us to test our assumptions of the key drivers, processes and pathways for carbon and nutrient cycling in grasslands exposed to multiple environmental perturbations.

Here, we combine new data from a long-term nutrient manipulation experiment on two contrasting upland grasslands (acidic and calcareous), both N-P co-limited to differing degrees (one more P limited, one more N limited within the co-NP range), with the mechanistic C-N-P plant-soil biogeochemical model; N14CP [Davies et al., 2016b]. We use this model and data to simulate the long-term nutrient manipulation experiment in both grasslands and then use the calibrated model to determine the long-term consequences of differing nutrient limitation on plant and soil C, N and P. To do so, we allow modelled P-access conditions to vary and used the combinations of P-access variables that most closely represented empirical data to simulate the grasslands.

Specifically, we aim to first explore how variation in P acquisition parameters, that control access to organic and inorganic sources of P in the model, may help account for differing responses to N and P additions in the empirical data on aboveground biomass carbon and soil C, N and P pools. Secondly, we explore the effects of long-term anthropogenic N deposition at the site and the effects of experimental nutrient additions (N and P) on plant and soil variables of the simulated acidic and calcareous grasslands. This will help improve our understanding of how similarly P-limited or N-P co-limited grasslands may respond to similar conditions. We hypothesise that 1) flexible P-access within the model may help in alleviating P limitation and that 2) grasslands of contrasting nutrient limitation respond to N deposition and nutrient treatment in dissimilar ways, with N deposition exacerbating nutrient limitation in more P-limited grasslands, in turn leading to declining productivity and carbon sequestration.

https://doi.org/10.5194/bg-2020-392
Preprint. Discussion started: 9 November 2020

2. Methods

2.1. Field experiment description

Wardlow Hay Cop (henceforth referred to as Wardlow) is a long-term experimental grassland site in the Peak District National Park (UK) [Morecroft et al., 1994]. There are two distinct grassland communities occurring in close proximity; acidic (National vegetation classification U4e) and calcareous (NVC CG2d) semi-natural grasslands (Table S2). Both grasslands share a carboniferous limestone hill but the calcareous grassland sits atop a thin humic ranker [Horswill et al., 2008] and occurs predominantly on the hill brow. In contrast, the acidic grassland occurs in the trough of the hill, allowing the accumulation of wind-blown loess and the formation of a deeper soil profile. As such, the acidic grassland shares the same limestone bedrock but sits atop a palaeo-argillic brown earth soil [Horswill et al., 2008].

The biomass in both grasslands show signs of both N and P-limitation, though they differ in the relative strength of limitation by N and P. The acidic grassland is co-limited in N and P, as positive biomass growth responses are observed with additions of both nutrients [Phoenix et al., 2003]. The calcareous grassland, however, is more strongly P-limited, showing increased productivity only with the addition of P [Carroll et al., 2003], though N and P co-limitation has been observed [Phoenix et al., 2003].

Nutrients (N and P) have been experimentally added to investigate the effects of elevated N deposition and the influence of P limitation [Morecroft et al., 1994]. Nitrogen treatments simulate additional N deposition to the background level and also act to exacerbate P limitation [Johnson et al., 1999; Phoenix et al., 2004], whereas the P treatment acts to alleviate it. Nutrients are added as solutions of distilled water and applied as fine spray by backpack sprayer, and have been applied monthly since 1995, and since 2017 bi-monthly. Nutrient additions are in the form of NH_4NO_3 for nitrogen and $NaH_2PO_4.H_2O$ for phosphorus. Nitrogen is applied at rates of 0 (distilled water control –

0N), 3.5 (low nitrogen – LN) and 14 g N m^{-2} yr^{-1} (high nitrogen – HN). The P treatment is applied at a rate of 3.5 g P m^{-2} yr^{-1} (phosphorus – P).

Data collected from the Wardlow grasslands for the purpose of this work are; aboveground biomass C, SOC, and total N, which is assumed to be equivalent to modelled SON. This new data is combined with total P data that was collected by Horswill et al. at the site [Horswill et al., 2008]. Summaries of these data are available within the supplementary material (Table S4) and details of their collection and conversion to model-compatible units in supplementary section S1.

2.2. Summary of model processes

2.2.1. N14CP model summary

The N14CP ecosystem model is an integrated C-N-P biogeochemical cycle model that simulates NPP, C, N and P flows and stocks between and within plant biomass and soils, and their associated fluxes to the atmosphere and leachates [Davies et al., 2016b].

N14CP was originally developed and tested on 88 northern Europe plot-scale studies, including grasslands, where C, N and P data were available. All but one of the tested ecosystems exhibited N limitation [Davies et al., 2016b]. It has also been extensively and successfully blind-tested against SOC [Tipping et al., 2017] and NPP data from unimproved grassland sites across the UK (~500 and ~300 sites, respectively) [Tipping et al., 2019]. However, the model has not been extensively tested against sites known to exhibit P or N-P co-limitation. A full model description can be found in Davies et al., [2016b], however, a summary of the most relevant features is given here for convenience.

https://doi.org/10.5194/bg-2020-392
Preprint. Discussion started: 9 November 2020

2.2.2. Net primary productivity

N14CP simulations run on a quarterly time step and are spun up from the onset of the Holocene (10,000 BP in the model). Plant biomass is simulated in the model as two sets of pools of coarse and fine tissues containing C, N and P. NPP adds to these pools and is calculated on a quarterly basis, with growth occurring in this case in quarters 2 and 3 (spring and summer). The NPP is calculated on the basis of a single limiting factor (i.e. temperature, precipitation, N or P) in accordance with a Liebig's law of the minimum [Davies et al., 2016b]. However, nutrient co-limiting behaviour can occur in the model through increased access to organic P sources in the presence of sufficient N, and by having the rate of N fixation dependent on plant and microbial available P [Davies et al., 2016b].

To calculate NPP, first, a regression is used to estimate potential NPP based on climate as in Tipping et al. [2014]. The corresponding plant demand for N and P, driven by plant stoichiometric constraints, to achieve this potential NPP is then calculated and compared with available N and P in the model [Davies et al., 2016b; Tipping et al., 2017].

2.2.3. Plant available N and P

Plant available N is derived from fixation, the decomposition of coarse litter, decomposition of SOM pools and by atmospheric deposition. Plant available P comes from desorption of inorganic P from soil surfaces, the turnover of SOM, and the decomposition of coarse litter [Davies et al., 2016b]. The sorbed inorganic P pool builds over time with inputs of weathered P and sorption of any excess plant available inorganic P, and desorption occurs as a first order process.

Phosphorus enters the plant-soil system by weathering of parent material, the initial value of which (P_{Weath0} within the model) can be set to a default value, or made site-specific by calibrating this initial condition to soil observational data (as in methods section 2.3.3). In principle, P can be added in small quantities by atmospheric deposition [Ridame and Guieu, 2002] or by local redistribution

[Tipping et al., 2014]. For the purpose of this study, P deposition is set to zero as its net contribution to the total P pool in comparison to weathering is assumed to be minimal. Annual release of weathered P is determined by a first-order rate constant, which is temperature dependent. Where the mean temperature falls below 0 °C, it is assumed that no weathering occurs.

In the presence of sufficient N and where plant demand for P cannot be met by more accessible P sources, plants can access P from the soil organic phosphorus (SOP) pool via a cleaving parameter termed $P_{CleaveMax}$, which is the maximum quantity of cleavable P within a growing season (g m^{-2}). It is $P_{CleaveMax}$ and P_{Weath0} that we allow to vary to account for discrepancies in empirical data. Contributions of N and P toward the plant available pools are summarised in Figure 1.

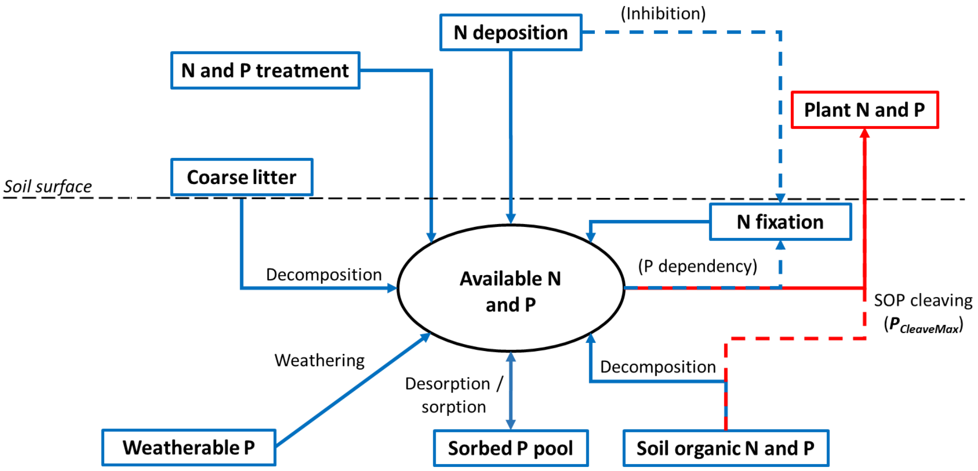


Figure 1: An illustration of the plant available N and P pools in the N14CP model. In the model, N can enter the available pool via atmospheric deposition, biological nitrogen fixation, coarse litter decomposition and decomposition of the soil organic matter pools. For P, the two main contemporary sources are the inorganic sorbed pool and from the turnover of soil organic matter. The former is derived initially from the weatherable supply of P, defined by its initial condition (P_{Weath0}). Solid lines indicate input to another pool and a dashed line indicates either a feedback or interaction with another pool. These interactions include the downregulation of N fixation by N deposition, the dependency of N fixation on P availability, and the cleaving of organic P by plants when N is sufficient and other P sources are inaccessible.

Phosphorus access within N14CP is determined by a hierarchal relationship, whereby plants and microbes access the most readily available P sources first and only move onto the next once it has been exhausted. Out of the P sources available to plants (Fig 1), organic P is the least bioavailable within the model hierarchy, hence a depletion in the SOP pool is indicative of severe P stress and low P availability.

Plant nutrient demand is defined by Plant Functional Type (PFT), which changes through time in accordance with ecosystem succession, and includes broadleaf woodland, coniferous woodland, shrubs (heather, heather grassland and montane habitats) and herbaceous plants (including neutral, acidic and calcareous grasslands). Each PFT has two stoichiometric end members, allowing the model to represent transitions from N-poor to N-rich species or an enrichment of the fine tissues within a single species (or a combination of both) [Davies et al., 2016b], dependent on available N. This allows a degree of flexibility in plant C:N ratios in response to environmental changes such as N deposition.

If the available nutrients cannot meet the calculated plant nutrient demand, the minimum calculated NPP based on either N or P availability is used, giving an estimation of the most limiting nutrient to plant growth. As the limiting nutrient of an ecosystem may not be static through time [Vitousek et al., 2010], and can change in response to external inputs of nutrients such as N deposition [Menge and Field, 2007], by looking at changes in the limiting nutrient, we can better explain model behaviour and its predictions of changes to C, N and P pools.

2.2.3. Soil processes – organic C, N and P

Detailed descriptions of C, N and P inputs, outputs and processes in the soil are explained in Davies et al. [2016b]. Nitrogen enters the system from N fixation and atmospheric N deposition. The former is related to P availability and based on literature values but is downregulated by N deposition (Figure 1).

A fraction of plant biomass is converted to litter in each quarterly time step and contributes a proportion of its C, N and P content to SOM, which is sectioned intro three pools (fast, slow and passive) depending on turnover rate [Davies et al., 2016b]. Carbon is lost as CO_2 following temperature-dependent decomposition and as dissolved organic carbon. Likewise, N and P are lost via dissolved organic N and P in a proportion consistent with the stoichiometry of each SOM pool. Inorganic N is lost via denitrification and inorganic P can be sorbed by soil surfaces. Both inorganic N and P can be leached in dissolved forms if they are in excess of plant demand.

2.3. Simulating the field manipulation experiment with the model

2.3.1. Nutrient applications

Nutrient treatments are treated in N14CP as individual plots in the simulations with differing amounts of inorganic N and P applied in line with the field experimental treatments. The N and P nutrient treatments are added to the bioavailable N and P pools of the model on a quarterly basis in line with the model's time-step. While Wardlow nutrient treatments are applied monthly and N14CP quarterly, the annual sum of applied N or P is equivalent, and nutrients are applied during all quarters.

2.3.2. Input drivers

Atmospheric N deposition, climate and PFT history need to be provided as annual time series to drive the model. As the acidic and calcareous sites are co-located, these input timeseries are shared for both grasslands. A summary of the data used for model input and model testing are provided in supplementary Tables S3 and S4 respectively. It is assumed in the model that anthropogenic N

https://doi.org/10.5194/bg-2020-392
Preprint. Discussion started: 9 November 2020

deposition was negligible prior to 1800 and the onset of the industrial revolution. After 1800, N deposition is assumed to have increased similarly across Europe [Schopp et al., 2003]. In N14CP, this trend is linearly extrapolated from the first year of data (1880) back to 1800 [Tipping et al., 2012]. Data regarding N deposition that is specific to Wardlow was incorporated between the years 2004 and 2014 and the Schöpp et al. [2003] anomaly scaled to represent the high N deposition of the site.

To provide climate forcing data, daily minimum, mean and maximum temperature and mean precipitation records beginning in 1960 were extracted from the UKPC09 Met office CEDA database (Table S3). The data nearest to Wardlow was calculated by triangulating latitude and longitude data and using Pythagoras' theorem to determine the shortest distance. These data were converted into mean quarterly temperature and precipitation. Prior to this, temperature was assumed to follow trends described in Davies et al. [2016b] and mean quarterly precipitation was derived from Met Office rainfall data between 1960 to 2016 and held constant.

To simulate the sites' land use history, PFT was defined on an annual basis using data on Holocene pollen stratigraphy of the White Peak region of Derbyshire [Taylor et al. 1994]. The defined PFT history represents an early colonisation of virgin soil by herbaceous plants following deglaciation (10,000 BP). A succession to broadleaf temperate forest develops and persists until a forest disturbance (but not clearance) by human settlers occurs in 5,190 BP, leaving an open forest mosaic characterised by hazel trees (defined in the model as shrub to distinguish it from forest). This open forest was deforested in 4100 BP to be used as rough grazing pasture for livestock, a practice that continues to this day.

2.3.3. Model parameters for the acidic and calcareous grasslands

The N14CP model has been previously calibrated and tested against a wide range of site data to provide a general parameter set that is applicable to temperate semi-natural ecosystems, without extensive site-specific calibration [Davies et al., 2016b]. The majority of those parameters are used here for both grasslands.

However, two parameters relating to P sources and processes were allowed to vary between the sites: the initial condition for the weatherable P pool, P_{Weath0}; and the rate of plant access to organic P sources, $P_{CleaveMax}$ (Figure 1).

We allowed P_{Weath0} to vary for each grassland as variation in a number of factors including lithology and topography mean that we should expect the flux of weathered P entering the plant-soil system to vary on a site-by-site basis Davies et al. [2016b]. Indeed, we should expect that P_{Weath0} differs between the acid and calcareous grasslands, as despite their proximity, they have differing lithology. Davies et al. [2016b], show that variation in this initial condition considerably helps explain variance in contemporary SOC, SON and SOP stocks between sites. However, it is difficult to set this parameter directly using empirical data, as information on lithology and P release is limited at the site scale.

We also allowed $P_{CleaveMax}$ to vary as this mechanism for P acquisition has been under-explored in previous modelling studies. This is the first time that this model has been knowingly applied to N-P co-limited or P-limited grasslands instead of N-limited sites. Soil organic P has been shown to be an important source of P to plants in P-stressed environments [Balemi and Negisho, 2012]. However, the rates of access to SOP and their controls are relatively poorly understood. We allowed the rate at which P can be cleaved from this pool ($P_{CleaveMax}$) to vary, to investigate how plant P acquisition might change when more readily accessible P forms become scarcer.

We ran a series of simulations systematically varying P_{Weath0} and $P_{CleaveMax}$ and comparing the results to observations, we simulated the two grasslands and their treatment blocks with a set of a 200 parameter combinations. This captured all combinations of 20 values of P_{Weath0} between 50 and 1000 g m^{-2} and 10 values of $P_{CleaveMax}$ between 0 to 1 g m^{-2} per growing season using a $\log_{10}$ spacing to focus on the lower range of $P_{CleaveMax}$ values. The P_{Weath0} range was set to capture the lower end of P_{Weath0}

estimates described in Davies et al. [2016b], which were more likely to be appropriate for these P-poor sites. We explored a range of values for $P_{CleaveMax}$, from zero where no access to organic sources is allowed, to 1 g m^{-2} per growing season – a rate in the order of magnitude of a fertilizer application.

The model outputs were compared to measured aboveground biomass C, SOC, SON (assumed equivalent to total N) and total P (Table S4) for each grassland. We tested how these parameter sets performed by calculating the error between the observations and model outputs of the same variables for each combination of $P_{CleaveMax}$ and P_{Weath0}. The sum of the absolute errors between modelled and observed plant C and soil C, N and P data were scaled (to account for differing numbers of observations) and summed to provide an F value (Equation 1) as an overall measure of error across multiple observation variables. The parameter combination with the lowest F value that still maintained the grassland's empirical response to nutrient additions for both the acidic and calcareous grasslands (Supplementary section S1.1.), was used within the analysis.

$$F = \left(\frac{SAE[C_{SOM}]}{\bar{C}_{SOM,Obs}}\right)/C_n + \left(\frac{SAE[N_{SOM}]}{\bar{N}_{SOM,Obs}}\right)/N_n + \left(\frac{SAE[P_{Total}]}{\bar{P}_{Total,Obs}}\right)/P_n \qquad \text{(Equation 1)}$$

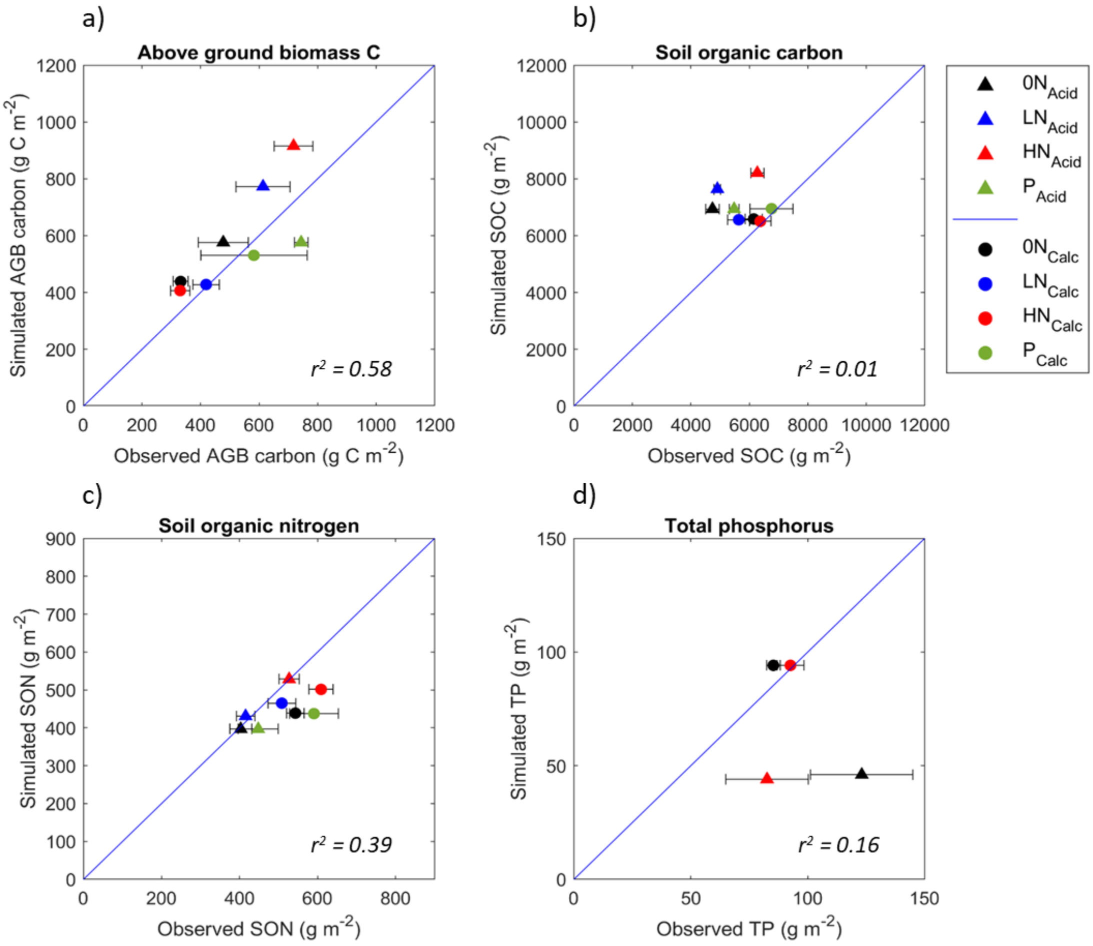
https://doi.org/10.5194/bg-2020-392
Preprint. Discussion started: 9 November 2020

3. Results

3.1. Varying phosphorus source parameters

The model calibration selected parameter values for P_{Weath0} and $P_{CleaveMax}$ that indicate contrasting use of P sources by the two grasslands, with the acidic grassland capable of acquiring more P from organic sources, having a $P_{CleaveMax}$ value of 0.3162 g m^{-2} $season^{-1}$ compared to the calcareous, with a value 10 times smaller at 0.0316 g m^{-2} $season^{-1}$. Conversely, inorganic P availability was greater in the calcareous grassland due to the larger weatherable pool of P, P_{Weath0}, at 300 g m^{-2} compared to 150 g m^{-2} in the acidic.

The outputs for the calibrated model are shown in Figure 2 against the observations for above-ground biomass C, soil organic C, and N for both the acidic and calcareous grasslands (Fig 2). The model estimates of above ground biomass C are broadly aligned with the observations: capturing variation between the grasslands and treatments (r^2=0.58), and on average overestimating the magnitude by 12.9% (SE ± 11.9) and 12.1% (SE ± 9.4) for the acidic and calcareous grasslands respectively (Fig 2a). Soil organic C on average was slightly overestimated (7.1% with SE ± 3.3) for the calcareous grassland (Fig 2b), with a larger average overestimate for the acidic grassland (39.9% with SE ± 6.8). However, in this latter case the variation between treatments was better captured. Simulated magnitudes of SON are well-aligned with observations for the acidic grassland, with an average error of 2.3% (SE ± 3.2), whilst the SON at the calcareous grassland was on average underestimated by 17.8% (SE ± 3.6) (Fig 2c). Finally, the model overestimated total soil P (defined in the model as organic P plus sorbed P) by an average of 6.0% (SE ± 4.3) for the calcareous but underestimated by 54.7% (SE ± 8.0) in the acidic grassland, which was the least accurately predicted variable out of those investigated (Fig 2d). Raw data used for Figure 2 are provided in supplementary tables S5 and S6.



381 **Figure 2**: A comparison of the observed values of a) aboveground biomass carbon, b) soil organic carbon, c) soil organic nitrogen and d) total soil phosphorus from both grasslands, with simulated values from the model. The blue line represents a 1 to 1 relationship and the closer the data points are to the line, the smaller the discrepancy between observed and modelled data. All data are in grams per metre squared and all treatments for which data were collected are presented. The horizontal error bars represent the standard error of the empirical data means. The r^2 value of regression models fitted to the data are presented to assess closeness to the 1 to 1 line.

3.2. The limiting nutrient through time

The model suggests that the acidic grassland NPP remained N-limited from 1800 through to 2020 under most nutrient treatments (Fig 3). Nitrogen deposition increased the potential NPP through time and the grassland moved toward co-limitation in the LN treatment (i.e. the N and P lines were closer) but remained N-limited (Fig 3b). In the HN treatment, the acidic grassland shifted to P limitation as N-limited NPP surpasses P-limited NPP (Fig 3c).

The calcareous grassland was also initially N-limited according to the simulation, but was driven through a prolonged (c. 100 year) state of apparent co-limitation until clearly reaching P-limitation in 1950, solely as a result of N deposition (Fig 3). In the 0N treatment, the grassland remained P-limited but the potential NPP values for N and P are similar, suggesting the grassland is close to co-limitation (Fig 3e). The LN and HN treatment amplified pre-existing P-limitation, lowering the potential NPP of the grasslands (Fig 3f, g). With the addition of P in 1995, P limitation is alleviated, and the ecosystem transitions to a more productive N-limited grassland (Figure 3h).

Another way to interpret the extent of nutrient limitation within N14CP with specific reference to P-demand, is to assess the rate of P cleaving through time. These data corroborate the N and P-limited NPP data, showing that in the calcareous grassland, the maximum amount of cleavable P is accessed by plants in the 0N, LN and HN treatments from approximately 1900 through to the end of the experimental period in 2020 (Fig S1, Table S14), highlighting its consistent state of P or N-P co-limitation.

Conversely, while P is cleaved in the 0N control treatment in the acidic grassland, it occurs at approximately one third of the total rate, hence the grassland is not entirely P-limited (Fig S1, Table S10). The LN treatment increases the rate of SOP cleaving and HN causes it to reach its maximum value, confirming the shift to P limitation suggested by the NPP data (Fig S1, Table S10). Soil organic P cleaving does not occur in the P-treated plots of either grassland.

https://doi.org/10.5194/bg-2020-392
Preprint. Discussion started: 9 November 2020

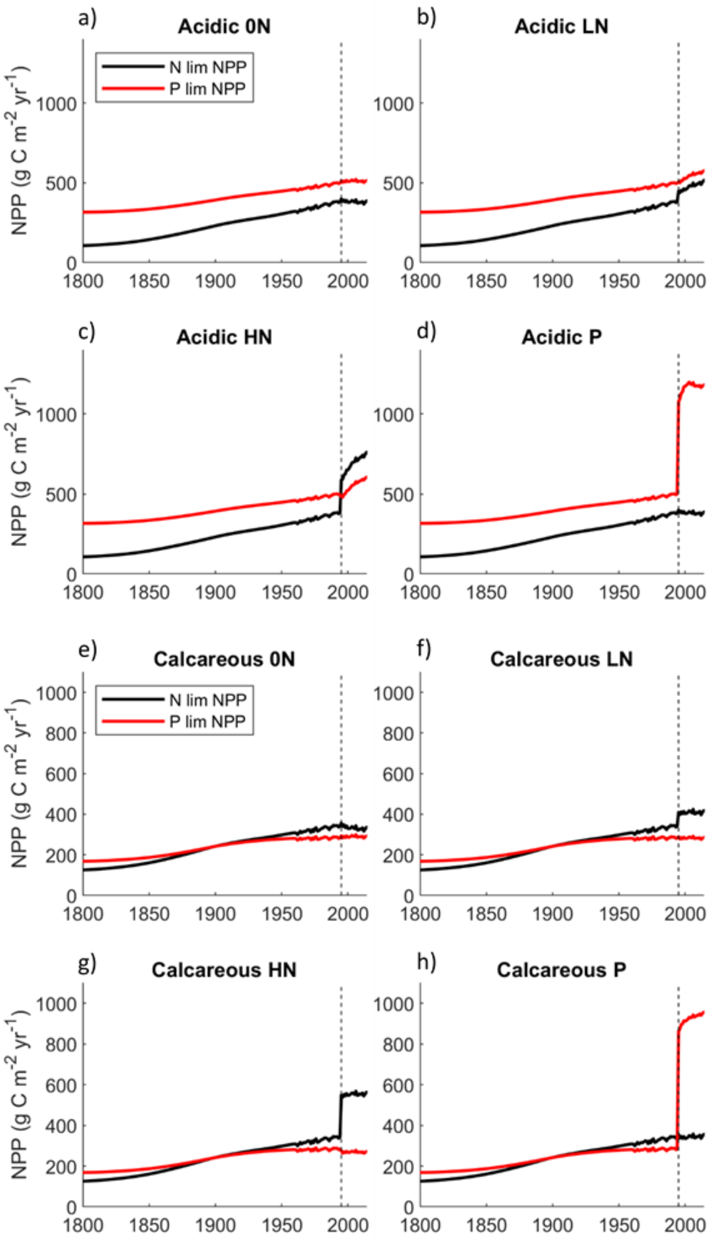


Figure 3: Plots showing the nutrient most limiting productivity for all nutrient treatments in both grasslands. The vertical dashed line is the year of first nutrient addition within the model (1995). The value of the lines represents the maximum amount of productivity attainable given the availability of N and P separately. Due to a Liebig's law of the minimum approach to plant growth, it is the lowest of the two lines that dictates the limiting nutrient of the grassland and represents actual modelled productivity. Where lines share a value, it can be considered in a state of N-P co-limitation.

407

3.3. Modelled trends and responses to nutrient additions

The model allows the temporal trends and responses to nutrient additions to be further explored. Figure 4 provides the temporal responses for the treatments, and Figure 5 a full nutrient budget for the year 2020. Full data for changes in soil C, N and P and plant biomass C pools since 1800 for both grasslands are included in supplementary Table S15. All data used for determining responses of biomass C and soil organic C, N and P pools to experimental nutrient additions are in supplementary Tables S16 (acidic) and S17 (calcareous).

3.3.1. Acidic grassland

The modelled time series suggest that in the 0N (control) treatment for the acidic grassland, background levels of atmospheric N deposition between the period 1800-2020 resulted in an almost four-fold increase in biomass C, a near-twofold increase in SOC and SON and increased the size of the SOP pool by almost a fifth (Fig 4).

Since initiated in 1995, all carbon and nitrogen pools responded positively to N but not P treatments (Fig 5a, c, Tables S7, S8). The LN and HN treatments further increased aboveground biomass C by 36.2% and 61.7% (Fig 4a) and increased the size of the total SOC pool by 11.5% and 20.6% respectively (Fig 4c). Similarly, the total SON pool in the acidic grassland increased by 9.7% in the LN treatment and 36.6% in the HN (Fig 4e).

Responses of the total SOP pool are in contrast to those of the SOC and SON pools, with LN and HN slightly decreasing SOP by 4.4% and 9.1% respectively, while P addition substantially increased the size of the SOP pool by 76.7% (Fig 4g). Nitrogen treatments facilitated access to SOP from both subsoil and topsoil, increasing plant available P and facilitating its uptake into biomass material (Fig 5e, Table S9).

3.3.2. Calcareous grassland

Model simulations for the calcareous grassland also suggest N deposition between 1800 and 2020 considerably increased aboveground biomass C, SOC and SON pools (Fig. 4), but to a lesser extent than in the acidic grassland. Soil organic C and SON increased by almost half and biomass C more than doubled. Soil organic P accumulated at a faster rate than in the acidic grassland, increasing by about a third (Fig 4, Table S15).

Responses of the aboveground biomass C and SOC pools in the calcareous grassland differ greatly to those of the acidic, declining with N addition and increasing with P addition (Fig 4). This response was ubiquitous to all C pools, with declines in subsoil, topsoil and biomass C (Fig 5b, Table S11). Biomass C declined by 2.4% and 7.3% with LN and HN addition (Fig 4b) and SOC declined by 0.5% and 1.4% with the same treatments (Fig 4d). Phosphorus addition increased biomass C and SOC by 22.0% and 6.1% respectively (Fig 4b, d).

Nitrogen treatments increased the size of subsoil, topsoil and available N pools, but led to small declines in biomass N (Fig 5d, Table S12). The P treatment slightly reduced subsoil and topsoil SON compared to the control yet increased available N and biomass N, to the extent where biomass N is greater in the P than HN treatment (Fig 5d, Table S12). Total SON increased by 6.4% and 15.0% with LN and HN respectively and declined by 0.2% with P treatment (Fig 4f).

The response of the calcareous P pools mirrors that of carbon, with declines in subsoil SOP, topsoil SOP, available P and biomass P with LN and HN addition (Fig 5f, Table S13). The calcareous grassland SOP pool declined by 0.2% with LN and 0.5% with HN addition, with an increase of 20.0% upon addition of P (Fig 4h). The P treatment substantially increased total ecosystem P in the calcareous grassland, particularly in the topsoil sorbed pool (Fig 5f, Table S13).

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Preprint. Discussion started: 9 November 2020

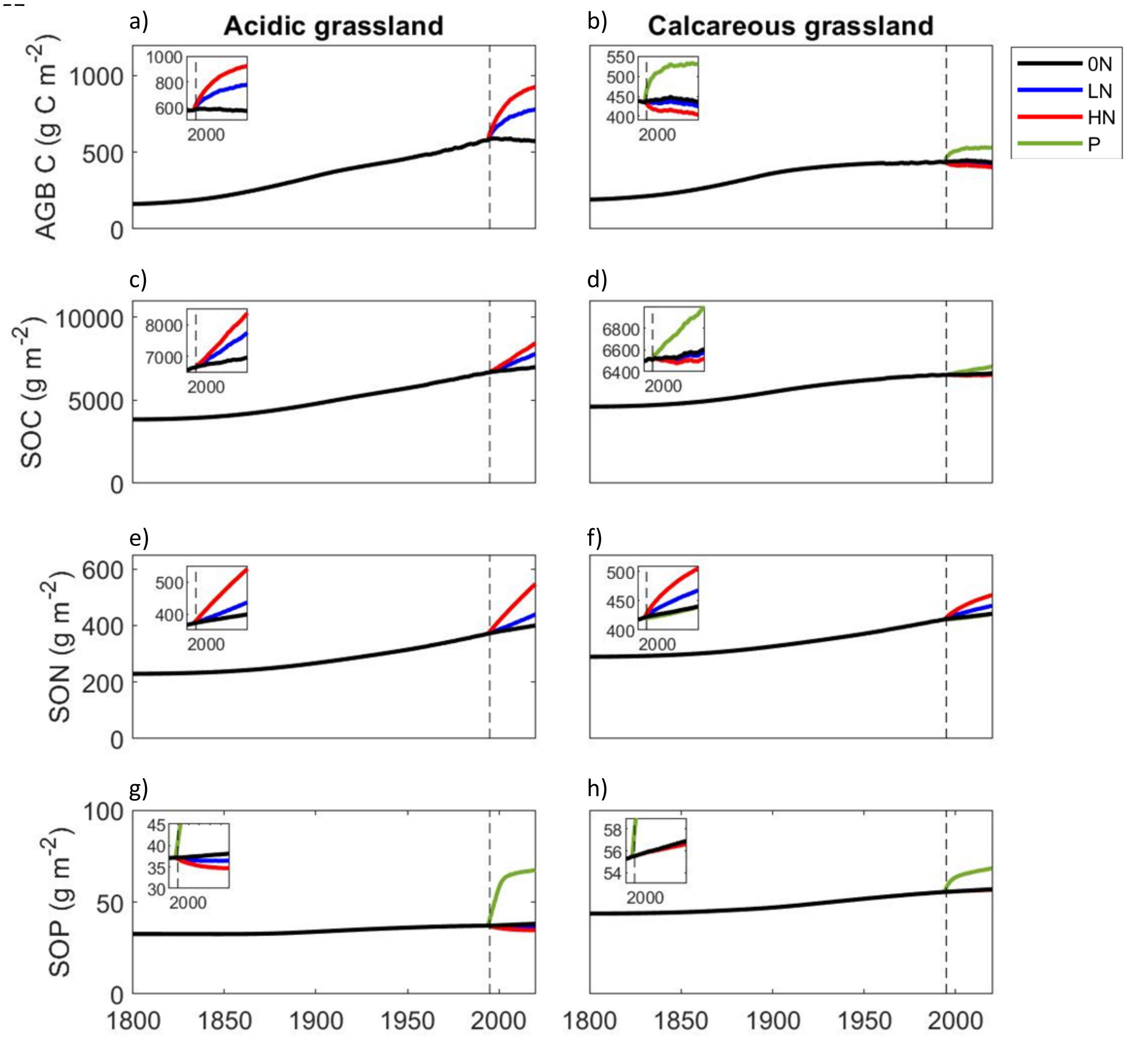

Figure 4: Time series plots of aboveground biomass C, soil organic C, N and P for the acidic (panels a, c, e and g respectively) and calcareous (panels b, d, f and h respectively) grasslands from 1800 to present day. The vertical dashed line represents the year of first nutrient addition (1995) and marks the beginning of experimental nutrient additions. The inset subplots show data from 1990 to 2020 to capture the experimental period (1995-2020) and highlight changes occurring as a result of nutrient additions rather than background N deposition. All nutrient treatments at Wardlow are represented in all panels though not all lines are visible if they do not differ from 0N. Both grassland share a y axis.

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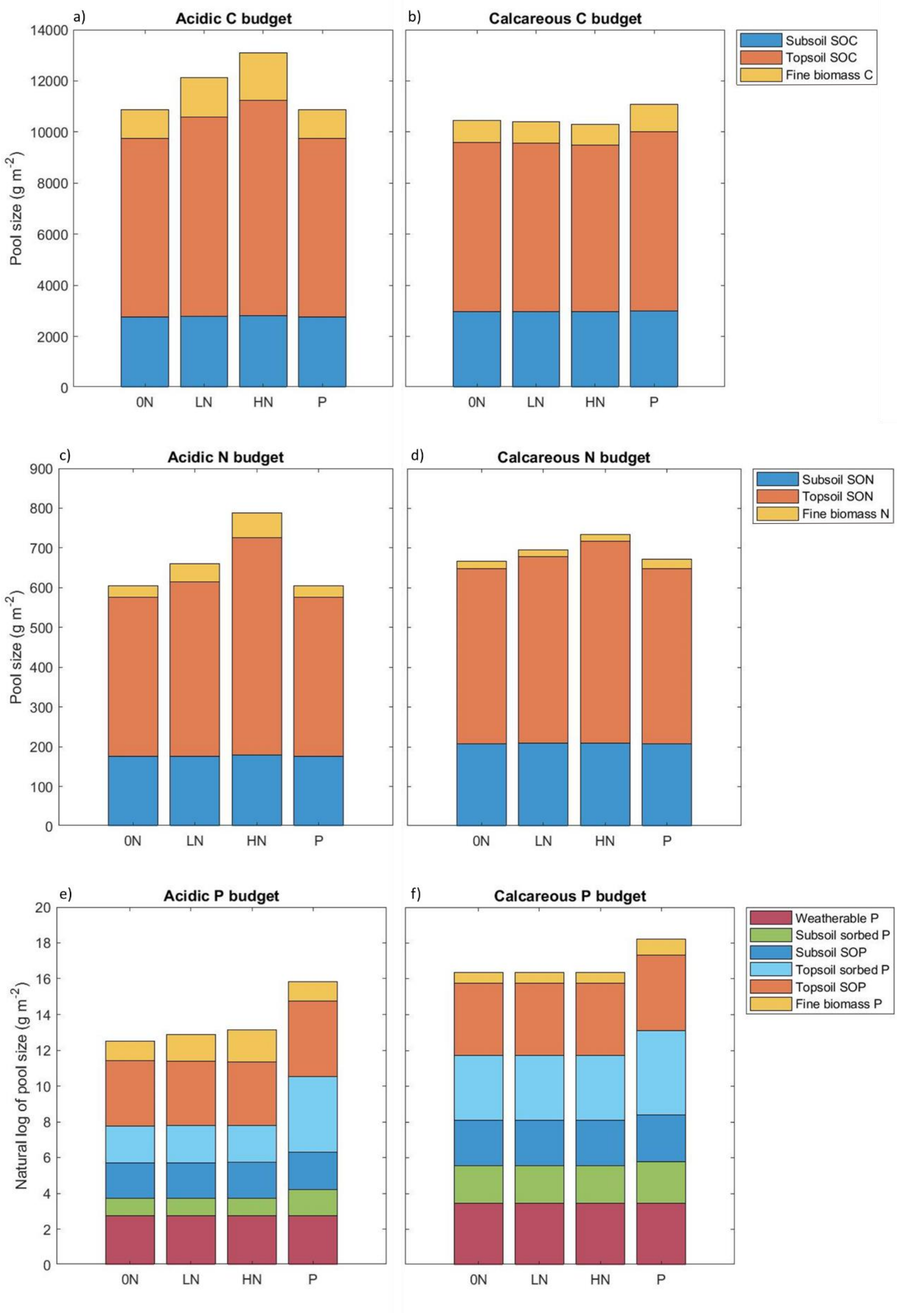

Figure 5: Modelled carbon, nitrogen and phosphorus budgets for the acidic (panels a, c and e) and calcareous (panels b, d, f) grasslands for the year 2020. Modelled sizes of C and N pools are in grams per metre squared, and P pools are presented as $\log_n$ grams per metre squared. Temporary pools such as available N and P and fixed N are not presented here to avoid 'double counting' in other pools and wood litter C, N and P are not presented due to their negligible sizes.

4. Discussion

4.1. Summary of findings

This is the first instance in which N14CP, and to the best of our knowledge; any other integrated C-N-P cycle model, has explicitly modelled N-P co-limited ecosystems and investigated their responses to N deposition and additional nutrient treatments.

The model suggests that the acidic grassland was characterised by high access to organic P, with comparatively low inorganic P availability, whereas the calcareous grassland was the opposite, with low organic and high inorganic P availability. The selected combinations of $P_{CleaveMax}$ and P_{Weath0} resulted in responses to nutrient addition consistent with N limitation in the modelled acidic, and P limitation in the modelled calcareous grassland. This aligned with our empirical understanding of the two real grasslands with co-N-P limitation being more towards either N or P limitation.

The modelling highlighted the contrasting impacts of experimental nutrient treatments on these two grasslands, and provided a means for decoupling the effects of deposition and experimental nutrient manipulation. Most notably, the responses of plant biomass C and SOC to N and P addition were in contrast to one another. In the simulations, N addition led to a small decline in biomass and SOC in the calcareous grassland but a substantial increase in the acidic. Nitrogen addition caused SOP to decline in both grasslands as N treatment exacerbated plant P demand, and increasing P limitation in the calcareous grassland.

4.2. Simulating grassland C, N and P pools by varying plant access to P sources

Although N14CP was not able to replicate a co-limited response for the acidic site, it produced behaviours akin to the most dominant limiting nutrient for both grasslands across multiple variables, with an average discrepancy between observed and modelled data of only 6.6% (SE ± 9.1) and 1.2% (SE ± 4.4) for the acidic and calcareous grasslands respectively across all variables (Table S5). The model's performance suggests that current C-N-P cycle models that employ a Liebig's law of the minimum can provide a broad representation of multiple variables, provided the ecosystem in question's limiting nutrient leans towards N or P limitation.

This was achievable in the case of N14CP by varying two P-cycling conditions used by the model, confirming that P acquisition, of both organic and inorganic forms, is a key determinant of contemporary soil carbon and nutrient stocks and flows [Davies et al., 2016b]. In addition, it confirms SOP could be a valuable source of P to plants in P-stressed environments, and we encourage further study aimed at quantifying SOP access by plants [Janes-Bassett et al., 2020; Phoenix et al., 2020].

The differences between P access of the two modelled grasslands could reflect the relative availability of different P sources at Wardlow. The acidic grassland forms in a hillside depression where loess has accumulated, distancing the plant community from the limestone beneath. The plant rooting zone of the acidic grassland is not in contact with the bedrock, so roots almost exclusively occur in the presence of organic P sources which can be cleaved and utilised by plants [Caldwell, 2005; Margalef et al., 2017]. Conversely, the calcareous soil rarely exceeds 10 cm depth, and the rooting zone extends to the limestone beneath. This provides plants with greater access to weatherable calcium phosphate [Smits et al., 2012].

The rate of organic P access was sufficiently high in the acidic grassland to temporarily overcome P limitation induced by anthropogenic N deposition. Due to its lower $P_{CleaveMax}$, the calcareous grassland was unable to meet additional P demand driven by N addition, and thus remained P-limited. It should be noted that the model grossly underestimates the acidic TP observations (Fig 2d), as few parameter sets where simultaneously able to simulate the magnitude of the empirical TP pool and the N-limited response of the acidic grassland to nutrient manipulations. Data that distinguishes between organic and inorganic forms of P would help in understanding this discrepancy.

N14CP has a number of mechanisms to account for N and P interdependence, meaning that in principle, it is capable of simulating N-P co-limited behaviour. Indeed, we found signs of N-P co-limited behaviour in both grasslands as nutrient treatment altered the limiting nutrient. Available N in the calcareous grassland was marginally greater in the P than 0N treatment (but less than LN and HN) (Fig 5d, Table S12), suggesting plants may be using surplus P to acquire N when it becomes limiting. Calcareous biomass N was also highest in the P treatment, though this reflects an absolute increase in N resulting from stimulated growth, and not a substantial acquisition of N from another pool (Fig 5d, Table S12). Similar behaviour was found in the modelled acidic grassland, where LN and HN treatments increased N availability, promoting access to available P (Table S9) and facilitating growth under N addition when it was largely P-limited (Fig 3c).

Nitrogen fixation remained unaffected by nutrient treatment in both grasslands (Tables S8, S12). This may be an unintended outcome of another N-P interaction within N14CP, whereby N fixation is downregulated by atmospheric N deposition [Gundale et al., 2013]. However, when N deposition exceeds fixation (as at Wardlow), fixation is essentially nullified (as in Tables S8, S12), meaning deposition becomes the sole source of N to the grassland. This in effect, removes the dependence of N acquisition on P availability, and could make modelling 'true' N-P co-limitation [Harpole et al., 2011] under high levels of N deposition challenging. This could be further explored by allowing N fixation limits in the model to adapt to P nutrient conditions.

4.3. The limiting nutrient through time

There is some evidence to suggest that modelled transitions of the limiting nutrient may be representative of historical nutrient limitation at Wardlow. Recent (post 1995) strengthening of P limitation (Fig 3g), transition to N limitation in the P-treated calcareous plots (Fig 3h), and transition to P limitation in the acidic HN treatment (Fig 3c), are likely to be accurate representations of the trends in nutrient limitations at the Wardlow grasslands.

Strengthening P limitation in both the acidic and calcareous grasslands under increased N input is supported by observations of increased root surface phosphatase enzyme activity in LN and HN treatments [Johnson et al., 1999; Phoenix et al., 2004] that indicate increased P demand. Furthermore, N deposition acidifies soil [Horswill et al., 2008], potentially reducing the availability of mineral P by facilitating the formation of iron and aluminium complexes which act to immobilise P [Kooijman et al., 1998]. Indeed, the model simulated reductions in plant available P for the calcareous grassland in response to the LN and HN treatments (Table S13), further supporting an exacerbated state of P limitation.

4.4. Modelled trends and responses to nutrient additions

4.4.1. Biomass C and SOC

Changes in plant biomass and SOC within N14CP are closely interlinked, due to detrital inputs of biomass being the primary source of SOC accumulation [Davies et al., 2016b]. As such, changes in SOC integrate long term trends in net primary productivity in systems where external nutrients are supplied. In the acidic grassland, biomass in the 0N treatment begins to decrease as a result of reducing N deposition following successful legislation to decrease atmospheric N pollution in the UK [Dirnbock et al., 2018]. The provision of additional N in the LN and HN treatments led to large increases in biomass accumulation as the model's limiting nutrient stimulated plant growth. Despite its P-limited condition under the HN treatment (Fig 3c), the acidic grassland continued to accumulate biomass with N addition as the grassland's greater access to topsoil SOP (Table S9) allowed it to acquire sufficient P to stimulate additional growth but not necessarily to alleviate P limitation. This is consistent with the acidic grassland at Wardlow, where N treatment stimulated root surface phosphatases, likely supplying more SOP to plants [D Johnson et al., 1999].

In the acidic grassland, LN and HN addition increased SOC almost linearly (Fig 4c). Similar increases in N-limited grassland SOC under N addition have been shown, resulting from significant increases in below-ground carbon input from litter, roots [He et al., 2013] and detrital inputs [Fornara et al., 2013], mechanisms similar to those reported by the model. Similarly, Tipping et al. [2017] used N14CP to show that N deposition onto N-limited UK ecosystems ubiquitously increased SOC storage by an average of 1.2 kgCm^{-2} (c. 10%) between 1750 and 2010 [Tipping et al., 2017].

Biomass C and SOC in the calcareous grassland responded positively to P addition, via similar mechanisms to the N-response in the acidic grassland. However, in contrast to the acidic grassland, N addition caused declines in calcareous biomass and SOC, the former of which has been observed at the calcareous grassland at Wardlow [Carroll et al., 2003]. Reductions in calcareous biomass C (and consequently SOC) in the model are a combined result of reductions in bioavailable P (Table S13), occurring via N-driven increases in stoichiometric P demand, in addition to an inability to access sufficient P from the SOP pool (Table S14). Plants therefore cannot meet P demand and new biomass is insufficient to replace senesced plant material, decreasing net biomass C input to the SOC pool.

Our results are consistent with findings by Li et al. [2018], who show that N fertilisation of an N-P co-limited grassland reduced SOC stocks by 5-12%, which they attribute to changes in community composition toward a higher proportion of forbs, whose lower tissue C:N increases the decomposability of litter input to the soil, and more rapid microbial degradation of SOC [Li et al., 2018]. However, there is little consensus regarding the fate of SOC under N and P addition in combination or solely P addition [Stiles et al., 2017]. Soil organic C has been found to increase with N and P addition [He et al., 2013], decrease with P [Scott et al., 2015; Luo et al., 2019] and show no net effect on SOM [Fornara et al., 2013].

In addition to affecting soil C influx, C efflux can be significantly altered by N deposition. Nutrient fertilisation can lead to decreases in plant tissue C:N and C:P, [Heyburn et al., 2017], increasing the relative availability of nutrients to below-ground microbes and facilitating degradation of SOM [Wild et al., 2014]. Furthermore, N deposition can reduce relative abundances of soil microbes and their enzymes responsible for cellulose and chitin degradation [DeForest et al., 2004; M Waldrop et al., 2004; Tian et al., 2019], slowing SOC decomposition, including in P-limited soils [Tian et al., 2019]. Such intricate interactions between soil microbes and N-driven acidification are not detailed within N14CP, therefore, our conclusion that N addition decreases P-limited SOC stocks is attributable to reduced C input rather than increased C output.

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Preprint. Discussion started: 9 November 2020

4.4.2. SON and SOP

Accumulation of SON is similar for both model grasslands, though it accumulates faster under N limitation, as microbial N mineralisation within the model may occur more rapidly to meet the increased demand for N. Much of the additional N bypasses immobilisation processes and rapidly accumulates. Consistent with this, both grasslands at Wardlow accumulated large quantities of simulated N deposition (up to 89% in the calcareous and 38% in the acidic grassland) [Phoenix et al., 2003]. The differences in rates of modelled versus empirical grassland SON accumulation are likely due to edaphic, topographical, and hydrological differences between the empirical grasslands that the model cannot replicate [Phoenix et al., 2003], or a representation of an initial versus long-term response.

Nitrogen addition increases plant demand for P and can shift ecosystems toward a state of P limitation or increase the severity of limitation where it already exists [Menge and Field, 2007; An et al., 2011; Goll et al., 2012]. Consistent with this, both simulated grasslands saw SOP decline with LN and HN treatment, worsening P limitation in the calcareous grassland, and depleting the SOP pool in the acidic. While SOP declined in both grasslands, the responses of available and biomass P to nutrient treatments differed markedly between the grasslands. Due to the higher rate of $P_{CleaveMax}$ in the acidic grassland, more P accumulated in the plant-available pool and hence P does not become the limiting factor under N treatments (Table S9). Conversely, available and biomass P decline under LN and HN addition in the calcareous grassland (Table S13), highlighting how calcareous $P_{CleaveMax}$ capability is insufficient to meet increased P demand.

5. Conclusions

We have shown that by varying two P-acquisition parameters within N14CP, we can account for contrasting responses of two N-P co-limited grasslands to long-term nutrient manipulation with reasonable accuracy. This suggests that current measures to account for co-limitation within the model are to some extent sufficient and widely applicable, at least to N-P co-limited ecosystems that are close to N or P limitation. Flexible organic P access allowed the modelled acidic grassland to acquire sufficient P to match the available N from chronic deposition and prevent 'anthropogenic P limitation'. However, the model suggests that this is an unsustainable strategy, as the SOP pool rapidly degrades, and if N additions are sustained, P limitation becomes likely. Conversely in the calcareous grassland, which was less able to access organic P, additional N provision exacerbated pre-existing P limitation.

We further show that anthropogenic N deposition since the onset of the industrial revolution has had a substantial impact on the C, N and P pools of both the acidic and calcareous grasslands, to the extent where almost half of contemporary soil carbon and nitrogen in the model could be from, or caused by, N deposition. Experimental N and P addition had contrasting impacts on the simulated grasslands. In the acidic grassland, N treatment stimulated plant access to soil organic P pools, promoting plant growth and soil carbon sequestration. However, in the calcareous grassland, further N addition simultaneously increased plant P demand and reduced its availability, decreasing plant carbon input to the soil and leading to degradation of soil carbon. Our work therefore suggests that as N deposition shifts more ecosystems toward a state of P limitation or strengthens it where it already occurs [Goll et al., 2012], we may see reductions in sequestration to the point where declines in grassland SOC stocks - one of our largest and most labile carbon pools – may occur.

Data availability: Data archiving is underway with the NERC's Environmental Information Data Centre (EIDC) and a DOI will be available once this process is complete. All data to be archived is present in the supplementary information for review purposes.

Author contributions:

CRT: Conceptualistion, data curation, formal analysis, investigation, methodology, project administration, software, validation, visualisation, writing – original draft preparation, writing – review and editing

VJB: Conceptualisation, formal analysis, investigation, methodology, supervision, software, writing – review and editing

GKP: Conceptualisation, methodology, funding acquisition, project administration, resources, supervision, writing – review and editing

BK: Investigation, methodology, supervision, writing – review and editing

IPH: Funding acquisition, methodology, resources, supervision, writing – review and editing

JD: Conceptualisation, formal analysis, investigation, resources, methodology, supervision, project administration, software, writing – review and editing

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