~~Carbon storage in phosphorus limited grasslands may decline in response to elevated nitrogen deposition: a long-term field manipulation and modelling study~~ Organic phosphorus cycling may control grassland responses to 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 two P-limited grasslands; one acidic and one limestone, occurring on contrasting soils and explore their ~~in different states of nutrient limitation. We explored the~~ responses to~~of~~ a long-term nutrient-manipulation experiment ~~on two P-limited grasslands, one acidic and one limestone, occurring on contrasting soils that are P-limited to different extents N-P co-limited grasslands; an acidic grassland that responds positively to N and P additionof stronger N-limitation and a calcareous limestone grassland of stronger P-limitation,~~. We do this by combining data with an integrated C-N-P cycling model (N14CP). ~~To~~ We explore the role of P-access mechanisms ~~in determining ecosystem state, we allowed P-access to vary~~by allowing these to vary in the modelling framework, and comparing~~ed~~ ~~the~~ model plant-soil C-N-P outputs to ~~plant-soil C-N-P~~ empirical data. Combinations of organic P access and inorganic P availability most closely representing empirical data were used to simulate the grasslands and quantify their temporal response to nutrient manipulation. The model suggested that access to organic P is a key determinant of grassland nutrient limitation and responses to experimental N and P manipulation. A high rate of organic P access allowed the acidic grassland to overcome N-induced P limitation~~ N addition~~, increasing~~s have increased~~ ~~C stocks in the acidic grassland~~biomass C input to soil and promoting SOC sequestration in response to N addition. Conversely, poor accessibility of organic P for the limestone grassland~~ but decreased them in the calcareous, where~~ meant N provision exacerbated P-limitation and reduced biomass input to the soil, reducing soil carbon storage. ~~Furthermore, p~~Plant acquisition of organic P may therefore play an important role in reducing P-limitation, and determining responses to anthropogenic changes in nutrient availability~~ as both simulated grasslands increased organic P uptake to meet P demand~~. We conclude that grasslands ~~of~~ differing in their access to organic P~~ limiting nutrients~~ may respond to N deposition in contrasting ways, and stress that should ~~as~~ N deposition~~-~~ shift~~s~~ ecosystems toward stronger 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 organic 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~~ with anthropogenic sources ~~of fixed N~~contributing 210 Tg of fixed N per year to the global N cycle, ~~now~~ surpassing naturally fixed N ~~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~~mobilisation of potentially toxic metals [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~~sequestration.

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 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 by nutrients in 31 out of 42 sites, most commonly through co-limitation of N and P ~~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 P limitation, alone or through co-limitation with N, ~~co-limitation of N and P~~ could constrain up to 82~~39~~% of the natural 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~~ limestone 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 P-limited ecosystems~~ with some form of P limitation, (including N-P co-limitation,)N-P co-limited and P-limited ecosystems diffe~~ differently to N-limited ones, yet there is little understanding of how N deposition impacts ~~P and N-P co-limited ecosystems~~these systems.

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]~~ may require substantial investments of N, suggesting that ~~in some areas with P depleted soils,~~increased N supply ~~N~~ may facilitate enhanced P uptake [Vance *et al.*, 2003; Long *et al.*, 2016; Chen *et al.*, 2020]. Indeed, previous work from long-term experimental grasslands~~It~~ has shown strong effects of~~been shown that~~ N deposition on plant enzyme production [Johnson *et al.* 1999; Phoenix *et al.* 2004]~~ and activity [Keane *et al.* 2020]~~, whereby the production of additional extracellular phosphatase enzymes was stimulated~~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~~. While it is n~~'~~ot clear if~~T~~ this response is~~could be~~ driven by exacerbated P-limitation resulting from N deposition or extra N availability making elevated enzyme production possible, such changes in plant physiology may promote cleaving of P from organic soil pools. Over time, the accumulation of plant-available P from organic sources may provide a mechanism by which plants exposed to high levels of N deposition may overcome P limitation [Chen *et al*. 2020].

By using the integrated C-N-P cycle model N14CP, Janes-Bassett *et al*. [2020] suggest that the role of organic P cycling in models may be poorly represented, as the model failed to simulate empirical yield data in agricultural soils with low P fertiliser input. Organic P access is therefore likely an important means of nutrient acquisition for plants in high N and low P soils [Chen *et al*. 2020], yet our understanding of organic P cycling in semi-natural ecosystems is fairly limited [Janes-Bassett *et al*. 2020]. ~~These~~ Such 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, or the role of organic P access in determining nutrient limitation, likely mirroring the relatively fewer empirical studies of these systems.

By ~~C~~combining process-based models with empirical data from long-term nutrient-manipulation experiments, we may simultaneously improve our understanding of empirical nutrient limitation, the role(s) of organic P acquisition, and their interactions with anthropogenic nutrient pollution. In particular, this approach ~~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 ~~use such an approach by~~ combin~~inge~~e new data from a long-term nutrient manipulation experiment on two ~~contrasting~~ P-limited upland grasslands (acidic and ~~calcareous~~limestone)~~,~~ occurring on contrasting soils, ~~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 these experimental data to explore the role of organic P access in determining ecosystem nutrient limitation ~~,~~ and grassland responses to long-term nutrient manipulations~~ to better understand the potential responses of similar grasslands to anthropogenic nutrient inputsTo 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.~~

~~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 of empirical grassland C, N and P pools to N and P additions ~~in the empirical data on aboveground biomass carbon and soil C, N and P pools~~. ~~Secondly, we~~Second, we explore the effects of long-term anthropogenic N deposition ~~at the site~~ and ~~the effects of~~ experimental N and P~~nutrient~~ additions ~~(N and P)~~ on plant and soil variables of the simulated acidic and ~~calcareous~~ limestone grasslands. This will help improve our understanding of organic P process attribution within the model and may suggest how similarly nutrient limited ~~P-limited or N-P co-limited~~ grasslands could~~may~~ respond to similar conditions.

We hypothesise that 1) access to organic P will be an important determinant of ecosystem nutrient limitation, 2)~~lexible~~ increased organic P availability may alleviate P limitation resulting from N deposition and 3) ~~P-access within the model may help in alleviating P limitation and that 2)~~ grasslands capable of accessing sufficient P from organic forms may overcome P limitation resulting from ~~of contrasting nutrient limitation respond to~~ N deposition and nutrient treatments, ~~in dissimilar ways,~~ whereas grasslands lacking such accessibility will not~~with N deposition exacerbating nutrient limitation in more P-limited grasslands, in turn leading to declining productivity and carbon sequestration.~~.

2. Methods

2.1. Field experiment description

The empirical data is from 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~~ limestone (NVC CG2d) semi-natural grasslands (Table S2). Both grasslands share a carboniferous limestone hill but the ~~calcareous~~ limestone 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 of a ~~. 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., 2003].~~ Despite contrasting soil types, both the acidic and limestone grasslands are largely P-limited [Morecroft *et al*. 1994; Carroll *et al*. 2003], though occasional N and P co-limitation can occur [Phoenix *et al*. 2003] and more recently, positive growth responses in solely N-treated ~~acidic~~ plots have been observed, in line with the latest understanding that long-term N loading may increase P supply by increasing phosphatase enzyme activity [Johnson et al. 1999; Phoenix et al.2004; Chen et al. 2020]~~.~~. ~~Such a response may reflect the differences in relative availability of organic P forms between the grasslands, typically more accessible in the acidic than limestone soil. Prolonged N addition may therefore facilitate enhanced access to P through phosphatase enzyme activity in the~~

~~acidic more so than in the limestone grassland [Johnson *et al.* 1999; Phoenix *et al.* 2004; Chen *et al.* 2020].~~

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~~and the P treatment acts to alleviate P limitation ~~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 net primary productivity (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, N14CP ~~the model~~ has not been extensively tested against sites known to exhibit P ~~or N-P co-~~limitation, especially where these are explicitly manipulated by long term experimental treatments. While the importance of modelled weatherable P (P_{Weath0}) and historic N deposition on N-limited C, N and P have been investigated [Davies *et al*. 2016b], the potential influence of organic P on ecosystem nutrient limitation and responses to nutrient perturbations have yet to be explored.

Here, we modify N14CP to add experimental N and P additions to simulate a long-term nutrient manipulation experiment similar to that at the limestone and acidic grasslands at Wardlow, and we use empirical data from ~~the~~ Wardlow ~~LTE~~ to explore the role of organic P cleaving in determining ecosystem state. ~~n.~~ 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.

2.2.2. Net primary productivity and nutrient limitations

~~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~~ representing both above and belowground plant C, N and P, with belowground biomass for each plant functional type (PFT) represented by a root fraction~~, with corresponding root fractions representing belowground biomass, which.~~ NPP adds to ~~these pools and is calculated~~ these on a quarterly basis~~,~~ with growth occurring ~~in this case~~ in quarters 2 and 3 (spring and summer). In N14CP, NPP depends on a single limiting factor, in accordance with Liebig's law of the minimum. The factors that can limit growth in the model include available N and P, temperature or precipitation, the latter two being provided as input driver data (see section 2.3.2).

First, the potential maximum NPP limited by climate is calculated using regression techniques, as in Tipping *et al*. [2014]. The corresponding plant demand for N and P to ~~, 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]. This demand is defined by ~~plant functional type (PFT)~~ PFT stoichiometry, which changes through time in accordance with ecosystem succession (see section 2.3.2).~~, and includes broadleaf woodland, coniferous woodland, shrubs (heather, heather grassland and montane habitats) and herbaceous plants (including neutral, acidic and limestone grasslands).~~ Stoichiometry of coarse tissue is constant ~~Each~~but each PFT's fine tissue 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.~~

~~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, n~~Nutrient co-limiting behaviour can occur in the model through increased access to organic P sources in the presence of sufficient N (see 2.2.3), and by having the rate of N fixation dependent on plant and microbial available P [Davies *et al*., 2016b]. The initial rate of N fixation is based on literature values for a given PFT but is downregulated by anthropogenic N deposition and related to P availability. The degree to which P availabilty limits this maximum rate of fixation is determined by a constant; K_{Nfix} [Davies *et al.* 2016b]. This means that while modelled NPP is limited by availability of a single nutrient, co-limitation may occur through P limitation of N fixation [Danger *et al.* 2008].

2.2.3. Plant and soil N and P cycling ~~available N and P~~

A simplified summary of key pools and processes regarding plant-soil nutrient cycling are detailed in Figure 1. Details such as initial base cation pools, their effects on soil pH, and most parameter names have been omitted for clarity but are available from ~~Davies *et al.*'s~~the original model development study [Davies *et al.* 2016b]. Key changes for the purpose of this work are highlighted in red.

Plant available N is derived from biological fixation, the decomposition of coarse litter and ~~,~~ ~~decomposition of~~ SOM ~~pools~~, ~~and by~~ atmospheric deposition and direct N application. Plant available P also comes from SOM and coarse litter decomposition, direct treatment, ~~-~~desorption of inorganic P from soil surfaces, and sometimes cleaving of organic P ~~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). From this initial pool, annual releases of weathered P are determined by first-order rate constants that are temperature dependent, with the assumption that no weathering occurs below 0 degrees Celsius. This weathered P can then contribute toward plant-available P in soil water or be sorbed to soil surfaces. 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.

The size of the available P pool is determined by summing: P retained within plant biomass prior to litterfall, inorganic P from decomposition, dissolved organic P and P cleaved from SOP by plants. Accessibility of each P form is determined by a hierarchal relationship in the order mentioned above, whereby plants and microbes access the most readily available P sources first and only move onto the next once it has been exhausted.

When N is in sufficient supply and more bioavailable P forms have been exhausted from the total available pool, simulated plants can access P from SOM via an implicit representation of extracellular P-cleaving enzymes with a parameter termed P_{Cleave}. While empirical data quantifying this parameter is scarce, N14CP constrains P_{Cleave} by utilising a maximum SOM C:P ratio; $[C:P]_{fixlim}$, that ensures SOM stoichiometry is not unrealistically disrupted by excessive removal of organic P (Equation 1).

$$P_{Cleave} = SOP - \frac{SOC}{[C:P]_{fixlim}} \qquad \text{Equation 1}$$

The functioning of the P_{Cleave} parameter, including its stoichiometric constraint, remains the same in this work but we have introduced a modifier to adjust the rate at which plants can access this P source. This parameter; $P_{CleaveMax}$, represents the maximum amount (g m^{-2} $season^{-1}$) of cleaved P that plants can acquire from the available P pool to satiate P demand. ~~How we use empirical data to calibrate this value and what the value means for ecosystems is detailed in section 2.3.3.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.~~

~~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]. Soil organic P (SOP) is simulated alongside SOC and SON using C:N:P stoichiometries of coarse and fine plant biomass. Decomposition of SOP, and ~~it's~~its contribution to the available P pool, is subject to the same turnover rate constants as for SOC and SON.

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.

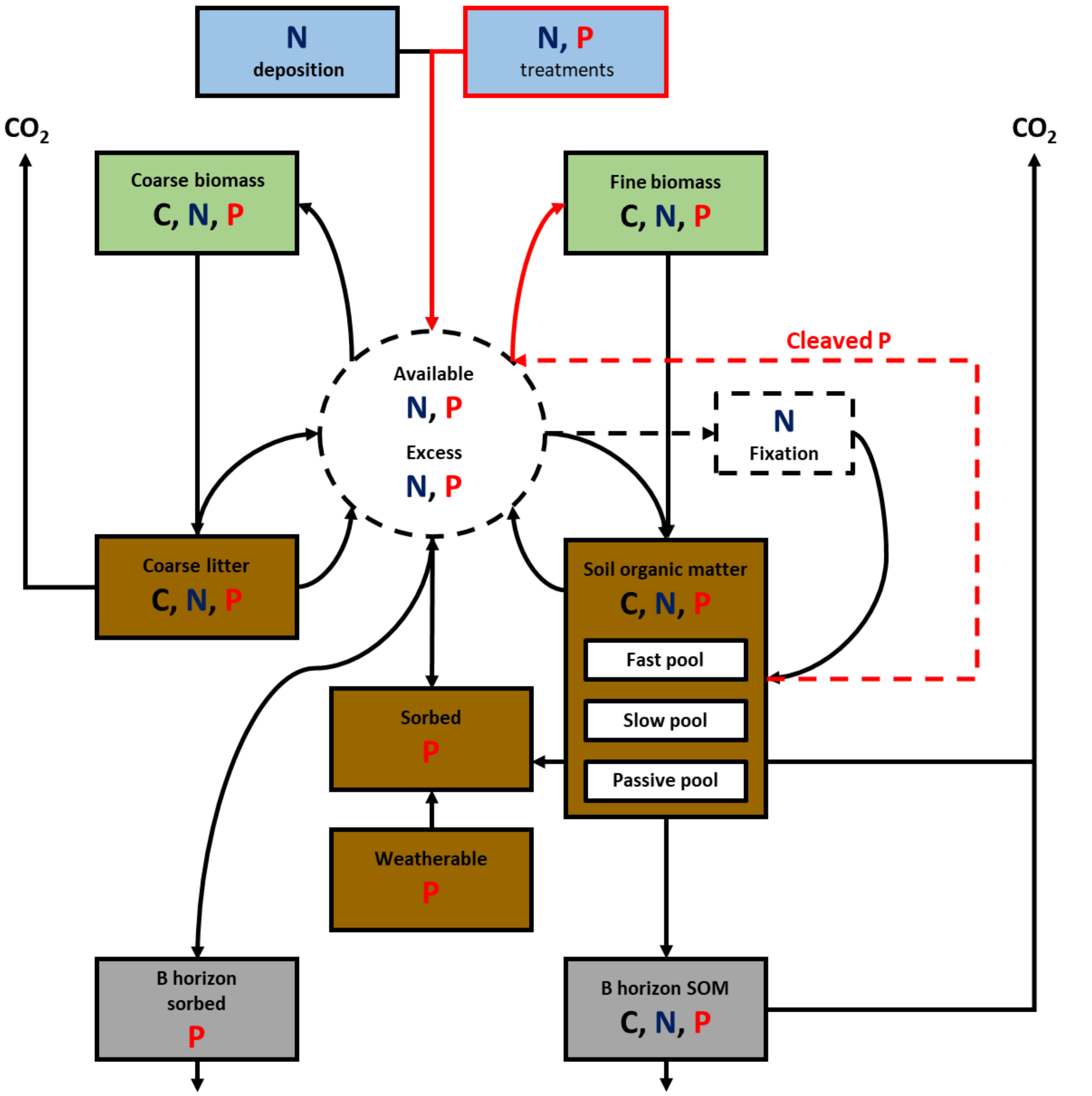

Figure 1: A simplified schematic of the key flows and pools of C, N and P within N14CP, adapted from the full schematic available in Davies *et al.* (2016a). Red lines highlight modifications to N14CP for the purpose of this work, including adding experimental nutrients and allowing uptake of cleaved P to be more flexible~~e than in N14CP~~. ~~n illustration of the plant available N and P pools in the N14CP model.~~ Solid lines indicate input to another pool and a dashed line indicates either a feedback or interaction with another pool. In the model, N can enter the available pool via atmospheric deposition, nutrient treatments, biological ~~nitrogen~~ fixation, and decomposition of coarse litter ~~decomposition~~ and SOM~~decomposition of the soil organic matter pools~~. For P, the two main ~~contemporary~~ sources are the inorganic sorbed pool and from the turnover of SOM~~soil organic matter~~. The former is derived initially from the weatherable supply of P, defined by its initial condition (P_{Weath0}). P can also be added to this pool experimentally as with N.~~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.~~ The dashed line going from available N and P to N fixation represents the downregulation of N fixation by N deposition and the dependency of N fixation on P availability. The cleaving of organic P from SOM and its incorporation into the plant-available nutrient pool, is represented by the dashed red line and its uptake by plants, determined by $P_{CleaveMax,}$ shown with a solid red line.

~~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.~~

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~~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.~~

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~~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

We use data from ~~both~~the Wardlow limestone and acidic grasslands to explore the potential role organic P access may have in determining grassland nutrient limitation when exposed to long-term N deposition and more recently, experimental nutrient manipulation. We use environmental input data collated from Wardlow to drive model processes. Empirical data regarding contemporary soil C, N and P for the contrasting grasslands is used to calibrate the initial size of the weatherable P pool within the model, and to allow access to organic cleaved P to vary to account for patterns in the data. We do n~~'~~ot aim to perfectly replicate the Wardlow grasslands but rather use the unique opportunity that Wardlow provides to ~~inform our understanding of poorly-understood model processes~~test our understanding of such P-limited ecosystems and how our conceptualisation of P access mechanisms within the model may affect them. In addition, we can use the model-~~-~~simulated grasslands to investigate the potential effects of long-term N deposition and nutrient manipulation on ~~ecosystems which differ in their limiting nutrient.~~ecosystems which may differ in their relative availability of different P forms.

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 (section 2.1). 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

N14CP simulations run on a quarterly time step and are spun up from the onset of the Holocene (10,000 BP in the model). This is to capture the length of time required for soil formation following deglaciation in north west Europe and is not an attempt to truly model this long term period. Instead, it allows us to form initial conditions for modern day simulations that takes in what we know about the site's history and forcings.

To use this spin up phase and simulate contemporary soil C, N and P stocks, we use a variety of input driver data. Inputs nearer the present are more accurately defined based on site-scale measurements and assumptions are made regarding past conditions. This approach of spinning up to present-day observations avoids the assumption that ecosystems are in a state of equilibrium, which is likely inaccurate for ecosystems exposed to long-term anthropogenic changes in C, N and P availability. ~~The most important i~~Input driver data ~~are~~include ~~plant functional type~~PFT history~~, which is analogous to land-use history~~, climatic data and N deposition data. A summary of the data used for model input is provided in supplementary Table S3. To simulate the sites' PFT history, we used data on Holocene pollen stratigraphy of the White Peak region of Derbyshire [Taylor *et al*. 1994], which captures important information regarding Wardlow's land-use history for the entire duration of the model spin up phase.

~~These data suggest a PFT history for the site that represents an early colonisation of virgin soil by herbaceous plants following deglaciation (10,000 BP) followed by succession to broadleaf temperate forest. This develops and persists until a forest disturbance (but not clearance) by humans 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.~~

~~Atmospheric N deposition, climate and PFT history~~Input drivers ~~need to be~~are provided as annual time series to drive the model and ~~. A~~as the acidic and ~~calcareous~~ limestone 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 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~~ limestone 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~~ limestone 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 allowed the maximum rate at which plants could access cleaved P (P~~$\sim\sim_{CleaveMax}$~~) to vary, to investigate how plant P acquisition might change when more readily accessible P forms become scarcer, a We also allowed~~ ~~$P_{CleaveMax}$ to vary as this mechanism for P acquisitionwhich has been under-explored in previous modelling studies [Janes-Bassett *et al.* 2020]. This is the first time that this model has been knowingly applied to ecosystems of a largely P-limited rather than N-limited N-P co-limited or P-limited grasslands instead of N-limited sites nature. Soil organic P has been shown to be an important source of P to plants in P-stressed environments [Balemi and Negisho, 2012; Chen *et al.* 2020], yet. However, the rates of access to SOP and their controls are relatively poorly understood. We therefore use a similar data-driven calibration for $P_{CleaveMax}$ as we do for P_{Weath0}. 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.~~

As this is the first time that N14CP has been knowingly applied to ecosystems of a largely P-limited nature, we also allowed the maximum rate at which plants could access cleaved P ($P_{CleaveMax}$) to vary, to investigate how plant P acquisition might change when more readily accessible P forms become scarcer. Empirical quantification of organic P access is poor [Janes-Bassett *et al.* 2020], hence we use a similar data-driven calibration for $P_{CleaveMax}$ as we do for P_{Weath0}.

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 2~~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~~ limestone grasslands (Supplementary section S1.1.), was used within the analysis.

$$F = \left(\frac{SAE[C_{SOM}]}{\bar{C}_{SOM,Obs}}\right)/C_n + \left(\frac{SAE[N_{SOM}]}{\bar{N}_{SOM,Obs}}\right)/N_n + \left(\frac{SAE[P_{Total}]}{\bar{P}_{Total,Obs}}\right)/P_n \qquad \text{(Equation 2~~1~~)}$$

Plant biomass C data were excluded from the cost function to allow for blind testing of the model's performance against empirical observations. As the variable most responsive to nutrient additions, both in terms of rapidity and magnitude of the response, we deemed these the most rigorous data to use for separate testing. We included soil C, N and P data from all nutrient treatments rather than just the control to ensure that the selected parameter combination could better account for patterns in empirical data. For instance, we know that empirical N treatments can increase plant ~~phosphatase~~and soil enzyme activity in both Wardlow grasslands, [Johnson *et al*. 1999; Phoenix *et al*. 2004; Keane *et al*. 2020] which a calibration to control-only data may not have captured.

While the cost function is a useful tool in allowing the model to simulate the magnitude of contemporary C, N and P pools, it does n~~'~~ot allow us to capture all necessary information to accurately simulate grasslands of contrasting nutrient limitation. The pattern of grassland response, i.e. how a variable responds to nutrient treatment, is an important consideration and is determined in the model by the most limiting nutrient. Consequently, the parameter combination with the lowest F value, that still maintained a grassland's empirical response to nutrient additions (Supplementary section S1.1.), was used within the analysis.

3. Results

Below, we first present data regarding the results of the calibration of P_{Weath0} and $P_{CleaveMax}$ for each grassland, and how simulated grassland C, N and P using these parameter combinations compares to the empirical data (section 3.1, Figure 2). Second, we explore how the limiting nutrient of the modelled grasslands has changed through time in response to N deposition and experimental treatment (section 3.2, Figure 3). Third, we explore how C, N and P pools in the simulated grasslands have responded to N deposition and nutrient treatment within the model, and include empirical data to contextualise changes (section 3.3, Figure 4). Finally, we present the C, N and P budgets for both modelled grasslands to examine changes in C, N and P pools more closely, in order to better our mechanistic understanding of changes in nutrient flows within the model (section 3.3, Figure 5).

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.~~

The model calibration selected parameter values for P_{Weath0} and $P_{CleaveMax}$ that indicate contrasting use of P sources by the two simulated 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 limestone, with a value 10 times smaller at 0.0316 g m^{-2} season^{-1}. Conversely, inorganic P availability was greater in the limestone 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 selected parameter combinations resulted in the model simulating the acidic grassland as N-limited and the limestone as P-limited, with reasonable congruence between observed and modelled data. 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 limestone grasslands (Fig 2). Raw data used for Figure 2 are provided in supplementary tables S5 and S6.

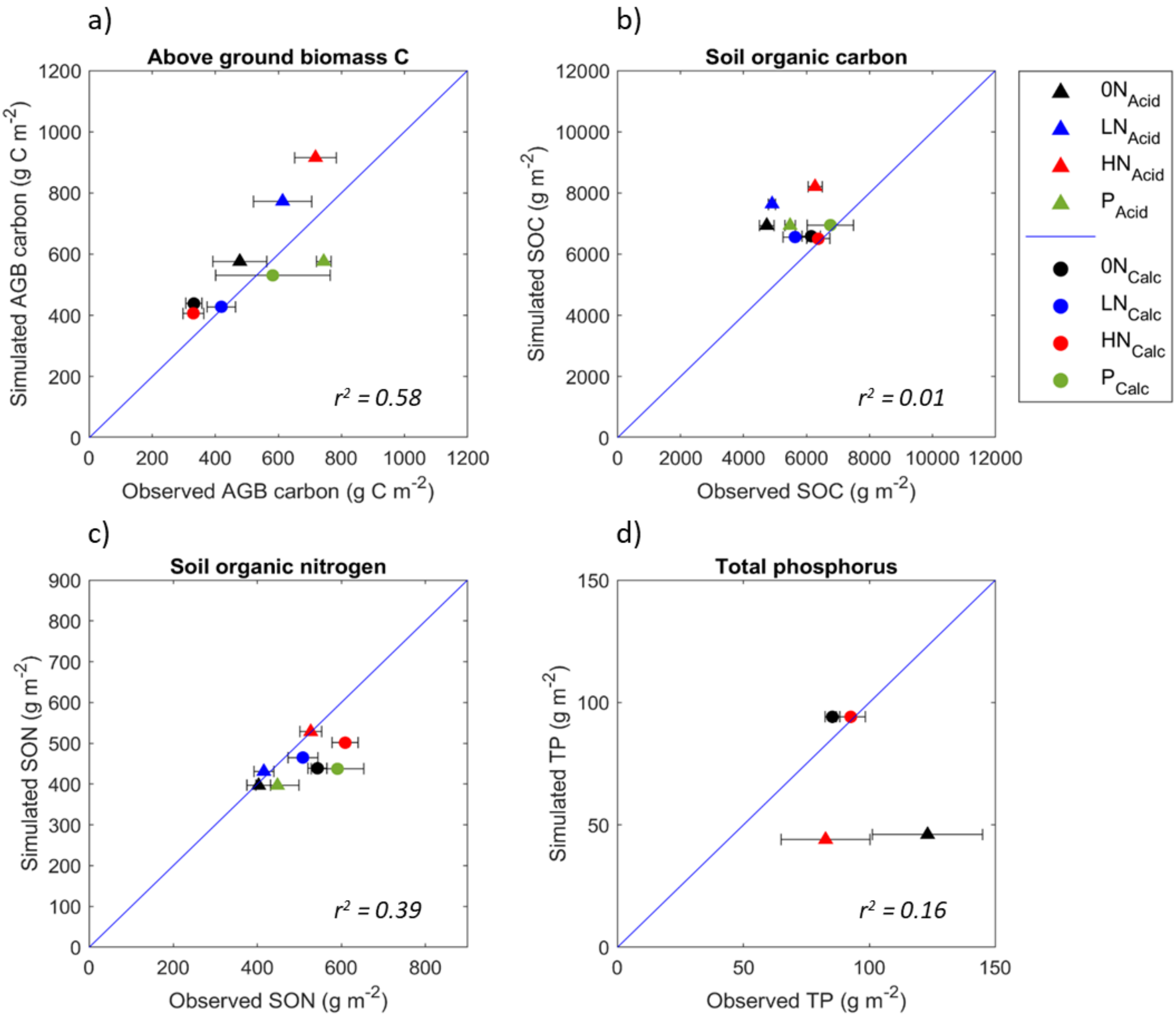
Overall, N14CP more accurately simulated the magnitude of limestone grassland C, N and P pools than the acidic, and it generally captured the pattern of responses to nutrient treatment, albeit this is not always supported by high r^2 values. The model estimates of above ground biomass C are broadly aligned 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 limestone grasslands respectively (Fig 2a).

Soil organic C on average was slightly overestimated (7.1% with SE ± 3.3) for the limestone 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. Despite a low r^2 value for SOC (0.01), the model broadly captured the patterns we observe in the empirical data, with N addition increasing SOC in the acidic and P addition increasing SOC in the limestone. However, the intermediate increase in SOC with P in the acidic grassland is not captured by the model, nor is the magnitude of the negative effect of LN treatment on limestone SOC.

Simulated magnitudes of SON are well-aligned with observations for the acidic grassland, with an average error of 2.3% (SE ± 3.2), whilst SON for the limestone grassland was on average underestimated by 17.8% (SE ± 3.6) (Fig 2c). The variation between treatments was better captured for acidic than limestone SON but was overall reasonable (r^2 = 0.39).

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 limestone 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). With only two empirical data points for TP across only two nutrient treatments, it is difficult to discern the relationship between treatments and TP so an r^2 value is of little relevance here.


a)
Above ground biomass C
Simulated AGB carbon (g C m-2)
Observed AGB carbon (g C m-2)
r2 = 0.58
b)
Soil organic carbon
Simulated SOC (g m-2)
Observed SOC (g m-2)
r2 = 0.01
0NAcid
LNAcid
HNAcid
PAcid
0NCalc
LNCalc
HNCalc
PCalc
c)
Soil organic nitrogen
Simulated SON (g m-2)
Observed SON (g m-2)
r2 = 0.39
d)
Total phosphorus
Simulated TP (g m-2)
Observed TP (g m-2)
r2 = 0.16

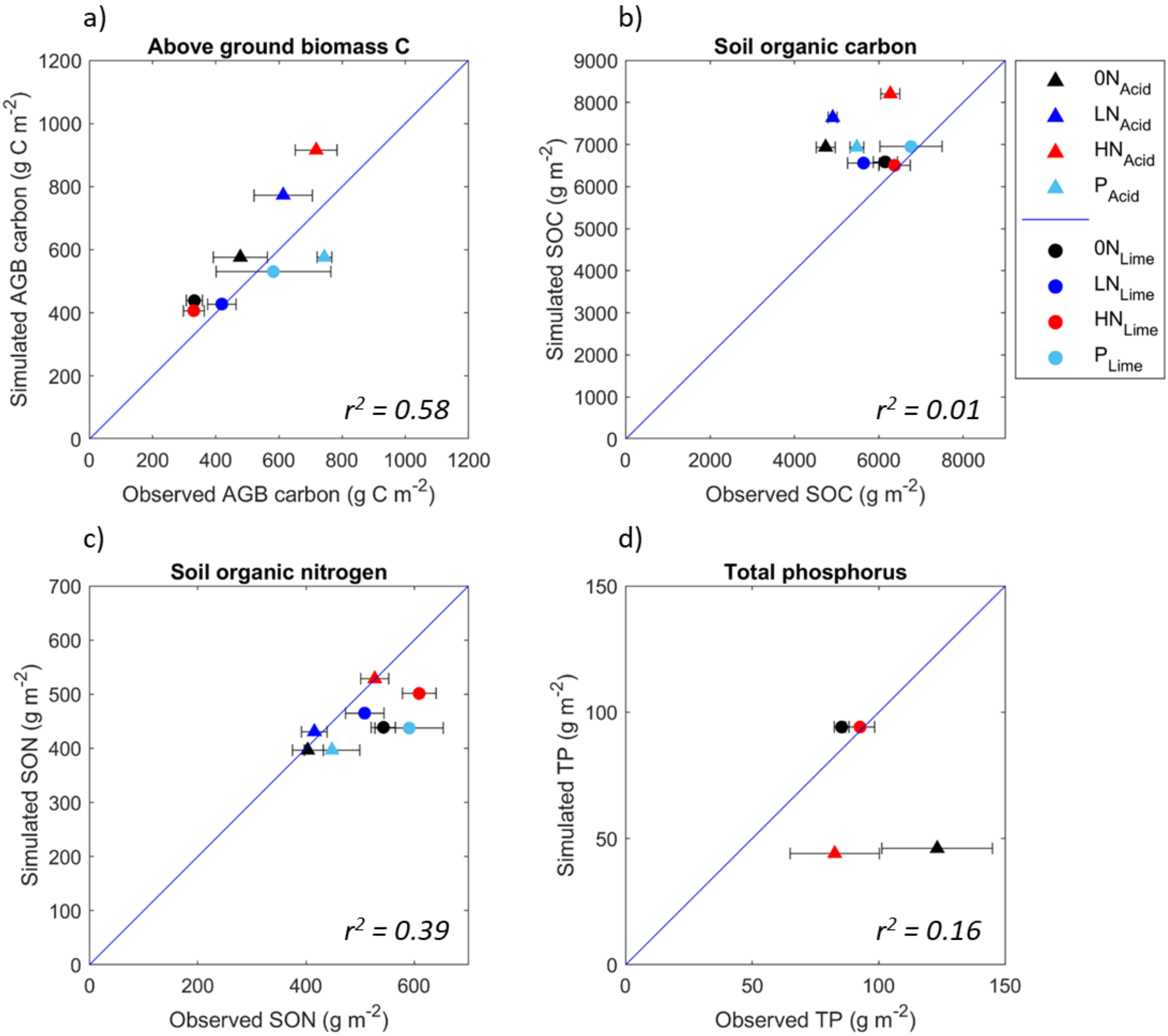


Figure 2: A comparison of the observed values of a) aboveground biomass carbon, b) soil organic carbon, c) soil organic nitrogen and d) total soil phosphorus from both grasslands, with simulated values from the model. The blue line represents a 1 to 1 relationship and the closer the data points are to 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 give an overall indication of the direction of response of each variable to nutrient addition, hence a low value is not necessarily indicative of poor model fit~~are presented to assess closeness to the 1 to 1 line.~~

3.2. The limiting nutrient through time

M~~The m~~odelled ~~suggests that the~~ acid~~ic~~ 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 simulated ~~calcareous~~ limestone 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~~ limestone 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 P is used 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 access to cleaved P~~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.

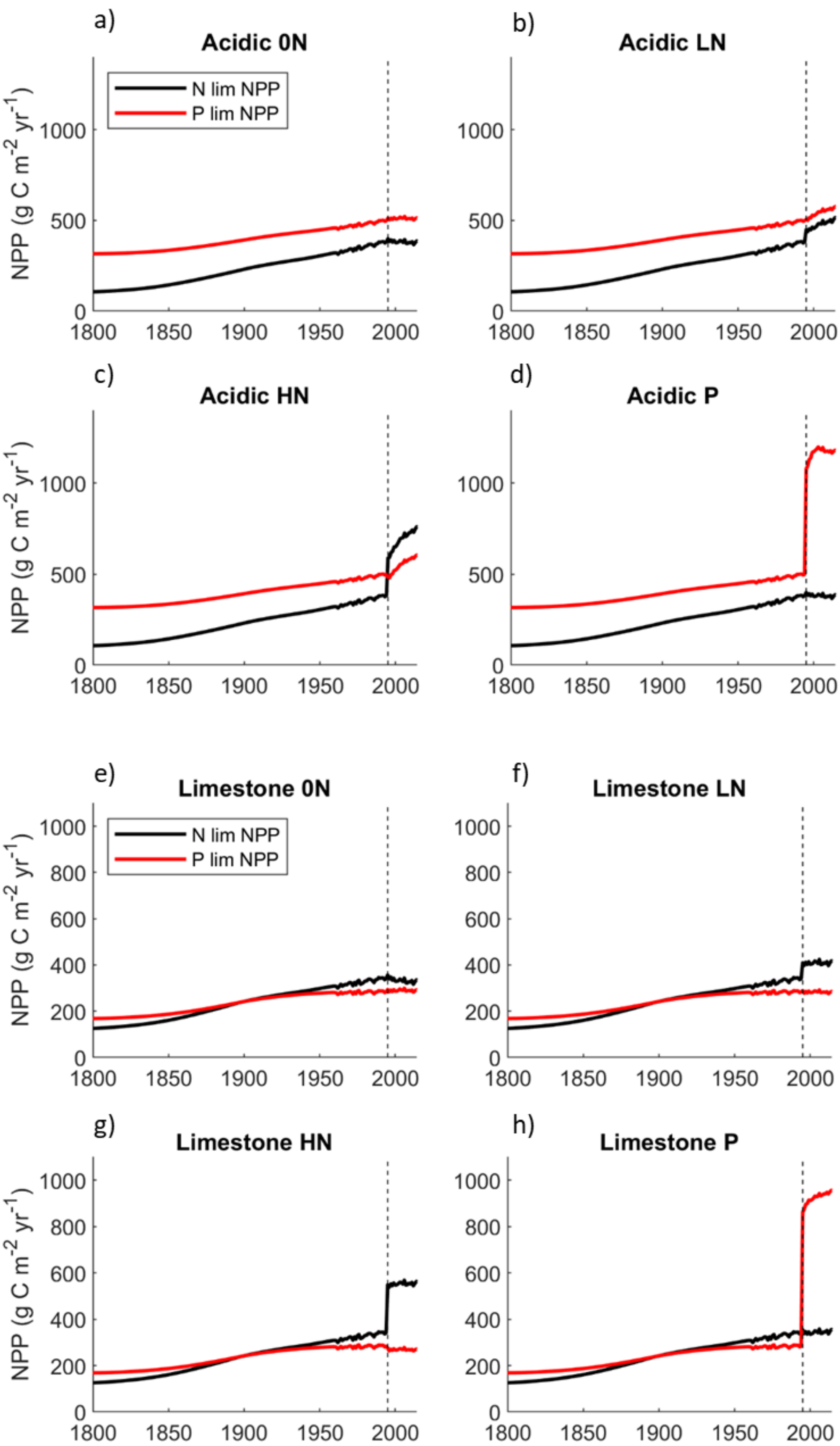


Figure 3: Plots showing the nutrient most limiting productivity for all nutrient treatments in both simulated grasslands. The vertical dashed line is the year of first nutrient addition within the model (1995). The value of the lines represents the maximum amount of productivity attainable given the 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.

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 the onset of large-scale N deposition (1800 within the model) ~~-~~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~~limestone).

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~~ C and ~~nitrogen~~ N 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).

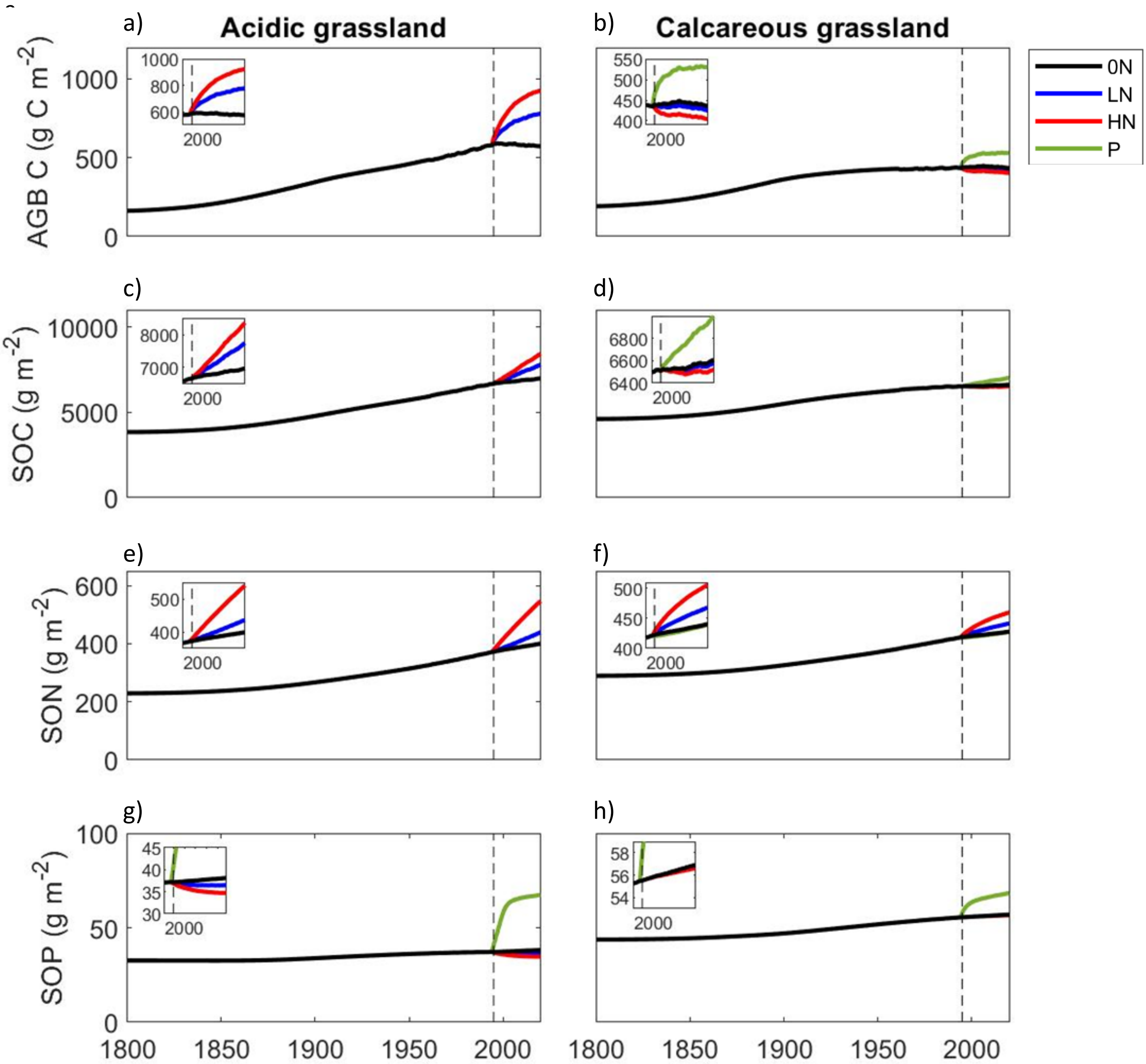
3.3.2. ~~Calcareous~~ Limestone grassland

Model simulations for the ~~calcareous~~ limestone 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~~ limestone 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~~ limestone 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~~ limestone 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~~ limestone grassland, particularly in the topsoil sorbed pool (Fig 5f, Table S13).


a)
Acidic grassland
b)
Calcareous grassland
0N
LN
HN
P
AGB C (g C m-2)
c)
d)
SOC (g m-2)
e)
f)
SON (g m-2)
g)
h)
SOP (g m-2)
1800
1850
1900
1950
2000

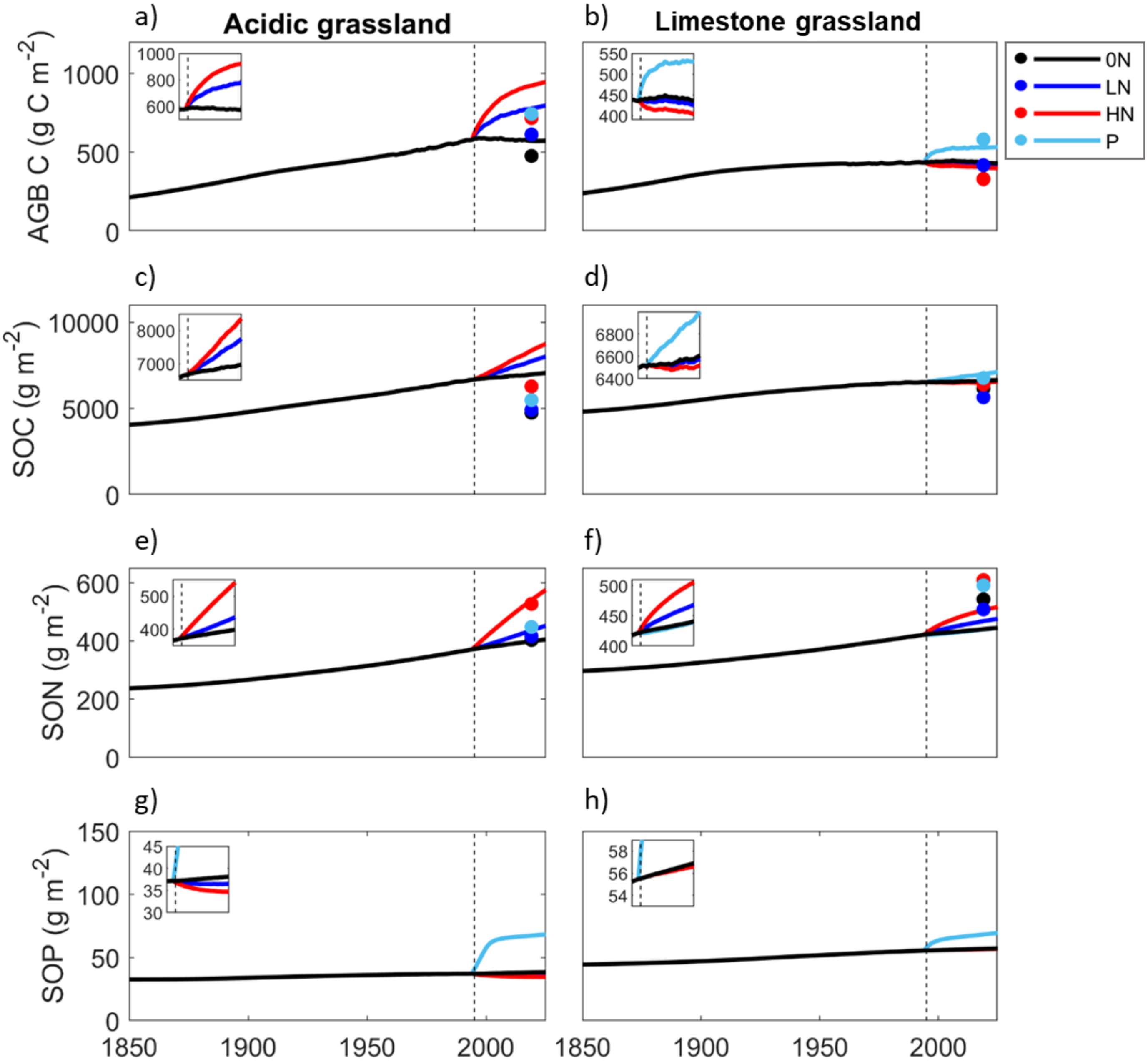


Figure 4: Time series plots of aboveground biomass C, soil organic C, N and P for the acidic (panels a, c, e and g respectively) and ~~calcareous~~limestone modelled grasslands (panels b, d, f and h respectively). ~~grasslands from 1800 to present day.~~ The vertical dashed line represents the ~~year of first nutrient addition~~first year of nutrient addition (1995) and marks the beginning of the experimental period ~~nutrient additions~~. The inset subplots ~~show data from 1990 to 2020 to capture~~focus on ~~the~~ this 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. Empirical data from figure 2 are plotted on the respective panels, with the exception of panels g and h, where empirical data is incompatible with modelled data (total P versus organic P).

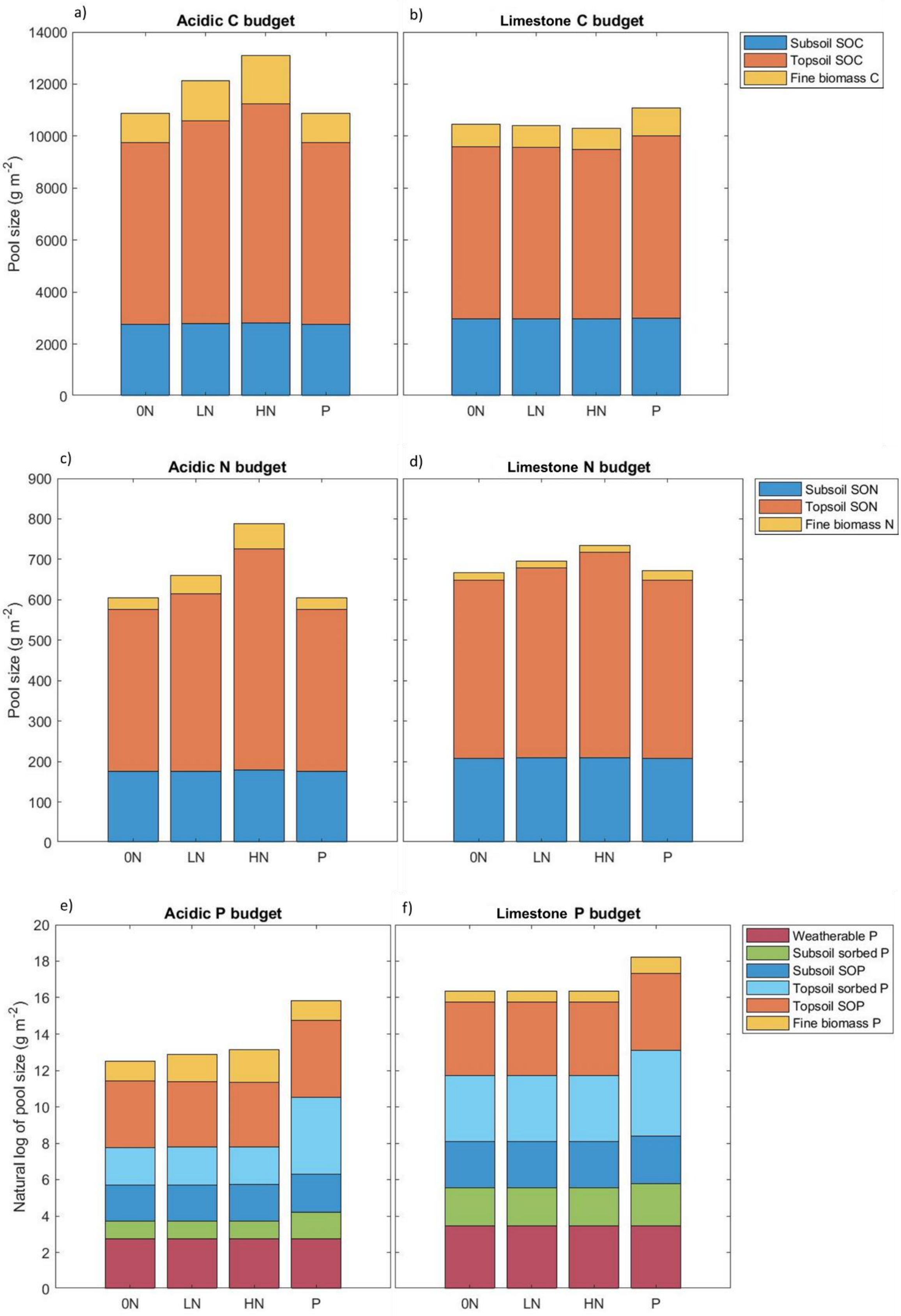


Figure 5: Modelled ~~carbon, nitrogen and phosphorus~~C, N and P budgets for the acidic (panels a, c and e) and ~~calcareous~~ limestone (panels b, d, f) grasslands for the year 2020. Modelled sizes of C and N pools are in grams per metre squared, and P pools are presented as $\log_n$ grams per metre 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~~contrasting grasslands by varying plant access to P sources

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 P-limited ecosystems and investigated their responses to N deposition and additional nutrient treatments. By using empirical data from long-term experimental grasslands to drive and calibrate N14CP, we could test the model's ability to simulate two contrasting P-limited grasslands, and how organic P access may affect this ability. While the purpose of this work was not to explicitly reproduce the Wardlow grasslands within N14CP, by comparing data from Wardlow to the simulated grasslands, we can simultaneously develop our understanding of the model's representation of under-studied P cycling processes and contextualise what this may mean for empirical systems such as Wardlow.

The model suggests that the acidic grassland was characterised by high access to organic P, with comparatively low inorganic P availability, whereas the limestone grassland was the opposite, with low organic and high inorganic P availability. These simulated differences could reflect the relative availability of different P sources at Wardlow. As the acidic grassland formed in a hillside depression, loess has accumulated, thickening the soil profile and distancing the plant community from the limestone bedrock. The plant rooting zone of the acidic grassland is therefore not in contact with the bedrock, and 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 limestone grassland soil rarely exceeds 10 cm depth, and the rooting zone extends to the limestone beneath, providing plants with greater access to weatherable calcium phosphate [Smits *et al*., 2012].

Such parameter combinations allowed for reasonable congruence between empirical and simulated data, with an average discrepancy of only 6.6% (SE ± 9.1) and 1.2% (SE ± 4.4) for the acidic and limestone grasslands respectively across all variables (Table S5). However, model performance differed greatly between the two grasslands. For instance, the model accurately captured the magnitude of limestone C, N and P data and their expected P-limited responses to nutrient treatment, but was less effective at simulating the acidic grassland. N14CP did no~~'~~t simulate an increase in biomass C or SOC with P addition in the acidic grassland, instead simulating a solely N-limited grassland. ~~-~~While this may be expected of a model that employs a law-of-the-minimum approach, 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~~ positive responses to LN, HN and P treatment, as observed in the empirical data from 2017 (section 2.2.2).

The overestimation of acidic C pools and underestimation of total P suggests that ~~too m~~the model is simulating that too much organic P is being accessed by plants in response to N addition and transferred into plant biomass pools (Fig 2d). Few parameter sets where simultaneously able to simulate the magnitude of the empirical TP pool and the positive response of biomass to N addition in the acidic grassland. This may also be due to limitations in the empirical P data, as P data used for calibrating P cycling were available for only two nutrient treatments and represented total soil P, not organic P. While we acknowledge the technical and theoretical issues associated with distinguishing between organic and inorganic P pools [Lajtha *et al.* 1999; Barrow *et al.* 2020], such distinctions would help in understanding this discrepancy and likely improve the model's ability to simulate P-limited systems, particularly when organic P availability may be important.

Additionally, N14CP's representation of organic P cleaving likely underestimates the ability of soil to rapidly occlude and protect organic P that enters solution. For example, inositol phosphate, a major constituent of organic P, has been found to be used extensively by plants grown in sand but is hardly accessed by plants grown in soil [Adams and Pate 1992]. Such organic phosphates become strongly bound to oxides in the soil, protecting them from attack by phosphatase enzymes [Barrow 2020]. This may be particularly prevalent in the acidic grassland at Wardlow where N deposition has resulted in acidification and base cation depletion [Horswill *et al.* 2008], potentially enhancing the formation of iron and aluminium complexes and immobilising P [Kooijman *et al*., 1998]. As the model lacks a mechanism for increasing access to secondary mineral P forms comparable to organic P-cleaving, the uptake of organic P by the acidic grassland is very ~~no doubt~~likely exaggerated.

The model's inability to simulate a positive response to both N and P addition in the acidic grassland may be an unintended consequence of the downregulation of N fixation by N deposition included within N14CP [Davies *et al*. 2016b]. While this representation is appropriate [Gundale *et al*. 2013], 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 behaviour akin to~~'true'~~ N-P co-limitation [Harpole *et al*., 2011] under high levels of N deposition challenging. This 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 by calibrating access to both organic and inorganic P sources [Davies *et al*. 2016b], provided the ecosystem in question's limiting nutrient leans towards N or P limitation. Furthermore, where access to organic P forms is likely to be lower, as in the limestone grassland, model performance may improve. This could be further explored by allowing N fixation limits in the model to adapt to P nutrient conditions or by attenuating the suppression of N deposition on N fixation, to represent acclimatisation of N-fixers to greater N availability [Zheng *et al*. 2018].

Ultimately, differences in modelled accessibility to organic forms of P enabled N14CP to distinguish between the two empirical grasslands, and simulate the magnitude and pattern of data with reasonable accuracy, albeit with the previously mentioned caveats.

4.2. Consequences of differential P access on ecosystem C, N and P

While the model's estimation of $P_{CleaveMax}$ for the acidic grassland is likely overestimated, the model experiment has highlighted that differences in organic versus inorganic P availability are a key determinant of an ecosystem's nutrient limitation, and consequently, how they respond to changes in anthropogenic N and P availability. For instance, while being exposed to the same background level of N deposition and the same magnitude of experimental treatment, the modelled acidic grassland was able to stimulate growth in response to LN and HN treatment whereas the modelled limestone grassland was negatively affected by it.

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 limestone grassland, and depleting the SOP pool in the acidic. As P cleaved from organic pools is the least bioavailable within the model hierarchy (methods 2.2.3), this is indicative of increasing P stress in both grasslands. 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 limestone grassland (Table S13), highlighting how the grassland's $P_{CleaveMax}$ capability is insufficient to meet increased P demand.

Such high access to organic P sources in the modelled acidic grassland likely led it to respond to nutrient enrichment in an N-limited manner, increasing productivity in response to N deposition and LN and HN treatments as the model's limiting nutrient stimulated plant growth. Detrital C inputs from plant biomass are the primary source of SOC accumulation within N14CP [Davies *et al*., 2016b] and as such, changes in SOC integrate long term trends in net primary productivity in systems where external nutrients are supplied. The provision of additional N in the modelled LN and HN treatments therefore led to large increases in biomass accumulation and consequently, almost linearly increased SOC (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].

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]. Our simulated acidic grassland therefore supports the hypothesis that prolonged N deposition may increase SOP access to such an extent that P limitation is alleviated and growth can be stimulated [Chen *et al*. 2020]. Organic P release from SOM and its potential immobilisation, is poorly represented in models and we encourage further study aimed at quantifying these processes [Chen *et al*. 2020; Janes-Bassett *et al*., 2020; Phoenix *et al*., 2020]. However, such high rates of SOP access only occurred under experimental LN and HN treatments, and in reality, such rapid degradation of SOP may eventually degrade the pool to such an extent that P limitation soon returns.

Conversely, biomass C and SOC in the modelled limestone grassland responded positively to P addition, via similar mechanisms to the N-response in the modelled acidic grassland. However, in contrast to the acidic grassland, N addition caused declines in limestone biomass and SOC, the former of which has been observed at the limestone grassland at Wardlow [Carroll *et al*., 2003]. Reductions in limestone 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. This implies that in ecosystems where plants are not well-adapted to acquiring organic forms of P [Phoenix *et al*. 2020], or where organic P is scarce, N deposition may worsen pre-existing P limitation and reduce ecosystem C stocks [Goll *et al*. 2012; Li *et al.* 2018].

4.3. ~~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 additional forb biomass lowering litter C:N and increasing its decomposability [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.~~

~~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.~~

~~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.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.~~

~~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).~~

~~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]. 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.~~

~~Nitrogen fixation remained unaffected by nutrient treatment in both grasslands (Tables S8, S12).~~

~~4.3. 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.~~

~~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.~~

Model limitations

While N14CP is a fairly simple ecosystem model by design, it is one of the first process-based biogeochemical models to explicitly incorporate P with the C and N cycles for semi-natural ecosystems, and to simulate NPP and soil C, N and P dynamics, for which it has been extensively tested [Davies *et al.* 2016a; Davies *et al.* 2016b; Tipping *et al.* 2017; Tipping *et al.* 2019; Janes-Bassett *et al.* 2020]. Previous work with N14CP has identified the need to enhance its ability to simulate organic P cycling [Janes-Bassett *et al.* 2020], which we aimed to do in this study using long-term experimental data from contrasting P-limited grasslands.

N14CP's simplified representation of plant nutrient pools and plant control over nutrient uptake, is largely controlled by stoichiometric demand [Davies *et al.* 2016a], and does not incorporate many plant strategies for P acquisition [Vance *et al.* 2003]. Indeed, by allowing $P_{CleaveMax}$ to vary to account for empirical data, we attempt to somewhat increase plant control over organic P uptake. We acknowledge earlier that such an approach likely underestimates the ability of soil surfaces and microbes to protect newly-cleaved P from plant uptake. As such, where we may expect access to organic P to be high, such as the acidic grassland at Wardlow, such modelled representation of plant-mediated P access may lead to unrealistic depletions in soil P and increases in biomass and soil C, and we would encourage further work aimed at improving model-representation of plant controls on organic P cycling [Fleischer *et al.* 2019].

While we feel incorporating a suite of plant strategies for acquiring P would represent over-parameterisation, we acknowledge that a modelled equivalent to $P_{CleaveMax}$ for accessing inorganic P forms is lacking, such as carbon-based acid exudation to increase mineral P weathering [Achat *et al.* 2016; Phoenix *et al.* 2020], which likely contributes toward the poor representation of the acidic total P pool. Biota-enhanced P weathering and nutrient redistribution by mycorrhizal hyphae are important for nutrient cycling [Quirk *et al.* 2012], and fungal community structure and function is strongly influenced by perturbations in the C and N cycles [Moore *et al.* 2020]. Such processes are not included within N14CP as the extent to which weathering can be controlled by such mechanisms and the manner in which these can be represented in C-N-P cycle models is debated [Davies *et al.* 2016b].

Currently, N14CP assumes C to be in unlimited supply, with its uptake by plants and consequent input into soil pools controlled by C:N:P stoichiometry, hence C availability has little effect on N and P dynamics within the model. Increasing atmospheric CO_2 may increase nutrient availability, as plants may reallocate additional carbon resources toward nutrient acquisition [Keane *et al.* 2020] or elevated CO_2 (eCO_2) may increase limitation of other nutrients such as N [Luo *et al.* 2004]. The inclusion of eCO_2 into N14CP poses a particularly enticing research opportunity, and we aim to use this study as a foundation for future work to include this process.

5. Conclusions

We have shown that by varying two P-acquisition parameters within N14CP, we can account for contrasting responses of two P-limited grasslands ~~which differ in their relative strength of P limitation~~of differing soil P chemistry, and with reasonable accuracy ~~two N-P co-limited grasslands to long-term nutrient manipulation with reasonable accuracy~~. However, such coarse representation of organic P cycling in the model likely overestimates the ability of plants to use newly-cleaved P and limits our ability to simulate grasslands where N and P interact to control plant productivity, including the potential for N inputs to alleviate P limitation ~~of a more N-P co-limited nature~~.

Differences in organic P access was a key factor distinguishing the contrasting responses of the modelled grasslands to nutrient manipulation, with ~~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~~ high plant access~~organic P access~~ allowing~~ed~~ the ~~modelled~~ acidic grassland to acquire sufficient P to match ~~the~~ available N from chronic deposition and prevent 'anthropogenic P limitation'. In the acidic grassland, N treatment stimulated plant access of ~~to soil~~ organic P~~P pools~~, promoting ~~plant~~ growth and C sequestration. 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~~may return. ~~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~~ limestone grassland, which was less able to access organic P, additional N provision exacerbated pre-existing P limitation by simultaneously increasing plant P demand and reducing ~~its~~P bioavailability. This reduced productivity and consequently C input to soil pools declined, resulting in SOC ~~respiration~~degradation exceeding its replacement.

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 modelled acidic and limestone grasslands, to the extent where almost half of contemporary soil C and N in the model could be from, or caused by, N deposition.

~~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 with sufficient access to organic P, long-term N addition may alleviate P limitation. Where organic P access is limited,~~ as~~ N deposition could shift~~shifts~~ more ecosystems toward a state of P limitation or strengthen~~s~~ it where it already occurs [Goll *et al*., 2012], ~~we~~ reducing productivity ~~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

Competing interests: The authors declare that they have no competing interests.

Personal acknowledgements: We thank Jonathan Leake for his insightful interpretation of our findings and for constructive feedback on early versions of the work. In addition, we are grateful for technical assistance from Irene Johnson, Heather Walker and Gemma Newsome, without whom there would be no carbon and nitrogen data for model input. We are grateful to the Met Office UK and the Centre for Ecology and Hydrology for use of their meteorlogical and deposition data respectively. Finally, we wish to extend our thanks to James Fisher for his earlier work on Wardlow carbon data, which prompted additional investigation into the grassland's carbon stocks.

Site access: Shaun Taylor at Natural England.

Funding: This work was funded by the Natural Environment Research Council award NE/N010132/1 to GKP and NERC award NE/N010086/1 to IPH of the 'Phosphorus Limitation and Carbon dioxide Enrichment' (PLACE) project. This work was also funded through 'Adapting to the Challenges of a Changing Environment' (ACCE), a NERC-funded doctoral training partnership to CRT: ACCE DTP NE/L002450/1.

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