

1   **Title:** Grassland responses to elevated CO<sub>2</sub> determined by microbial competition for phosphorus.

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16

17    **Abstract**

18    **Rising atmospheric CO<sub>2</sub> has stimulated plant productivity, with terrestrial ecosystems**  
19    **currently absorbing nearly one-third of anthropogenic CO<sub>2</sub> emissions. Increases in**  
20    **photosynthesis can subsequently lead to increased carbon (C) storage in plants and soil.**  
21    **However, there is growing evidence that nitrogen (N) availability constrains elevated CO<sub>2</sub>**  
22    **(eCO<sub>2</sub>) responses, yet we know much less about the role of phosphorus (P) limitation on**  
23    **productivity under eCO<sub>2</sub>. This is important because P-limited ecosystems are globally**  
24    **widespread, and the biogeochemical cycles of N and P differ fundamentally. We conducted**  
25    **a free-air-CO<sub>2</sub>-enrichment (FACE) experiment for three years on two contrasting P-**  
26    **limited grasslands in the Peak District National Park of northern England under long-**  
27    **term nutrient manipulation. Here we show that competition between plants and microbes**  
28    **for P can determine plant productivity responses to eCO<sub>2</sub>. In a limestone grassland,**  
29    **aboveground productivity increased (16%) and microbial biomass P remained unchanged,**  
30    **whereas in an acidic grassland, aboveground productivity and P uptake declined (11%**  
31    **and 20%, respectively), but P immobilisation into microbial biomass increased (36%).**  
32    **Our results demonstrate that strong competition with microbes can cause plant P uptake**  
33    **to decline under eCO<sub>2</sub>, with implications for the future productivity of P-limited**  
34    **ecosystems in response to climate change.**

35

37 The ability of terrestrial ecosystems to sequester more C as CO<sub>2</sub> levels rise represents potentially the  
38 single most important biogeochemical feedback limiting climate change<sup>1</sup>. This ability can be  
39 constrained substantially by nutrient availability<sup>1-4</sup>, with Earth system models indicating that  
40 nitrogen (N) limitation reduces this capacity for greater C sequestration by as much as 58%<sup>5</sup>.  
41 However, between a third and a half of terrestrial ecosystems are limited by P, not N<sup>6,7</sup>. Despite the  
42 spatial extent of P limitation, we know very little about how P limitation affects ecosystem  
43 responses to eCO<sub>2</sub>, thus significantly limiting our ability to predict future rates of C uptake by the  
44 terrestrial biosphere<sup>8,9</sup>.

45 Crucially, understanding of eCO<sub>2</sub> responses in N-limited ecosystems does not transfer to P-limited  
46 ecosystems because the mechanisms differ so greatly. While C can be utilised by soil microbes to  
47 fix atmospheric N, there is no biological equivalent that may increase ecosystem P stock, which is  
48 reliant principally on weathering of mineral P. Thus, total ecosystem P stocks will only increase if  
49 weathering of P-containing minerals is enhanced. Where the primary minerals have already been  
50 weathered, and secondary mineral P is insoluble, competition between plants and microbes for P  
51 may control productivity responses. Similarly, in acidic soils inorganic P is likely bound to  
52 abundant iron (Fe) and aluminium (Al)<sup>10</sup>, to which plant and microbe access may be extremely  
53 limited and thus competition for organic P is more intense. Furthermore, unlike the N cycle, soil  
54 microbial biomass (MBP) is typically a larger store of P than above ground plant biomass (AGBP),  
55 especially during ecosystem retrogression<sup>11</sup>, where ecosystems become increasingly P-limited  
56 during soil development as they age. Thus, changes in competition between plants and microbes  
57 could potentially control the response of P limited ecosystems to eCO<sub>2</sub>, but this has not been  
58 investigated to date.

59 Many of the strategies employed by plants to acquire P require C, either through mycorrhizal  
60 symbiosis, or the production of C-rich compounds such as organic acids to mobilise organic- and  
61 mineral-bound P, or root surface enzymes such as phytases and phosphatases to mineralise organic

62 P sources<sup>12</sup>. Therefore, the expected increase in C entering ecosystems via photosynthesis under  
 63 eCO<sub>2</sub> may then provide a mechanism by which P limited productivity is alleviated, consequently  
 64 increasing C sequestration. However, it has been shown that changes in C input can shift investment  
 65 by soil microbes away from C acquisition to N and P acquisition under eCO<sub>2</sub><sup>13</sup>, and the  
 66 consequences for competition between plants and microbes is unclear<sup>14</sup>.

67 Despite growing recognition of the global importance of P limitation, few studies have quantified  
 68 changes in P cycling under eCO<sub>2</sub>. In an Australian Eucalypt forest<sup>15</sup> of proven P-limitation, net  
 69 primary productivity did not increase in response to eCO<sub>2</sub>, with forest maturity or P-limitation  
 70 proposed as potential explanations<sup>16,17</sup>. Meta-analysis of eCO<sub>2</sub> productivity responses indicated that  
 71 P availability is likely an important constraint<sup>18</sup>, but without direct experimental evidence from  
 72 proven P-limited ecosystems, major uncertainty surrounds eCO<sub>2</sub> responses of P-limited ecosystems.  
 73 This holds especially true for grasslands, which represent 20% of terrestrial net primary  
 74 productivity<sup>19</sup> and are the most spatially extensive P-limited ecosystem in temperate regions<sup>7</sup>.

75 To address this, we established a Free Air Carbon dioxide Enrichment (FACE) experiment, where  
 76 intact soil-turf monoliths were exposed to either ambient CO<sub>2</sub> (ca. 410 ppm) or eCO<sub>2</sub> of 600 ppm.  
 77 The monoliths used for the experiment were extracted from a long-term N and P manipulation study  
 78 established in 1995 on two adjacent naturally P-limited grasslands<sup>20</sup>: a limestone grassland and an  
 79 acidic grassland, which represent different stages of ecosystem retrogression. The limestone soil sits  
 80 over a potential source of readily weatherable mineral P in calcium phosphates (Ca; Ca-P). This  
 81 contrasts with the acidic soil, where Ca is 95% less abundant, and inorganic P is likely bound to  
 82 abundant Fe and Al, to which plant and microbe access may be extremely limited, and thus  
 83 competition for organic P is more intense<sup>10</sup>. In addition to control (no nutrient addition) plots,  
 84 nutrient inputs have been manipulated through long-term (ca. 25 years) additions of P (35 kg P ha<sup>-1</sup>  
 85 y<sup>-1</sup> (P)), and two levels of N addition (35 kg N ha<sup>-1</sup> y<sup>-1</sup> (LN) and 140 kg N ha<sup>-1</sup> y<sup>-1</sup> (HN)). Addition  
 86 of P alleviates the P-limitation of productivity and N additions simulate atmospheric N deposition:  
 87 in line with the latest understanding of N loading impacts<sup>27</sup>, these are now also alleviating P-

88 limitation, potentially by more weathering of Ca-P in the limestone soil, and possible stimulation of  
89 organic P mineralisation by microbial phosphatases<sup>13,21</sup>. CO<sub>2</sub> fumigation began in 2018 with the  
90 FACE system supplying eCO<sub>2</sub> during the hours of daylight from April until November in each year  
91 for three years (2018-2020).

## 92 *Aboveground biomass responses to eCO<sub>2</sub>*

93 There were contrasting responses to eCO<sub>2</sub> of aboveground primary productivity between the two  
94 ecosystems (grassland x CO<sub>2</sub> interaction;  $F_{1,60} = 8.60$ ,  $p < 0.01$ ; Fig. 1). Cumulative above ground  
95 biomass production in the limestone grassland increasing 16% in response to eCO<sub>2</sub>, ( $p = 0.03$ ; Fig.  
96 1) whilst in the acidic grassland it declined by 11% ( $p = 0.05$ , Fig. 1). These contrasting responses  
97 were consistent through years 2-3 (2019-2020) and multiple harvests within these years (Fig. 1;  $F_{1,449} = 14.67$   $p < 0.0001$ , and Extended Data Fig. 1). The increased productivity in the limestone  
98 grassland is broadly in line with previously reported eCO<sub>2</sub> responses<sup>22,23</sup>, where swards increased by  
100 7- 20% in the first 3-4 years of fumigation. The decline in productivity in the acidic grassland was  
101 unexpected but not unprecedented: of 139 studies in a meta-analysis, two grassland systems showed  
102 significant declines in productivity under eCO<sub>2</sub><sup>17</sup>. The mechanisms for driving these previously  
103 reported reductions are not understood, but our outdoor study of grasslands supplied with eCO<sub>2</sub> after  
104 several decades of different nitrogen and phosphorus addition treatments, provide this mechanistic  
105 understanding of the contrasting plant responses for the first time.

## 106 *Nutrient treatment response of biomass*

107 Aboveground biomass increased with both nitrogen and phosphorus additions in both ecosystems  
108 ( $F_{1,60} = 23.15$ ,  $p < 0.0001$ , Extended Data Fig. 1). Total productivity was in the order  $0N < LN < HN$   
109  $< P$  across both grasslands, though the acidic grassland was less sensitive to N addition (grassland x  
110 nutrient,  $F_{3,60} = 1.32$ ,  $p > 0.2$ ). Productivity only increased under HN (by 29%) in the acidic grassland  
111 relative to controls ( $p < 0.005$ ), whereas LN increased productivity in the limestone (by 39%;  
112  $p < 0.007$ ), but did not further increase under HN. In both grasslands, P addition gave the highest

shoot productivity, confirming this as the most limiting nutrient in these ecosystems. While N-loading may exacerbate P-demand, the increase in productivity arising from the N treatments is in line with current understanding that N addition can alleviate P-limitation in the long-term, by enhancing weathering of Ca-P, and mineralisation of organic P by microbial phosphatases<sup>13,21</sup>. Reflecting this, across all the nutrient treatments, positive relationships were observed between available P and above ground biomass production in both grasslands (Fig. 2).

#### *Competition for P drives contrasting productivity responses*

Three growing seasons of eCO<sub>2</sub>, caused no change in the limestone grassland MBP ( $p > 0.5$ ), contrasting with the 36% increase in MBP in the acidic grassland across all nutrient treatments ( $p < 0.03$ , Extended Data Fig. 2). These responses were reflected in strong eCO<sub>2</sub> effects on the ratios of above ground plant biomass P (AGBP) to MBP in the two grasslands ( $F_{1,185} = 6.78$ ,  $p = 0.01$ ). Whilst eCO<sub>2</sub> tended to increase AGBP:MBP in the limestone grassland this was not significant, in the acidic grassland it highly significantly decreased it ( $p < 0.003$ ). These contrasts gave a significant interaction between CO<sub>2</sub> treatment and grassland type ( $F_{1,185} = 8.94$ ,  $p = 0.004$ , Fig. 3). The AGBP:MBP responses to eCO<sub>2</sub> were apparent from the end of the first year of enrichment and persisted throughout the experiment (Fig. 3).

Increases in microbial P pools causing immobilisation, can reduce plant productivity<sup>24</sup>. The decline in the AGBP:MBP under eCO<sub>2</sub> in the acidic grassland implies that competition between soil microbes and plants for P intensified, increased plant growth limitation by P, and reduced shoot productivity. It has long been thought that, in the short term at least, microbes are the better competitors<sup>25</sup> for limiting nutrients, and microbial N uptake can be an order of magnitude greater than by plants in grasslands<sup>26</sup>. None-the-less, while competition for P can be intense<sup>27</sup>, it is less well-understood<sup>28</sup> and our work suggests an important role for this in mediating eCO<sub>2</sub> productivity responses. The contrasting, lack of decline in AGBP:MBP under eCO<sub>2</sub> in the limestone grassland shows no evidence of microbial-driven exacerbation of plant P-limitation and allowed the positive

138 plant productivity and shoot P content response to eCO<sub>2</sub> in this grassland (see NPP and AGBP,  
 139 Table 1).

140 There was no evidence of CO<sub>2</sub> × nutrient treatment interactions on plant productivity (Supp  
 141 Fig 3;  $p < 0.7$ ). Thus, the contrasting responses of the two grasslands appear to be driven by  
 142 differences in microbial immobilisation even in plots receiving P additions. In the acidic  
 143 grassland, eCO<sub>2</sub> still reduced plant productivity in plots receiving P, probably because the  
 144 increase in immobilisation was greatest in this treatment; with microbial biomass P  
 145 increased by a factor of 1.7 (Table 1). In this context, and in contrast to previous studies<sup>9,29</sup>,  
 146 the ecosystems had undergone nutrient manipulation for > 25 years, thus the CO<sub>2</sub> effects on  
 147 each grassland-nutrient combination represented responses of grasslands that have largely  
 148 stabilised to their new nutrient status rather than responding to a recent step-change in  
 149 nutrient loading. Competition between plants and microbes is not just controlled by the  
 150 microbes. Differences in plant species present between the two grasslands could also play a  
 151 role. For example, low-P specialist groups such as sedges are abundant in the limestone  
 152 grassland<sup>10</sup>, which could explain some of the differences between grasslands. However,  
 153 plant community composition changed substantially with long-term nutrient manipulations,  
 154 including an increase in sedge abundance in the N treatments and a decline with P addition.  
 155 Despite these changes, a consistent positive eCO<sub>2</sub> biomass response was seen across all  
 156 nutrient treatments. This suggests that it is the key differences in the soil biogeochemistry  
 157 and potential for P to be immobilised under eCO<sub>2</sub> that explains the overall differences  
 158 between the grasslands.

159 The key to the differences in the outcome of microbial-plant competition may lie in  
 160 differences in soil P chemistry between the two grasslands. In both cases, recently-fixed C  
 161 that enters soil via roots and associated mycorrhizal fungi<sup>30</sup> includes enzymes and organic  
 162 acids, that liberate P needed to sustain a positive plant growth response<sup>31</sup> to eCO<sub>2</sub>. In terms  
 163 of differences in organic P cycling, the lower soil pH in the acidic grassland may have

164 resulted in reduced total microbial phosphatase activity; it has been shown that reductions in  
165 the abundance of alkaline phosphatases in low pH soils cannot necessarily be compensated  
166 by increased acid phosphatase activities<sup>32</sup>. This may in turn promote greater competition  
167 between plants and microbes for P<sup>33</sup>. There are also important differences in mineral P  
168 pools. In the limestone grassland soil, abundant calcium phosphates (Ca-P) offer potential  
169 new P inputs, the weathering of which may be enhanced by eCO<sub>2</sub> increasing organic acid  
170 exudates<sup>31</sup> and arbuscular mycorrhizal fungal (AMF) activity<sup>34</sup>. In contrast, in the acid  
171 grassland, Ca is 95% less abundant, and inorganic P predominantly bound to Fe and Al<sup>10</sup>, to  
172 which plant and microbial access may be extremely limited<sup>35</sup>. Greater microbial biomass P  
173 will thus promote stronger competition for organic P, and may facilitate adsorption and  
174 occlusion of microbial biomass-derived P into the low-availability soil P pools. As well as  
175 lysing microbial cells, chloroform fumigation may liberate some organic P from soil pools.  
176 Therefore, the increase in ‘chloroform-labile P’ in the acidic grassland soils reflects greater  
177 amounts of P immobilised in live microbial biomass and potentially some P immobilised by  
178 microbes into organic matter pools. In the longer-term, the latter could become a substantial  
179 sink for P further reducing plant productivity under eCO<sub>2</sub>. In summary, the limestone and  
180 acidic soils represent early and later stages of ecosystem retrogression respectively through  
181 loss of mineral P (especially Ca-P), acidification, and enrichment of highly recalcitrant Fe  
182 and Al secondary P minerals<sup>11,35</sup>. Our findings are likely to be applicable to other P-limited  
183 ecosystems at these different stages of ecosystem retrogression. Furthermore, because the  
184 contrasting eCO<sub>2</sub> responses of the two grasslands are consistent across the different nutrient  
185 treatments, this suggests the mechanisms hold true irrespective of nutrient status, suggesting  
186 a very strong controlling role of soil microbes and chemistry.

#### 187 *P-limited ecosystem responses to eCO<sub>2</sub>*

188 Grasslands represent 20% of global terrestrial net primary productivity<sup>36</sup> and make major  
189 contributions to soil C stocks. They are also the most spatially extensive P-limited



ecosystem in temperate regions<sup>7</sup>. More broadly, greater than 40% of terrestrial ecosystems globally may be P-limited, highlighting the need to understand the impact of eCO<sub>2</sub> on the productivity of these ecosystems. Overall, the quantity, pathways, function and fate of C below ground controls the future C gain in plants and soils under eCO<sub>2</sub>. We have shown that two P-limited grasslands show directly opposing above-ground biomass responses to eCO<sub>2</sub>, and that these responses may be driven by competition for the limiting P-resource between plants and soil microbes. These contrasting responses appear to be controlled by differences in soil chemistry, giving new insight into how P-limited ecosystems of contrasting stages of pedogenesis may respond to eCO<sub>2</sub>. Below-ground interactions between plants, microbes and soil strongly dictate the fate of the C and potential sequestration, therefore current climate models which do not consider plant-microbe competition for limiting resources may under- or over-estimate terrestrial C sequestration dependent on the outcome of the competition<sup>37</sup>. Multiple studies of long-term nutrient and CO<sub>2</sub> manipulations have been invaluable for our understanding of N-limited productivity responses to eCO<sub>2</sub><sup>38</sup>, and we hope that more studies will now do the same regarding P limitation. It is essential that plant microbial competition, and their interactions with soil chemistry, are more clearly understood to better predict how ecosystems will respond to eCO<sub>2</sub> and climate change<sup>39</sup>.

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## 222 **Author Contributions Statement**

223

224 IPH, GKP, JBK and JRL designed the eCO<sub>2</sub> experiment. GKP, IPH, FM, JBK and CRT  
225 installed the FACE system and the mesocosms. JBK and CRT oversaw the operation and  
226 maintenance of the experiment. Lab analyses were undertaken by JBK and CRT and JBK  
227 performed the data analyses. JBK, GKP and IPH wrote the original draft of the manuscript  
228 and all authors contributed to subsequent revisions.

229

## 230 **Competing Interests Statement**

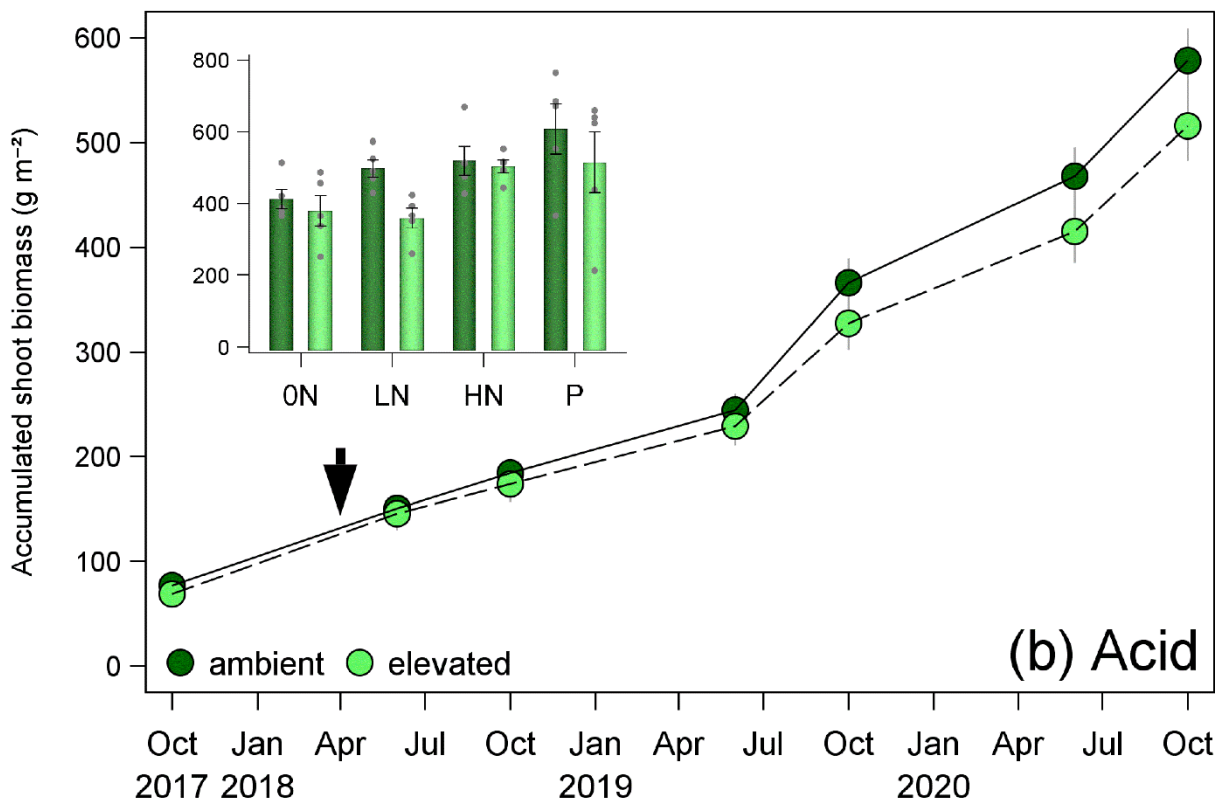
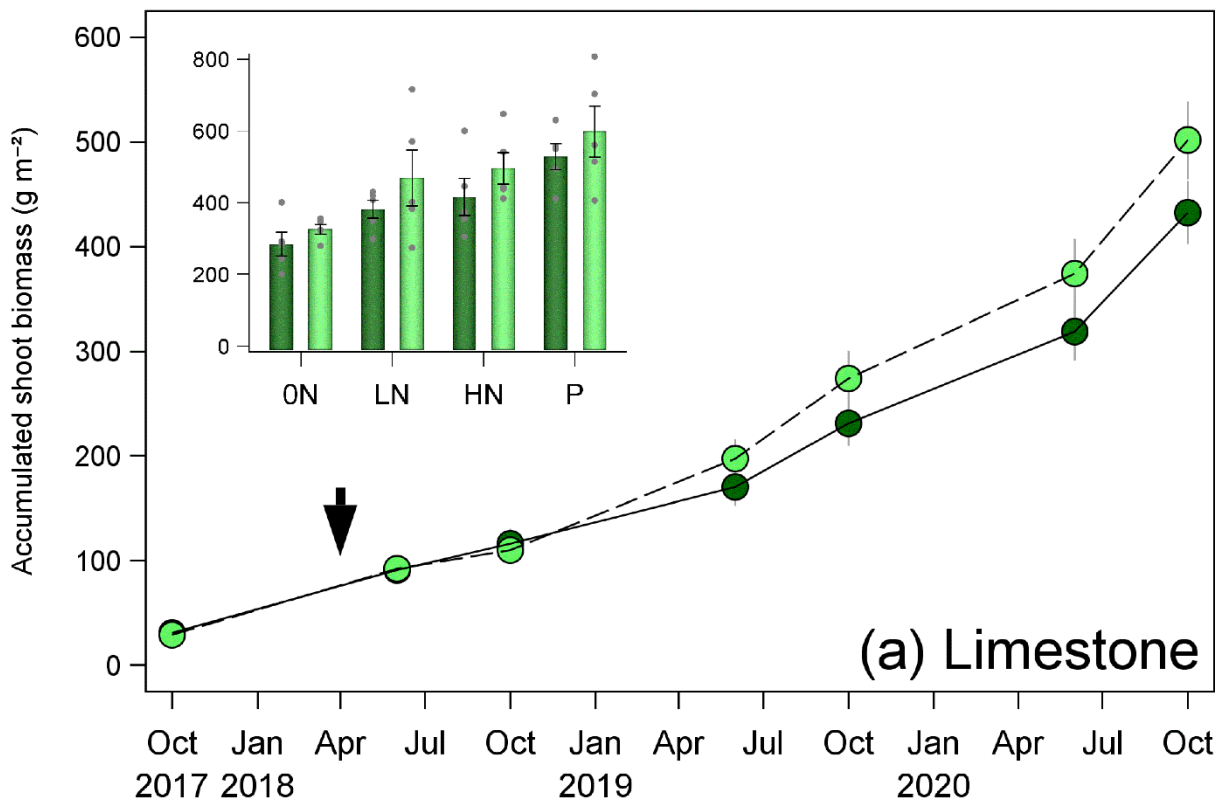
231 The authors declare no competing interests

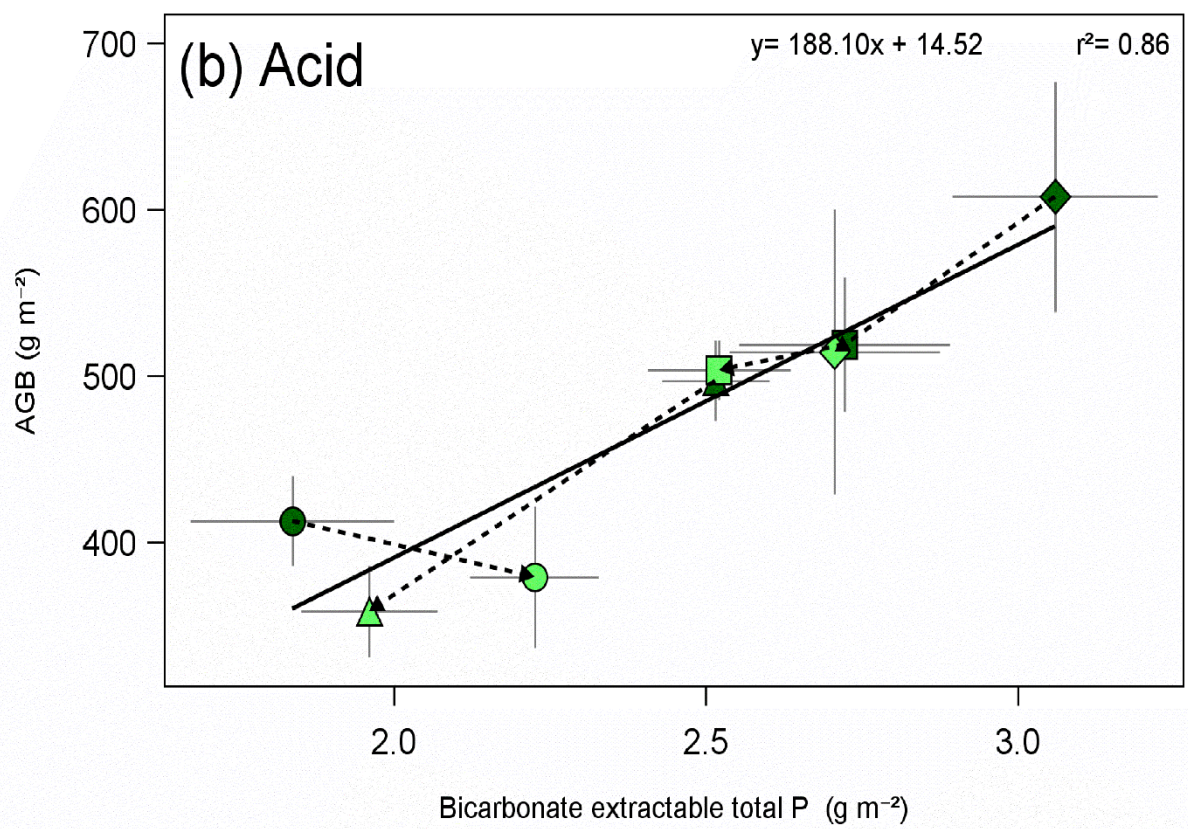
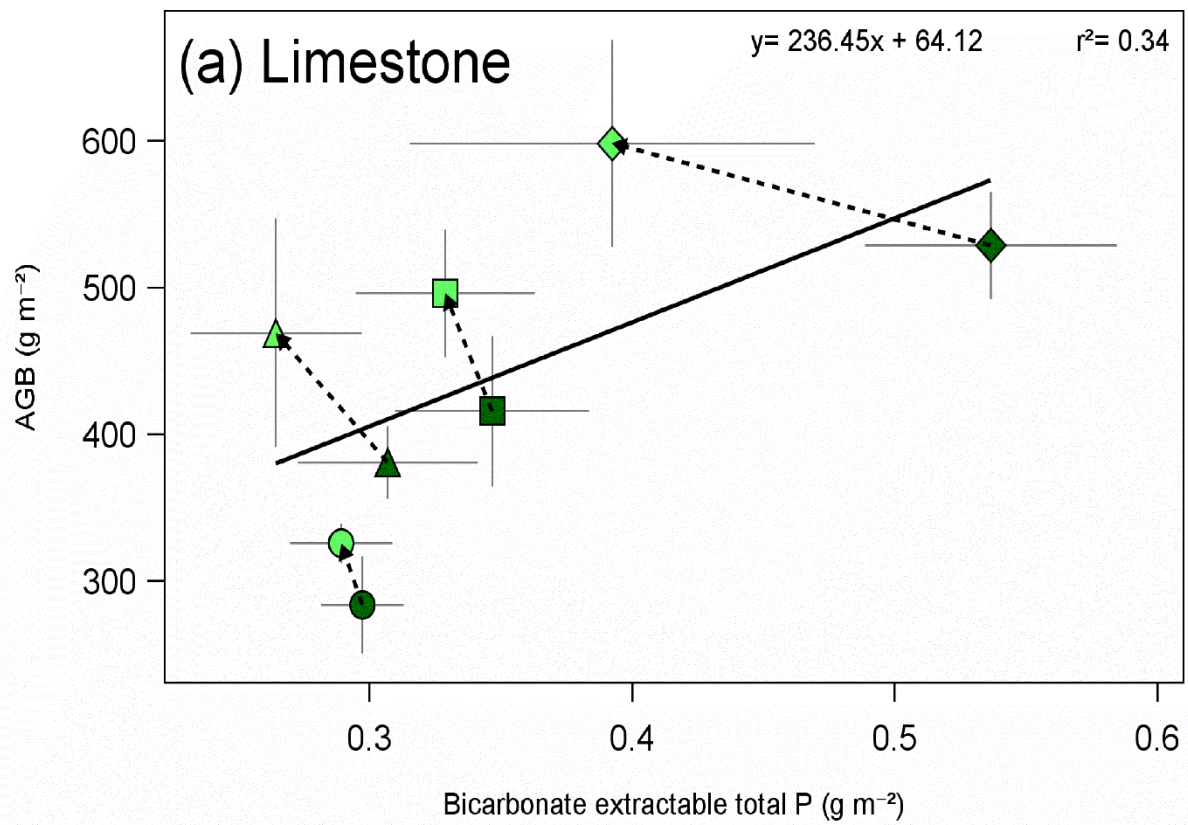
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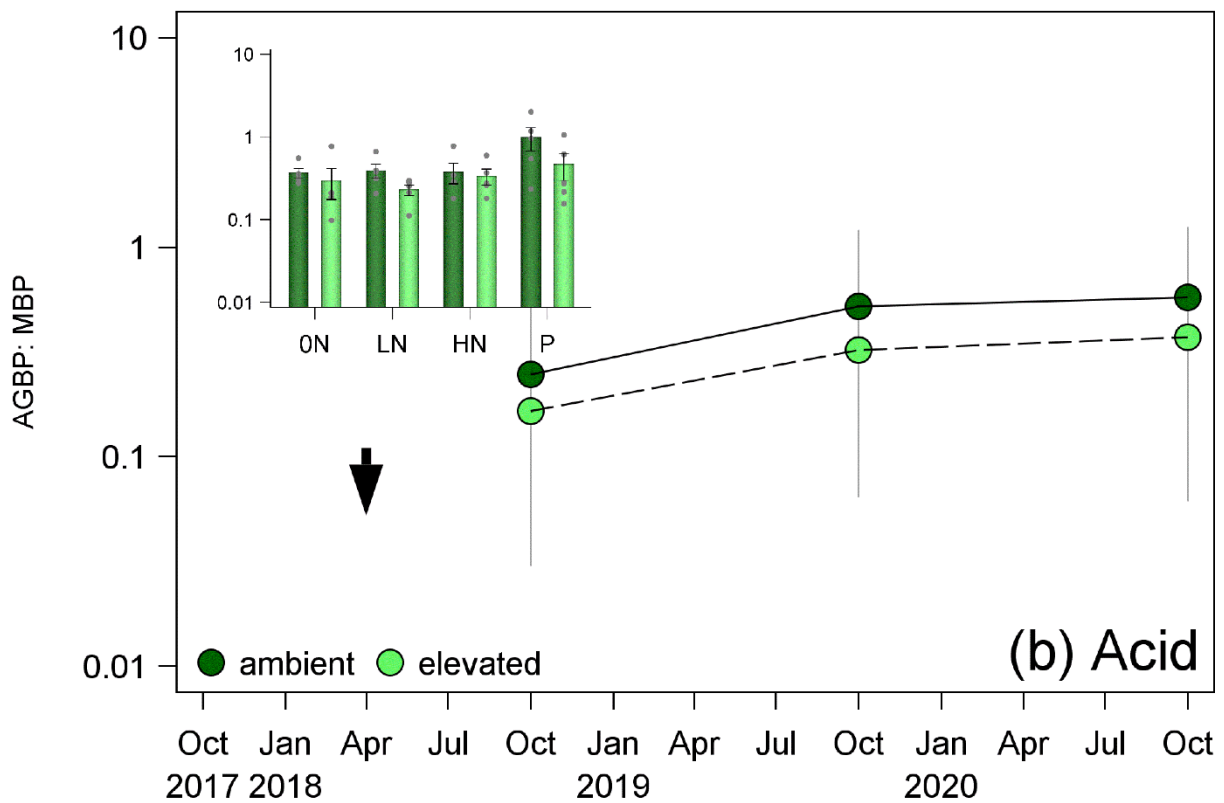
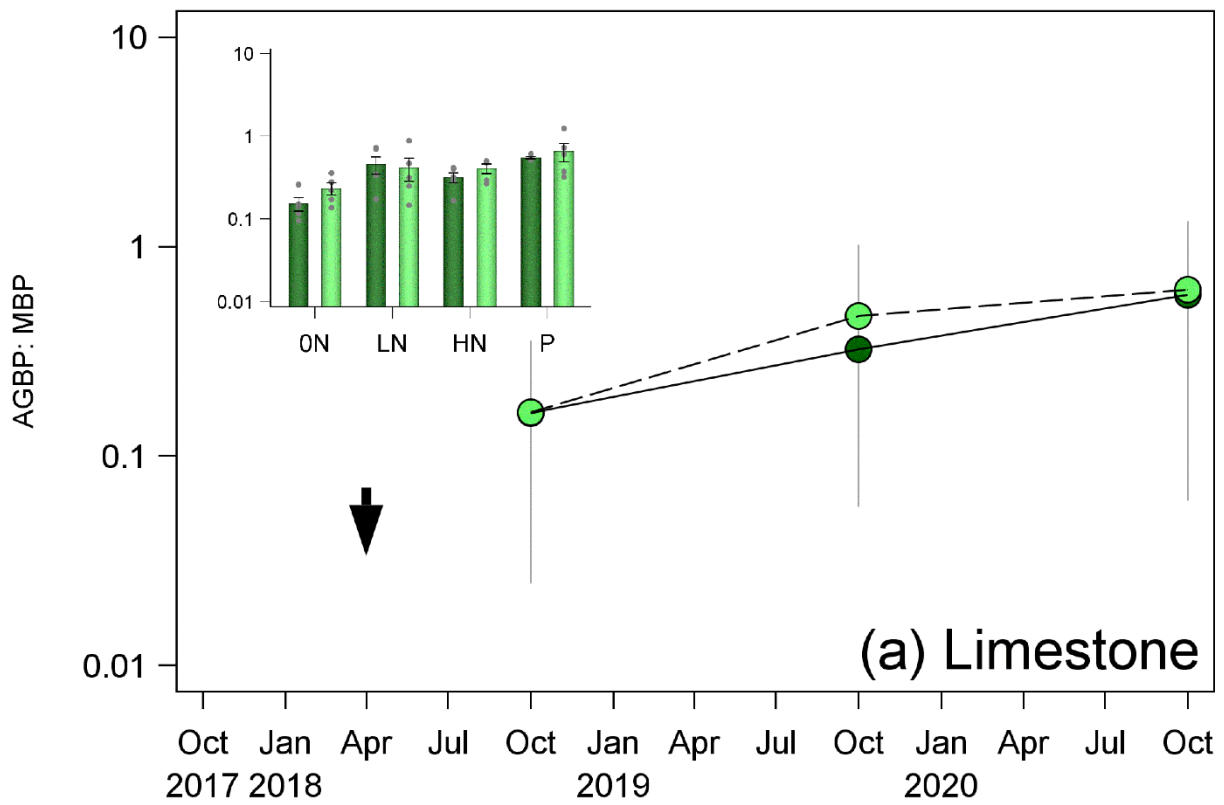
233 **Tables**

234 **Table 1 Summary of productivity and soil and microbial phosphorus pools. The mean**  
235 **(*n*= 5, with standard errors in parentheses) annual net primary production (NPP),**  
236 **aboveground biomass phosphorus (AGBP) and microbial biomass phosphorus (MBP)**  
237 **stocks from three years of CO<sub>2</sub> fumigation (a= ambient, e= elevated) from the acid and**  
238 **limestone grasslands, subjected to four nutrient amendment treatments (0N= control,**  
239 **LN= 3.5 g N m<sup>-2</sup> y<sup>-1</sup>, N m<sup>-2</sup> y<sup>-1</sup>, HN= 14 g N m<sup>-2</sup> y<sup>-1</sup>, P= 3.5 g P m<sup>-2</sup> y<sup>-1</sup>)**

Grassland	Nutrient	CO <sub>2</sub>	NPP (g m <sup>-2</sup> )							AGB P (mg m <sup>-2</sup> )							MBP (mg m <sup>-2</sup> )						
			2018		2019		2020		Mean	2018		2019		2020		Mean	2018		2019		2020		Mean
Limestone	0N	a	41.00	(7.98)	80.42	(11.49)	162.37	(21.82)	94.60 (13.76)	39.21	(7.39)	74.59	(23.46)	243.22	(49.52)	119.01 (26.79)	577.78	(51.62)	524.55	(61.93)	600.37	(37.57)	567.57 (50.37)
		e	41.44	(6.72)	104.13	(11.80)	180.05	(7.00)	108.54 (8.51)	38.71	(5.24)	114.26	(17.75)	221.54	(9.41)	124.83 (10.80)	494.60	(77.40)	440.00	(90.15)	468.03	(70.11)	467.54 (79.22)
	LN	a	73.26	(13.10)	120.23	(10.76)	187.38	(10.08)	126.96 (11.31)	66.94	(12.18)	123.27	(13.90)	249.11	(21.74)	146.44 (15.94)	366.02	(58.10)	245.94	(67.36)	434.02	(68.86)	348.66 (64.77)
		e	86.55	(21.86)	154.43	(38.62)	227.98	(29.17)	156.32 (29.88)	69.38	(17.45)	128.08	(27.39)	268.20	(36.12)	155.22 (26.99)	374.35	(52.80)	390.25	(104.79)	410.56	(69.94)	391.72 (75.84)
	HN	a	84.51	(20.81)	126.92	(26.74)	204.24	(18.58)	138.56 (22.04)	73.37	(18.18)	102.52	(10.76)	252.21	(29.91)	142.70 (19.62)	404.50	(59.69)	346.63	(53.36)	430.24	(34.83)	393.79 (49.29)
		e	81.47	(7.92)	171.53	(26.56)	243.07	(10.36)	165.36 (14.95)	66.47	(5.64)	172.39	(29.79)	294.52	(15.66)	177.79 (17.03)	392.39	(36.15)	319.62	(49.93)	467.89	(61.35)	393.30 (49.14)
	P	a	144.73	(6.36)	131.58	(20.68)	252.24	(32.67)	176.19 (19.91)	343.03	(17.31)	373.18	(89.99)	825.90	(103.02)	514.04 (70.11)	937.27	(71.73)	713.78	(65.88)	895.57	(114.38)	848.87 (83.99)
		e	113.53	(7.85)	225.78	(33.61)	258.96	(40.35)	199.42 (27.27)	207.57	(11.84)	559.72	(87.36)	669.42	(122.43)	478.91 (73.88)	705.89	(93.14)	595.64	(41.28)	1060.08	(347.06)	787.20 (160.49)
	0N	a	90.20	(13.09)	134.38	(15.51)	188.36	(16.54)	137.65 (15.05)	85.12	(17.87)	148.99	(22.42)	262.85	(35.59)	165.65 (25.29)	435.19	(85.24)	485.16	(152.21)	610.47	(79.42)	510.27 (105.62)
		e	96.98	(12.64)	107.51	(16.15)	174.86	(20.73)	126.45 (16.51)	95.73	(15.72)	103.50	(21.53)	223.99	(22.06)	141.08 (19.77)	563.06	(125.73)	435.28	(172.00)	1155.20	(209.33)	717.84 (169.02)
	LN	a	121.84	(17.18)	182.69	(8.30)	192.69	(21.90)	165.74 (15.80)	121.74	(17.14)	187.39	(32.63)	268.91	(37.96)	192.68 (29.25)	680.99	(135.02)	413.75	(79.57)	563.57	(177.19)	552.77 (130.59)
		e	69.94	(5.60)	134.20	(18.96)	154.50	(5.72)	119.55 (10.09)	52.89	(3.78)	130.57	(20.66)	175.64	(4.14)	119.70 (9.53)	584.39	(149.11)	682.07	(398.24)	614.30	(114.72)	626.92 (220.69)
Acid	HN	a	106.64	(19.77)	200.05	(26.85)	212.33	(16.58)	173.01 (21.06)	99.91	(19.31)	157.98	(20.40)	244.30	(24.20)	167.40 (21.31)	655.00	(154.56)	357.14	(111.15)	701.90	(108.13)	571.35 (124.61)
		e	107.69	(11.18)	190.56	(12.13)	205.34	(19.68)	167.86 (14.33)	97.55	(13.59)	183.81	(14.81)	224.46	(23.64)	168.61 (17.35)	753.36	(94.60)	701.90	(225.59)	487.49	(192.65)	647.58 (170.94)
	P	a	142.68	(10.32)	208.33	(38.07)	256.70	(23.71)	202.57 (24.03)	421.38	(38.37)	515.33	(173.14)	767.59	(63.17)	568.10 (91.56)	674.55	(89.92)	691.65	(273.81)	733.60	(198.73)	699.93 (187.49)
		e	113.32	(32.51)	181.27	(26.33)	219.97	(34.94)	171.52 (31.26)	318.34	(66.20)	434.07	(104.78)	561.94	(89.93)	438.12 (86.97)	1129.69	(256.07)	1140.56	(138.16)	1286.02	(147.50)	1185.43 (180.58)







243

244

245 **Figure Legends**

246 **Fig. 1** Contrasting aboveground shoot productivity responses to CO<sub>2</sub>. Limestone (a) and  
247 acidic (b) grasslands exposed to ambient (dark green circles and bars) or 600 ppm CO<sub>2</sub> (light  
248 green circles and bars), from April 2018 (denoted by vertical arrows). The main panels show  
249 the cumulative productivity over the study period, averaging across nutrient treatments  
250 (mean  $\pm$  1 SE, n= 20). Inset bar charts show the final accumulated shoot biomass (mean  $\pm$  1  
251 SE, n=5) in relation to the four multi-decadal nutrient treatments (0N= control, LN= 3.5 g N  
252 m<sup>-2</sup> y<sup>-1</sup>, HN= 14 g N m<sup>-2</sup> y<sup>-1</sup>, P= 3.5 g P m<sup>-2</sup> y<sup>-1</sup>).

253 **Fig. 2:** Relationship between bicarbonate-extractable total P (as determined by ICP-  
254 OES) and aboveground biomass. Please note this measure of available P differs from  
255 Olsen P by including bicarbonate-extractable organic P in addition to inorganic P. Two  
256 grasslands, limestone (a) and acidic (b) were exposed to ambient CO<sub>2</sub> (dark green  
257 symbols) or eCO<sub>2</sub> (600 ppm, light green symbols) under four nutrient manipulations  
258 (0N= control (circles), LN= 3.5 g N m<sup>-2</sup> y<sup>-1</sup> (triangles), HN= 14 g N m<sup>-2</sup> y<sup>-1</sup> (squares),  
259 P= 3.5 g P m<sup>-2</sup> y<sup>-1</sup> (diamonds)). Dashed arrows show the directional change in soil P  
260 availability and aboveground productivity under eCO<sub>2</sub> within each grassland-nutrient  
261 combination. The overall positive relationship between aboveground productivity and  
262 soil available P for each grassland is described by a linear regression (solid black line)  
263 and the formula is displayed in the top right of each panel. Data points are means for  
264 each nutrient-grassland-CO<sub>2</sub> combination (n= 5  $\pm$  1SE).

265

266 **Fig. 3:** Contrasting ratio (on a log scale) of aboveground plant biomass P (AGBP) to  
267 soil microbial biomass P (MBP). A limestone (a) and acidic grassland (b) were exposed  
268 to ambient (dark green circles and bars) or 600 ppm CO<sub>2</sub> (light green circles and bars),  
269 from April 2018. The main panels show time series measurements (mean  $\pm$  1 SE, n=  
270 20), inset bar charts giving overall means ( $\pm$  1 SE, n=5) for each nutrient treatment



271 (0N= control, LN= 3.5 g N m<sup>-2</sup> y<sup>-1</sup>, HN= 14 g N m<sup>-2</sup> y<sup>-1</sup>, P= 3.5 g P m<sup>-2</sup> y<sup>-1</sup>). Vertical  
272 arrows denote start of CO<sub>2</sub> fumigation.  
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412

## 413    **Methods**

### 414    *Nutrient manipulation*

415    Sets of 10 intact soil-turf monoliths of area 0.35 x 0.35 m, were taken from each treatment  
416    plot of a long-term grassland nutrient manipulation experiment that was established in 1995  
417    at Wardlow, Peak District National Park, UK<sup>20</sup>. The limestone grassland (NVC  
418    classification

419    *Festuca-Avenula* CG2d) occurs on a shallow ranker (~ 10 cm A horizon down to limestone  
420    parent material) that is transitioning from a humic rendzina due to leaching<sup>10</sup>. The acidic  
421    grassland (*Festuca-Agrostis-Galium* U4e) is on a cryptic podzol, with an organic-rich A  
422    horizon ca. 10 cm deep above a loessic mineral horizon extending to at least 70 cm depth  
423    (though rooting and biological activity is largely limited to the top 15 cm). On each  
424    grassland, replicate 9 m<sup>2</sup> experimental plots have received either no treatment (natural P  
425    limitation, application of distilled water only (0N)), monthly applications of P at 35 kg P ha<sup>-1</sup>  
426    y<sup>-1</sup> (P), or N at 35 and 140 kg N ha<sup>-1</sup> y<sup>-1</sup> (LN and HN, respectively)) and were continued  
427    throughout the study.

428    The monoliths were taken during February and March 2017, being excavated to the bedrock  
429    (~10 cm) in the limestone grassland and to below the main rooting depth in the acidic  
430    grassland (~20 cm). They were transported in polypropylene boxes to the Bradfield  
431    Environment Laboratory research station, also in the Peak District National Park. Since  
432    roots readily reach the underlying limestone in shallow rendzina soils, a base of limestone  
433    chippings, sourced from a quarry on the same limestone as Wardlow (Bee Low limestone,  
434    Dove Hole Quarry, Derbyshire UK), was applied to the bottom of the limestone mesocosms.

435    At the Bradfield research station, the mesocosms were set flush with the ground surface in  
436    holes dug out of the soil to ensure they were thermally buffered, and the surface of the turfs  
437    matched the surrounding vegetation. The mesocosm sides were solid so there was no direct



438 contact with the surrounding soils, and the base freely drained through holes covered with a  
439 mesh voile (to stop particulate loss and root outgrowth, or ingrowth of roots from  
440 surrounding plants). The Bradfield research station (ca. 390 m asl) is less than 20 km from  
441 Wardlow (ca. 350 m asl) and has similar climate.

#### 442 *CO<sub>2</sub> enrichment*

443 Mesocosms were assigned to form groups of 8, comprising one of each of the four nutrient  
444 treatments from both grasslands, and these were placed within five miniFACE or five  
445 control rings of 1.6 m diameter that were supported ca. 20 cm above the ground surface. The  
446 mesocosms in control rings experienced ambient CO<sub>2</sub> concentrations, whilst the miniFACE  
447 system<sup>40</sup> Each ring consisted of PVC tubes with laser drilled micro-holes. The processors in  
448 the FACE ring control units received CO<sub>2</sub> information from sensors (GTM222, Vaisala,  
449 Finland) installed in the centre of each ring. Microprocessors, linked to automated pressure  
450 regulators, controlled the pressure inside the releasing pipes and therefore delivery of CO<sub>2</sub> to  
451 the rings. Fumigation started in 2018, with fossil fuel-derived CO<sub>2</sub> supplied by BOC  
452 Cryospeed (BOC Limited, Manchester, UK). During the hours of daylight, CO<sub>2</sub> enrichment  
453 was set to a target of 600 ppm, which continued from the beginning of April until the end of  
454 October for three year (2018-2020). The FACE system achieved a mean of  $598 \pm 0.07$  ppm  
455 across all elevated plots over the experiment, with the 5% and 95% quantiles being 555 and  
456 643 ppm respectively.

#### 457 *Biomass harvesting*

458 Aboveground biomass was harvested twice each year, at the end of June and during  
459 September. Plant biomass was cut at 2.5 cm and 5 cm height in the limestone and acidic  
460 grasslands respectively and oven dried at 70 °C until constant weight. Dried plant material  
461 was first homogenised using a food processor then milled to a fine powder (IKA 10 Mill,  
462 IKA®-Werke GmbH & Co.KG, Staufen, Germany).

463    *Soil collection*

464    Soil was sampled once a year from each mesocosm contemporaneously with the September  
465    aboveground biomass harvest. Triplicate 2 cm diameter soil cores were taken from random  
466    locations within each mesocosm and in the acid grassland these were divided into the A and  
467    B horizons. Soil was passed first through a 10 mm sieve and then roots were removed by  
468    hand. The remaining soil was then passed through a 2 mm sieve in preparation for chemical  
469    analyses, with a subsample placed in the oven at 105 °C to determine soil moisture content.

470    *Determination of soil and plant biomass P content*

471    Plant biomass underwent hydrogen peroxide acid digest modified from Grimshaw (1987)<sup>41</sup>  
472    and Leake (1988)<sup>42</sup>. Dried plant material (20– 50 mg) was added to a glass digest tube and 1  
473    cm<sup>3</sup> of 100% H<sub>2</sub>SO<sub>4</sub> was added. The tubes were gently shaken and left overnight in a fume  
474    cupboard to ‘pre-digest’. Custom-made glass ‘cold fingers’ were used to cover the tubes,  
475    allowing acid condensate to safely reflux with the reagents. Two digest blanks per 30  
476    samples were prepared for colorimetry, which consisted of 1 cm<sup>3</sup> H<sub>2</sub>SO<sub>4</sub> which underwent  
477    the same subsequent procedure as the plant sample digests. Samples were heated in a  
478    heating block (Grant heat block, BT5D model) to 350 °C. Before reaching the target  
479    temperature (and at approximately 250 °C) samples were briefly removed from the block  
480    and swirled to mix the digestate. The tubes were allowed to reach 350 °C and remained at  
481    temperature for 15 minutes. Tubes were removed from the blocks, swirled again and  
482    allowed to partially cool.

483    Samples were clarified by addition of H<sub>2</sub>O<sub>2</sub> while solutions were still hot, following a delay  
484    of 30 –60 seconds to prevent loss of sample by spitting. An initial volume of 800 µl H<sub>2</sub>O<sub>2</sub>  
485    was added to each tube and boiled off in the heating block until most visible evaporation  
486    had ceased. Samples were cooled again and another 200 µl of hydrogen peroxide was added  
487    and boiled off. This latter addition was repeated once more to ensure all samples were clear

488 and no more plant sample remained adhered to the tubes. The digest solutions were diluted  
489 to 10 ml by adding 9 ml of ultra-high purity (UHP) water. Total P in the digest solutions was  
490 then determined using an adapted version of the molybdate blue reaction<sup>43,44</sup>.

#### 491 *Determination of soil microbial biomass P*

492 Soil microbial P (MBP) was determined using the chloroform-fumigation method of  
493 Brookes et al (1982)<sup>45</sup>. Gravimetric water content of soil was determined from oven drying a  
494 4 g fresh weight subsample for 48 hours at 105°C. Two further 4 g fresh weight aliquots  
495 were weighed into acid washed (HCl) 50 cm<sup>3</sup> beakers: one for fumigation and one for non-  
496 fumigation. One aliquot was placed in a vacuum desiccator with a beaker containing boiling  
497 chips and 20 ml chloroform (CHCl<sub>3</sub>). The desiccator was evacuated until chloroform boiled  
498 three times, venting between the first two times and left in darkness for 24 hours. After  
499 incubation, CHCl<sub>3</sub> was removed by drawing a vacuum and venting 5 times.

500 Both fumigated and unfumigated soils were extracted in 50 ml of 0.5 M sodium bicarbonate  
501 (NaHCO<sub>3</sub>; pH 8.5). Extracts were shaken for 1 hour on a rotary shaker and filtered through  
502 pre-leached Whatman 44 filter paper. Extracts were stored at -18 °C until quantification of P  
503 was determined using Inductively Coupled Plasma - Optical Emission Spectrometry  
504 (ICPOES; Thermo Fisher iCAP PRO). Soil MBP was calculated by subtracting the  
505 concentration of P in the non-fumigated aliquot from the fumigated aliquot.

#### 506 *Statistical analyses*

507 Linear mixed effects models were used to test for fixed effects of eCO<sub>2</sub>, nutrient treatment,  
508 and grassland, with block as a random factor, on aboveground biomass, cumulative  
509 aboveground biomass, aboveground biomass P, soil microbial P and AGB:MBP ratio. A log  
510 transformation was performed on AGBP:MBP before analysis. Differences between groups  
511 were assessed using least square means. All figures and analyses were performed using SAS  
512 9.4 (SAS Institute, Cary, NC, USA).

513 **Data availability**

514 Data used to produce this paper are available via the EIDC data repository

515 <https://doi.org/10.5285/35921c93-2d9e-4e35-8de5-adbfc37641b4>

516

517 **Methods References**

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