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1   **Title:** Grassland responses to elevated CO_2 determined by microbial competition for phosphorus.

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Abstract

Rising atmospheric CO_2 has stimulated plant productivity, with terrestrial ecosystems currently absorbing nearly one-third of anthropogenic CO_2 emissions. Increases in photosynthesis can subsequently lead to increased carbon (C) storage in plants and soil. However, there is growing evidence that nitrogen (N) availability constrains elevated CO_2 (eCO_2) responses, yet we know much less about the role of phosphorus (P) limitation on productivity under eCO_2. This is important because P-limited ecosystems are globally widespread, and the biogeochemical cycles of N and P differ fundamentally. We conducted a free-air-CO_2-enrichment (FACE) experiment for three years on two contrasting P-limited grasslands in the Peak District National Park of northern England under long-term nutrient manipulation. Here we show that competition between plants and microbes for P can determine plant productivity responses to eCO_2. In a limestone grassland, aboveground productivity increased (16%) and microbial biomass P remained unchanged, whereas in an acidic grassland, aboveground productivity and P uptake declined (11% and 20%, respectively), but P immobilisation into microbial biomass increased (36%). Our results demonstrate that strong competition with microbes can cause plant P uptake to decline under eCO_2, with implications for the future productivity of P-limited ecosystems in response to climate change.

Main Text

The ability of terrestrial ecosystems to sequester more C as CO_2 levels rise represents potentially the single most important biogeochemical feedback limiting climate change[1]. This ability can be constrained substantially by nutrient availability[1-4], with Earth system models indicating that nitrogen (N) limitation reduces this capacity for greater C sequestration by as much as 58%[5]. However, between a third and a half of terrestrial ecosystems are limited by P, not N[6,7]. Despite the spatial extent of P limitation, we know very little about how P limitation affects ecosystem responses to eCO$_2$, thus significantly limiting our ability to predict future rates of C uptake by the terrestrial biosphere[8,9].

Crucially, understanding of eCO$_2$ responses in N-limited ecosystems does not transfer to P-limited ecosystems because the mechanisms differ so greatly. While C can be utilised by soil microbes to fix atmospheric N, there is no biological equivalent that may increase ecosystem P stock, which is reliant principally on weathering of mineral P. Thus, total ecosystem P stocks will only increase if weathering of P-containing minerals is enhanced. Where the primary minerals have already been weathered, and secondary mineral P is insoluble, competition between plants and microbes for P may control productivity responses. Similarly, in acidic soils inorganic P is likely bound to abundant iron (Fe) and aluminium (Al)[10], to which plant and microbe access may be extremely limited and thus competition for organic P is more intense. Furthermore, unlike the N cycle, soil microbial biomass (MBP) is typically a larger store of P than above ground plant biomass (AGBP), especially during ecosystem retrogression[11], where ecosystems become increasingly P-limited during soil development as they age. Thus, changes in competition between plants and microbes could potentially control the response of P limited ecosystems to eCO$_2$, but this has not been investigated to date.

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

3

62 P sources[12]. Therefore, the expected increase in C entering ecosystems via photosynthesis under

63 eCO_2 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_2[13], and the

66 consequences for competition between plants and microbes is unclear[14].

67 Despite growing recognition of the global importance of P limitation, few studies have quantified

68 changes in P cycling under eCO_2. In an Australian Eucalypt forest[15] of proven P-limitation, net

69 primary productivity did not increase in response to eCO_2, with forest maturity or P-limitation

70 proposed as potential explanations[16,17]. Meta-analysis of eCO_2 productivity responses indicated that

71 P availability is likely an important constraint[18], but without direct experimental evidence from

72 proven P-limited ecosystems, major uncertainty surrounds eCO_2 responses of P-limited ecosystems.

73 This holds especially true for grasslands, which represent 20% of terrestrial net primary

74 productivity[19] and are the most spatially extensive P-limited ecosystem in temperate regions[7].

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_2 (ca. 410 ppm) or eCO_2 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[20]: 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[10]. 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^{-1}

85 y^{-1} (P)), and two levels of N addition (35 kg N ha^{-1} y^{-1} (LN) and 140 kg N ha^{-1} y^{-1} (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[27], these are now also alleviating P-

4

88   limitation, potentially by more weathering of Ca-P in the limestone soil, and possible stimulation of

89   organic P mineralisation by microbial phosphatases[13,21]. CO_2 fumigation began in 2018 with the

90   FACE system supplying eCO_2 during the hours of daylight from April until November in each year

91   for three years (2018-2020).

Aboveground biomass responses to eCO_2

93   There were contrasting responses to eCO_2 of aboveground primary productivity between the two

94   ecosystems (grassland x CO_2 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_2, ($p = 0.03$; 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; $F_{1,}$

98    $_{449} = 14.67$ $p < 0.0001$, and Extended Data Fig. 1). The increased productivity in the limestone

99   grassland is broadly in line with previously reported eCO_2 responses[22,23], 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_2[17]. The mechanisms for driving these previously

103   reported reductions are not understood, but our outdoor study of grasslands supplied with eCO_2 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.

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

113   shoot productivity, confirming this as the most limiting nutrient in these ecosystems. While N-

114   loading may exacerbate P-demand, the increase in productivity arising from the N treatments is in

115   line with current understanding that N addition can alleviate P-limitation in the long-term, by

116   enhancing weathering of Ca-P, and mineralisation of organic P by microbial phosphatases[13,21].

117   Reflecting this, across all the nutrient treatments, positive relationships were observed between

118   available P and above ground biomass production in both grasslands (Fig. 2).

119   *Competition for P drives contrasting productivity responses*

120   Three growing seasons of eCO_2, caused no change in the limestone grassland MBP ($p > 0.5$),

121   contrasting with the 36% increase in MBP in the acidic grassland across all nutrient treatments ($p <$

122   0.03, Extended Data Fig. 2). These responses were reflected in strong eCO_2 effects on the ratios of

123   above ground plant biomass P (AGBP) to MBP in the two grasslands ($F_{1,185} = 6.78$, $p = 0.01$). Whilst

124   eCO_2 tended to increase AGBP:MBP in the limestone grassland this was not significant, in the

125   acidic grassland it highly significantly decreased it ($p < 0.003$). These contrasts gave a significant

126   interaction between CO_2 treatment and grassland type ($F_{1, 185} = 8.94$, $p = 0.004$, Fig. 3). The

127   AGBP:MBP responses to eCO_2 were apparent from the end of the first year of enrichment and

128   persisted throughout the experiment (Fig. 3).

129   Increases in microbial P pools causing immobilisation, can reduce plant productivity[24]. The decline

130   in the AGBP:MBP under eCO_2 in the acidic grassland implies that competition between soil

131   microbes and plants for P intensified, increased plant growth limitation by P, and reduced shoot

132   productivity. It has long been thought that, in the short term at least, microbes are the better

133   competitors[25] for limiting nutrients, and microbial N uptake can be an order of magnitude greater

134   than by plants in grasslands[26]. None-the-less, while competition for P can be intense[27], it is less

135   well-understood[28] and our work suggests an important role for this in mediating eCO_2 productivity

136   responses. The contrasting, lack of decline in AGBP:MBP under eCO_2 in the limestone grassland

137   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_2 in this grassland (see NPP and AGBP,

139   Table 1).

140   There was no evidence of $CO_2 \times$ 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_2 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[9,29],

146   the ecosystems had undergone nutrient manipulation for > 25 years, thus the CO_2 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[10], 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_2 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_2 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[30] includes enzymes and organic

162   acids, that liberate P needed to sustain a positive plant growth response[31] to eCO_2. 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[32]. This may in turn promote greater competition

167 between plants and microbes for P[33]. 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$_2$ increasing organic acid

170 exudates[31] and arbuscular mycorrhizal fungal (AMF) activity[34]. In contrast, in the acid

171 grassland, Ca is 95% less abundant, and inorganic P predominantly bound to Fe and Al[10], to

172 which plant and microbial access may be extremely limited[35]. 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$_2$. 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[11,35]. 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$_2$ 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$_2$*

188 Grasslands represent 20% of global terrestrial net primary productivity[36] and make major

189 contributions to soil C stocks. They are also the most spatially extensive P-limited

190   ecosystem in temperate regions[7]. More broadly, greater than 40% of terrestrial ecosystems

191   globally may be P-limited, highlighting the need to understand the impact of eCO_2 on the

192   productivity of these ecosystems. Overall, the quantity, pathways, function and fate of C

193   below ground controls the future C gain in plants and soils under eCO_2. We have shown that

194   two P-limited grasslands show directly opposing above-ground biomass responses to eCO_2,

195   and that these responses may be driven by competition for the limiting P-resource between

196   plants and soil microbes. These contrasting responses appear to be controlled by differences

197   in soil chemistry, giving new insight into how P-limited ecosystems of contrasting stages of

198   pedogenesis may respond to eCO_2. Below-ground interactions between plants, microbes and

199   soil strongly dictate the fate of the C and potential sequestration, therefore current climate

200   models which do not consider plant-microbe competition for limiting resources may under-

201   or over-estimate terrestrial C sequestration dependent on the outcome of the competition[37].

202   Multiple studies of long-term nutrient and CO_2 manipulations have been invaluable for our

203   understanding of N-limited productivity responses to eCO_2[38], and we hope that more studies

204   will now do the same regarding P limitation. It is essential that plant microbial competition,

205   and their interactions with soil chemistry, are more clearly understood to better predict how

206   ecosystems will respond to eCO_2 and climate change[39].

222   **Author Contributions Statement**

223

224   IPH, GKP, JBK and JRL designed the eCO_2 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

232

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_2 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^{-2} y^{-1}, N m^{-2} y^{-1}, HN= 14 g N m^{-2} y^{-1}, P= 3.5 g P m^{-2} y^{-1})**

Grassland	Nutrient	CO$_2$	NPP (g m^{-2}) 2018	2019	2020	Mean	AGB P (mg m^{-2}) 2018	2019	2020	Mean	MBP (mg m^{-2}) 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)
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)	435.19 (85.24)	485.16 (152.21)	610.47 (79.42)	510.27 (105.62)
		e	96.98 (12.64)	107.56 (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)
	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)

240

12

241

(a) Limestone

y= 236.45x + 64.12   r²= 0.34

(b) Acid

y= 188.10x + 14.52   r²= 0.86

AGB (g m⁻²)

Bicarbonate extractable total P (g m⁻²)
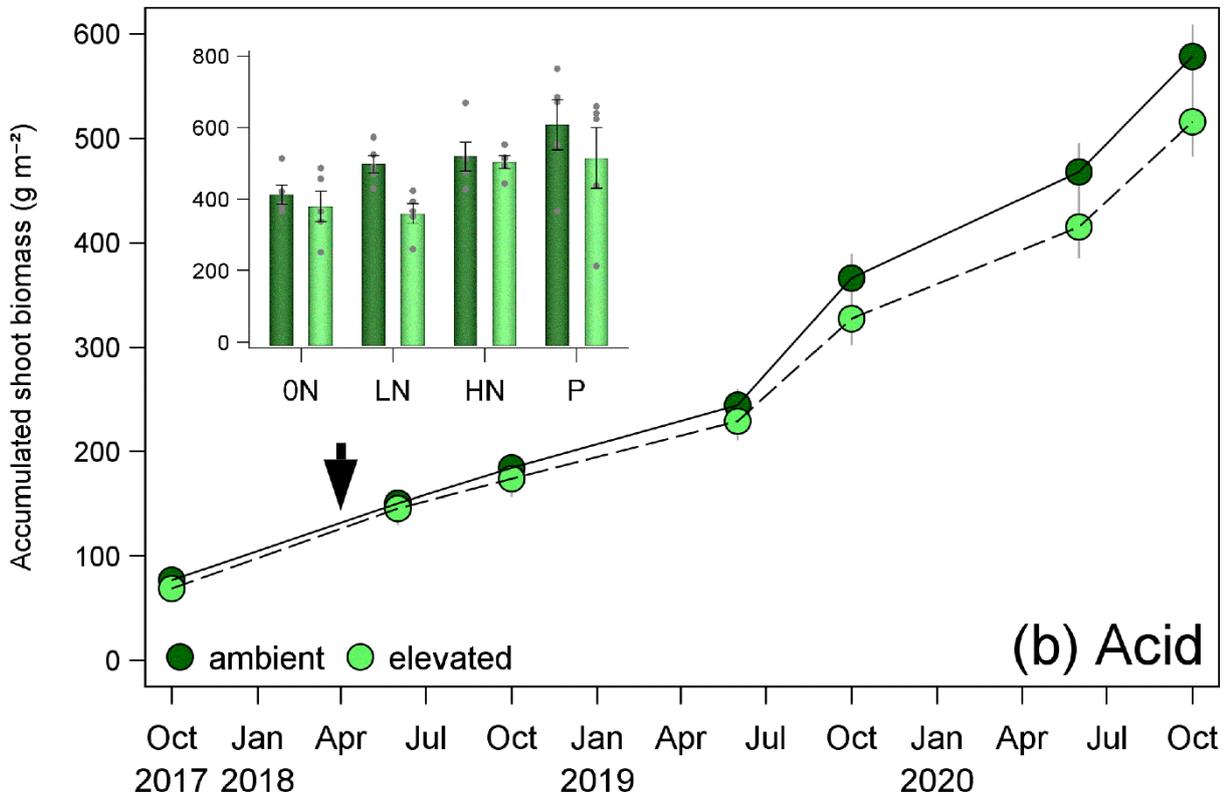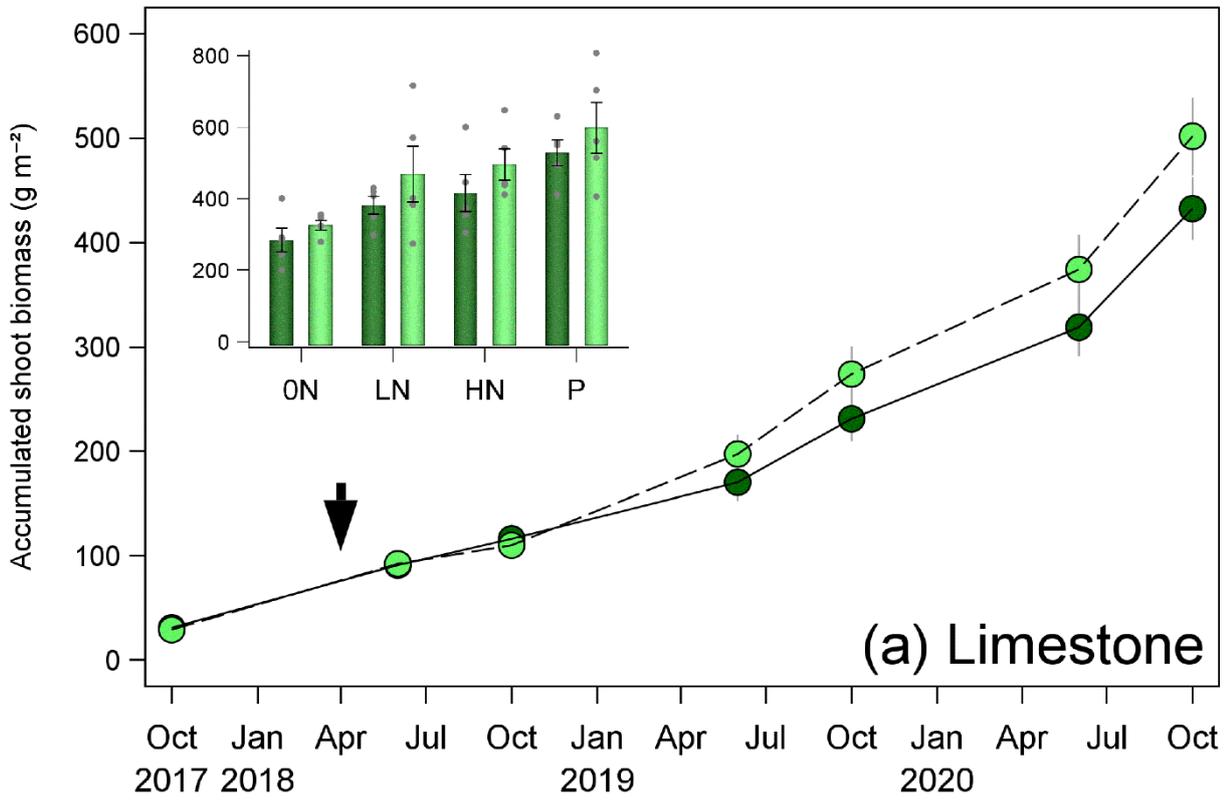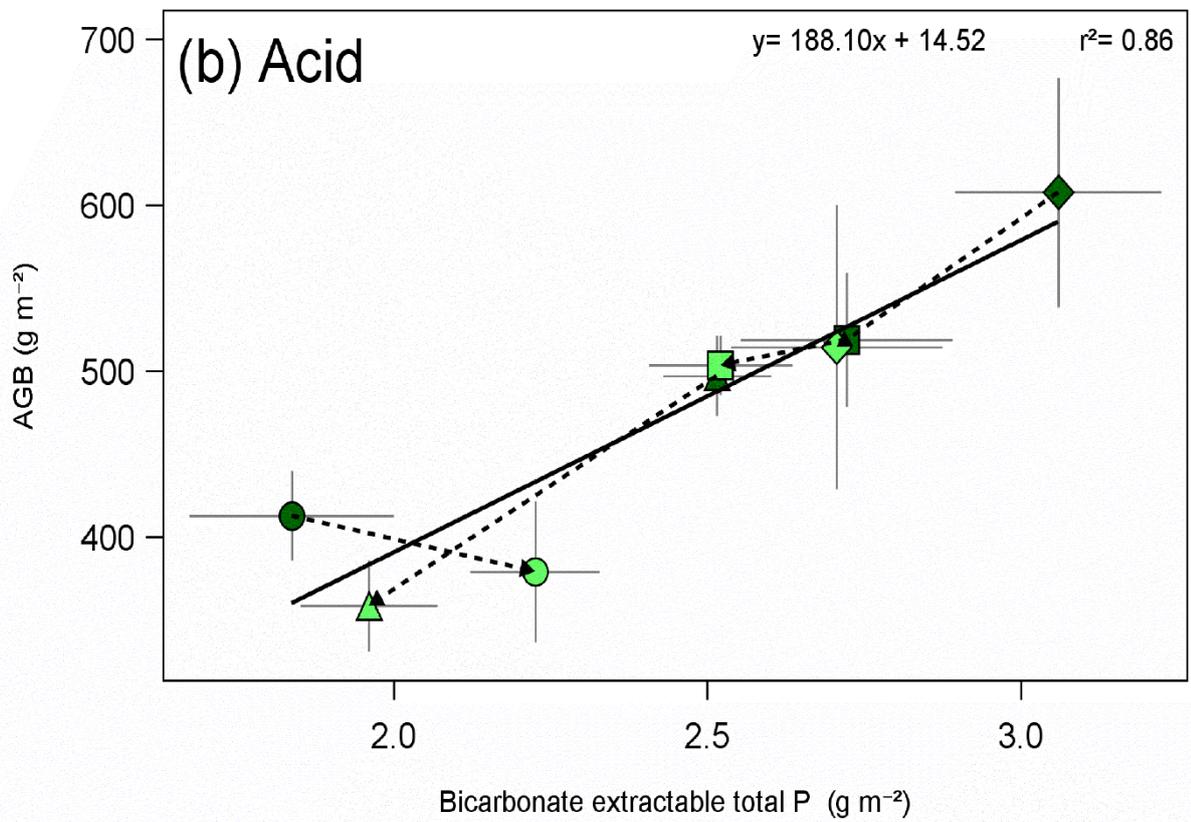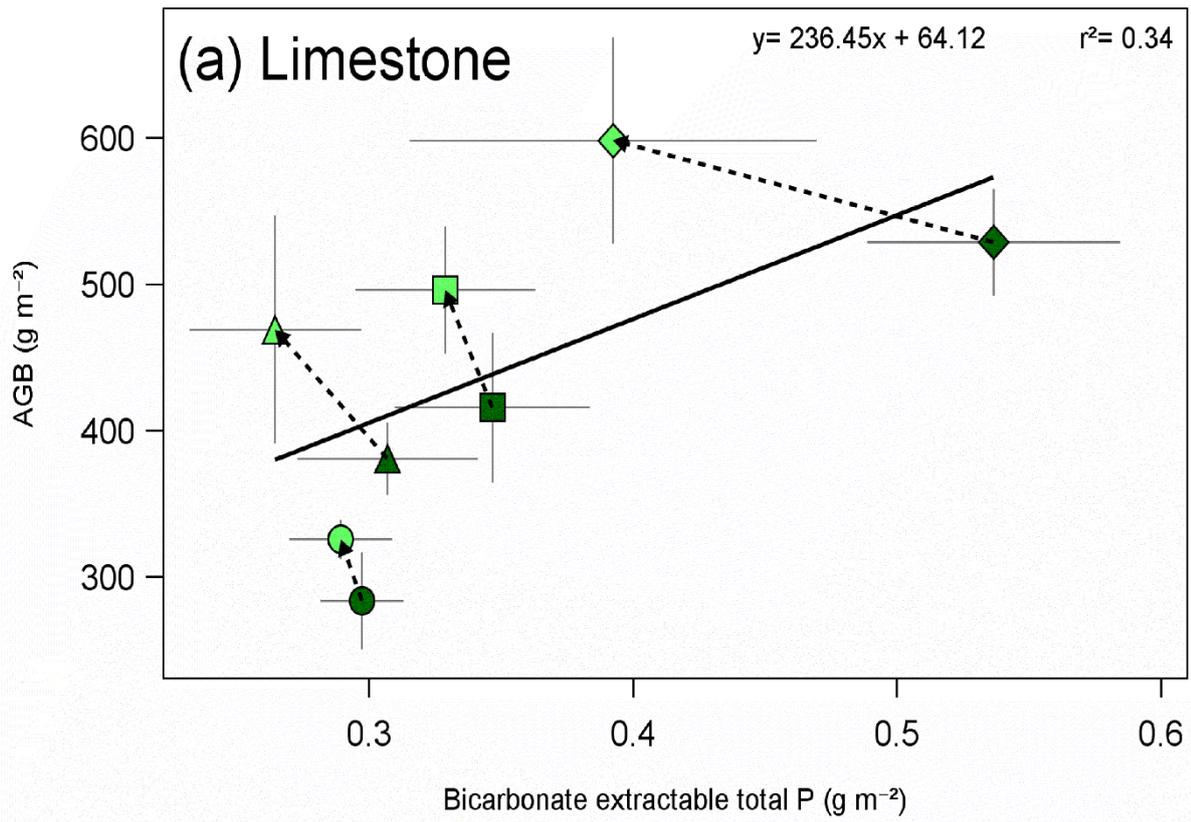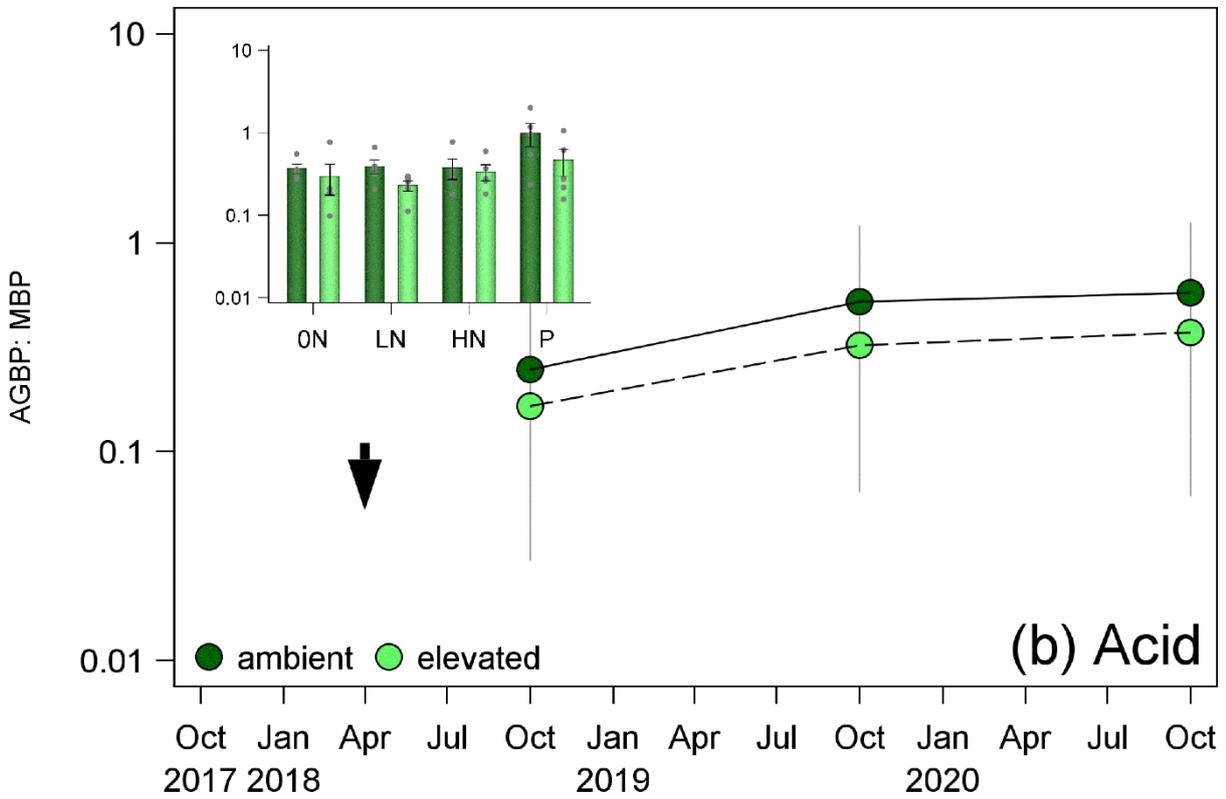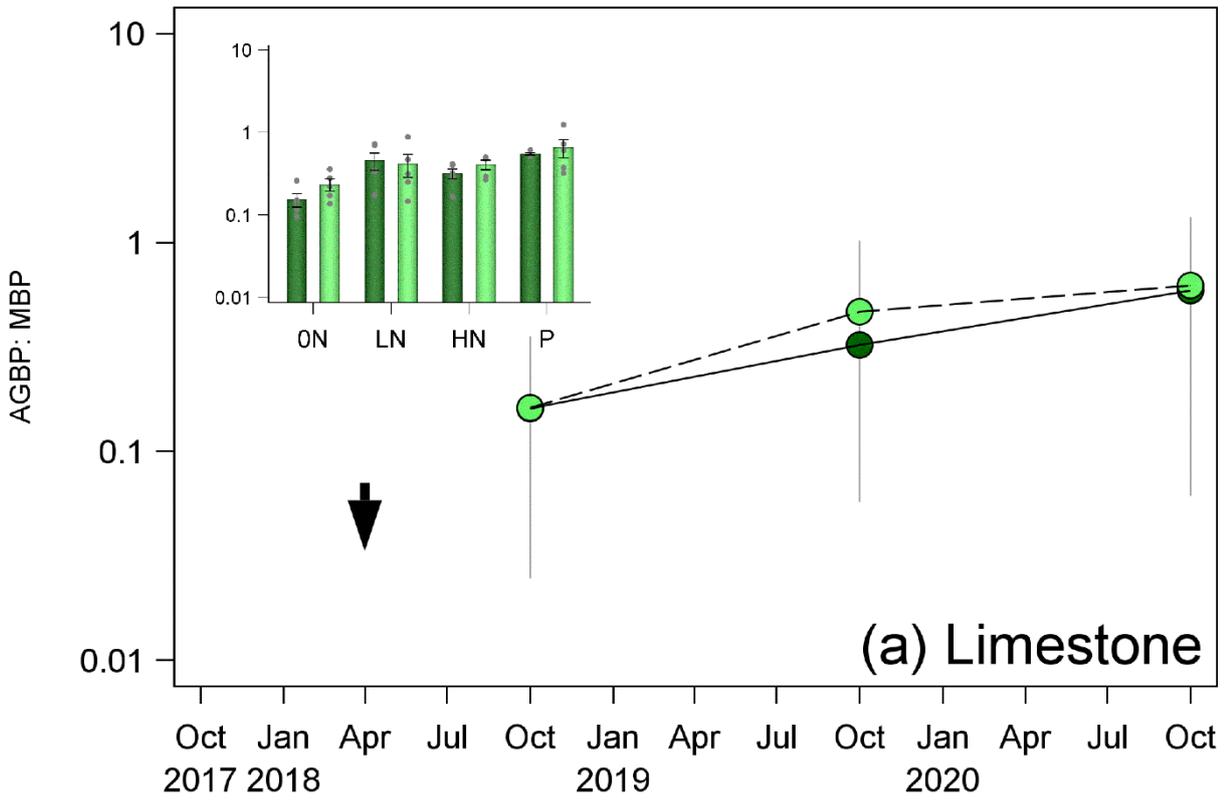
245   **Figure Legends**

246   **Fig. 1 Contrasting aboveground shoot productivity responses to CO_2. Limestone (a) and**

247   **acidic (b) grasslands exposed to ambient (dark green circles and bars) or 600 ppm CO_2 (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 ± 1 SE, n= 20). Inset bar charts show the final accumulated shoot biomass (mean ± 1**

251   **SE, n=5) in relation to the four multi-decadal nutrient treatments (0N= control, LN= 3.5 g N**

252   **m^{-2} y^{-1}, HN= 14 g N m^{-2} y^{-1}, P= 3.5 g P m^{-2} y^{-1}).**

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_2 (dark green**

257   **symbols) or eCO2 (600 ppm, light green symbols) under four nutrient manipulations**

258   **(0N= control (circles), LN= 3.5 g N m-2 y-1 (triangles), HN= 14 g N m-2 y-1 (squares),**

259   **P= 3.5 g P m-2 y-1 (diamonds)). Dashed arrows show the directional change in soil P**

260   **availability and aboveground productivity under eCO_2 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_2 combination (n= 5 ± 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_2 (light green circles and bars),**

269   **from April 2018. The main panels show time series measurements (mean ± 1 SE, n=**

270   **20), inset bar charts giving overall means (± 1 SE, n=5) for each nutrient treatment**

271 **(0N= control, LN= 3.5 g N m^{-2} y^{-1}, HN= 14 g N m^{-2} y^{-1}, P= 3.5 g P m^{-2} y^{-1}). Vertical**

272 **arrows denote start of CO$_2$ fumigation.**

273

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274 **References**

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Methods

Nutrient manipulation

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

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

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

At the Bradfield research station, the mesocosms were set flush with the ground surface in holes dug out of the soil to ensure they were thermally buffered, and the surface of the turfs 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_2 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_2 concentrations, whilst the miniFACE

447   system[40] Each ring consisted of PVC tubes with laser drilled micro-holes. The processors in

448   the FACE ring control units received CO_2 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_2 to

451   the rings. Fumigation started in 2018, with fossil fuel-derived CO_2 supplied by BOC

452   Cryospeed (BOC Limited, Manchester, UK). During the hours of daylight, CO_2 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 ± 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).

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)[41]

472 and Leake (1988)[42]. Dried plant material (20– 50 mg) was added to a glass digest tube and 1

473 cm^3 of 100% H_2SO_4 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^3 H_2SO_4 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_2O_2 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_2O_2

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[43,44].

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)[45]. 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^3 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 ($CHCl_3$). 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, $CHCl_3$ 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 ($NaHCO_3$; 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_2, 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

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