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. Phosphorus (P) limited ecosystems are widespread, yet there is limited understanding of how these ecosystems may respond to anthropogenic deposition of nitrogen (N), and the interconnected effects on the biogeochemical cycling of carbon (C), N and P. 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 responses to a long-term nutrient-manipulation experiment. We do this by combining data with an integrated C-N-P cycling model (N14CP). We explore the role of P-access mechanisms by allowing these to vary in the modelling framework, and comparing model plant-soil C-N-P outputs to 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, increasing biomass C input to soil and promoting SOC sequestration in response to N addition. Conversely, poor accessibility of organic P for the limestone grassland meant N provision exacerbated P-limitation and reduced biomass input to the soil, reducing soil carbon storage. Plant acquisition of organic P may therefore play an important role in reducing P-limitation, and determining responses to anthropogenic changes in nutrient availability. We conclude that grasslands differing in their access to organic P may respond to N deposition in contrasting ways, and where access is limited, soil organic carbon stocks could decline. and stress that should N deposition shift ecosystems toward stronger P-limitation, a globally important carbon sink risks degradation.

1. Introduction

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52 Grasslands represent up to a third of terrestrial net primary productivity (NPP) [Hoekstra *et al.*, 2005]

53 and potentially hold over 10% of the total organic carbon stored within the biosphere [Jones and

54 Donnelly, 2004]. The ecosystem services provided by grasslands, such as carbon storage, are highly

55 sensitive to perturbations in their nutrient cycling, including the perturbation of nitrogen (N) inputs

56 from atmospheric deposition [Phoenix *et al.*, 2012].

57 Since the onset of the industrial revolution, human activity has doubled the global cycling of N, with

58 anthropogenic sources contributing 210 Tg of fixed N per year to the global N cycle, surpassing

59 naturally fixed N by 7 Tg N yr^{-1} [Fowler *et al.*, 2013]. Much of this additional N is deposited on terrestrial

60 ecosystems from atmospheric sources. This magnitude of N deposition results in a range of negative

61 impacts on ecosystems (including grasslands) such as reductions in biodiversity [Bobbink *et al.*, 2010;

62 Southon *et al.*, 2013], acidification of soil, and the mobilisation of potentially toxic metals [Carroll *et*

63 *al.*, 2003; Horswill *et al.*, 2008; Phoenix *et al.*, 2012]

64 Despite large anthropogenic fluxes of N, most terrestrial ecosystems on temperate post-glacial soils

65 are thought to be N-limited (biomass production is most restricted by N availability) [Vitousek and

66 Howarth, 1991; Du *et al.*, 2020], as weatherable sources of phosphorus (P) remain sufficiently large to

67 meet plant P demand [Vitousek and Farrington, 1997; Menge *et al.*, 2012]. Both empirical and

68 modelling studies have shown that pollutant N, when deposited on N-limited ecosystems, can increase

69 productivity [Tipping *et al.*, 2019] and soil organic carbon (SOC) storage [Tipping *et al.*, 2017], largely

70 as a result of stimulated plant growth. This suggests that while there are negative consequences of N

71 deposition, there may also be benefits from enhanced plant productivity and increases in carbon

72 sequestration.

73 Whilst most research focuses on N-limited ecosystems [LeBauer and Treseder, 2008], a number of

74 studies have highlighted that P limitation and N-P co-limitation are just as prevalent, if not more

75   widespread, than N limitation [Fay *et al*., 2015; Du *et al*., 2020; Hou *et al*., 2020]. In a meta-analysis of

76   grassland nutrient addition experiments spanning five continents, Fay *et al*. [2015] found that

77   aboveground annual net primary productivity was limited by nutrients in 31 out of 42 sites, most

78   commonly through co-limitation of N and P [Fay *et al*., 2015]. Similarly, P additions in 652 field

79   experiments increased aboveground plant productivity by an average of 34.9% [Hou *et al*., 2020],

80   while it is estimated that P limitation, alone or through co-limitation with N, could constrain up to

81   82% of the natural terrestrial surface's productivity [Du *et al*., 2020].

82   Furthermore, P limitation may be exacerbated by N deposition [Johnson *et al*., 1999; Phoenix *et al*.,

83   2004], or become increasingly prevalent as previously N-limited ecosystems transition to N-sufficient

84   states [Goll *et al*., 2012]. For example, in parts of the Peak District National Park, UK, N deposition has

85   exceeded 3 g m^{-2} yr^{-1}, with further experimental additions of 3.5 g m^{-2} yr^{-1} leading to decreases rather

86   than increases in productivity of limestone grasslands [Carroll *et al*., 2003]. This makes P limitation

87   critical to understand in the context of global carbon and nutrient cycles. By definition, N deposition

88   should impact P-limited ecosystems differently to N-limited ones, yet there is little understanding of

89   how N deposition impacts these systems.

90   While N deposition may worsen P limitation in some instances, plant strategies for P acquisition may

91   require substantial investments of N, suggesting that increased N supply may facilitate enhanced P

92   uptake [Vance *et al.,* 2003; Long *et al*., 2016; Chen *et al*., 2020]. Indeed, previous work from long-term

93   experimental grasslands has shown strong effects of N deposition on plant enzyme production

94   [Johnson *et al.* 1999; Phoenix *et al.* 2004], whereby the production of additional extracellular

95   phosphatase enzymes was stimulated. While it is not clear if this response is driven by exacerbated P-

96   limitation resulting from N deposition or extra N availability making elevated enzyme production

97   possible, such changes in plant physiology may promote cleaving of P from organic soil pools. Over

98   time, the accumulation of plant-available P from organic sources may provide a mechanism by which

99   plants exposed to high levels of N deposition may overcome P limitation [Chen *et al.* 2020].

100   By using the integrated C-N-P cycle model N14CP, Janes-Bassett *et al.* [2020] suggest that the role of

101   organic P cycling in models may be poorly represented, as the model failed to simulate empirical yield

102   data in agricultural soils with low P fertiliser input. Organic P access is therefore likely an important

103   means of nutrient acquisition for plants in high N and low P soils [Chen *et al.* 2020], yet our

104   understanding of organic P cycling in semi-natural ecosystems is fairly limited [Janes-Bassett *et al.*

105   2020]. Such interdependencies of the C, N and P cycles make understanding an ecosystem's response

106   to perturbations in any one nutrient cycle challenging, particularly when ecosystems are not solely

107   limited in N. This highlights the need for integrated understanding of plant-soil nutrient cycling across

108   the C, N and P cycles, and in ecosystems that are not solely N-limited.

109   Process-based models have a role to play in addressing this, as they allow us to test our mechanistic

110   understanding and decouple the effects of multiple drivers. There has been increasing interest in

111   linking C with N and P cycles in terrestrial ecosystem models [Wang *et al*., 2010; Achat *et al*., 2016;

112   Jiang *et al*., 2019] as the magnitude of the effects that anthropogenic nutrient change can have on

113   biogeochemical cycling are realised [Yuan *et al*., 2018]. Yet, few modelling studies have explicitly

114   examined the effects of P limitation, or the role of organic P access in determining nutrient limitation,

115   likely mirroring the relatively fewer empirical studies of these systems.

116   By combining process-based models with empirical data from long-term nutrient-manipulation

117   experiments, we may simultaneously improve our understanding of empirical nutrient limitation, the

118   role(s) of organic P acquisition, and their interactions with anthropogenic nutrient pollution. In

119   particular, this approach offers a valuable opportunity for understanding ecosystem responses to

120   environmental changes that may only manifest after extended periods of time, such as with changes

121   in soil organic C, N and P pools, which typically occur on decadal timescales [Davies *et al*., 2016a, Janes-

122   Bassett *et al*., 2020]. Here, we combine new data from a long-term nutrient manipulation experiment

123   on two P-limited upland grasslands (acidic and limestone) occurring on contrasting soils, with the

124   mechanistic C-N-P plant-soil biogeochemical model; N14CP [Davies *et al*., 2016b].

125   We use these experimental data to explore the role of organic P access in determining ecosystem

126   nutrient limitation and grassland responses to long-term nutrient manipulations. Specifically, we aim

127   to explore how variation in P acquisition parameters, that control access to organic and inorganic

128   sources of P in the model, may help account for differing responses of empirical grassland C, N and P

129   pools to N and P additions. Second, we explore the effects of long-term anthropogenic N deposition

130   and experimental N and P additions on plant and soil variables of the simulated acidic and limestone

131   grasslands. This will help improve our understanding of organic P process attribution within the model

132   and may suggest how similarly nutrient limited grasslands could respond to similar conditions.

133   We hypothesise that 1) access to organic P will be an important determinant of ecosystem nutrient

134   limitation, 2) increased organic P availability may alleviate P limitation resulting from N deposition and

135   3) grasslands capable of accessing sufficient P from organic forms may overcome P limitation resulting

136   from N deposition and nutrient treatments, whereas grasslands lacking such accessibility will not.

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2. Methods

2.1. Field experiment description

The empirical data is from Wardlow Hay Cop (henceforth referred to as Wardlow), a long-term experimental grassland site in the Peak District National Park (UK) [Morecroft *et al*., 1994]. Details of empirical data collection are available in supplementary section 1. There are two distinct grassland communities occurring in close proximity; acidic (National vegetation classification U4e) and limestone (NVC CG2d) semi-natural grasslands (Table S2). Both grasslands share a carboniferous limestone hill but the 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 palaeo-argillic brown earth [Horswill *et al*., 2008].

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 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].

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 the P treatment acts to alleviate P limitation. 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).

165   Data collected from the Wardlow grasslands for the purpose of this work are; aboveground biomass

166   C, SOC, and total N, which is assumed to be equivalent to modelled SON. This new data is combined

167   with total P data that was collected by Horswill *et al*. at the site [Horswill *et al*., 2008]. Summaries of

168   these data are available within the supplementary material (Table S4) and details of their collection

169   and conversion to model-compatible units in supplementary section S1.

170

171   **2.2. Summary of model processes**

172   2.2.1. N14CP model summary

173   The N14CP ecosystem model is an integrated C-N-P biogeochemical cycle model that simulates net

174   primary productivity (NPP), C, N and P flows and stocks between and within plant biomass and soils,

175   and their associated fluxes to the atmosphere and leachates [Davies *et al*., 2016b]. N14CP was

176   originally developed and tested on 88 northern Europe plot-scale studies, including grasslands,

177   where C, N and P data were available. All but one of the tested ecosystems exhibited N limitation

178   [Davies *et al*., 2016b]. It has also been extensively and successfully blind-tested against SOC [Tipping

179   *et al*., 2017] and NPP data from unimproved grassland sites across the UK [Tipping *et al*., 2019].

180   However, N14CP has not been extensively tested against sites known to exhibit P limitation,

181   especially where these are explicitly manipulated by long term experimental treatments. While the

182   importance of modelled weatherable P (P_{Weath0}) and historic N deposition on N-limited C, N and P

183   have been investigated [Davies *et al.* 2016b], the potential influence of organic P on ecosystem

184   nutrient limitation and responses to nutrient perturbations have yet to be explored.

185   Here, we modify N14CP to add experimental N and P additions to simulate a long-term nutrient

186   manipulation experiment similar to that at the limestone and acidic grasslands at Wardlow, and we

187   use empirical data from Wardlow to explore the role of organic P cleaving in determining ecosystem

188   state. A full model description can be found in Davies *et al*., [2016b], however, a summary of the

189   most relevant features is given here for convenience.

190   2.2.2. Net primary productivity and nutrient limitations

191   Plant biomass is simulated in the model as two sets of pools of coarse and fine tissues representing

192   both above and belowground plant C, N and P, with belowground biomass for each plant functional

193   type (PFT) represented by a root fraction. NPP adds to these on a quarterly basis with growth

194   occurring in quarters 2 and 3 (spring and summer). In N14CP, NPP depends on a single limiting

195   factor, in accordance with Liebig's law of the minimum. The factors that can limit growth in the

196   model include available N and P, temperature or precipitation, the latter two being provided as

197   input driver data (see section 2.3.2).

198   First, the potential maximum NPP limited by climate is calculated using regression techniques, as in

199   Tipping *et al.* [2014]. The corresponding plant demand for N and P to achieve this potential NPP is

200   then calculated [Davies *et al.*, 2016b; Tipping *et al.*, 2017]. This demand is defined by PFT plant

201   functional type stoichiometry, which changes through time in accordance with ecosystem succession

202   (see section 2.3.2). Stoichiometry of coarse tissue is constant but the fine tissue of each plant

203   functional type each PFT's fine tissue has two stoichiometric end members,. This allowsing the

204   model to represent transitions from N-poor to N-rich plant communities species or an enrichment of

205   the fine tissues within a single species plants (or a combination of both) [Davies *et al.*, 2016b],

206   dependent on available N. This allows a degree of flexibility in plant C:N ratios in response to

207   environmental changes such as N deposition. If the available nutrients cannot meet the calculated

208   plant nutrient demand, the minimum calculated NPP based on either N or P availability is used,

209   giving an estimation of the most limiting nutrient to plant growth.

210   Nutrient co-limiting behaviour can occur in the model through increased access to organic P sources

211   in the presence of sufficient N (see 2.2.3), and by having the rate of N fixation dependent on plant

212   and microbial available P [Davies *et al.*, 2016b]. The initial rate of N fixation is based on literature

213   values for a given plant functional type and is downregulated by anthropogenic N deposition, but

214   not soil N content more generally, as it is assumed that atmospherically deposited N is readily

215   available to N-fixers. Nitrogen fixation in the model is also related to P availability. The degree to

216   which P availabilty limits this maximum rate of fixation is determined by a constant; K_{Nfix} [Davies et

217   al. 2016b]. ~~The initial rate of N fixation is based on literature values for a given PFT but is~~

218   ~~downregulated by anthropogenic N deposition and related to P availability. The degree to which P~~

219   ~~availabilty limits this maximum rate of fixation is determined by a constant; K_{Nfix} [Davies *et al.*~~

220   ~~2016b].~~ This means that while modelled NPP is limited by availability of a single nutrient, co-

221   limitation may occur through P limitation of N fixation [Danger *et al.* 2008].

222

223   2.2.3. Plant and soil N and P cycling

224   A simplified summary of key pools and processes regarding plant-soil nutrient cycling are detailed in

225   Figure 1. Details such as initial base cation pools, their effects on soil pH, and most parameter names

226   have been omitted for clarity but are available from the original model development study [Davies *et*

227   *al.* 2016b]. Key changes for the purpose of this work are highlighted in red.

228   Plant available N is derived from biological fixation, the decomposition of coarse litter and SOM,

229   atmospheric deposition and direct N application. Fine plant litter enters the SOM pool directly due

230   to its rapid rate of turnover whereas coarse litter contributes N and P through decomposition and

231   does not join the SOM pool. Plant available P also comes from SOM and coarse litter decomposition,

232   direct treatment, desorption of inorganic P from soil surfaces, and sometimes cleaving of organic P

233   [Davies *et al*., 2016b]. The sorbed inorganic P pool builds over time with inputs of weathered P and

234   sorption of any excess plant available inorganic P, and desorption occurs as a first order process.

235   Phosphorus enters the plant-soil system by weathering of parent material, the initial value of which

236   (P_{Weath0} within the model) can be set to a default value, or made site-specific by calibrating this initial

237   condition to soil observational data (as in methods section 2.3.3). From this initial pool, annual

238   releases of weathered P are determined by first-order rate constants that are temperature

239   dependent, with the assumption that no weathering occurs below 0 degrees Celsius. This weathered

240   P can then contribute toward plant-available P in soil water or be sorbed to soil surfaces. ~~In principle,~~

241   ~~P can be added in small quantities by atmospheric deposition [Ridame and Guieu, 2002] or by local~~

242   ~~redistribution [Tipping *et al.*, 2014].~~ In principle, P can be added in small quantities by atmospheric

243   deposition [Ridame and Guieu, 2002] but for the purpose of this work, P deposition is set to zero in

244   the model. While the contribution of P through atmospheric deposition is increasingly realised

245   [Aciego et al. 2017], we cannot account for the losses of P that may also occur through landscape

246   redistribution [Tipping et al. 2014]. ~~For the purpose of this study, P deposition is set to zero as its net~~

247   ~~contribution to the total P pool in comparison to weathering is assumed to be minimal.~~

248   The size of the available P pool is determined by summing: P retained within plant biomass prior to

249   litterfall, inorganic P from decomposition, dissolved organic P and P cleaved from SOP by plants.

250   Accessibility of each P form is determined by a hierarchal relationship in the order mentioned above,

251   whereby plants and microbes access the most readily available P sources first and only move onto

252   the next once it has been exhausted.

253   When N is in sufficient supply and more bioavailable P forms have been exhausted from the total

254   available pool, simulated plants can access P from SOM via an implicit representation of extracellular

255   P-cleaving enzymes with a parameter termed P_{Cleave}. While empirical data quantifying this parameter

256   is scarce, N14CP constrains P_{Cleave} by utilising a maximum SOM C:P ratio; $[C:P]_{fixlim}$, that ensures SOM

257   stoichiometry is not unrealistically disrupted by excessive removal of organic P (Equation 1).

258

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

260

261   The functioning of the P_{Cleave} parameter, including its stoichiometric constraint, remains the same in

262   this work but we have introduced a modifier to adjust the rate at which plants can access this P

263   source. This parameter; $P_{CleaveMax}$, represents the maximum amount (g m^{-2} season^{-1}) of cleaved P that

264   plants can acquire from the available P pool to satiate P demand.

265   A fraction of plant biomass is converted to litter in each quarterly time step and contributes a

266   proportion of its C, N and P content to SOM, which is sectioned intro three pools (fast, slow and

267   passive) depending on turnover rate [Davies *et al.*, 2016b]. Soil organic P (SOP) is simulated

268   alongside SOC and SON using C:N:P stoichiometries of coarse and fine plant biomass. Decomposition

269   of SOP, and its contribution to the available P pool, is subject to the same turnover rate constants as

270   for SOC and SON.

271   Carbon is lost as CO_2 following temperature-dependent decomposition and as dissolved organic

272   carbon. Likewise, N and P are lost via dissolved organic N and P in a proportion consistent with the

273   stoichiometry of each SOM pool. Inorganic N is lost via denitrification and inorganic P can be sorbed

274   by soil surfaces. Both inorganic N and P can be leached in dissolved forms if they are in excess of

275   plant demand.

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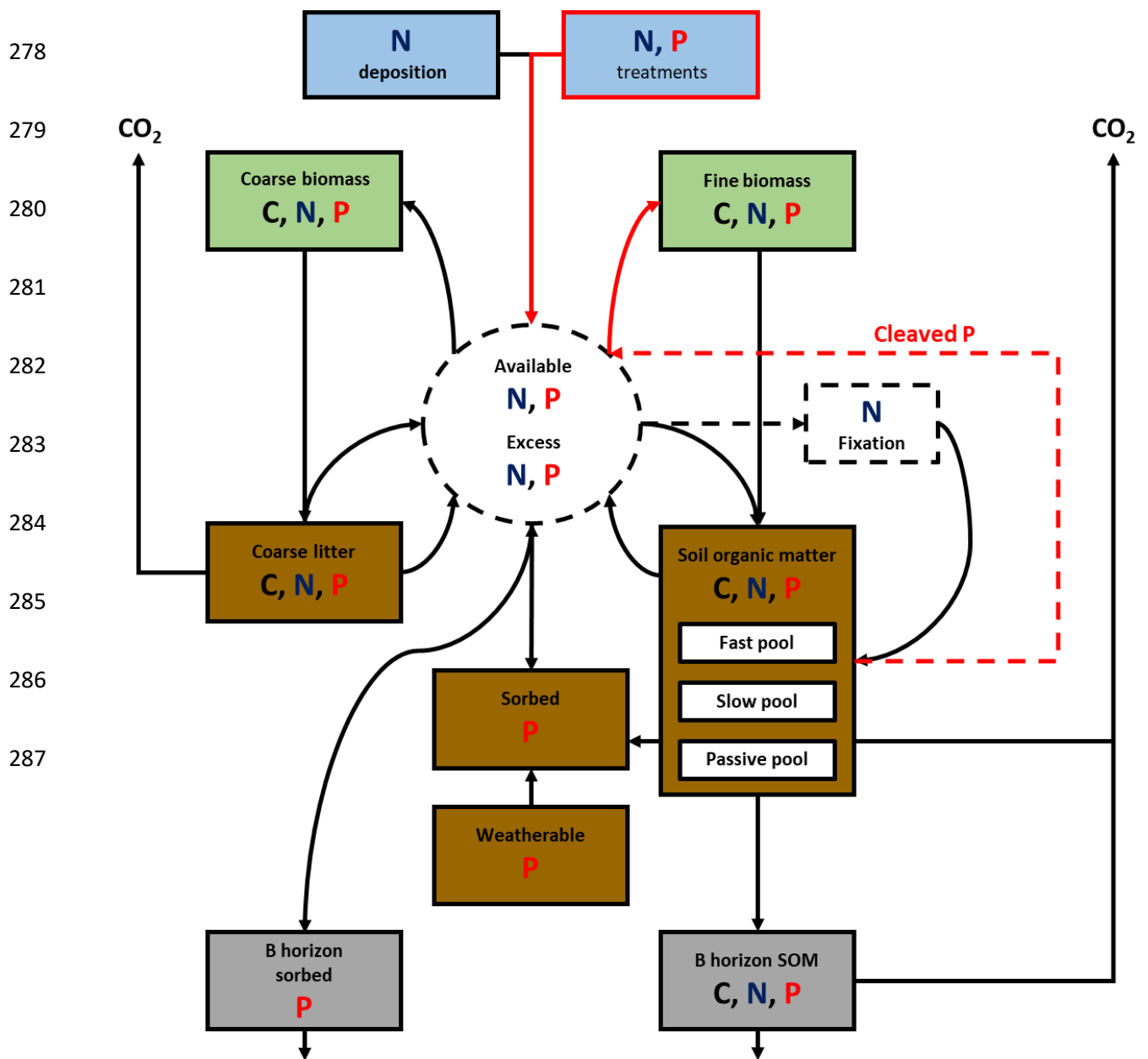
Figure 1: A simplified schematic of the key flows and pools of C, N and P within N14CP, adapted from the full schematic available in Davies *et al.* (2016a). Red lines highlight modifications to N14CP for the purpose of this work, including adding experimental nutrients and allowing uptake of cleaved P to be more flexible. 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 fixation, and decomposition of coarse litter and SOM. For P, the two main sources are the inorganic sorbed pool and from the turnover of SOM. 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. 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.

2.3. Simulating the field manipulation experiment with the model

We use data from 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 not aim to perfectly replicate the Wardlow grasslands but rather use the unique

opportunity that Wardlow provides to 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 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 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.

310   2.3.2. Input drivers

311   N14CP simulations run on a quarterly time step and are spun up from the onset of the Holocene

312   (10,000 BP in the model). This is to capture the length of time required for soil formation following

313   deglaciation in north west Europe and is not an attempt to truly model this long term period.

314   Instead, it allows us to form initial conditions for modern day simulations that takes in what we

315   know about the site's history and forcings.

316   To use this spin up phase and simulate contemporary soil C, N and P stocks, we use a variety of input

317   driver data. Inputs nearer the present are more accurately defined based on site-scale

318   measurements and assumptions are made regarding past conditions. This approach of spinning up

319   to present-day observations avoids the assumption that ecosystems are in a state of equilibrium,

320   which is likely inaccurate for ecosystems exposed to long-term anthropogenic changes in C, N and P

321   availability. Input driver data include ~~PFT~~ plant functional type history, climatic data and N

322   deposition data. A summary of the data used for model input is provided in supplementary Table S3.

323   To simulate the sites' ~~PFT~~ plant functional type history, we used data on Holocene pollen

324   stratigraphy of the White Peak region of Derbyshire [Taylor *et al*. 1994], which captures important

325   information regarding Wardlow's land-use history for the entire duration of the model spin up

326   phase.

327   Input drivers are provided as annual time series to drive the model and as the acidic and limestone

328   sites are co-located, these input timeseries are shared for both grasslands. It is assumed in the

329   model that anthropogenic N deposition was negligible prior to 1800 and the onset of the industrial

330   revolution. After 1800, N deposition is assumed to have increased similarly across Europe [Schopp *et*

331   *al*., 2003]. In N14CP, this trend is linearly extrapolated from the first year of data (1880) back to 1800

332   [Tipping *et al*., 2012]. Data regarding N deposition that is specific to Wardlow was incorporated

333   between the years 2004 and 2014 and the Schöpp *et al.* [2003] anomaly scaled to represent the high

334   N deposition of the site.

335 To provide climate forcing data, daily minimum, mean and maximum temperature and mean

336 precipitation records beginning in 1960 were extracted from the UKPC09 Met office CEDA database

337 (Table S3). The data nearest to Wardlow was calculated by triangulating latitude and longitude data

338 and using Pythagoras' theorem to determine the shortest distance. These data were converted into

339 mean quarterly temperature and precipitation. Prior to this, temperature was assumed to follow

340 trends described in Davies *et al*. [2016b] and mean quarterly precipitation was derived from Met

341 Office rainfall data between 1960 to 2016 and held constant.

342

343 2.3.3. Model parameters for the acidic and limestone grasslands

344 The N14CP model has been previously calibrated and tested against a wide range of site data to

345 provide a general parameter set that is applicable to temperate semi-natural ecosystems, without

346 extensive site-specific calibration [Davies *et al*., 2016b]. The majority of those parameters are used

347 here for both grasslands. However, two parameters relating to P sources and processes were

348 allowed to vary between the sites: the initial condition for the weatherable P pool, P_{Weath0}; and the

349 rate of plant access to organic P sources, $P_{CleaveMax}$ (Figure 1). We allowed P_{Weath0} to vary for each

350 grassland as variation in a number of factors including lithology and topography mean that we

351 should expect the flux of weathered P entering the plant-soil system to vary on a site-by-site basis

352 [Davies *et al*. 2016b]. Indeed, we should expect that P_{Weath0} differs between the acid and limestone

353 grasslands, as despite their proximity, they have differing lithology. Davies *et al*. [2016b], show that

354 variation in this initial condition considerably helps explain variance in contemporary SOC, SON and

355 SOP stocks between sites. However, it is difficult to set this parameter directly using empirical data,

356 as information on lithology and P release is limited at the site scale.

357 As this is the first time that N14CP has been knowingly applied to ecosystems of a largely P-limited

358 nature, we also allowed the maximum rate at which plants could access cleaved P ($P_{CleaveMax}$) to vary,

359 to investigate how plant P acquisition might change when more readily accessible P forms become

360   scarcer. Empirical quantification of organic P access is poor [Janes-Bassett *et al.* 2020], hence we use

361   a similar data-driven calibration for $P_{CleaveMax}$ as we do for P_{Weath0}.

362   We ran a series of simulations systematically varying P_{Weath0} and $P_{CleaveMax}$ and comparing the results

363   to observations., W~~w~~e simulated the two grasslands and their treatment blocks with a set of 200

364   parameter combinations. This captured all combinations of 20 values of P_{Weath0} between 50 and 1000

365   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

366   on the lower range of $P_{CleaveMax}$ values. The P_{Weath0} range was set to capture the lower end of P_{Weath0}

367   estimates described in Davies *et al*. [2016b], which were more likely to be appropriate for these P-

368   poor sites. We explored a range of values for $P_{CleaveMax}$, from zero where no access to organic sources

369   is allowed, to 1 g m^{-2} per growing season – a rate in the order of magnitude of a fertilizer application.

370   The model outputs were compared to measured, SOC, SON and total P (Table S4) for each grassland.

371   We tested how these parameter sets performed by calculating the error between the observations

372   and model outputs of the same variables for each combination of $P_{CleaveMax}$ and P_{Weath0}. The sum of

373   the absolute errors between modelled and observed soil C, N and P data were scaled (to account for

374   differing numbers of observations) and summed to provide an F value (Equation 2) as an overall

375   measure of error across multiple observation variables. ~~The parameter combination with the lowest~~

376   ~~F value that still maintained the grassland's empirical response to nutrient additions for both the~~

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

378

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

379

380

381

382 Plant biomass C data were excluded from the cost function to allow for blind testing of the model's

383 performance against empirical observations. As the variable most responsive to nutrient additions,

384 both in terms of rapidity and magnitude of the response, we deemed these the most rigorous data

385 to use for separate testing. We included soil C, N and P data from all nutrient treatments rather than

386 just the control to ensure that the selected parameter combination could better account for

387 patterns in empirical data. For instance, we know that empirical N treatments can increase plant and

388 soil enzyme activity in both Wardlow grasslands, [Johnson *et al.* 1999; Phoenix *et al.* 2004; Keane *et*

389 *al.* 2020] which a calibration to control-only data may not have captured.

390 While the cost function is a useful tool in allowing the model to simulate the magnitude of

391 contemporary C, N and P pools, it does not allow us to capture all necessary information to

392 accurately simulate grassland responses to long-term nutrient manipulations of contrasting nutrient

393 limitation. The pattern of grassland response, i.e. how a variable responds to nutrient treatment, is

394 an important consideration and is determined in the model by the most limiting nutrient.

395 Consequently, the parameter combination with the lowest F value, that still maintained a grassland's

396 empirical response to nutrient additions, (Supplementary section S1.1.), was used within the

397 analysis.

398

399

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). Raw empirical data is available in table S1 in section 2 of the supplementary material. 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 simulated grasslands, with the acidic grassland capable of acquiring more P from organic sources, having a $P_{CleaveMax}$ value of 0.32~~162~~ g m^{-2} season^{-1} compared to the limestone, with a value 10 times smaller at 0.03~~16~~ 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.

426   Overall, N14CP more accurately simulated the magnitude of limestone grassland C, N and P pools

427   than the acidic, and it generally captured the pattern of responses to nutrient treatment, albeit this

428   is not always supported by high r^2 values. The model estimates of above ground biomass C are

429   broadly aligned with the observations: capturing variation between the grasslands and treatments

430   (r^2 = 0.58), and on average overestimating the magnitude by 12.9% (SE ± 11.9) and 12.1% (SE ± 9.4)

431   for the acidic and limestone grasslands respectively (Fig 2a).

432   Soil organic C on average was slightly overestimated (7.1% with SE ± 3.3) for the limestone grassland

433   (Fig 2b), with a larger average overestimate for the acidic grassland (39.9% with SE ± 6.8). However,

434   in this latter case the variation between treatments was better captured. Despite a low r^2 value for

435   SOC (0.01), the model broadly captured the patterns we observe in the empirical data, with N

436   addition increasing SOC in the acidic and P addition increasing SOC in the limestone. However, the

437   intermediate increase in SOC with P in the acidic grassland is not captured by the model, nor is the

438   magnitude of the negative effect of LN treatment on limestone SOC.

439   Simulated magnitudes of SON are well-aligned with observations for the acidic grassland, with an

440   average error of 2.3% (SE ± 3.2), whilst SON for the limestone grassland was on average

441   underestimated by 17.8% (SE ± 3.6) (Fig 2c). The variation between treatments was better captured

442   for acidic than limestone SON but was overall reasonable (r^2 = 0.39).

443   Finally, the model overestimated total soil P (defined in the model as organic P plus sorbed P) by an

444   average of 6.0% (SE ± 4.3) for the limestone but underestimated by 54.7% (SE ± 8.0) in the acidic

445   grassland, which was the least accurately predicted variable out of those investigated (Fig 2d). With

446   only two empirical data points for TP across only two nutrient treatments, it is difficult to discern the

447   relationship between treatments and TP so an r^2 value is of little relevance here.

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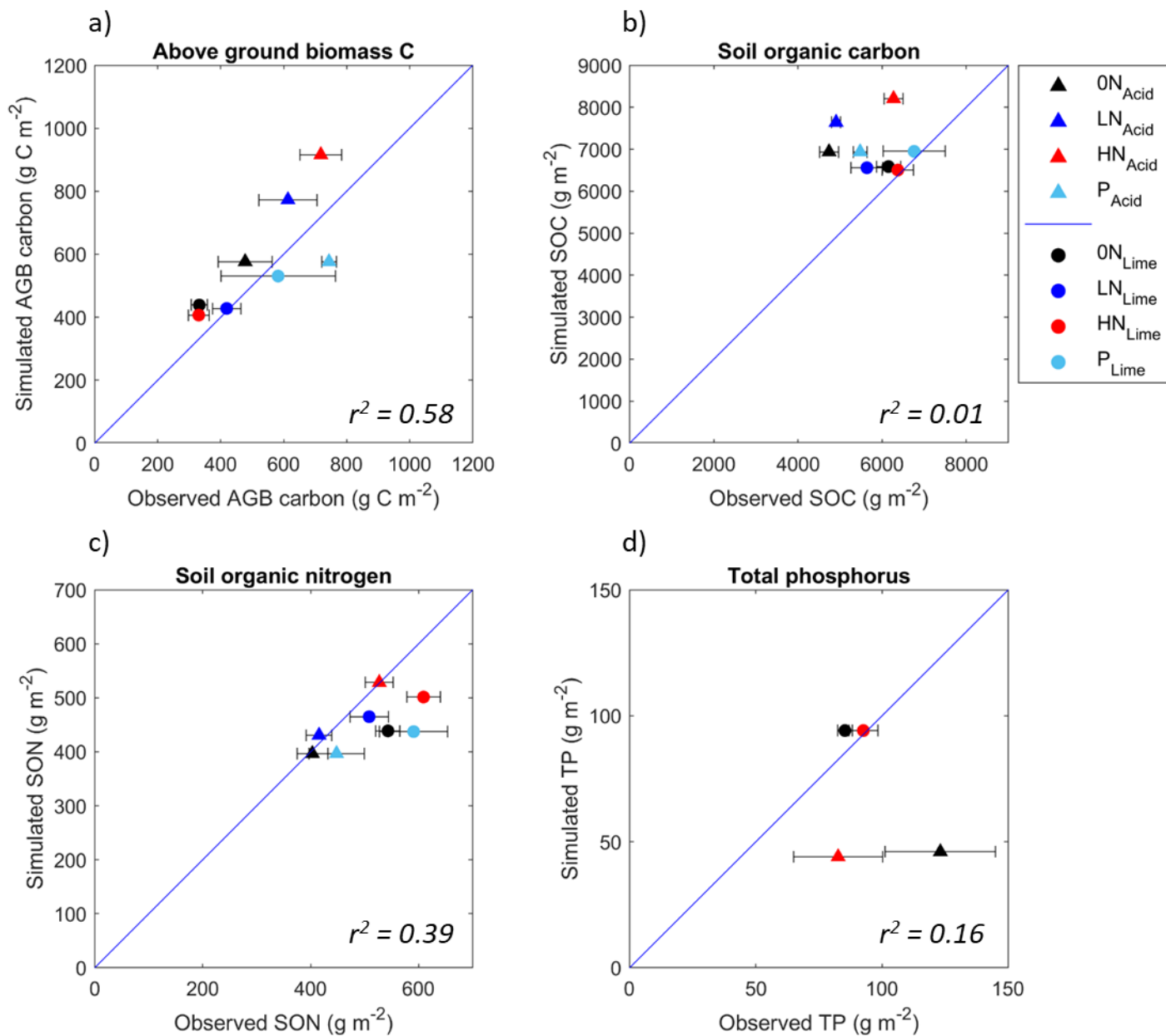
Figure 2: A comparison of the observed values of a) aboveground biomass carbon, b) soil organic carbon, c) soil organic nitrogen and d) total soil phosphorus from both grasslands, with simulated values from the model. The blue line represents a 1 to 1 relationship and the closer the data points are to 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

3.2. The limiting nutrient through time

Modelled acid 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 limestone grassland was also initially N-limited, 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 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 limitation.

Conversely, while cleaved P is used in the 0N 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 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.
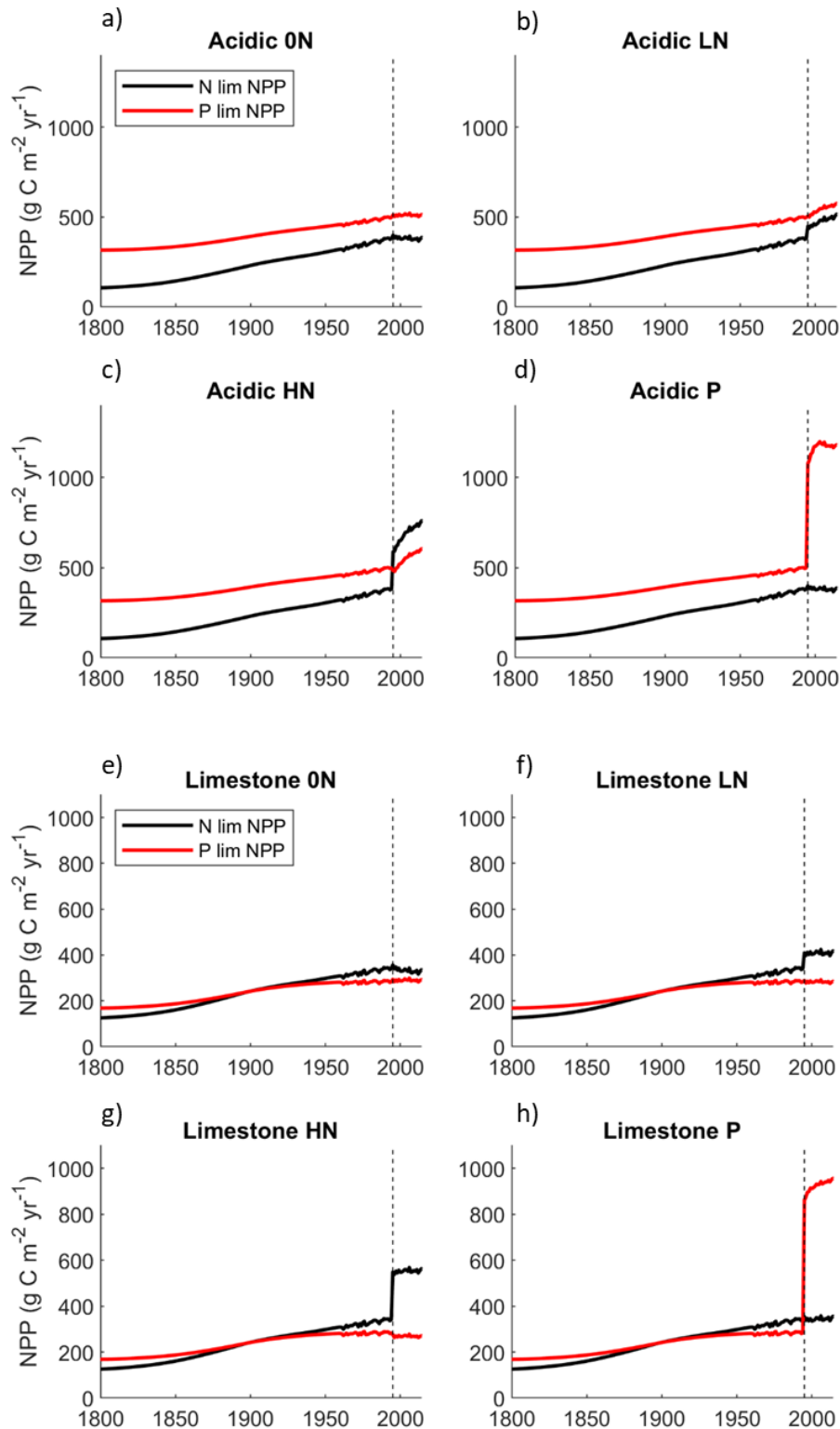
Figure 3: Plots showing the nutrient most limiting productivity for all nutrient treatments in both simulated grasslands. The vertical dashed line is the year of first nutrient addition within the model (1995). The value of the lines represents the maximum amount of productivity attainable given the 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 (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 C and 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 SOP pool are in contrast to those of the SOC and SON pools, with LN and HN 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).

502   3.3.2. Limestone grassland

503   Model simulations for the limestone grassland also suggest N deposition between 1800 and 2020

504   considerably increased aboveground biomass C, SOC and SON pools (Fig. 4), but to a lesser extent

505   than in the acidic grassland. Soil organic C and SON increased by almost half and biomass C more

506   than doubled. Soil organic P accumulated at a faster rate than in the acidic grassland, increasing by

507   about a third (Fig 4, Table S15).

508   Responses of the aboveground biomass C and SOC pools in the limestone grassland differ greatly to

509   those of the acidic, declining with N addition and increasing with P addition (Fig 4). This response

510   was ubiquitous to all C pools, with declines in subsoil, topsoil and biomass C (Fig 5b, Table S11).

511   Biomass C declined by 2.4% and 7.3% with LN and HN addition (Fig 4b) and SOC declined by 0.5%

512   and 1.4% with the same treatments (Fig 4d). Phosphorus addition increased biomass C and SOC by

513   22.0% and 6.1% respectively (Fig 4b, d).

514   Nitrogen treatments increased the size of subsoil, topsoil and available N pools, but led to small

515   declines in biomass N (Fig 5d, Table S12). The P treatment slightly reduced subsoil and topsoil SON

516   compared to the control yet increased available N and biomass N, to the extent where biomass N is

517   greater in the P than HN treatment (Fig 5d, Table S12). Total SON increased by 6.4% and 15.0% with

518   LN and HN respectively and declined by 0.2% with P treatment (Fig 4f).

519   The response of the limestone P pools mirrors that of carbon, with declines in subsoil SOP, topsoil

520   SOP, available P and biomass P with LN and HN addition (Fig 5f, Table S13). The limestone grassland

521   SOP pool declined by 0.2% with LN and 0.5% with HN addition, with an increase of 20.0% upon

522   addition of P (Fig 4h). The P treatment substantially increased total ecosystem P in the limestone

523   grassland, particularly in the topsoil sorbed pool (Fig 5f, Table S13).

524

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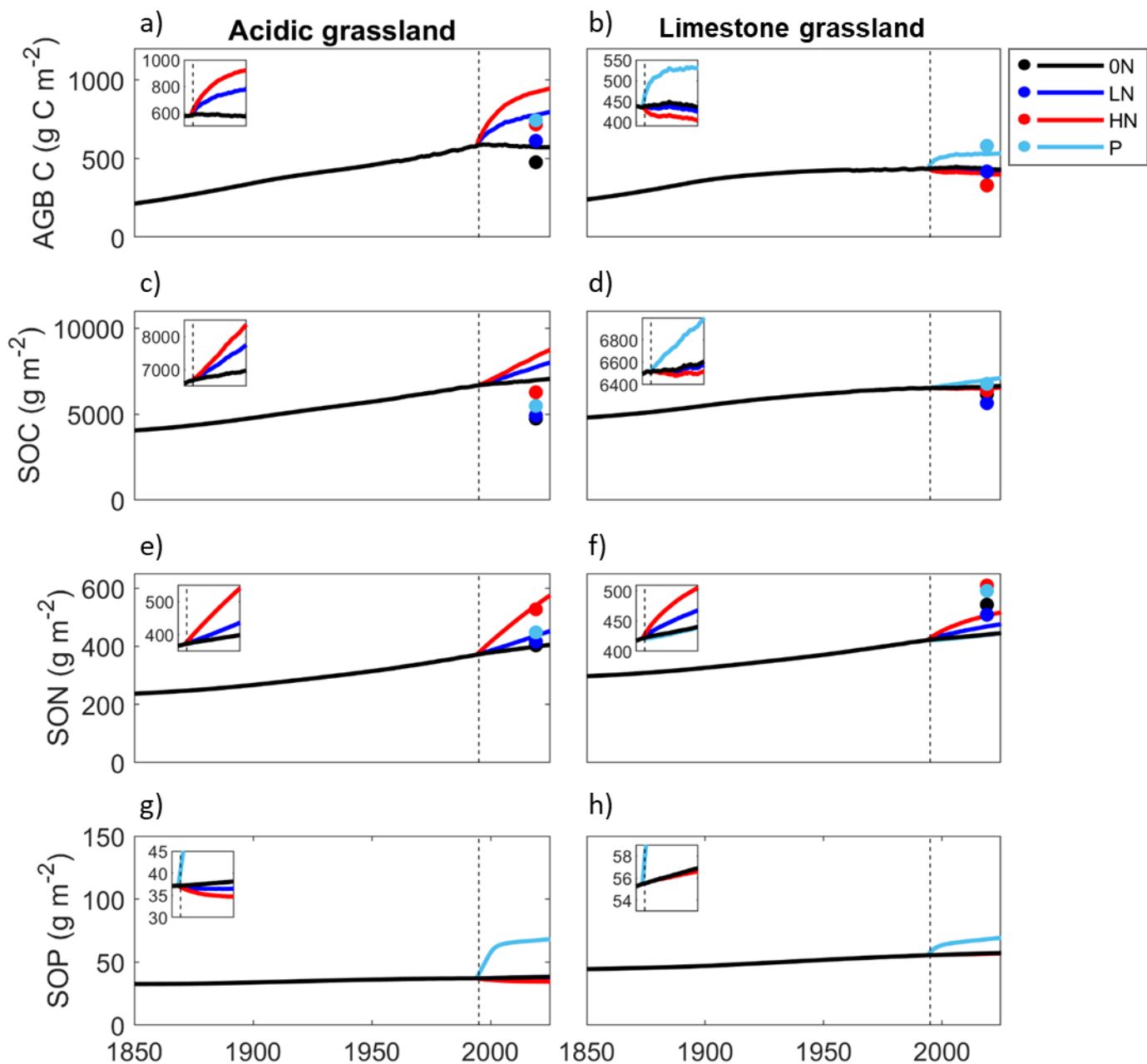
Figure 4: Time series plots of aboveground biomass C, soil organic C, N and P for the acidic (panels a, c, e and g respectively) and limestone modelled grasslands (panels b, d, f and h respectively). The vertical dashed line represents the first year of nutrient addition (1995) and marks the beginning of the experimental period. The inset subplots focus on 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).
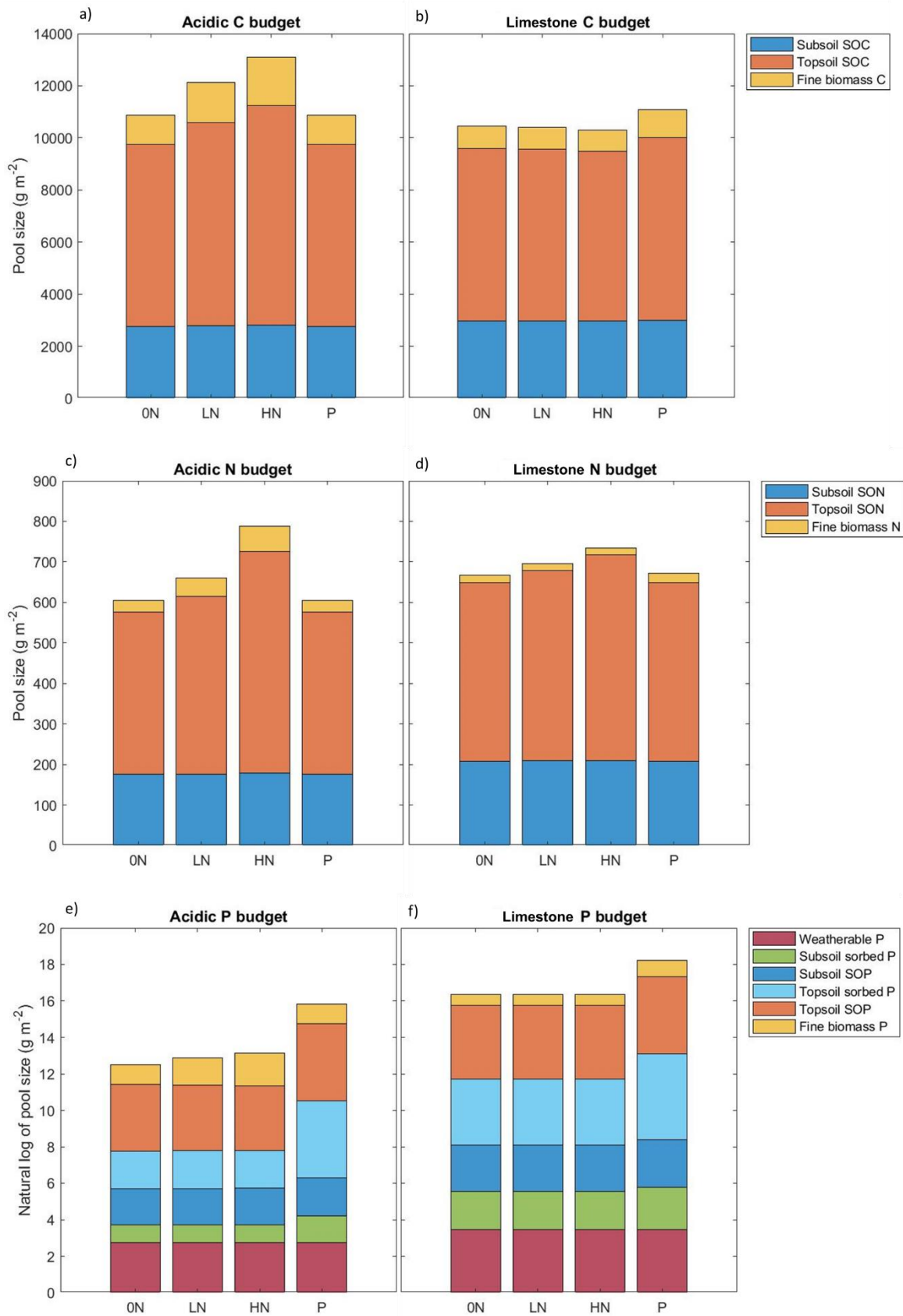
Figure 5: Modelled C, N and P budgets for the acidic (panels a, c and e) and limestone (panels b, d, f) grasslands for the year 2020. Modelled sizes of C and N pools are in grams per metre squared, and P pools are presented as $\log_n$ grams per metre squared. Temporary pools such as available N and 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.

529 **4. Discussion**

530 **4.1. Simulating contrasting grasslands by varying plant access to P sources**

531 This is the first instance in which N14CP, and to the best of our knowledge, any other integrated C-N-

532 P cycle model, has explicitly modelled P-limited ecosystems and investigated their responses to N

533 deposition and additional nutrient treatments. By using empirical data from long-term experimental

534 grasslands to drive and calibrate N14CP, we could test the model's ability to simulate two

535 contrasting P-limited grasslands, and how organic P access may affect this ability. While the purpose

536 of this work was not to explicitly reproduce the Wardlow grasslands within N14CP, by comparing

537 data from Wardlow to the simulated grasslands, we can simultaneously develop our understanding

538 of the model's representation of under-studied P cycling processes and contextualise what this may

539 mean for empirical systems such as Wardlow.

540 The model suggests that the acidic grassland was characterised by high access to organic P, with

541 comparatively low inorganic P availability, whereas the limestone grassland was the opposite, with

542 low organic and high inorganic P availability. These simulated differences could reflect the relative

543 availability of different P sources at Wardlow. As the acidic grassland formed in a hillside depression,

544 loess has accumulated, thickening the soil profile and distancing the plant community from the

545 limestone bedrock. The plant rooting zone of the acidic grassland is therefore not in contact with the

546 bedrock, and roots almost exclusively occur in the presence of organic P sources which can be

547 cleaved and utilised by plants [Caldwell, 2005; Margalef *et al.*, 2017]. Conversely, the limestone

548 grassland soil rarely exceeds 10 cm depth, and the rooting zone extends to the limestone beneath,

549 providing plants with greater access to weatherable calcium phosphate [Smits *et al.*, 2012].

550 Such parameter combinations allowed for reasonable congruence between empirical and simulated

551 data, with an average discrepancy of only 6.6% (SE ± 9.1) and 1.2% (SE ± 4.4) for the acidic and

552 limestone grasslands respectively across all variables (Table S5). However, model performance

553 differed greatly between the two grasslands. For instance, the model accurately captured the

554   magnitude of limestone C, N and P data and their expected P-limited responses to nutrient

555   treatment, but was less effective at simulating the acidic grassland. N14CP did not simulate an

556   increase in biomass C or SOC with P addition in the acidic grassland, instead simulating a solely N-

557   limited grassland. While this may be expected of a model that employs a law-of-the-minimum

558   approach, N14CP has a number of mechanisms to account for N and P interdependence, meaning

559   that in principle, it is capable of simulating positive responses to LN, HN and P treatment, as

560   observed in the empirical data from 2017 (section 2.2.2).

561   The overestimation of acidic C pools and underestimation of total P suggests that the model is

562   simulating that too much organic P is being accessed by plants in response to N addition and

563   transferred into plant biomass pools (Fig 2d). Few parameter sets where simultaneously able to

564   simulate the magnitude of the empirical TP pool and the positive response of biomass to N addition

565   in the acidic grassland. This may also be due to limitations in the empirical P data, as P data used for

566   calibrating P cycling were available for only two nutrient treatments and represented total soil P, not

567   organic P. While we acknowledge the technical and theoretical issues associated with distinguishing

568   between organic and inorganic P pools [Lajtha *et al.* 1999; Barrow *et al.* 2020], such distinctions

569   would help in understanding this discrepancy and likely improve the model's ability to simulate P-

570   limited systems, particularly when organic P availability may be important.

571   Additionally, N14CP's representation of organic P cleaving likely underestimates the ability of soil to

572   rapidly occlude and protect organic P that enters solution. For example, inositol phosphate, a major

573   constituent of organic P, has been found to be used extensively by plants grown in sand but is hardly

574   accessed by plants grown in soil [Adams and Pate 1992]. Such organic phosphates become strongly

575   bound to oxides in the soil, protecting them from attack by phosphatase enzymes [Barrow 2020].

576   This may be particularly prevalent in the acidic grassland at Wardlow where N deposition has

577   resulted in acidification and base cation depletion [Horswill *et al.* 2008], potentially enhancing the

578   formation of iron and aluminium complexes and immobilising P [Kooijman *et al.*, 1998].

579 In addition to physico-chemical processes reducing P availability, in P-limited grassland soils,

580 microbial processes may be dominant drivers of ecosystem P fluxes [Bünemann et al. 2012]. For

581 instance, while mineralisation of organic P may increase inorganic P in solution [Schneider *et al.*

582 2017], this can be rapidly and almost completely immobilised by microbes, particularly when soil P

583 availability is low [Bünemann et al. 2012]. As the model lacks a mechanism for increasing access to

584 secondary mineral P forms comparable to organic P-cleaving, and microbial P immobilisation is

585 incompletely represented for P-limited conditions, it is possible that the uptake of organic P by the

586 acidic grassland in the model is exaggerated.

587 ~~As the model lacks a mechanism for increasing access to secondary mineral P forms comparable to~~

588 ~~organic P-cleaving, the uptake of organic P by the acidic grassland is very likely exaggerated.~~

589 The model's inability to simulate a positive response to both N and P addition in the acidic grassland

590 may be an unintended consequence of the downregulation of N fixation by N deposition included

591 within N14CP [Davies *et al.* 2016b]. While this representation is appropriate [Gundale *et al.* 2013],

592 when N deposition exceeds fixation (as at Wardlow), fixation is essentially nullified (as in Tables S8,

593 S12), meaning deposition becomes the sole source of N to the grassland. This in effect, removes the

594 dependence of N acquisition on P availability, and could make modelling behaviour akin to N-P co-

595 limitation [Harpole *et al*., 2011] under high levels of N deposition challenging. This suggests that

596 current C-N-P cycle models that employ a Liebig's law of the minimum can provide a broad

597 representation of multiple variables by calibrating access to both organic and inorganic P sources

598 [Davies *et al.* 2016b], provided the ecosystem in question's limiting nutrient leans towards N or P

599 limitation. Furthermore, where access to organic P forms is likely to be lower, as in the limestone

600 grassland, model performance may improve. This could be further explored by allowing N fixation

601 limits in the model to adapt to P nutrient conditions or by attenuating the suppression of N

602 deposition on N fixation, to represent acclimatisation of N-fixers to greater N availability [Zheng *et*

603 *al.* 2018].

604 Ultimately, differences in modelled accessibility to organic forms of P enabled N14CP to distinguish

605 between the two empirical grasslands, and simulate the magnitude and pattern of data with

606 reasonable accuracy, albeit with the previously mentioned caveats.

607

608 **4.2. Consequences of differential P access on ecosystem C, N and P**

609 While the model's estimation of $P_{CleaveMax}$ for the acidic grassland is likely overestimated, the model

610 experiment has highlighted that differences in organic versus inorganic P availability are a key

611 determinant of an ecosystem's nutrient limitation, and consequently, how they respond to changes

612 in anthropogenic N and P availability. For instance, while being exposed to the same background

613 level of N deposition and the same magnitude of experimental treatment, the modelled acidic

614 grassland was able to stimulate growth in response to LN and HN treatment whereas the modelled

615 limestone grassland was negatively affected by it.

616 Nitrogen addition increases plant demand for P and can shift ecosystems toward a state of P

617 limitation or increase the severity of limitation where it already exists [Menge and Field, 2007; An *et*

618 *al.*, 2011; Goll *et al.*, 2012]. Consistent with this, both simulated grasslands saw SOP decline with LN

619 and HN treatment, worsening P limitation in the limestone grassland, and depleting the SOP pool in

620 the acidic. As P cleaved from organic pools is the least bioavailable within the model hierarchy

621 (methods 2.2.3), this is indicative of increasing P stress in both grasslands. While SOP declined in

622 both grasslands, the responses of available and biomass P to nutrient treatments differed markedly

623 between the grasslands. Due to the higher rate of $P_{CleaveMax}$ in the acidic grassland, more P

624 ~~accumulated in the~~was in plant-available forms ~~pool~~ and hence P does not become the limiting

625 factor under N treatments (Table S9). Conversely, available and biomass P decline under LN and HN

626 addition in the limestone grassland (Table S13), highlighting how the grassland's $P_{CleaveMax}$ capability is

627 insufficient to meet increased P demand.

628   Such high access to organic P sources in the modelled acidic grassland likely led it to respond to

629   nutrient enrichment in an N-limited manner, increasing productivity in response to N deposition and

630   LN and HN treatments as the model's limiting nutrient stimulated plant growth. Detrital C inputs

631   from plant biomass are the primary source of SOC accumulation within N14CP [Davies *et al*., 2016b]

632   and as such, changes in SOC integrate long term trends in net primary productivity in systems where

633   external nutrients are supplied. The provision of additional N in the modelled LN and HN treatments

634   therefore led to large increases in biomass accumulation and consequently, almost linearly increased

635   SOC (Fig 4c).

636   Similar increases in N-limited grassland SOC under N addition have been shown, resulting from

637   significant increases in below-ground carbon input from litter, roots [He *et al*., 2013] and detrital

638   inputs [Fornara *et al*., 2013], mechanisms similar to those reported by the model. Similarly, Tipping

639   *et al*. [2017] used N14CP to show that N deposition onto N-limited UK ecosystems ubiquitously

640   increased SOC storage by an average of 1.2 kgCm^{-2} (c. 10%) between 1750 and 2010 [Tipping *et al*.,

641   2017].

642   Despite its P-limited condition under the HN treatment (Fig 3c), the acidic grassland continued to

643   accumulate biomass with N addition as the grassland's greater access to topsoil SOP (Table S9)

644   allowed it to acquire sufficient P to stimulate additional growth but not necessarily to alleviate P

645   limitation. This is consistent with the acidic grassland at Wardlow, where N treatment stimulated

646   root surface phosphatases, likely supplying more SOP to plants [Johnson *et al*., 1999]. Our simulated

647   acidic grassland therefore supports the hypothesis that prolonged N deposition may increase SOP

648   access to such an extent that P limitation is alleviated and growth can be stimulated [Chen *et al.*

649   2020]. Organic P release from SOM and its potential immobilisation, is poorly represented in models

650   and we encourage further study aimed at quantifying these processes [Chen *et al.* 2020; Janes-

651   Bassett *et al*., 2020; Phoenix *et al*., 2020]. However, such high rates of SOP access only occurred

652   under experimental LN and HN treatments, and in reality, such rapid degradation of SOP may

653   eventually degrade the pool to such an extent that P limitation soon returns.

654   Conversely, biomass C and SOC in the modelled limestone grassland responded positively to P

655   addition, via similar mechanisms to the N-response in the modelled acidic grassland. However, in

656   contrast to the acidic grassland, N addition caused declines in limestone biomass and SOC, the

657   former of which has been observed at the limestone grassland at Wardlow [Carroll *et al*., 2003].

658   Reductions in limestone biomass C (and consequently SOC) in the model are a combined result of

659   reductions in bioavailable P (Table S13), occurring via N-driven increases in stoichiometric P demand,

660   in addition to an inability to access sufficient P from the SOP pool (Table S14). Plants therefore

661   cannot meet P demand and new biomass is insufficient to replace senesced plant material,

662   decreasing net biomass C input to the SOC pool. This suggests that in P-limited limestone grasslands

663   such as at Wardlow, where access to organic P forms may be comparatively limited, N deposition

664   may worsen pre-existing P limitation and reduce ecosystem C stocks [Goll *et al.* 2012, Li *et al.*

665   2018]. This implies that in ecosystems where plants are not well-adapted to acquiring organic forms

666   of P [Phoenix *et al.* 2020], or where organic P is scarce, N deposition may worsen pre-existing P

667   limitation and reduce ecosystem C stocks [Goll *et al.* 2012; Li *et al.* 2018].

668

669   **4.3. Model limitations**

670
671   While N14CP is a fairly simple ecosystem model by design, it is one of few models to integrate the C,

672   N and P cycles for semi-natural ecosystems and has been extensively tested against empirical NPP

673   and soil C, N and P data it is one of the first process-based biogeochemical models to explicitly

674   incorporate P with the C and N cycles for semi-natural ecosystems, and to simulate NPP and soil C, N

675   and P dynamics, for which it has been extensively tested [Davies *et al.* 2016a; Davies *et al.* 2016b;

676   Tipping *et al.* 2017; Tipping *et al.* 2019; Janes-Bassett *et al.* 2020]. Previous work with N14CP has

677   identified the need to enhance its ability to simulate organic P cycling [Janes-Bassett *et al.* 2020],

678   which we aimed to do in this study by using long-term experimental data from contrasting P-limited

679   grasslands.

680   N14CP's simplified representation of plant nutrient pools and plant control over nutrient uptake, is

681   largely controlled by stoichiometric demand [Davies *et al.* 2016a], and does not incorporate many

682   plant strategies for P acquisition [Vance *et al.* 2003]. Indeed, by allowing $P_{CleaveMax}$ to vary to account

683   for empirical data, we attempt to somewhat increase plant control over organic P uptake. We

684   acknowledge earlier that such an approach likely underestimates the ability of soil surfaces and

685   microbes to protect newly-cleaved P from plant uptake. As such, where we may expect access to

686   organic P to be high, such as the acidic grassland at Wardlow, such modelled representation of

687   plant-mediated P access may lead to unrealistic depletions in soil P and increases in biomass and soil

688   C, and we would encourage further work aimed at improving model-representation of plant controls

689   on organic P cycling [Fleischer *et al.* 2019].

690   While we feel incorporating a suite of plant strategies for acquiring P would represent over-

691   parameterisation, we acknowledge that a modelled equivalent to $P_{CleaveMax}$ for accessing inorganic P

692   forms is lacking, such as carbon-based acid exudation to increase mineral P weathering [Achat *et al.*

693   2016; Phoenix *et al.* 2020], which likely contributes toward the poor representation of the acidic

694   total P pool. Biota-enhanced P weathering and nutrient redistribution by mycorrhizal hyphae are

695   important for nutrient cycling [Quirk *et al.* 2012], and fungal community structure and function is

696   strongly influenced by perturbations in the C and N cycles [Moore *et al.* 2020]. Such processes are

697   not included within N14CP as the extent to which weathering can be controlled by such mechanisms

698   and the manner in which these can be represented in C-N-P cycle models is debated [Davies *et al.*

699   2016b].

700   Currently, N14CP assumes C to be in unlimited supply, with its uptake by plants and consequent

701   input into soil pools controlled by C:N:P stoichiometry, hence C availability has little effect on N and

702   P dynamics within the model. Increasing atmospheric CO_2 may increase nutrient availability, as

703   plants may reallocate additional carbon resources toward nutrient acquisition [Keane *et al.* 2020] or

704   elevated CO_2 (eCO_2) may increase limitation of other nutrients such as N [Luo *et al.* 2004]. The

705   inclusion of eCO_2 into N14CP poses a particularly enticing research opportunity, and we aim to use

706   this study as a foundation for future work to include this process.

707

5. Conclusions

708

709 We have shown that by varying two P-acquisition parameters within N14CP, we can account for

710 contrasting responses of two P-limited grasslands ~~of differing soil P chemistry,~~ and with reasonable

711 accuracy. However, such coarse representation of organic P cycling in the model likely overestimates

712 the ability of plants to use newly-cleaved P and limits our ability to simulate grasslands where N and

713 P interact to control plant productivity, including the potential for N inputs to alleviate P limitation.

714 Differences in organic P access was a key factor distinguishing the contrasting responses of the

715 modelled grasslands to nutrient manipulation, with high plant access allowing the acidic grassland to

716 acquire sufficient P to match available N from chronic deposition and prevent 'anthropogenic P

717 limitation'. In the acidic grassland, N treatment stimulated plant access of organic P, promoting

718 growth and C sequestration. However, the model suggests that this is an unsustainable strategy, as

719 the SOP pool rapidly degrades, and if N additions are sustained, P limitation may return. Conversely

720 in the limestone grassland, which was less able to access organic P, additional N provision

721 exacerbated pre-existing P limitation by simultaneously increasing plant P demand and reducing P

722 bioavailability. This reduced productivity and consequently C input to soil pools declined, resulting in

723 SOC degradation exceeding its replacement.

724 We further show that anthropogenic N deposition since the onset of the industrial revolution has

725 had a substantial impact on the C, N and P pools of both the modelled acidic and limestone

726 grasslands, to the extent where almost half of contemporary soil C and N in the model could be

727 from, or caused by, N deposition.

728 Our work therefore suggests that with sufficient access to organic P, long-term N addition may

729 alleviate P limitation. Where organic P access is limited, N deposition could shift more ecosystems

730 toward a state of P limitation or strengthen it where it already occurs [Goll *et al.*, 2012], reducing

731 productivity to the point where declines in grassland SOC stocks - one of our largest and most labile

732 carbon pools – may occur.

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

736

737 *Author contributions:*

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

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

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

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

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

749

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751

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