

This is a repository copy of *Grassland responses to elevated CO2 determined by plant–microbe competition for phosphorus*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/201449/>

Version: Submitted Version

---

**Article:**

Keane, Ben [orcid.org/0000-0001-7614-8018](https://orcid.org/0000-0001-7614-8018), Hartley, Iain, Taylor, Christopher R et al. (4 more authors) (2023) *Grassland responses to elevated CO2 determined by plant–microbe competition for phosphorus*. *Nature Climate Change*. ISSN 1758-678X

<https://doi.org/10.1038/s41561-023-01225-z>

---

**Reuse**

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:

<https://creativecommons.org/licenses/>

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

1 **Title:** Microbial competition for phosphorus determines grassland responses to elevated CO<sub>2</sub>

2 **Authors:** J. Ben Keane<sup>1,5</sup>, Iain P. Hartley<sup>4</sup>, Christopher R. Taylor<sup>1,6</sup>, Jonathan R. Leake<sup>1</sup>, Marcel R.  
3 Hoosbeek<sup>2</sup>, Franco Miglietta<sup>3</sup>, Gareth K. Phoenix<sup>1</sup>.

4 **1** Plants Photosynthesis and Soil, School of Biosciences, University of Sheffield, Western Bank,  
5 Sheffield, S10 2TN

6 **2** Soil Chemistry, Wageningen University, PO Box 47, 6700AA, Wageningen, The Netherlands

7 **3** Istituto Di Biometeorologia - Consiglio Nazionale Delle Ricerche, Sede centrale: via Giovanni  
8 Caproni 8, Firenze, 50145, Italia

9 **4** Geography, Faculty of Environment, Science and Economy, University of Exeter, Amory  
10 Building, Rennes Drive, Exeter, EX4 4RJ, UK

11 **5** Department of Environment and Geography, Wentworth Way, University of York, Heslington,  
12 York, YO10 5NG, UK

13 **6** Soil and Ecosystem Ecology, Earth and Environmental Sciences, University of Manchester,  
14 Manchester, UK

15  
16 **Rising atmospheric CO<sub>2</sub> has stimulated plant productivity, with terrestrial ecosystems**  
17 **currently absorbing nearly one-third of anthropogenic CO<sub>2</sub> emissions<sup>1</sup>. There is growing**  
18 **evidence that nitrogen (N) availability constrains eCO<sub>2</sub> responses<sup>1-4</sup>, yet we know much less**  
19 **about the role of phosphorus (P). This is important because P-limited ecosystems are globally**  
20 **widespread, and the biogeochemical cycles of N and P differ fundamentally. Using a free-**  
21 **airCO<sub>2</sub>-enrichment (FACE) experiment on two contrasting P-limited grasslands, we show that**  
22 **competition between plants and microbes for P can determine plant productivity responses to**  
23 **eCO<sub>2</sub>. In a limestone grassland, aboveground productivity increased (16%) and microbial**  
24 **biomass P remained unchanged, whereas in an acidic grassland, aboveground productivity**  
25 **and P uptake declined (11% and 20%, respectively), but P immobilisation into microbial**  
26 **biomass increased (36%). Our results demonstrate that strong competition with microbes can**  
27 **cause plant P uptake to decline under eCO<sub>2</sub>, with implications for the future productivity of P-**  
28 **limited ecosystems.**

29 The ability of terrestrial ecosystems to sequester more C as CO<sub>2</sub> levels rise represents potentially the  
30 single most important biogeochemical feedback limiting climate change<sup>1</sup>. This ability can be

31 constrained substantially by nutrient availability<sup>1-4</sup>, with Earth system models indicating that  
32 nitrogen (N) limitation reduces this capacity for greater C sequestration by as much as 58%<sup>5</sup>.  
33 However, between a third and a half of terrestrial ecosystems are limited by P, not N<sup>6,7</sup>. Despite the  
34 spatial extent of P limitation, we know very little about how P limitation affects ecosystem  
35 responses to eCO<sub>2</sub>, thus significantly limiting our ability to predict future rates of C uptake by the  
36 terrestrial biosphere<sup>8,9</sup>.

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

51 Many of the strategies employed by plants to acquire P require C, either through mycorrhizal  
52 symbiosis, or the production of C-rich compounds such as organic acids to mobilise organic- and  
53 mineral-bound P, or root surface enzymes such as phytases and phosphatases to mineralise organic  
54 P sources<sup>12</sup>. Therefore, the expected increase in C entering ecosystems via photosynthesis under  
55 eCO<sub>2</sub> may then provide a mechanism by which P limited productivity is alleviated, consequently  
56 increasing C sequestration. However, it has been shown that changes in C input can shift investment  
57 by soil microbes away from C acquisition to N and P acquisition under eCO<sub>2</sub><sup>13</sup>, and the  
58 consequences for competition between plants and microbes is unclear<sup>14</sup>.

59 Currently, only one FACE experiment has been established on an ecosystem of proven P-limitation.  
60 Importantly, this study in Eucalypt forest<sup>15</sup> in Australia, found that net primary productivity did not  
61 increase in response to eCO<sub>2</sub>, with forest maturity or P-limitation proposed as potential  
62 explanations<sup>16,17</sup>. Meta-analysis of eCO<sub>2</sub> productivity responses indicated that P availability is an

63 important constraint<sup>18</sup>, but without direct experimental evidence from P-limited ecosystems, major  
64 uncertainty surrounds eCO<sub>2</sub> responses of P-limited ecosystems that are widespread and prevalent  
65 globally. This holds especially true for grasslands, which represent 20% of terrestrial net primary  
66 productivity<sup>19</sup> and are the most spatially extensive P-limited ecosystem in temperate regions<sup>7</sup>.

67 To address this, we established a Free Air Carbon dioxide Enrichment (FACE) experiment, where  
68 intact soil-turf monoliths were exposed to either ambient CO<sub>2</sub> (ca. 410 ppm) or eCO<sub>2</sub> of 600 ppm.  
69 The monoliths used for the experiment were extracted from a long-term N and P manipulation study  
70 established in 1995 on two adjacent naturally P-limited grasslands<sup>20</sup>: a limestone grassland and an  
71 acidic grassland, which represent different stages of ecosystem retrogression. The limestone soil sits  
72 over a potential source of readily weatherable mineral P in calcium phosphates (Ca; Ca-P). This  
73 contrasts with the acidic soil, where Ca is 95% less abundant, and inorganic P is likely bound to  
74 abundant Fe and Al, to which plant and microbe access may be extremely limited, and thus  
75 competition for organic P is more intense<sup>10</sup>. In addition to control (no nutrient addition) plots,  
76 nutrient inputs have been manipulated through long-term (ca. 25 years) additions of P (35 kg P ha<sup>-1</sup>  
77 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  
78 of P alleviates the P-limitation of productivity and N additions simulate atmospheric N deposition:  
79 in line with the latest understanding of N loading impacts<sup>27</sup>, these are now also alleviating  
80 P-limitation, potentially by more weathering of Ca-P in the limestone soil, and possible stimulation  
81 of organic P mineralisation by microbial phosphatases<sup>13,21</sup>. CO<sub>2</sub> fumigation began in 2018 with the  
82 FACE system supplying eCO<sub>2</sub> during the hours of daylight from April until November in each year  
83 for three years (2018-2020).

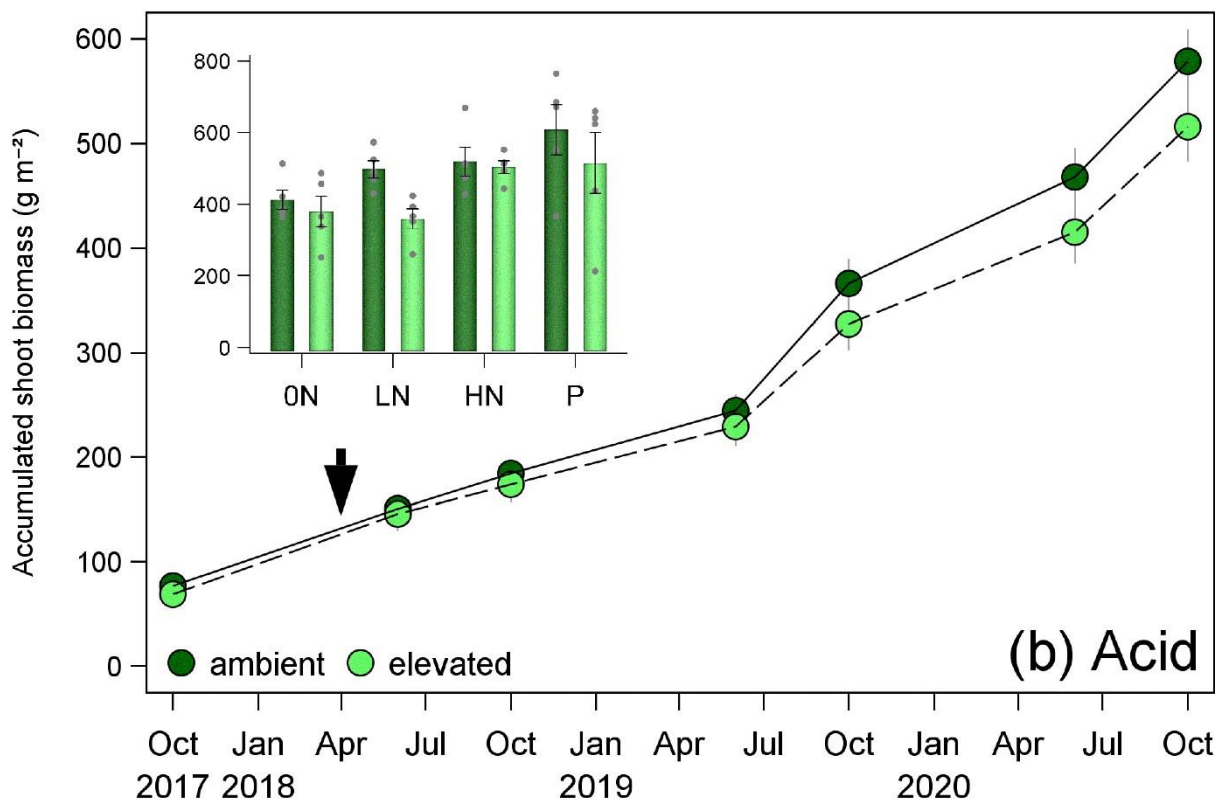
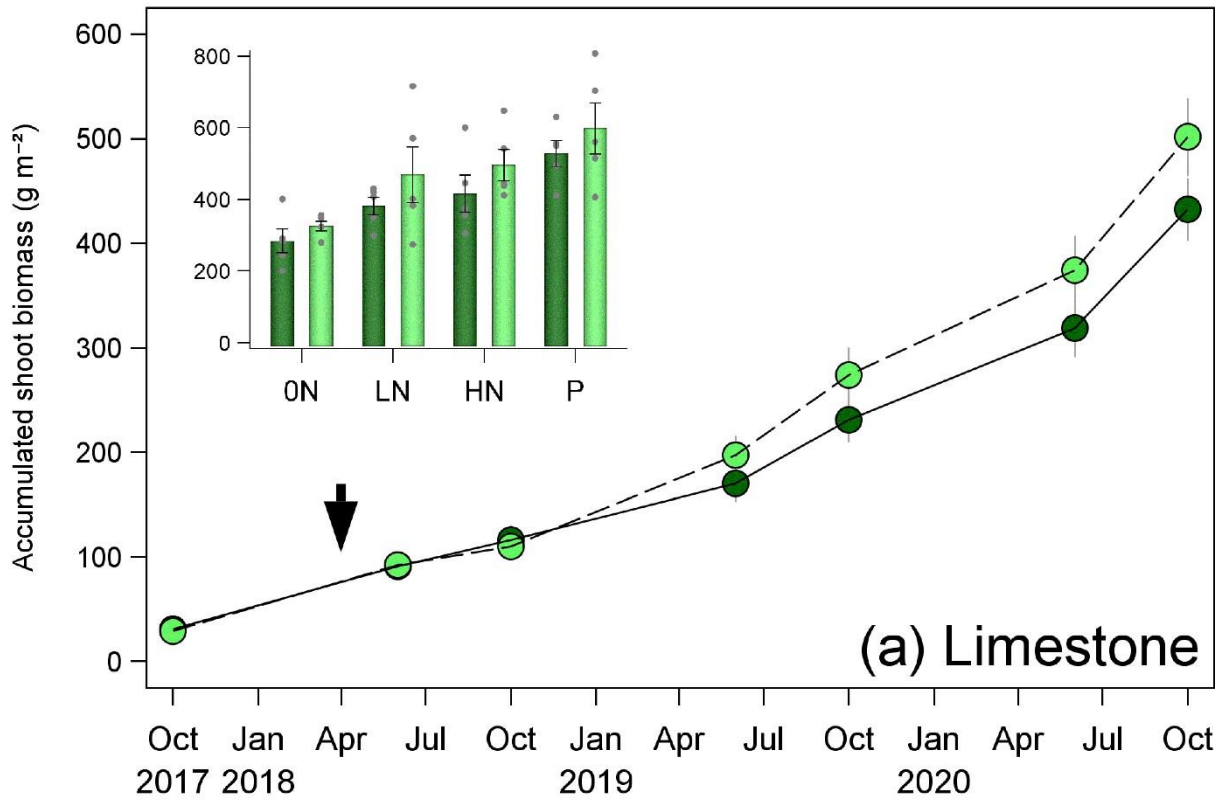
#### 84 *Aboveground biomass responses to eCO<sub>2</sub>*

85 There were consistent, contrasting responses to eCO<sub>2</sub> of aboveground primary productivity between  
86 the two ecosystems (grassland x CO<sub>2</sub> interaction;  $F_{1,60} = 8.60$ ,  $p < 0.01$ ; Fig. 1). This was due to  
87 cumulative above ground biomass production in the limestone grassland increasing 16% in response  
88 to eCO<sub>2</sub>, ( $p = 0.03$ ; Fig. 1) whilst in the acidic grassland it declined by 11% ( $p = 0.05$ , Fig. 1). These  
89 contrasting responses were consistent through years 2-3 (2019-2020) and multiple harvests within  
90 these years (Fig. 1;  $F_{1,449} = 14.67$   $p < 0.0001$ , and Supp Fig. 3). The increased productivity in the  
91 limestone grassland is broadly in line with previously reported eCO<sub>2</sub> responses<sup>22,23</sup>, where swards  
92 increased by 7- 20% in the first 3-4 years of fumigation. The decline in productivity in the acidic  
93 grassland was unexpected but not unprecedented: of 139 studies in a meta-analysis, two grassland  
94 systems showed significant declines in productivity under eCO<sub>2</sub><sup>17</sup>. The mechanisms for driving

95 these previously reported reductions are not understood, but our outdoor study of grasslands  
96 supplied with eCO<sub>2</sub> after several decades of different nitrogen and phosphorus addition treatments,  
97 provide this mechanistic understanding of the contrasting plant responses for the first time.

98 *Nutrient treatment response of biomass*

99 Aboveground biomass increased with both nitrogen and phosphorus additions in both ecosystems  
100 ( $F_{1,60} = 23.15$ ,  $p < 0.0001$ , Supp Fig.3). Total productivity was in the order  $0N < LN < HN < P$  across  
101 both grasslands, though the acidic grassland was less sensitive to N addition (grassland x nutrient,  
102  $F_{3,60} = 1.32$ ,  $p > 0.2$ ). Productivity only increased under HN (by 29%) in the acidic grassland relative  
103 to controls ( $p < 0.005$ ), whereas LN increased productivity in the limestone (by 39%;  $p < 0.007$ ), but  
104 did not further increase under HN. In both grasslands, P addition gave the highest shoot  
105 productivity, confirming this as the most limiting nutrient in these ecosystems. While N-loading  
106 may exacerbate P-demand, the increase in productivity arising from the N treatments is in line with  
107 current understanding that N addition can alleviate P-limitation in the long-term, by enhancing  
108 weathering of any Ca-P, and mineralisation of organic P by microbial phosphatases<sup>13,21</sup>.



109 **Fig. 1 Contrasting aboveground shoot productivity responses in (a) limestone and (b) acidic grasslands**  
110 **exposed to ambient CO<sub>2</sub> (dark green circles and bars) or 600 ppm CO<sub>2</sub> (light green circles and bars),**  
111 **supplied from April 2018 (denoted by vertical arrows). The main panels for each grassland show the**  
112 **cumulative productivity over the study period, averaging across nutrient treatments (mean ± SE, n=**  
113 **20). Inset bar charts show the final accumulated shoot biomass (mean ± SE, n=5) in relation to the four**  
114 **multi-decadal nutrient treatments (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>**  
115 **y<sup>-1</sup>).**

116 *Plant-microbe competition for P as a driver of contrasting productivity responses*

117 Three growing seasons of eCO<sub>2</sub>, caused no change in the limestone grassland MBP (p> 0.5),  
118 contrasting with the 36% increase in MBP in the acidic grassland across all nutrient treatments (p<  
119 0.03, Supplementary Fig. 4). These responses were reflected in strong eCO<sub>2</sub> effects on the ratios of  
120 above ground plant biomass P (AGBP) to MBP in the two grasslands (F<sub>1,185</sub>= 6.78, p= 0.01). Whilst  
121 eCO<sub>2</sub> tended to increase AGBP:MBP in the limestone grassland this was not significant, in the  
122 acidic grassland it highly significantly decreased it (p< 0.0001). These contrasts gave a significant  
123 interaction between CO<sub>2</sub> treatment and grassland type (F<sub>1, 185</sub>= 11.21, p= 0.001, Fig. 2). The  
124 AGBP:MBP responses to eCO<sub>2</sub> were apparent from the end of the first year of enrichment and  
125 persisted throughout the experiment (Fig. 2).

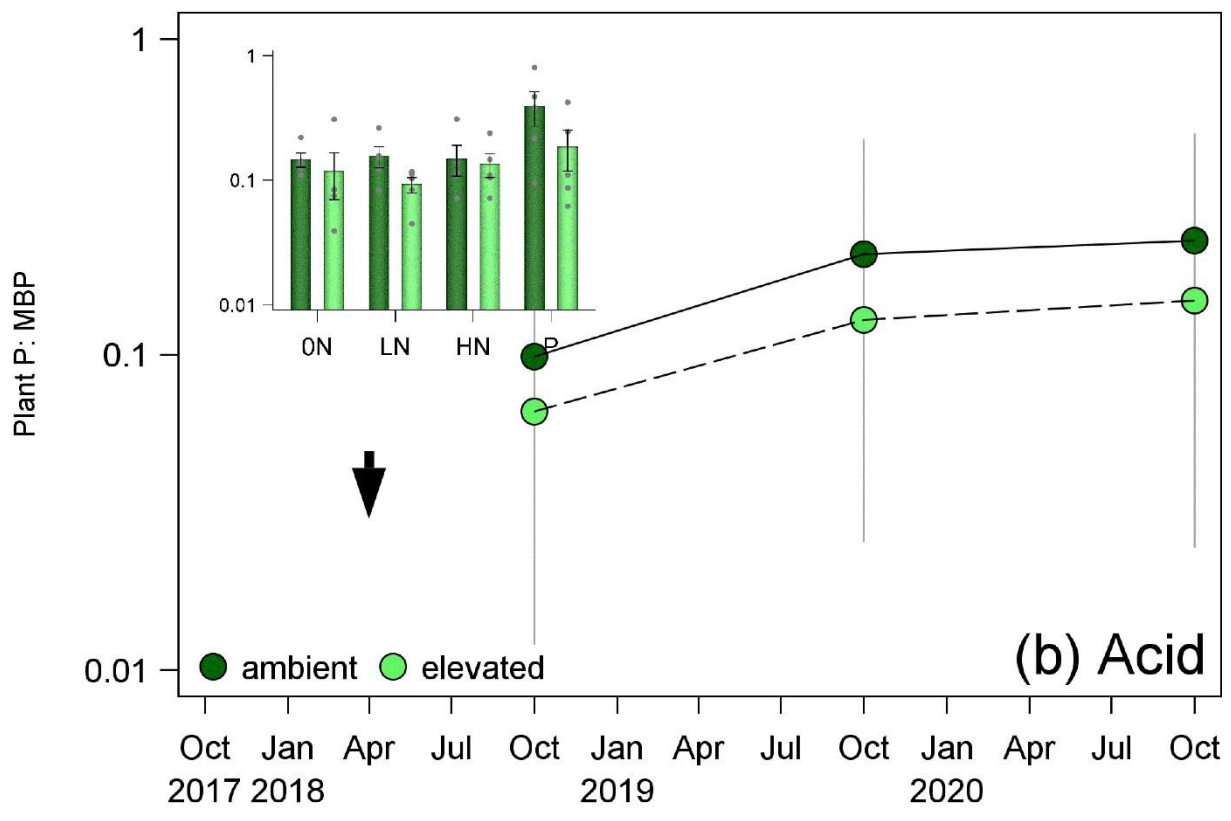
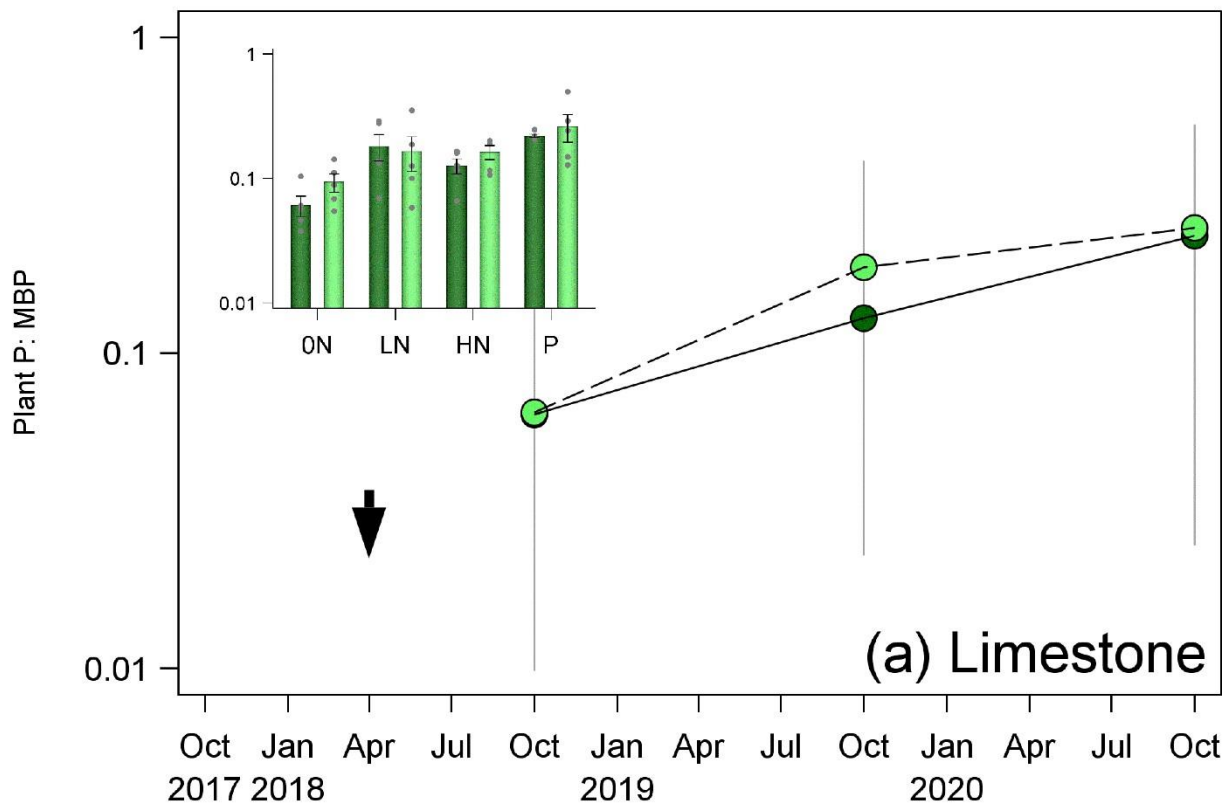
126 Increases in microbial P pools causing immobilisation, can reduce plant productivity<sup>24</sup>. The decline  
127 in the AGBP:MBP under eCO<sub>2</sub> in the acidic grassland implies that competition between soil  
128 microbes and plants for P intensified, increased plant growth limitation by P, and reduced shoot  
129 productivity. It has long been thought that, in the short term at least, microbes are the better  
130 competitors<sup>25</sup> for limiting nutrients, and microbial N uptake can be an order of magnitude greater  
131 than by plants in grasslands<sup>26</sup>. None-the-less, while competition for P can be intense<sup>27</sup>, it is less  
132 well-understood<sup>28</sup> and our work suggests an important role for this in mediating eCO<sub>2</sub> productivity  
133 responses. The contrasting, lack of decline in AGBP:MBP under eCO<sub>2</sub> in the limestone grassland  
134 shows no evidence of microbial-driven exacerbation of plant P-limitation and allowed the positive  
135 plant productivity and shoot P content response to eCO<sub>2</sub> in this grassland (see NPP and AGBP,  
136 Table 1).

137 **Table 1 The mean (n= 5, with standard errors in parentheses) annual net primary production (NPP),**  
138 **aboveground biomass phosphorus (AGBP) and microbial biomass phosphorus (MBP) stocks from**  
139 **three years of CO<sub>2</sub> fumigation (a= ambient, e= elevated) from the acid and limestone grasslands,**  
140 **subjected to four nutrient amendment treatments (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>,**  
141 **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> )								AGBP (mg m <sup>-2</sup> )				MBP (mg m <sup>-2</sup> )			
		2018		2019		2020		Mean		2018		2019		2020		Mean	
Acid	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)	1087.97 (213.10)	1212.89 (380.52)	1526.17 (198.55)	1275.68 (264.06)			
		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)	1407.64 (314.34)	1088.19 (430.01)	2887.99 (523.32)	1794.61 (422.56)			
	LN	a	121.84 (17.18)	182.69 (8.3)	192.69 (21.9)	165.74 (15.79)	121.74 (17.14)	187.39 (32.63)	268.91 (37.96)	192.68 (29.24)	1702.48 (337.56)	1034.37 (198.92)	1408.92 (442.97)	1381.92 (326.48)			
		e	69.94 (5.6)	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)	1460.98 (372.79)	1705.17 (995.59)	1535.74 (286.80)	1567.30 (551.73)			
	HN	a	106.64 (19.77)	200.05 (26.85)	212.33 (16.58)	173.01 (21.07)	99.91 (19.31)	157.98 (20.4)	244.30 (24.2)	167.40 (21.30)	1637.49 (386.40)	892.86 (277.86)	1754.76 (270.32)	1428.37 (311.53)			
		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)	1883.40 (236.50)	1754.74 (563.96)	1218.73 (481.62)	1618.96 (427.36)			
	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)	1686.37 (224.79)	1729.13 (684.53)	1834.00 (496.83)	1749.84 (468.72)			
		e	113.32 (32.51)	181.27 (26.33)	219.97 (34.94)	171.52 (31.26)	318.34 (66.2)	434.07 (104.78)	561.94 (89.93)	438.12 (86.97)	2824.24 (640.19)	2851.40 (345.40)	3215.05 (368.74)	2963.56 (451.44)			
	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)	1444.45 (129.06)	1311.37 (154.83)	1500.93 (93.92)	1418.92 (125.94)		
			e	41.44 (6.72)	104.13 (11.8)	180.05 (7)	108.54 (8.51)	38.71 (5.24)	114.26 (17.75)	221.54 (9.41)	124.83 (10.80)	1236.49 (193.49)	1099.99 (225.38)	1170.08 (175.28)	1168.86 (198.05)		
LN		a	73.26 (13.1)	120.23 (10.76)	187.38 (10.08)	126.96 (11.31)	66.94 (12.18)	123.27 (13.9)	249.11 (21.74)	146.44 (15.94)	915.04 (145.24)	614.84 (168.40)	1085.06 (172.16)	871.65 (161.93)			
		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)	935.88 (131.99)	975.62 (261.97)	1026.40 (174.85)	979.30 (189.61)			
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)	1011.25 (149.22)	866.56 (133.39)	1075.59 (87.08)	984.47 (123.23)			
		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)	980.97 (90.36)	799.06 (124.83)	1169.74 (153.37)	983.26 (122.85)			
P		a	144.73 (6.36)	131.58 (20.68)	252.24 (32.67)	176.19 (19.90)	343.03 (17.31)	373.18 (89.99)	825.90 (103.02)	514.04 (70.11)	2343.18 (179.32)	1784.44 (164.71)	2238.94 (285.94)	2122.19 (209.99)			
		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)	1764.72 (232.86)	1489.09 (103.20)	2650.19 (867.64)	1968.00 (401.23)			



146 **Fig. 2 Contrasting ratio (on a log scale) of aboveground plant biomass P (AGBP) to soil**  
147 **microbial biomass P (MBP) in (a) limestone and (b) acidic grassland (b) exposed to ambient CO<sub>2</sub>**  
148 **(dark green circles and bars) or 600 ppm CO<sub>2</sub> (red circles and bars), supplied from April**  
149 **2018. The main panels for each grassland show the time series measurements (mean ± SE, n=**  
150 **20), with the inset bar charts giving overall means (± SE, n=5) for each nutrient treatment (0N=**  
151 **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 arrows denote start**  
152 **of CO<sub>2</sub> fumigation.**

153 There was no evidence of CO<sub>2</sub> × nutrient treatment interactions on plant productivity (Supp  
154 Fig 3; p< 0.7). Thus, the contrasting responses of the two grasslands appear to be driven by  
155 differences in microbial immobilisation even in plots receiving P additions (see below). In  
156 the acidic grassland eCO<sub>2</sub> still reduced plant productivity in plots receiving P, probably  
157 because the increase in immobilisation was greatest in this treatment; with microbial biomass  
158 P increased by a factor of 1.7 (Table 1). In this context, and in contrast to previous studies<sup>9,29</sup>,  
159 the ecosystems had undergone nutrient manipulation for > 25 years, thus the CO<sub>2</sub> effects on  
160 each grassland-nutrient combination represented responses of grasslands that have largely  
161 stabilised to their new nutrient status rather than responding to a recent step-change in  
162 nutrient loading.

163 The key to the differences in the outcome of microbial-plant competition may lie in  
164 differences in soil P chemistry between the two grasslands. In both cases, recently-fixed C  
165 that enters soil via roots and associated mycorrhizal fungi<sup>30</sup> includes enzymes and organic  
166 acids, that liberate P needed to sustain a positive plant growth response<sup>31</sup> to eCO<sub>2</sub>. However,  
167 in the limestone grassland soil, abundant calcium phosphates (Ca-P) offer potential new P  
168 inputs, the weathering of which may be enhanced by eCO<sub>2</sub> increasing organic acid exudates<sup>31</sup>  
169 and arbuscular mycorrhizal fungal (AMF) activity<sup>32</sup>. In contrast, in the acid grassland, Ca is  
170 95% less abundant, and inorganic P predominantly bound to Fe and Al<sup>10</sup>, to which plant and  
171 microbial access may be extremely limited<sup>33</sup>. Greater microbial biomass P will thus promote  
172 stronger competition for organic P, and may facilitate adsorption and occlusion of microbial  
173 biomass-derived P into the low-availability soil P pools. In this context, the limestone and  
174 acidic soils represent early and later stages of ecosystem retrogression respectively through  
175 loss of mineral P (especially Ca-P), acidification, and enrichment of highly recalcitrant Fe  
176 and Al secondary P minerals<sup>11,33</sup>. Our findings are likely to be applicable to other P-limited  
177 ecosystems at these different stages of ecosystem retrogression. Furthermore, because the

178 contrasting eCO<sub>2</sub> responses of the two grasslands are consistent across the different nutrient  
179 treatments, this suggests the mechanisms hold true irrespective of nutrient status, suggesting a  
180 very strong controlling role of soil microbes and chemistry.

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

182 Grasslands represent 20% of global terrestrial net primary productivity<sup>34</sup> and make major  
183 contributions to soil C stocks. They are also the most spatially extensive P-limited ecosystem  
184 in temperate regions<sup>7</sup>. More broadly, greater than 40% of terrestrial ecosystems globally may  
185 be P-limited, highlighting the need to understand the impact of eCO<sub>2</sub> on the productivity of  
186 these ecosystems. Overall, the quantity, pathways, function and fate of C below ground  
187 controls the future C gain in plants and soils under eCO<sub>2</sub>. We have shown that two P-limited  
188 grasslands show directly opposing above-ground biomass responses to eCO<sub>2</sub>, and that these  
189 responses may be driven by competition for the limiting P-resource between plants and soil  
190 microbes. These contrasting responses appear to be controlled by differences in soil  
191 chemistry, giving new insight into how P-limited ecosystems of contrasting stages of  
192 pedogenesis may respond to eCO<sub>2</sub>. Below-ground interactions between plants, microbes and  
193 soil strongly dictate the fate of the C and potential sequestration, therefore current climate  
194 models which do not consider plant-microbe competition for limiting resources may under- or  
195 over-estimate terrestrial C sequestration dependent on the outcome of the competition<sup>35</sup>. It is  
196 essential that plant microbial competition, and their interactions with soil chemistry, are more  
197 clearly understood to better predict how ecosystems will respond to eCO<sub>2</sub> and climate  
198 change<sup>36</sup>.

199

200

## 201 **Acknowledgements**

202

203 This work was funded by the Natural Environment Research Council, (Grant No.  
204 NE/N0100086/1 to IPH, and NE/N010132/1 to GKP) as part of the Phosphorus Limitation  
205 And ecosystem responses to Carbon dioxide Enrichment (PLACE) project. We would like to  
206 thank Natural England for access to their Wardlow SSSI, Shaun Taylor (Natural England) for  
207 help with monolith extraction and transport, and the Peak Park authority for permission to

208 establish the mini-FACE experiment within the Peak District National Park. We are grateful  
209 to Dr Gary M<sup>c</sup>Clean for work in establishing the experiment, and Cheryl Hook, Irene Johnson  
210 and Esther Paton for P analyses.

211 This preprint has not undergone peer review (when applicable) or any post-submission  
212 improvements or corrections. The Version of Record of this article is published in Nature  
213 Geoscience, and is available online at <http://dx.doi.org/10.1038/s41561-023-01225-z> and  
214 <https://rdcu.be/dgu66>.

215 For the purpose of open access, the author has applied a Creative Commons Attribution (CC  
216 BY) licence to any Author Accepted Manuscript version arising.

217

## 218 Data availability

219 All data are available via the EIDC data repository (<https://eidc.ac.uk/>).

## 220 Author contributions

221

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

227 **References**

- 228 1 Zhongming, Z., Linong, L., Wangqiang, Z. & Wei, L. AR6 Climate Change 2021: The  
 229 Physical Science Basis. (2021).
- 230 2 Reich, P. B. *et al.* Nitrogen limitation constrains sustainability of ecosystem response to CO<sub>2</sub>.  
 231 *Nature* **440**, 922-925 (2006). <https://doi.org:10.1038/nature04486>
- 232 3 Zavalloni, C. *et al.* Exposure to warming and CO<sub>2</sub> enrichment promotes greater above-ground  
 233 biomass, nitrogen, phosphorus and arbuscular mycorrhizal colonization in newly established  
 234 grasslands. *Plant and Soil* **359**, 121-136 (2012). <https://doi.org:10.1007/s11104-012-1190-y>
- 235 4 Reich, P. B. & Hobbie, S. E. Decade-long soil nitrogen constraint on the CO<sub>2</sub> fertilization of  
 236 plant biomass. *Nature Climate Change* **3**, 278-282 (2013).  
 237 <https://doi.org:10.1038/nclimate1694>
- 238 5 Zaehle, S., Jones, C. D., Houlton, B., Lamarque, J. F. & Robertson, E. Nitrogen Availability  
 239 Reduces CMIP5 Projections of Twenty-First-Century Land Carbon Uptake. *Journal of*  
 240 *Climate* **28**, 2494-2511 (2015). <https://doi.org:10.1175/jcli-d-13-00776.1>
- 241 6 Goll, D. S. *et al.* Nutrient limitation reduces land carbon uptake in simulations with a model  
 242 of combined carbon, nitrogen and phosphorus cycling. *Biogeosciences* **9**, 3547-3569 (2012).  
 243 <https://doi.org:10.5194/bg-9-3547-2012>
- 244 7 Du, E. Z. *et al.* Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature*  
 245 *Geoscience* **13**, 221-+ (2020). <https://doi.org:10.1038/s41561-019-0530-4>
- 246 8 Zhang, Q., Wang, Y. P., Matear, R. J., Pitman, A. J. & Dai, Y. J. Nitrogen and phosphorous  
 247 limitations significantly reduce future allowable CO<sub>2</sub> emissions. *Geophysical Research*  
 248 *Letters* **41**, 632-637 (2014). <https://doi.org:10.1002/2013gl058352>
- 249 9 Ellsworth, D. S. *et al.* Elevated CO<sub>2</sub> does not increase eucalypt forest productivity on a  
 250 lowphosphorus soil. *Nature Climate Change* **7**, 279-+ (2017).  
 251 <https://doi.org:10.1038/nclimate3235>
- 252 10 Horswill, P., O'Sullivan, O., Phoenix, G. K., Lee, J. A. & Leake, J. R. Base cation depletion,  
 253 eutrophication and acidification of species-rich grasslands in response to long-term simulated  
 254 nitrogen deposition. *Environmental Pollution* **155**, 336-349 (2008).  
 255 <https://doi.org:10.1016/j.envpol.2007.11.006>
- 256 11 Turner, B. L. *et al.* Soil microbial biomass and the fate of phosphorus during long-term  
 257 ecosystem development. *Plant and Soil* **367**, 225-234 (2013).  
 258 <https://doi.org:10.1007/s11104012-1493-z>
- 259 12 Vance, C. P., Uhde-Stone, C. & Allan, D. L. Phosphorus acquisition and use: critical  
 260 adaptations by plants for securing a nonrenewable resource. *New Phytologist* **157**, 423-447  
 261 (2003). <https://doi.org:10.1046/j.1469-8137.2003.00695.x>
- 262 13 Keane, J. B. *et al.* Soil C, N and P cycling enzyme responses to nutrient limitation under  
 263 elevated CO<sub>2</sub>. *Biogeochemistry* **151**, 221-235 (2020). [https://doi.org:10.1007/s10533-](https://doi.org:10.1007/s10533-02000723-1)  
 264 [02000723-1](https://doi.org:10.1007/s10533-02000723-1)
- 265 14 Terrer, C., Vicca, S., Hungate, B. A., Phillips, R. P. & Prentice, I. C. Mycorrhizal association  
 266 as a primary control of the CO<sub>2</sub> fertilization effect. *Science* **353**, 72-74 (2016).  
 267 <https://doi.org:10.1126/science.aaf4610>
- 268 15 Drake, J. E. *et al.* Short-term carbon cycling responses of a mature eucalypt woodland to  
 269 gradual stepwise enrichment of atmospheric CO<sub>2</sub> concentration. *Global Change Biology* **22**,  
 270 380-390 (2016). <https://doi.org:10.1111/gcb.13109>
- 271 16 Jiang, M. K. *et al.* The fate of carbon in a mature forest under carbon dioxide enrichment.  
 272 *Nature* **580**, 227-+ (2020). <https://doi.org:10.1038/s41586-020-2128-9>
- 273 17 Terrer, C. *et al.* Nitrogen and phosphorus constrain the CO<sub>2</sub> fertilization of global plant  
 274 biomass. *Nature Climate Change* **9**, 684-+ (2019). <https://doi.org:10.1038/s41558-019-0545-2>
- 275 18 Jiang, M. K. *et al.* Low phosphorus supply constrains plant responses to elevated CO<sub>2</sub>: A  
 276 meta-analysis. *Global Change Biology* **26**, 5856-5873 (2020).

277 <https://doi.org:10.1111/gcb.15277>

278 19 Chapin, F. S., Matson, P. A., Mooney, H. A. & Vitousek, P. M. Principles of terrestrial  
279 ecosystem ecology. (2002).

280 20 Phoenix, G. K. *et al.* Effects of enhanced nitrogen deposition and phosphorus limitation on  
281 nitrogen budgets of semi-natural grasslands. *Global Change Biology* **9**, 1309-1321 (2003).  
282 <https://doi.org:10.1046/j.1365-2486.2003.00660.x>

283 21 Chen, J. *et al.* Long-term nitrogen loading alleviates phosphorus limitation in terrestrial  
284 ecosystems. *Global Change Biology* **26**, 5077-5086 (2020). <https://doi.org:10.1111/gcb.15218>

285 22 Hebeisen, T. *et al.* Growth response of *Trifolium repens* L and *Lolium perenne* L as  
286 monocultures and bi-species mixture to free air CO<sub>2</sub> enrichment and management. *Global*  
287 *Change Biology* **3**, 149-160 (1997). <https://doi.org:10.1046/j.1365-2486.1997.00073.x>

288 23 Kammann, C., Grunhage, L., Gruters, U., Janze, S. & Jager, H. J. Response of aboveground  
289 grassland biomass and soil moisture to moderate long-term CO<sub>2</sub> enrichment. *Basic and*  
290 *Applied Ecology* **6**, 351-365 (2005). <https://doi.org:10.1016/j.baae.2005.01.011>

291 24 Jonasson, S., Michelsen, A. & Schmidt, I. K. Coupling of nutrient cycling and carbon  
292 dynamics in the Arctic, integration of soil microbial and plant processes. *Applied Soil Ecology*  
293 **11**, 135-146 (1999). [https://doi.org:10.1016/s0929-1393\(98\)00145-0](https://doi.org:10.1016/s0929-1393(98)00145-0)

294 25 Hodge, A., Robinson, D. & Fitter, A. Are microorganisms more effective than plants at  
295 competing for nitrogen? *Trends in Plant Science* **5**, 304-308 (2000).  
296 [https://doi.org:10.1016/s1360-1385\(00\)01656-3](https://doi.org:10.1016/s1360-1385(00)01656-3)

297 26 Liu, Q. Y. *et al.* Nitrogen acquisition by plants and microorganisms in a temperate grassland.  
298 *Scientific Reports* **6** (2016). <https://doi.org:10.1038/srep22642>

299 27 Bunemann, E. K. *et al.* Rapid microbial phosphorus immobilization dominates gross  
300 phosphorus fluxes in a grassland soil with low inorganic phosphorus availability. *Soil Biology*  
301 *& Biochemistry* **51**, 84-95 (2012). <https://doi.org:10.1016/j.soilbio.2012.04.012>

302 28 Dijkstra, F. A., He, M. Z., Johansen, M. P., Harrison, J. J. & Keitel, C. Plant and microbial  
303 uptake of nitrogen and phosphorus affected by drought using N-15 and P-32 tracers. *Soil*  
304 *Biology & Biochemistry* **82**, 135-142 (2015). <https://doi.org:10.1016/j.soilbio.2014.12.021>

305 29 Reich, P. B. *et al.* Do species and functional groups differ in acquisition and use of C, N and  
306 water under varying atmospheric CO<sub>2</sub> and N availability regimes? A field test with 16  
307 grassland species. *New Phytologist* **150**, 435-448 (2001).  
308 <https://doi.org:10.1046/j.14698137.2001.00114.x>

309 30 Deneff, K. *et al.* Community shifts and carbon translocation within metabolically-active  
310 rhizosphere microorganisms in grasslands under elevated CO<sub>2</sub>. *Biogeosciences* **4**, 769-779  
311 (2007). <https://doi.org:10.5194/bg-4-769-2007>

312 31 Jin, J., Tang, C. X. & Sale, P. The impact of elevated carbon dioxide on the phosphorus  
313 nutrition of plants: a review. *Annals of Botany* **116**, 987-999 (2015).  
314 <https://doi.org:10.1093/aob/mcv088>

315 32 Quirk, J., Andrews, M. Y., Leake, J. R., Banwart, S. A. & Beerling, D. J. Ectomycorrhizal  
316 fungi and past high CO<sub>2</sub> atmospheres enhance mineral weathering through increased  
317 belowground carbon-energy fluxes. *Biology Letters* **10** (2014).  
318 <https://doi.org:10.1098/rsbl.2014.0375>

319 33 Peltzer, D. A. *et al.* Understanding ecosystem retrogression. *Ecological Monographs* **80**,  
320 509-529 (2010). <https://doi.org:10.1890/09-1552.1>

321 34 Chapin, F. S., Matson, P. A. & Vitousek, P. M. in *Principles of Terrestrial Ecosystem Ecology*  
322 259-296 (Springer, 2011).

323 35 Zhu, Q., Riley, W. J. & Tang, J. Y. A new theory of plant-microbe nutrient competition  
324 resolves inconsistencies between observations and model predictions. *Ecological Applications*  
325 **27**, 875-886 (2017). <https://doi.org:10.1002/eap.1490>

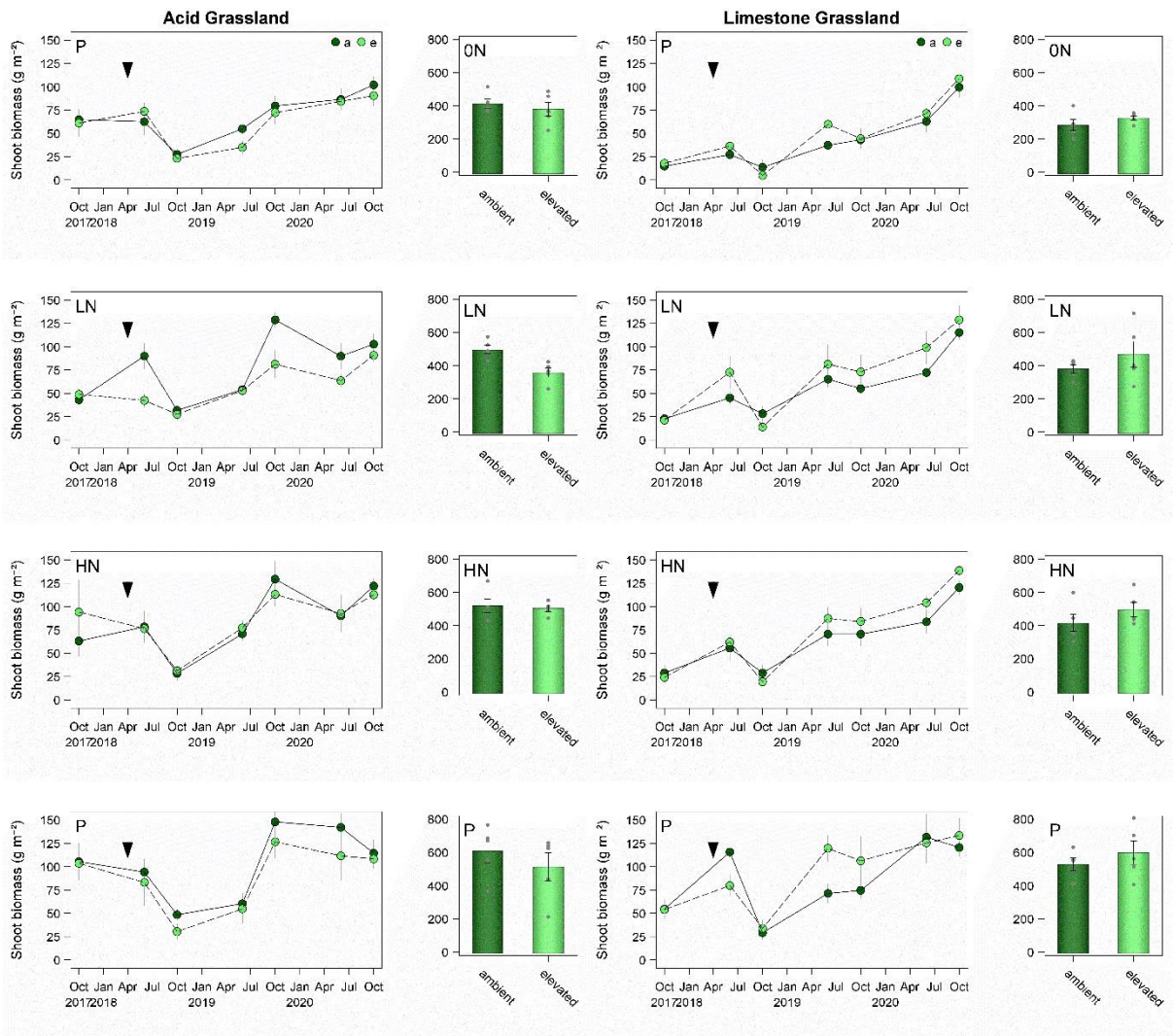
326 36 Sulman, B. N., Phillips, R. P., Oishi, A. C., Shevliakova, E. & Pacala, S. W. Microbe-driven  
327 turnover offsets mineral-mediated storage of soil carbon under elevated CO<sub>2</sub>. *Nature Climate*  
328 *Change* **4**, 1099-1102 (2014). <https://doi.org:10.1038/nclimate2436>

329 37 Miglietta, F. *et al.* Spatial and temporal performance of the MiniFACE (Free Air CO<sub>2</sub>  
330 Enrichment) system on bog ecosystems in northern and central Europe. *Environmental*  
331 *Monitoring and Assessment* **66**, 107-127 (2001). <https://doi.org/10.1023/a:1026495830251> 38  
332 Grimshaw, H. M. in *Chemical analysis in environmental research* (ed A. P. Rowland) 9295 (Abbots  
333 Ripton, 1987).



327 39 Leake, J. R. *The causes and effects of soil acidification by Calluna vulgaris L (Hull) with*  
328 *special reference to the role of mycorrhizas*, (1988).  
329 40 Murphy, J. & Riley, J. P. A modified single solution method for the determination of 330  
phosphate in natural waters. *Analytica chimica acta*. **27**, 31-36 (1962).  
331 [https://doi.org:10.1016/S0003-2670\(00\)88444-5](https://doi.org:10.1016/S0003-2670(00)88444-5)  
332 info:doi/10.1016/S0003-2670(00)88444-5  
333 41 Taylor, C. R. *A tale of two grasslands: The interactions of nutrient limitation,*  
*nitrogen 334 deposition and elevated CO2 on the biogeochemical cycling and*  
*biodiversity of two 335 contrasting UK grasslands*, (2021).  
336 42 Vance, E. D., Brookes, P. C. & Jenkinson, D. S. AN EXTRACTION METHOD FOR 337  
MEASURING SOIL MICROBIAL BIOMASS-C. *Soil Biol Biochem* **19**, 703-707 (1987).  
338 [https://doi.org:10.1016/0038-0717\(87\)90052-6](https://doi.org:10.1016/0038-0717(87)90052-6)  
339 43 Brookes, P. C., Powlson, D. S. & Jenkinson, D. S. MEASUREMENT OF MICROBIAL  
340 BIOMASS PHOSPHORUS IN SOIL. *Soil Biol Biochem* **14**, 319-329 (1982).  
341 [https://doi.org:10.1016/0038-0717\(82\)90001-3](https://doi.org:10.1016/0038-0717(82)90001-3)  
342  
343

343 **Supplementary Information**



344

345 **Supplementary Fig. 3** Contrasting aboveground productivity responses to ambient CO<sub>2</sub> (a- dark  
 346 green bars and filled circles) or elevated CO<sub>2</sub> at 600 ppm (e-light green bars and filled circles), in  
 347 acidic, (left hand column) and limestone grasslands, (right hand column). Data show mean (±  
 348 SE, n= 5) in time series and cumulative productivity (vertical bars, mean ± SE, n= 5) over the  
 349 study period. Vertical arrows denote the start of CO<sub>2</sub> fumigation.

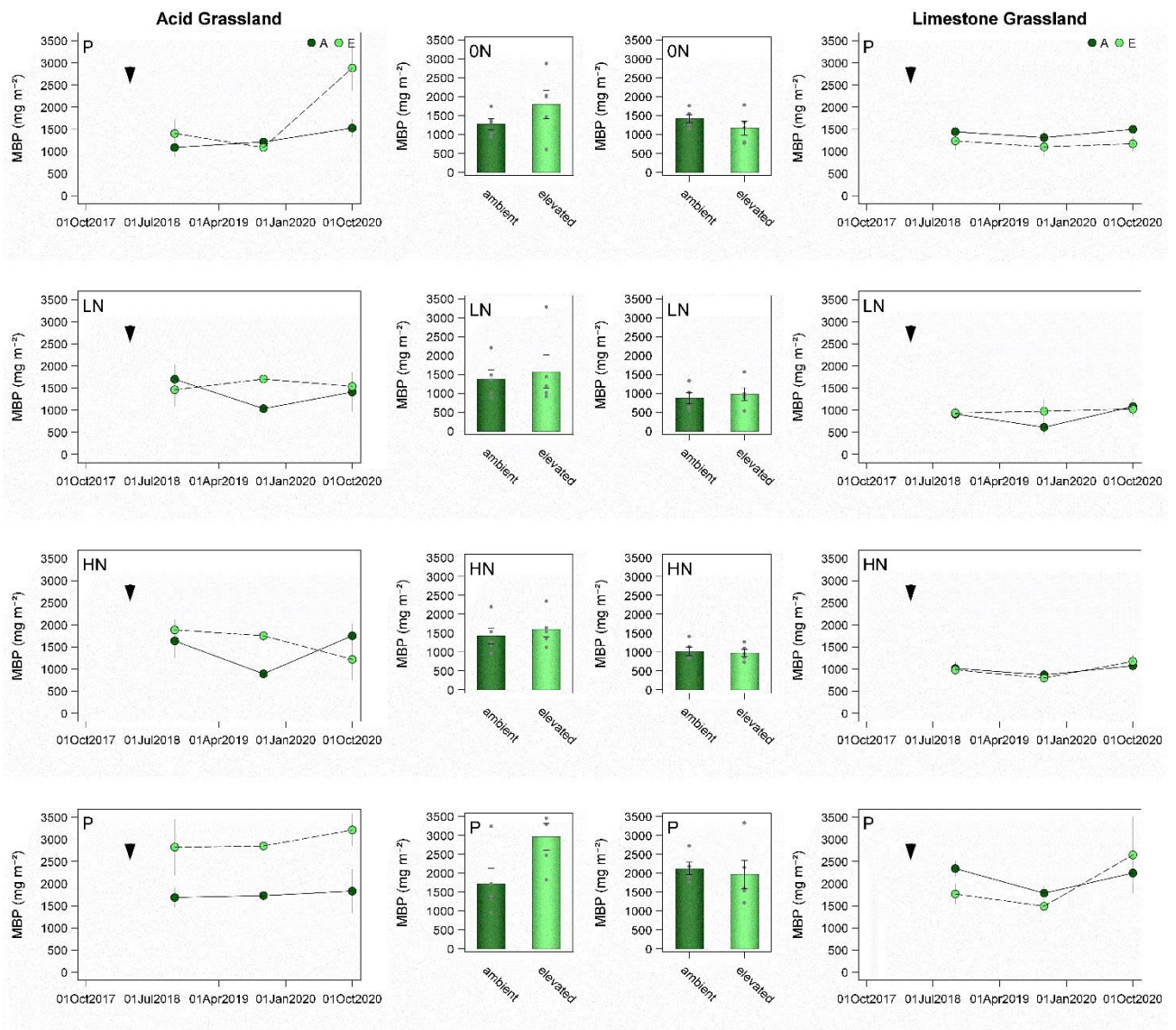
350

351

352

353

354



355

356 **Supplementary Fig. 4. Soil microbial biomass P (MBP) responses to ambient CO<sub>2</sub> (a- dark green**  
 357 **bars and filled circles) or elevated CO<sub>2</sub> at 600 ppm (e-light green bars and filled circles), in**  
 358 **acidic, (left hand column) and limestone grasslands, (right hand column). Data show means (±**  
 359 **SE) in time series (n= 5) and cumulative productivity (n= 5) over the study period. Vertical**  
 360 **arrows denote start of CO<sub>2</sub> fumigation.**

361

## 362 **Methods**

### 363 *Nutrient manipulation*

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

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

375 The monoliths were taken during February and March 2017, being excavated to the bedrock  
376 (~10 cm) in the limestone grassland and to below the main rooting depth in the acidic  
377 grassland (~20 cm). They were transported in polypropylene boxes to the Bradfield  
378 Environment Laboratory research station, also in the Peak District National Park. Since roots  
379 readily reach the underlying limestone in shallow rendzina soils, a base of limestone  
380 chippings, sourced from a quarry on the same limestone as Wardlow (Bee Low limestone,  
381 Dove Hole Quarry, Derbyshire UK), was applied to the bottom of the limestone mesocosms.

382 At the Bradfield research station, the mesocosms were set flush with the ground surface in  
383 holes dug out of the soil to ensure they were thermally buffered, and the surface of the turfs  
384 matched the surrounding vegetation. The mesocosm sides were solid so there was no direct  
385 contact with the surrounding soils, and the base freely drained through holes covered with a  
386 mesh voile (to stop particulate loss and root outgrowth, or ingrowth of roots from surrounding  
387 plants). The Bradfield research station (ca. 390 m asl) is less than 20 km from Wardlow (ca.  
388 350 m asl) and has similar climate.

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

390 Mesocosms were assigned to form groups of 8, comprising one of each of the four nutrient  
391 treatments from both grasslands, and these were placed within five miniFACE or five control  
392 rings of 1.6 m diameter that were supported ca. 20 cm above the ground surface. The mesocosms  
393 in control rings experienced ambient CO<sub>2</sub> concentrations, whilst the miniFACE system<sup>37</sup> Each  
394 ring consisted of PVC tubes with laser drilled micro-holes. The processors in the FACE ring  
395 control units received CO<sub>2</sub> information from sensors (GTM222, Vaisala, Finland) installed in the  
396 centre of each ring. Microprocessors, linked to automated pressure regulators, controlled the  
397 pressure inside the releasing pipes and therefore delivery of CO<sub>2</sub> to the rings. Fumigation started

398 in 2018, with fossil fuel-derived CO<sub>2</sub> supplied by BOC Cryospeed (BOC Limited, Manchester,  
399 UK). During the hours of daylight, CO<sub>2</sub> enrichment was set to a target of 600 ppm, which  
400 continued from the beginning of April until the end of October for three year (2018-2020). The  
401 FACE system achieved a mean of 598 ± 0.07 ppm across all elevated plots over the experiment,  
402 with the 5% and 95% quantiles being 555 and 643 ppm respectively.

#### 403 *Biomass harvesting*

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

#### 409 *Soil collection*

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

#### 416 *Determination of soil and plant biomass P content*

417 Plant biomass underwent hydrogen peroxide acid digest modified from Grimshaw (1987)<sup>38</sup> and  
418 Leake (1988)<sup>39</sup>. Dried plant material (20– 50 mg) was added to a glass digest tube and 1 cm<sup>3</sup> of  
419 100% H<sub>2</sub>SO<sub>4</sub> was added. The tubes were gently shaken and left overnight in a fume cupboard  
420 to ‘pre-digest’. Custom-made glass ‘cold fingers’ were used to cover the tubes, allowing acid  
421 condensate to safely reflux with the reagents. Two digest blanks per 30 samples were prepared  
422 for colorimetry, which consisted of 1 cm<sup>3</sup> H<sub>2</sub>SO<sub>4</sub> which underwent the same subsequent  
423 procedure as the plant sample digests. Samples were heated in a heating block (Grant heat  
424 block, BT5D model) to 350 °C. Before reaching the target temperature (and at approximately  
425 250 °C) samples were briefly removed from the block and swirled to mix the digestate. The  
426 tubes were allowed to reach 350 °C and remained at temperature for 15 minutes. Tubes were  
427 removed from the blocks, swirled again and allowed to partially cool.

428 Samples were clarified by addition of H<sub>2</sub>O<sub>2</sub> while solutions were still hot, following a delay  
429 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> was  
430 added to each tube and boiled off in the heating block until most visible evaporation had  
431 ceased. Samples were cooled again and another 200 µl of hydrogen peroxide was added and  
432 boiled off. This latter addition was repeated once more to ensure all samples were clear and  
433 no more plant sample remained adhered to the tubes. The digest solutions were diluted to 10  
434 ml by adding 9 ml of ultra-high purity (UHP) water. Total P in the digest solutions was then  
435 determined using an adapted version of the molybdate blue reaction<sup>40,41</sup>.

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

437 Soil microbial P (MBP) was determined using the chloroform-fumigation method of Vance et  
438 al (1987)<sup>42</sup>. Gravimetric water content of soil was determined from oven drying a 4 g fresh  
439 weight subsample for 48 hours at 105°C. Two further 4 g fresh weight aliquots were weighed  
440 into acid washed (HCl) 50 cm<sup>3</sup> beakers: one for fumigation and one for non-fumigation. One  
441 aliquot was placed in a vacuum desiccator with a beaker containing boiling chips and 20 ml  
442 chloroform (CHCl<sub>3</sub>). The desiccator was evacuated until chloroform boiled three times, venting  
443 between the first two times and left in darkness for 24 hours. After incubation, CHCl<sub>3</sub> was  
444 removed by drawing a vacuum and venting 5 times.

445 Both fumigated and unfumigated soils were extracted in 50 ml of 0.5 M sodium bicarbonate  
446 (NaHCO<sub>3</sub>; pH 8.5). Extracts were shaken for 1 hour on a rotary shaker and filtered through  
447 pre-leached Whatman 44 filter paper. Extracts were stored at -18 °C until quantification of P  
448 was determined using Inductively Coupled Plasma - Optical Emission Spectrometry (ICPOES;  
449 Thermo Fisher iCAP PRO). Soil MBP was calculated by subtracting the concentration of P in  
450 the non-fumigated aliquot from the fumigated aliquot and dividing by an adjustment factor of  
451 0.4 after Brookes et al. (1982)<sup>43</sup>.

#### 452 *Statistical analyses*

453 Linear mixed effects models were used to test for fixed effects of eCO<sub>2</sub>, nutrient treatment, and  
454 grassland, with block as a random factor, on aboveground biomass, cumulative

457 aboveground biomass, aboveground biomass P, soil microbial P and AGB:MBP ratio. A log  
458 transformation was performed on AGBP:MBP before analysis. Differences between groups  
459 were assessed using least square means. All figures and analyses were performed  
using SAS 460 9.4 (SAS Institute, Cary, NC, USA).