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1 **Drought-induced decline of productivity in the dominant grassland species**

2 ***Lolium perenne* L. depends on soil type and prevailing climatic conditions**

3

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23 **ABSTRACT**

24 Severe constraints on grasslands productivity, ecosystem functions, goods and services are
25 expected to result from projected warming and drought scenarios under climate change.
26 Negative effects on vegetation can be mediated via soil fertility and water holding capacity,
27 though specific mechanisms are fairly complex to generalise. In field drought experiments, it
28 can be difficult to disentangle a drought effect *per se* from potential confounding effects
29 related to vegetation or soil type, both varying along with climate. Furthermore, there is the
30 need to distinguish the long-term responses of vegetation and soil to gradual climate shift
31 from responses to extreme and stochastic climatic events. Here we address these limitations
32 by means of a factorial experiment using a single dominant grassland species (the perennial
33 ryegrass *Lolium perenne* L.) grown as a phytometer on two soils types with contrasted
34 physicochemical characteristics, placed at two elevation sites along a climatic gradient, and
35 exposed to early or late-season drought during the plant growing season.

36 Warmer site conditions and reduced precipitation along the elevational gradient
37 affected biogeochemistry and plant productivity more than the drought treatments alone,
38 despite the similar magnitude in volumetric soil moisture reduction. Soil type, as defined here
39 by its organic matter content (SOM), modulated the drought response in relation to local site
40 climatic conditions and, through changes in microbial biomass and activity, determined the
41 seasonal above and belowground productivity of *L. perenne*. More specifically, our combined
42 uni- and multivariate analyses demonstrate that microbes in a loamy soil with low SOM are
43 strongly responsive to change in climate, as indicated by a simultaneous increase in their
44 C,N,P pools at high elevation with cooler temperatures and wetter soils. Contrastingly,
45 microbes in a clay-loam soil with high SOM are mainly sensitive to temperature, as indicated
46 by a strong increase in microbial biomass under warmer temperatures at low elevation and a
47 concomitant increase in C:N, C:P and N:P ratios. High SOM promoted a better annual yield

48 of the phytometer grass under warmer climate and the effect of drought on productivity was
49 transient. In contrast, low SOM reduced cumulative yield under warmer conditions and root
50 production strongly decreased, enduring a lasting drought effect. Microbes in soils with high
51 organic matter remained more active during warmer and drier conditions, ensuring soil
52 fertility and stimulating a higher overall plant nutrient availability and productivity.

53 Our study highlights the important role of soil type for grassland responses to both
54 stochastic climatic extremes and long-term climate change. Management practices enhancing
55 SOM accumulation via organic residue incorporation seem a promising way to mitigate the
56 effects of increased temperature and drought on plants and soil microbes alike promoting
57 thereby a sustainable ecosystem functioning.

58

59 *Keywords:* climate change, rain shelter, microbial biomass, microbial activity, organic matter,
60 fertility

61 **1. Introduction**

62 Along with the pressure of climate change, grasslands are challenged to meet the
63 growing demand for ecosystem services and for providing human goods (EEA, 2012). Under
64 sufficient water and nutrient supply, grassland productivity could benefit from future climatic
65 scenarios, i.e. higher CO₂ concentrations, higher temperatures and longer vegetation periods
66 (Lüscher et al. 2005). However, sufficient water availability during the vegetation growing
67 season might be at stake under certain future climatic predictions. For central Europe, for
68 example, it is predicted that the occurrence of dry summers will increase by the year 2050
69 (IPCC 2013) and that extreme drought events could become more frequent and intense in
70 response to climate change (Ciais et al., 2005). In the western part of Switzerland, extreme
71 summer droughts are predicted to cause 70% decrease in precipitations by the end of the 21st
72 century (CH2011, 2011). Under such projected water deficits, we can then expect severe
73 constraints on grassland productivity (Smith, 2011) as well as negative effects on other
74 ecosystem functions, goods and services such as soil fertility, biodiversity and carbon (C)
75 storage (de Vries et al., 2012, Jentsch et al., 2011).

76 The response of grasslands to drought and the degree to which ecosystem services are
77 affected depend on the complex interactions among different factors involving plant
78 community diversity, soil properties, climatic conditions and land management (see for
79 example Deléglise et al. 2015, Thébault et al. 2014). Furthermore, duration, intensity and
80 timing of the drought, as well as the frequency of rainfall events are all very important factors
81 influencing the response of grassland communities to drought (Chou et al., 2008, Didiano et
82 al. 2016). For example, using a mesocosm experiment, De Boeck et al. (2011) found that
83 season of drought had a large effect on the magnitude of reduction in plant biomass and
84 growth, with early summer drought having the largest impact followed by spring and then

85 autumn droughts. This is likely because the early summer is a time of rapid plant development
86 with leaf area at its peak and high temperatures favouring transpiration.

87 Higher growing-season temperatures and more frequent occurrence of drought periods
88 have the capacity to alter C and nutrient cycling at ecosystem level, with a potential for
89 significant plant–soil feedback (Melillo et al., 2002). Higher temperatures may lead to
90 enhanced C losses from soils (Bond-Lamberty and Thomson, 2010) and faster nutrient
91 cycling (Ineson et al., 1998), yet these increases may be offset, or even reversed, by reduced
92 soil moisture (Davidson and Janssens, 2006). Drought stress and, more generally, lower soil
93 moisture can reduce plant nutrient uptake by decreasing nutrient mineralization (Fierer and
94 Schimel, 2002), nutrient diffusion and mass flow in soil (Chapin, 1991). When drought stress
95 is followed by rewetting, this often results in enhanced mineralization (Canarini and Dijkstra,
96 2015), which has been attributed to nutrient release from dead microbial biomass (Borken and
97 Matzner, 2009), or the decay of fine roots that have accumulated during the drying period
98 (Sanaullah et al. 2012). Duration and intensity of drought, as well as frequency of drought-
99 rewetting cycles will thus determine the net effect of drought stress on nutrient supply and
100 soil organic C storage (He and Dijkstra, 2014). Therefore, while the ecophysiological
101 response of plants to drought stress is rapid, soil fertility might respond differently and in a
102 more complex way because of potential buffering capacities (Fridley et al., 2011).

103 Elevation gradients within a confined geographical region can be conceived as a
104 natural climatic experiment where ecosystem processes can be primarily attributed to
105 variation in temperature and precipitation. Soil monolith transplantation from high to low
106 elevations has been shown to effectively simulate climate change (see for example Link et al.,
107 2003; Mills et al., 2014), including a combined effect of temperature and precipitation.
108 Alternatively, rain shelter experiments in grasslands have been increasingly used to single out
109 drought effects. For example, Gilgen and Buchmann (2009) applied rain shelters to three

110 grasslands at different elevations in Switzerland showing that there is not a general drought
111 response, due to the different grassland types and associated plant functional types, local
112 climate conditions and soil types. Such “space-for-time” substitutions make it difficult to
113 disentangle the drought effect *per se* from potential confounding effects related to vegetation
114 or soil type, which also vary along with climate. Another difficulty is disentangling long-term
115 climate shifts (interpreted from elevation gradient analysis) from short-term climatic extremes
116 (interpreted from drought treatments).

117 In an attempt to control for these confounding effects, we performed a pot experiment
118 using two types of soils with different soil organic matter content, i.e. a loamy low-organic
119 and a clay-loam high-organic soil, which were placed at two sites with different climatic
120 conditions, and were further subjected to the effects of early and late-season droughts during
121 the plant growing season. Perennial ryegrass (*Lolium perenne* L.) was grown in the pots as a
122 phytometer since it is one of the most important forage grasses in Europe, rendering high
123 yields and nutritional value. Moreover, because it has no distinct tolerance to drought, this
124 forage species is likely to be particularly affected by climate change (Westermeier et al.,
125 2016). With respect to plant productivity, we hypothesized that: (i) late-season drought has a
126 stronger negative impact than early season drought, but that local climatic conditions interact
127 with the timing in determining the direction and magnitude of the drought effect; (ii) long-
128 term warmer and drier conditions at low elevation have a stronger effect on plant growth than
129 short-term droughts, due to a combined reduction in assimilation capacity and in soil
130 microbial activity and bioavailability of nutrients. Furthermore, with respect to soil properties,
131 we hypothesized that (iii) drought effects on plant productivity are tempered in organic matter
132 rich soils due to a higher ability to retain soil moisture, and (iv) higher soil organic matter
133 content warrants positive effects of warmer climate in increasing microbial C, N and P pools,
134 rendering these soils less prone to drought stress.

135

136 2. Materials and methods

137

138 2.1. Experimental design

139 The pot experiment took place within an existing rainout shelter experiment, which was
140 installed in two permanent grasslands (sites) of Swiss Jura mountains in 2015-2016. One site
141 was located in Chésereux (N 46°24', E 6°10') at 540 m a.s.l., representing conditions of low
142 elevation (thereafter named “low site”), whereas the second site was located in St-George (N
143 46°30', E 6°15') at 945 m a.s.l representing conditions of high elevation (“high site”). Mean
144 annual temperatures for the low and high sites were 10.4 and 7.6 °C, respectively, and mean
145 annual precipitation were 1050 and 1290 mm, respectively. In this region, grasslands are
146 highly productive (9–12 Mg DM ha⁻¹ yr⁻¹) and are regularly grazed by cattle or mowed for
147 haymaking. The dominant plant species are the same in both sites: *Lolium perenne* L.,
148 *Trifolium repens* L., *Poa trivialis* L., *Poa pratensis* L., *Dactylis glomerata* L., *Phleum*
149 *pratense* L. and *Taraxacum officinale* (L.) Weber ex F.H. Wigg.

150 Briefly, the rainout shelter experiment which was used for the pot experiment consisted in a
151 complete randomized block design with five replicates (rain shelters of 12.0 x 6.4 m) set up in
152 both sites on a grassland surface of approximatively 1 ha. Drought was simulated in plots of
153 4.5 m² (0.9 m x 5 m) within the rain shelters which included also the control plots. The
154 periods of water stress were based on the seasonal dynamic of the growth (Figure 1), either
155 during the peak of growing season (treatment of early-season drought) or after the peak of
156 growing season (treatment of late-season drought) for a period of 8 weeks. Based on the last
157 30-year precipitation average at each site, during a period of two months (see Table 1) control
158 plots received 100% of precipitation whereas drought plots received 30% of precipitation

159 simulating the periods of water stress (i.e. early-season and late-season drought). Before and
160 after the period of water stress, drought plots received similar precipitation to the control plots
161 (C). Watering was done twice a week by means of a flow controller and a custom-made
162 watering stick, which was moved along the plots, thus mimicking a regular rain. The climatic
163 conditions measured by a meteorological station installed at each site during the three periods
164 of 8 weeks are given in Table 1. Mean vapor pressure deficit (VPD) was calculated for these
165 periods as well. VPD is a measure of the drying power of air, therefore it directly influences
166 the plant hydraulic capacity and, in turn, may impact rates of growth and overall plant health.
167 VPD is calculated based on air relative humidity and air temperature as the difference
168 between saturation and actual vapor pressure of air for a given temperature. A VPD threshold
169 of 1.5 kPa was chosen to represent the approximate value above which stomata close under
170 extremely dry air for most plant species (Sangines et al., 2018). This experimental design
171 would allow disentangling long-term climate shift (site effect), from short time climate
172 extreme (drought treatment effect).

173 The use of pots allowed to integrate in the experimental design the effect of soil type
174 in 2016. Soils have been collected in close proximity to each shelter and selected so as to be
175 representative of two contrasted features (e.g. fertility, SOM) of the same dominant soil at
176 these altitudes, which is a cambisol (IUSS Working Group WRB, 2006). Soils had similar
177 grassland species. At both site we took the top soil (0-30 cm). The low site had a low-organic
178 matter content (thereafter named “loamy low-organic” or ”LOM”), and the high site had a
179 higher organic matter content (thereafter named “clay-loam high-organic” or “HOM”).
180 Besides the difference in organic matter content, these two soils differ also in their particle
181 size fractions (determined on air-dried soils by sieving and the hydrometer method after
182 removal of organic fraction with 30% H₂O₂ – Guy and Bauder, 1986), in their fertility and
183 pH, being the HOM soil characterized by a finer texture and higher values of inorganic N,

184 available P and pH as compared to the LOM soil (Table 2 and Supplementary table S1).
185 Available water for plants in the soil (%) is comparable between both soils and it is calculated
186 as difference in water content at pF=4.2 and pF=2.5 measured by means of a pressure plate
187 (N=10). Water content ranged between 34% and 33% at pF=2.5 (field capacity), and between
188 18% and 19% at pF=4.2 (permanent wilting point) for LOM and HOM soil, respectively.

189 In January 2016, both soils were collected up to a depth of 30 cm, larger stones and
190 coarse organic residues removed, then used to fill pots of 9.3 l volume (diameter 20 cm,
191 height 24.3 cm), which were stored under a roof at ambient winter temperature. In parallel, in
192 a glasshouse under optimal growth conditions, shallow (3 cm) turfs of same diameter as the
193 pots were prepared using each of the soils and sown with the phytometer *Lolium perenne* L.
194 (variety Salamandra 4n, Schweizer and Steffen, Switzerland) at a density of 0.7 g of seeds per
195 pot. Following germination, the *Lolium* turfs were placed on top of pots, fertilised with a
196 solution of N 27.5% (as ammonium nitrate), corresponding to 30 kg N ha⁻¹, and kept cool
197 prior to their transfer to the field. This initial fertilization simulates a field common practice at
198 the time of sowing (or after plant emergence). Another reason to apply this fertilizer was to
199 get rapidly a dense plant cover and to standardize the initial plant available nutrient content in
200 the two soils so as to avoid confounding effects at the start of the experiment. Altogether, the
201 experimental design consisted in two sites (low vs high site), three drought treatments (plots:
202 control, early-season and late-season droughts), two soil types (pots: loamy low-organic-LOM
203 vs clay-loam high organic-HOM) and five replicates (rain shelters). In total there were 60
204 pots.

205 By end of March, once the morning frost events ceased at each site, the pots were
206 transferred to the rain shelters. The timing of the entire procedure was calculated so as to have
207 a delay of about 2-3 weeks between the placement in the low and high sites, and to match the
208 vegetation seasonality at these two sites (Figure 1). In each recipient plot, two pits were

209 prepared and randomly received a pot with each soil type. Pots received the same watering
210 regime as the entire plot.

211

212 *Table 1 Climate characteristics at the low and high elevation sites during the different periods of the*
 213 *experiment in 2016 and average 30-year rainfall, measured by a meteorological station installed at*
 214 *each site or nearby.*

Site	Periods (8 weeks)	Average rainfall (1981-2010) (mm)	Mean air temperature (°C)	Mean air humidity (%)	Mean vapor pressure deficit (VPD) (kPa)	Number of hours with mean VPD > 1.5 kPa
Low site 540 m a.s.l	Early-season drought	143	10.5	66.3	0.48	28
	Late-season drought	154	18.0	69.4	0.78	215
	End of growing season	144	20.0	63.2	0.99	315
High site 945 m a.s.l	Early-season drought	234	9.9	80.0	0.27	3
	Late-season drought	213	16.7	72.1	0.59	48
	End of growing season	235	15.6	72.9	0.57	68

215 *Precipitation applies for the conditions outside the rain shelters.*

216

217

218

219 *Table 2 Main physico-chemical characteristics of the soil (0-30 cm) used for the experiment.*

Soil type	Total C (%)	Total N (%)	pH _{H2O}	Clay (%)	Silt (%)	Sand (%)	Inorganic N mg kg ⁻¹	Available P mg kg ⁻¹	Available water (%)
Loamy low-organic (LOM)	2.25	0.24	5.8	20	41	39	16	9	15.9
Clay-loam high-organic (HOM)	5.45	0.48	7.5	36	42	22	27	51	14.2

220

221

222

223 2.2. Analytical methods

224 2.2.1 Aboveground and belowground plant biomass

225 The standing crop of *Lolium perenne* was cut in all pots with scissors at time T1 (i.e.,
226 at the end of the early-season drought), T2 (i.e., at the end of the late-season drought) and T3
227 (i.e., at the end of the growing season period) (see Figure 1). Plant material was dried at 65 °C
228 to constant weight. At final harvest (time T3 – see Figure 1), pots were extracted from the
229 field plots and the surface (0-12 cm) and deep (12-22 cm) soil layers were separated. Soil
230 samples were gently sieved (2 mm mesh) in the field to separate roots, which were washed in
231 the laboratory before being dried at 65 °C and weighed. Soil was stored in a cooled box for
232 temporary storage at 4 °C until further analyses, which started the day after the sampling.

233

234 2.2.2. Soil respiration (R_s), soil temperature and moisture

235 All measurements were done four times, at the beginning of the drought treatment,
236 when vegetation started to grow (T0), and immediately after the harvest of plant biomass, i.e.,
237 at the end of the early-season (T1) and late-season (T2) drought periods, and at the end of the
238 growing season (T3) (see Figure 1). On the day of measurements, all pots were shaded
239 beforehand so as to avoid increase of soil temperature during the measurement session, which
240 took place early in the morning after sunrise (between 8 am and 11 am). A customized funnel
241 lid was tightly put on the pot and the measurement chamber was fixed on top of this lid. We
242 used an infrared gas analyzer (LiCor Li-8100) connected to a 10-cm survey chamber (LiCor
243 8100-102). Each measurement lasted 3 minutes, with a dead band of 30 seconds. Respiration
244 fluxes were calculated from the increase in CO₂ concentration over time using an exponential
245 regression and correcting for atmospheric pressure and temperature, as well as for the total

246 volume under the chamber in each pot and the surface of the pot. Soil moisture, here
247 corresponding to the water volumetric content (FieldScout TDR 300) and temperature were
248 recorded in each pot, down to 12 cm, immediately after the measurement.

249

250 2.2.3. *Soil properties*

251 Soil samples were analysed at the final harvest (T3 – see Figure 1) for the top soil
252 layer (0-12 cm) corresponding to the main rooting zone. The water content was determined
253 gravimetrically by drying soil subsamples at 105 °C to a constant weight. Total carbon (C)
254 and nitrogen (N) content in the bulk soil were analysed after ball milling. Soil C and N were
255 determined under high-temperature oxidation using an elemental analyser (CE Instruments
256 model NA2500 Nitrogen Carbon Analyser) and expressed as percent of dry soil weight. For
257 the determination of microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), pairs of c. 5 g of
258 fresh soil were weighed for each replicate and one sample from each pair was immediately
259 extracted in 25 ml solution of 0.5 M K₂SO₄, whereas the other sample was put in a vacuum
260 desiccator and subjected to chloroform vapour. After one day of fumigation, the fumigated
261 soil sample was extracted with the same solution. Total C and N concentrations in the extracts
262 of fumigated and non-fumigated samples were analysed by a TOC/TN analyser (Shimadzu
263 TOC-V). To determine the soil available phosphorus (P) and the microbial phosphorus
264 (P_{mic}), 3 g of fumigated and non-fumigated fresh soil was extracted with 40 ml of 0.5 M
265 NaHCO₃ (Olsen method). Inorganic phosphorus content of the extracts was analysed by
266 colorimetry using a spectrophotometer at 890 nm (Olsen et al. 1954). Microbial biomass C, N
267 and P were estimated as the differences between the amounts of C, N and P after and before
268 fumigation using an extractability factor of 0.45 for C (Vance et al., 1987), 0.54 for N
269 (Brookes et al., 1985) and 0.4 for P (Brookes et al., 1982). Microbial biomass C, N and P and
270 soil available P are expressed as mg.kg⁻¹ oven dry soil. Ammonium (N-NH₄) and nitrate (N-

271 NO₃) concentrations were determined by continuous flow analyses using an automated
272 analyser (SEAL AA3 HR Autoanalyser) after extraction of 5 g of fresh soil with 30 ml of 1 M
273 KCl and the results expressed as mg·kg⁻¹ oven dry soil.

274

275 *2.5. Statistical analysis*

276 Statistical analyses were performed for all measured variables either at the different
277 sampling times T1, T2 and T3 (volumetric soil moisture, soil temperature, above-ground
278 biomass of *L. perenne*, soil respiration) or only at the final harvest T3 (total root biomass of *L.*
279 *perenne* and root biomass at two depths, microbial biomass C, N and P, soil NH₄, NO₃,
280 extractable P and C/N, C/P and N/P ratios). Overall ANOVA tests (three-way) and tests for
281 each soil separately (two-way) are given in the figures and in Supplementary table S2. In the
282 two-way ANOVA, two factors, i.e. site (S: experimental sites at low and high elevation) and
283 watering regime (D: control, early-season and late-season drought), as well as their interaction
284 were tested with block as a random factor. In the three-way ANOVA, soil was given as
285 additional factor (G: LOM and HOM soil types), including interactions. Redundancy analysis
286 (RDA) was done on the multivariate data from the final harvest (standardized) for each soil
287 type, with drought treatment (categorical variable), soil temperature and soil moisture given
288 as explanatory variables. Canonical axes and explanatory variables were tested subsequently
289 with permutation tests. Assumptions of normality and homoscedasticity of the residuals in all
290 final models were verified visually using diagnostic plots, with log transformations being
291 applied to data when necessary. All analyses were carried out using R 3.4.2 (R development
292 core team, 2017).

293

294 **3. Results**

295 *3.1 Soil temperature and moisture and air VPD*

296 Considering the comparable range of plant water availability of the two soils and their
297 moisture content at pF 2.5 and 4.2 (Table 1), it can be concluded that changes in measured
298 soil volumetric water content (TDR) reflect well the plant available water. Over the growing
299 season, both soil types placed at the high site retained on average, significantly more soil
300 water compared to the low site (LOM soil: +9%, +39, +61, and HOM soil: +16%, +19% and
301 +81%, at T1, T2 and T3, respectively; Supplementary figure S3). Both early-season and late-
302 season droughts strongly and significantly decreased mean soil volumetric moisture at both
303 sites (LOM soil: -30% and -60%, and HOM soil: -39% and -43% at T1 and T2, respectively).
304 The overall wetter HOM than LOM soils (+ 7%) provided for their significantly ($P < 0.05$)
305 higher moisture content at T2 during the second part of the growing season and buffered
306 significantly the negative effect of the drought treatment (Soil×Drought, $P < 0.001$). With
307 respect to temperature, during the early growing season there was no temperature difference
308 between the two elevation sites for both soil types (on average 13.1°C) (Supplementary figure
309 S4). Later in the season, however, both soils became increasingly warmer ($P < 0.001$) at the
310 low site compared to the high site (for both LOM and HOM soils: +5.5 °C, +10.7 °C during
311 late-season drought (T2) and end of growing season (T3), respectively). In addition, late-
312 season drought (T2) induced slightly warmer conditions in both soil types (+ 0.9 °C; $P <$
313 0.05). HOM soils were on average slightly warmer than LOM soils (+0.2 °C; $P < 0.05$) during
314 the entire growing season.

315 The mean air temperature during the early-season drought period was +0.6 °C higher
316 at the low site (Table 1), and during the late-season drought and the following two months it
317 was +1.3 °C and +4.4 °C higher, respectively. However, the water stress for plants, as
318 quantified by the number of hours with mean VPD > 1.5 kPa, increased at the low site during
319 the late-season drought and the following two months.

320

321 3.2 Above and belowground dry matter yield

322 Yield of *Lolium perenne* was significantly different between the two soils at all
323 sampling periods (overall +43% in HOM soils, $P<0.001$), with a strong interaction with site at
324 the early-season drought (Figure 2). Yield did vary for the same soil type depending on site
325 elevation and season. For both soil types, there was more yield at the low site at the end of the
326 early-season drought period (Figure 2-T1; $P<0.001$), but the trend reversed later in the
327 subsequent harvests as the production at the high site increased (Figure 2-T3; $P<0.05$ for
328 LOM soil, $P<0.1$ for HOM soil). Drought had a weaker effect than site. Yield slightly
329 decreased in both soil types during the late-season drought (Figure 2-T2, $P<0.05$), but this
330 effect carried over until the end of the growing season only in the LOM soil at the high site
331 (Figure 2-T3, interaction, $P<0.05$). With respect to the cumulative yield from the three
332 harvests, it was different for both sites only in the HOM soil, with more yield at the low site
333 (although marginally significant, $P<0.1$) and drought effect persisted only in the LOM soil
334 ($P<0.01$). Interestingly, in the LOM soil, the early-season drought had a positive effect on
335 aboveground plant production (Figure 2-T1; $P<0.01$).

336 Root production was also different depending on soil type (Supplementary figure 5:
337 +15% total root biomass in HOM soils, $P<0.05$, with interaction with site). Total root biomass
338 in the LOM soil was significantly lower at the low site ($P<0.05$), while in the HOM soil there
339 was no difference between the two sites. Drought effect was only weakly significant, with
340 interaction ($P<0.01$) showing an increase of roots in the LOM soil in the late-season drought
341 at the high site (both at 0-12 and 12-22 cm depth – data not shown), while in the HOM soil
342 this increase occurred only in the deeper soil layer (data not shown). In both soil types, only a
343 minor fraction of total roots was found in the deeper layer (data not shown).

344

345 *3.3 Soil respiration*

346 Soil respiration was significantly different at all sampling periods depending on soil
347 type ($P < 0.001$) and interactions with site were observed (Figure 3). Soils respired more at
348 the low site at the end of the early-season (Figure 3-T1; +25%, $P < 0.05$ for HOM soil) and
349 late-season (Figure 3-T2; LOM: +101% and HOM: +57%, $P < 0.01$) drought periods, as well
350 as at the end of the growing season (Figure 3-T3; LOM and HOM: +140%, $P < 0.001$).
351 Drought treatment effect on respiration was marked in HOM soils already during the early-
352 season drought (Figure 3-T1; -26%, $P < 0.01$), but late-season drought impacted negatively the
353 soil respiration most significantly in the LOM soil (Figure 3-T2; -11% and -25% in early and
354 late season drought, respectively, $P < 0.05$). For the LOM soil a legacy effect was still visible
355 at the end of the growing season (Figure 3-T3; -16% and -6% in early and late season
356 drought, respectively, $P < 0.1$).

357

358 *3.4 Microbial biomass*

359 Soil type significantly affected microbial biomass C and N ($P < 0.05$) and there were
360 highly significant interactions with site for all microbial biomass nutrients (Figure 4). In each
361 soil type site effect was generally highly significant for microbial biomass C, N and P (Figure
362 4; $P < 0.01$ with the only exception of microbial P in HOM soil). The microbial biomass C, N
363 and P pools increased in the LOM soil when it was placed at the high site and in the HOM
364 soil when it was placed at the low site. The drought effect was observed only for microbial
365 biomass C in HOM soil (Figure 4a; $P < 0.01$). The ratios of microbial C:N, C:P and N:P
366 increased significantly in HOM soil when transplanted at the low site (Supplementary figure
367 S6; $P < 0.01$), while there were no significant changes in the LOM soil.

368

369 *3.5 Soil available ammonium, nitrate and phosphorus*

370 Soil type had an effect on soil nitrate ($P < 0.05$) and available P ($P < 0.001$), with strong
371 interaction with site (Supplementary figure S7). LOM soil type did not show any significant
372 pattern with respect to ammonium, nitrate and phosphorus. In HOM soil, nitrate showed a
373 decrease under drought ($P < 0.05$) and concentration of available P was significantly reduced
374 at low site ($P < 0.001$), while ammonium was affected neither by site nor by drought.

375

376 *3.6 Multivariate analysis*

377 RDA scatter plots with LOM and HOM soils (Figure 5) shows that soil moisture and
378 soil temperature are opposed along the first canonical axis, while drought treatment lies along
379 axis 2. The general pattern was that total above-ground biomass was correlated to soil
380 moisture, while soil respiration was correlated to temperature. Nitrate lies along axis 2 and it
381 is more related to drought treatments, likewise the ratio above-belowground biomass. Most
382 interestingly, microbial biomass N and P, as well as to some extent microbial biomass C, were
383 related to soil moisture in the LOM soil, while in the HOM soil they were related to
384 temperature. In HOM soil, microbial C:N, C:P and N:P ratios were strongly correlated to
385 temperature, but not in the LOM.

386

387 **4. Discussion**

388 *4.1 Seasonality of biomass productivity under different climatic conditions*

389 In accordance with our first hypothesis, we found that local climatic conditions
390 interact with the timing of drought occurrence to determine the direction and magnitude of

391 shifts in plant productivity, based on the different stages of plant development. The higher
392 mean air temperature at the low site during the early-season drought period favoured the
393 growth of *L. perenne*, but later in the season the water stress for plants in relation to higher
394 temperatures (e.g. VPD) increased drastically, causing a reduction in plant growth. Indeed,
395 temperature has a beneficial role on the growth of temperate forage grasses, such as the
396 perennial ryegrass *L. perenne* (Wingler and Hennessy, 2016). Nevertheless, the consequences
397 of a prolonged water stress at landscape scale, as during the 2003 summer heat wave, were
398 estimated in a 20-30% decrease of grassland production in Switzerland (Keller and Fuhrer,
399 2004). At plant species level, the most immediate effect of water stress is the limitation of
400 plant growth through diminished leaf C fixation resulting from stomatal closure to prevent
401 excessive transpiration (Signarbieux and Feller, 2012). Such growth reduction in response to
402 experimental warming has also been observed in field-grown ryegrass (Nijs et al., 1996) and
403 natural subalpine grassland communities (Gavazov et al. 2014, Mariotte et al. 2013).
404 Furthermore, as hypothesized, late-season drought had a stronger negative impact than early-
405 season drought, albeit this effect was inferior to the season-long combined temperature and
406 precipitation shift along the elevational gradient, which determined the VPD for plant growth.
407 It has been shown by Craine et al. (2012) that the inter-annual timing of climate variability is
408 a factor determining the grassland productivity since, in addition to environmental conditions,
409 developmental factors determine the growth of grasses (Hurtado-Uria et al., 2013).
410 Noteworthy, the early-season drought seems to have promoted the growth of *L. perenne* in the
411 LOM soil (Figure 2-T1), which can be explained by the positive effect of drier soils on
412 temperature conduction (Seneviratne et al. 2006) at the onset of spring growth. When
413 considering the cumulative aboveground yield of *L. perenne*, the drought had a lasting effect
414 only in the LOM soil. Conversely, in the HOM soil, *L. perenne* could retain the advantage of

415 an earlier onset of the growing season with warmer climate at the low site, due to better
416 ability of the soil to retain moisture that mitigates the negative effects of drought.

417 With respect to the below-ground biomass, late-season drought, which had a strong
418 effect on soil moisture, promoted root growth at depth, especially within the LOM soil at the
419 high elevation site. It seems that under unfavourable climatic conditions, *L. perenne* was able
420 to invest relatively more resources belowground when deeper water resources were available.
421 Such root plasticity has also been reported in multispecies communities (Hernandez and
422 Picon-Cochard, 2016), in which vertical root complementarity was beneficial for water and
423 nutrient uptake. Although we did not specifically measure fine root production, we could
424 speculate that the observed depth increase of seasonal root biomass at the high site could have
425 been related to a change in relative root size and therefore modified the potential uptake of
426 nitrogen (Clarkson et al., 1986) and thus the recovery in plant biomass production.

427

428 *4.2 Effect on soil fertility and plant growth of long-term warmer and drier conditions vs*
429 *short-term drought*

430 In our experiment, the transplantation to a warmer and drier site had a stronger effect
431 on soil fertility and plant growth as compared to the short-term drought treatments, despite a
432 similar magnitude in soil moisture reduction. The soil microbial and chemical analyses were
433 done only at the end of the growing season and therefore they do not strictly represent the
434 response at the end of either of the two drought periods. However, they have the potential to
435 highlight any drought legacy effect on microbial growth and nutrient mineralization. It seems
436 that the legacy effect was absent, except for nitrate in the HOM soil, whose content was lower
437 when soils had experienced a drought. In contrast, the warmer and drier soil conditions at the
438 low site significantly reduced phosphorus availability and increased microbial C/P and N/P

439 ratios in the HOM soil. Jupp and Newman (1987) found that the phosphorus uptake by *L.*
440 *perenne* was reduced immediately after a period of drought due to reduced diffusion rate of
441 phosphorus to the root surface in the drying soil, whereas after three weeks the recovery of
442 uptake ability did occur. Although we could not evaluate the plant uptake and possible
443 limitation in the absence of chemical analyses of plant biomass, the decrease in productivity
444 at the end of the growing season (Figure 2-T3) for the warmer and drier HOM soils at the
445 lower site seem to reflect this drop of extractable P. Indeed, in a meta-analysis, He and
446 Dijkstra (2014) pointed to the increase in soil available N and a decrease in soil available P to
447 explain the increase in plant N:P in response to drought.

448 Overall our findings suggest that long-term (growing season) climatic conditions and the
449 warming-induced drying of air and soil affect soil biological activity and nutrient
450 bioavailability more than stochastic drought events and confirms our second hypothesis.
451 Considering that soil legacy effects have been demonstrated after repeated drought stress
452 (Legay et al., 2018), it is plausible that we only detected biogeochemical changes in response
453 to the long-term climate shift along the elevation gradient.

454 *4.3 Opposed soil temperature and soil moisture limitation for different soil types*

455 Soils are diverse in mountainous regions, in particular for what concerns soil organic
456 matter stocks and physicochemical characteristics, which makes predictions for their response
457 to climate and land use change particularly difficult (Hagedorn et al., 2010). The two
458 contrasting soil types responded differently to the two climate change treatments with respect
459 to most of the measured physical, chemical and biological variables (Supplementary table
460 S2). This was particularly noticeable for above-ground biomass and root biomass and it
461 related also to the differences in fertility, in particular with respect to available N and P (Table
462 2). Microbial activity (here expressed as soil respiration and microbial biomass growth)
463 differed significantly between the two soil types in response to a warmer and drier climate,

464 likely due to inherent differences in the ability of the soil to retain moisture. Given that the
465 rate of organic matter turnover in mountain grasslands is strongly dependent upon soil
466 moisture only when temperature is not limiting microbial activity (Mills et al., 2014), it is not
467 surprising that soil respiration rates in both soil types responded positively to the warmer
468 conditions at low elevation. The soil moisture reduction induced by the drought treatment
469 affected the respiration primarily in the LOM soil, especially during the late-season drought
470 when this soil remained drier than HOM. Furthermore, in the LOM soil, the drought treatment
471 had a legacy effect on soil respiration at the end of growing period (T3), two months after the
472 late-season drought ceased, which indicates that soil microbial pool was affected beyond the
473 drought period (Legay et al., 2018). The higher ability of HOM soil to retain moisture under
474 field conditions is likely related to the combined effect of higher organic matter and clay
475 contents, which together influence the soil structure. We presume that the soil structure of the
476 HOM soil is such that it allows a better retention of precipitation water due to a lower rate of
477 evaporation under drought, which could be explained by its pore's configuration. Overall,
478 high SOM provides a buffered habitat for microbes (McGonicle and Turner, 2017; Xu et al.
479 2018), which in accordance with our third hypothesis can further mitigate drought effects on
480 plant productivity due to a higher ability of the soil to retain moisture.

481 Interestingly, no direct drought effect was observed on microbial C, N and P pools
482 (except for C in HOM soil) at final harvest, but both soil types showed a strong site elevation
483 effect, with opposite directions with respect to soil temperature and moisture. A similar
484 limitation trade-off between soil temperature and moisture has been previously demonstrated
485 for soil respiration by Mills et al. (2014) who showed that, for the same type of soil
486 transplanted along an elevation gradient, moisture was limiting at the warm and dry extreme,
487 while it was temperature at the cold and moist extreme. These contrasting patterns are best
488 illustrated in the RDAs, which showed that the LOM soil was prone to moisture limitation

489 and thus could take advantage of the increased soil moisture at the higher site for increasing
490 microbial biomass. Differently, the HOM soil, which was richer in soil organic matter and
491 could sustain higher moisture, benefited from warmer soil conditions at the lower site in
492 increasing microbial biomass. The microbial biomass C:N, C:P and N:P stoichiometry
493 changed drastically only in the HOM soil, and their increase due to warmer climate was
494 indicative of nutrient limitation for the increased microbial biomass. Warmer conditions did
495 not affect soil extractable N for plant growth and only slightly reduced P-availability in HOM,
496 which in accordance with the fourth hypothesis, indicates that higher soil organic matter
497 content warrants positive effects of warmer and drier climate on microbial activity and
498 growth, rendering these soils less prone to drought stress.

499

500 5. Conclusion

501 In conclusion, our study shows that soils with different physicochemical characteristics
502 respond differently to drought in relation to site climatic conditions and that this impacts on
503 biogeochemistry and plant productivity. Overall, soil with high organic matter content was
504 less affected by drought than soil with low organic matter content. This indicates that soil type
505 and management practices, such as SOM accumulation and organic residue incorporation
506 (e.g. solid manure and/or slurry in pastures, or compost in cropland), represent a promising
507 way to both curtail greenhouse gas emissions and mitigate the effect of increased temperature
508 and drought on plant growth.

509

510 **Authors' contribution**

511 AB conceived the ideas, designed methodology and drafted the paper, AB, TG, SP, GM, JQ
512 collected the data, AB, PM, MM, TG, CS, LB and KG contributed to the interpretation of

513 results and writing. All authors commented critically on the final version and gave final
514 approval for publication.

515

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523

524 **Appendix A. Supplementary information**

525 Supplementary information related to this article can be found at [http:.....](http://...)

526

527

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686 CAPTIONS

687 Figure 1. Scheme of the seasonal dynamic of grassland biomass production for the two
688 sites (unpublished data available from Agroscope institute). The different periods of plant
689 growth along the season (each lasting for 8 weeks) are represented below the graph (Peak of
690 growing season, After peak of growing season and End of growing season) with their
691 respective sampling times (T1, T2 and T3). The 'peak of growing season' period has been
692 centred on the peak of the vegetation growth curve and determined the beginning of the
693 experiment at each site. T0 corresponds to the sampling time for initial conditions. The
694 growth curve, periods and sampling times are drawn in black for the low site (540 m a.s.l.)
695 and grey for the high site (945 m a.s.l.). Early-season drought was applied during the peak of
696 growing season, while late-season drought was applied after the peak of growing season.

697

698 Figure 2. Mean (\pm SE) above-ground biomass (N=5) of *Lolium perenne* after the
699 early-season drought (T1), the late-season drought (T2) and at the end of the growing season

700 (T3) for the two soil types and the two sites. The cumulative above-ground biomass is given
701 at the bottom (T1+T2+T3). Both soil types were transplanted each in the two sites at low (540
702 m a.s.l) and high (945 m a.s.l) elevation. Black hatching highlights when pots were
703 experiencing drought, either early-season (Early, T1) or late-season drought (Late, T2)
704 treatment, while grey hatching highlights pots that were previously subjected to drought. No
705 hatching corresponds to either control pots (C) or to pots which did not yet undergo the
706 drought treatment (e.g. Late in T1). ANOVA tests are given for each soil with site effect (S),
707 drought effect (D) and their interaction (SxD), and the overall test is given in italics with soil
708 effect (G) and interaction with S and D. Significance levels are $P<0.001$ (***), $P<0.01$ (**),
709 $P<0.05$ (*) and $P<0.10$ (.).

710

711

712 Figure 3. Mean (\pm SE) soil respiration of the pots (N=5) after the early-season drought
713 (T1), after the late-season drought (T2) and at the end of the growing season (T3) for the two
714 soil types and the two sites. See Figure 2 for additional information.

715

716 Figure 4. Mean and SE (N=5) soil (0-12 cm) microbial C (a), N (b) and P (c) at final
717 harvest (T3) in the control pots and in the pots with early-season and late-season drought
718 treatments for the two soil types and the two sites. See Figure 2 for additional information.

719

720

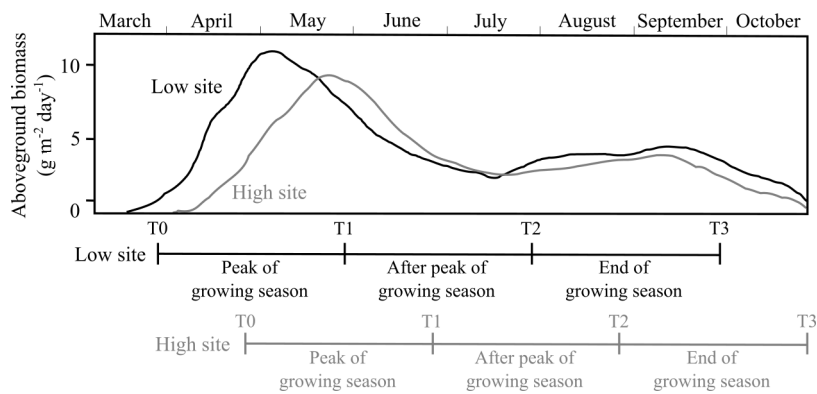
721 Figure 5. Redundancy analysis (RDA) of the soil samples (surface 0-12 cm layer of
722 the pot) for the loamy low-organic soil (graph on the left) and the clay-loam high-organic
723 (graph on the right) placed at the low (540 m a.s.l) and high (945 m a.s.l) sites. Samples are

724 labelled according to the recipient sites, CHX: Chésereux, SGT: St-George and to the
725 treatments, C: control pots, E: early-season drought treatment, L: late-season drought
726 treatment; thus, replicates have the same labels. Response variable are soil biochemical
727 properties and biomass measures. Explanatory variables are treatments (control, early-season
728 and late-season droughts), soil moisture and soil temperature. Left graph: axis 1: 21%
729 ($P<0.001$); axis 2: 7% ($P=0.10$), overall model is significant ($P<0.001$); soil temperature and
730 soil moisture are significant ($P<0.001$). Right graph: axis 1: 30% ($P<0.001$); axis 2: 4.4%
731 ($P=0.44$), overall model is significant ($P<0.001$); soil temperature and soil moisture are
732 significant ($P<0.001$).

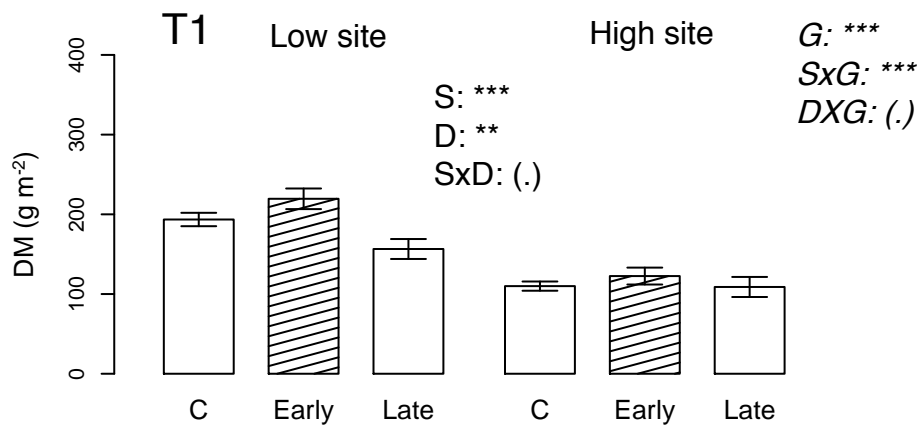
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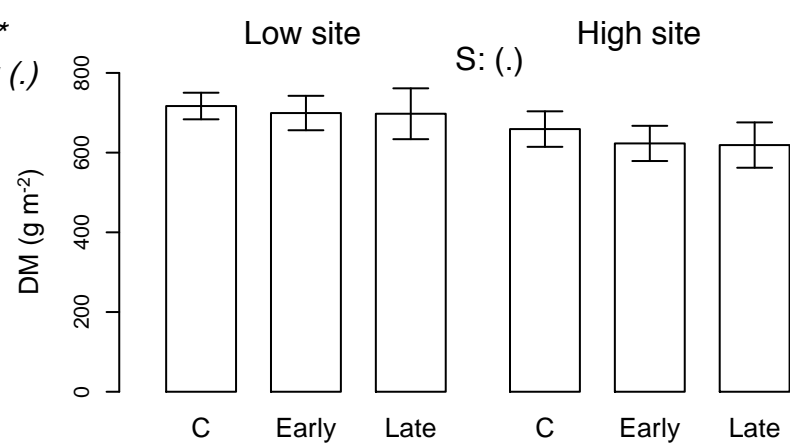
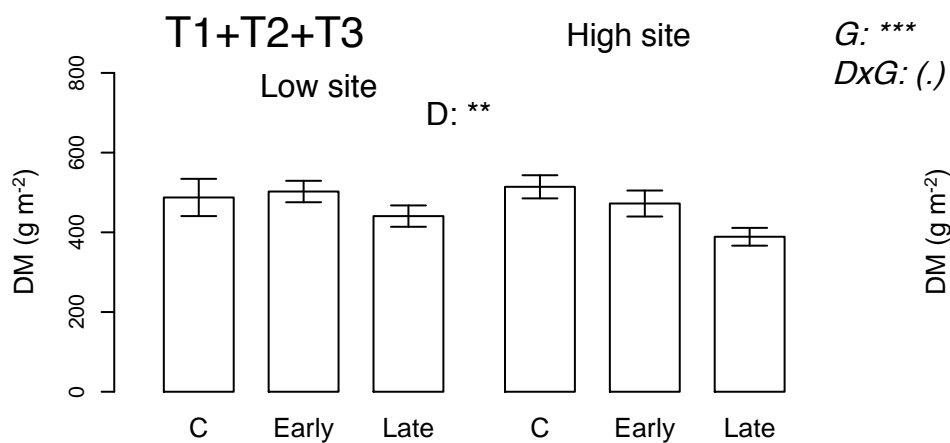
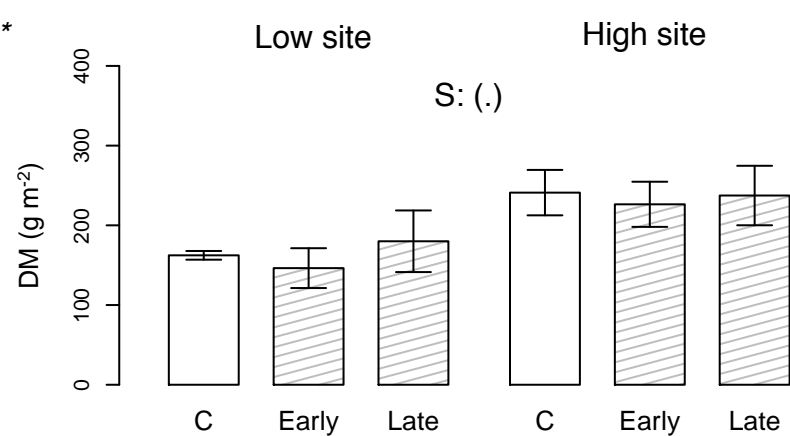
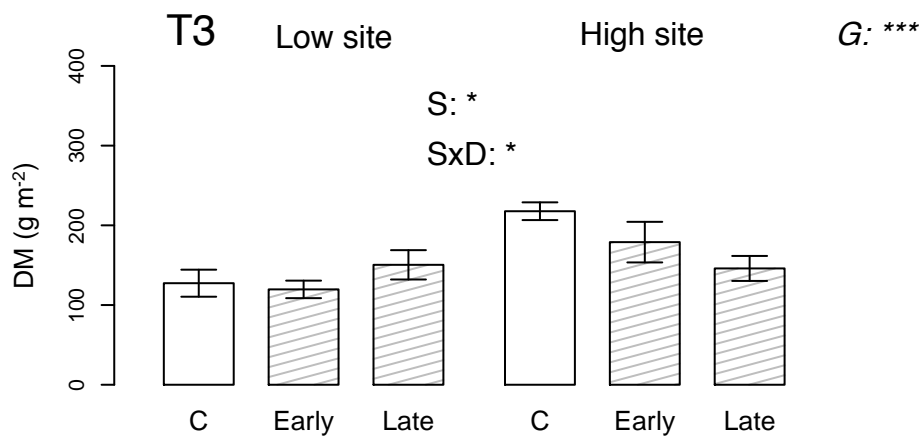
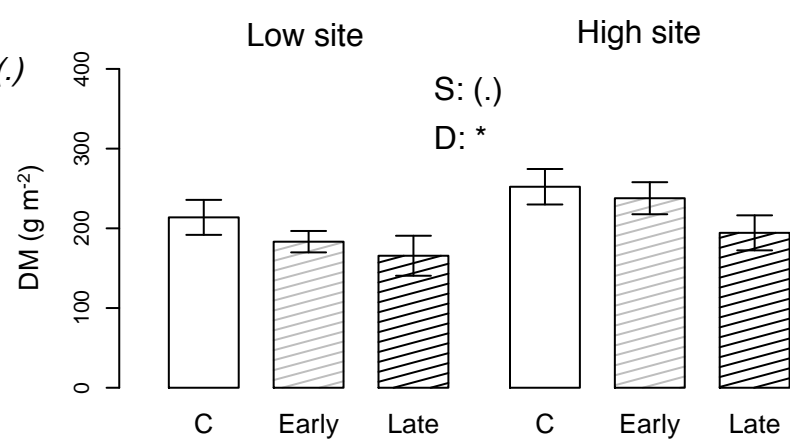
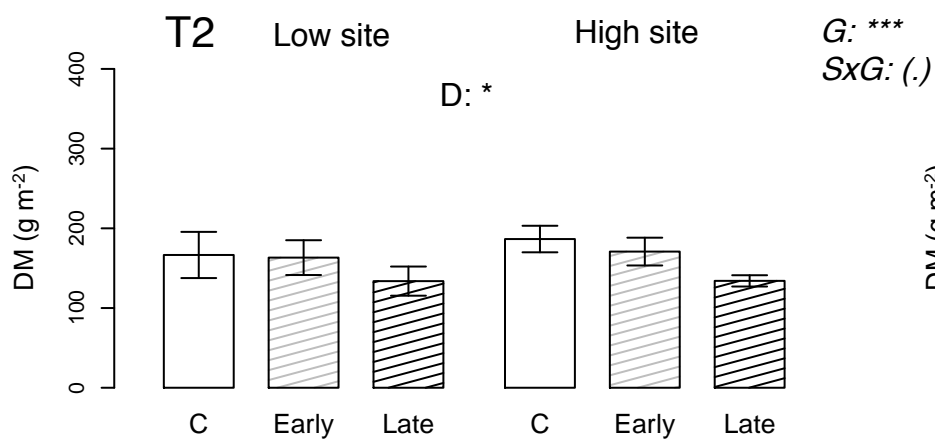
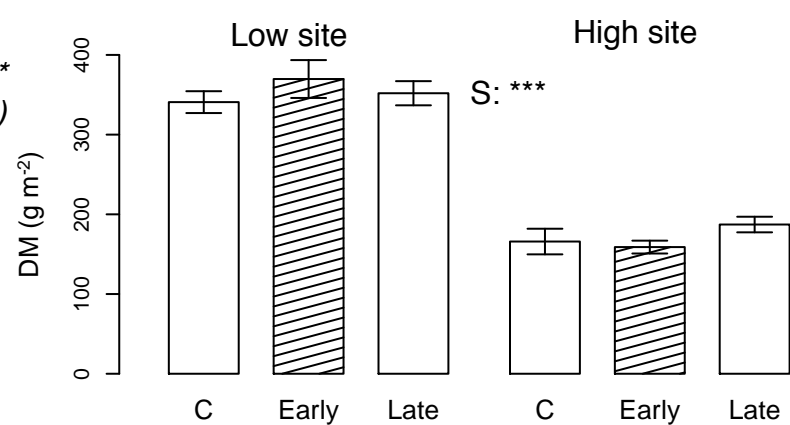
735



Loamy low-organic soil (LOM)

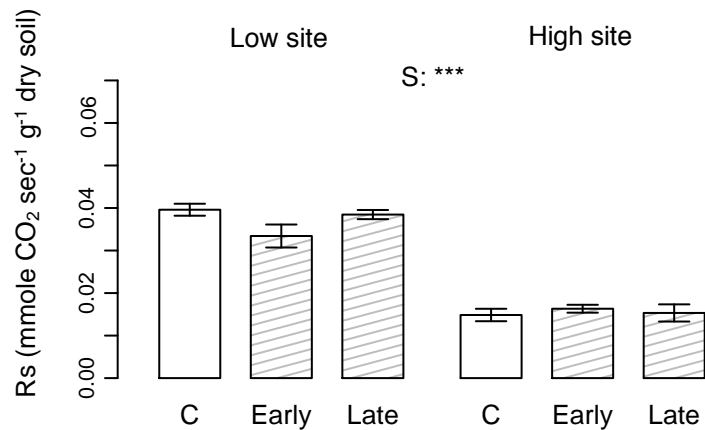
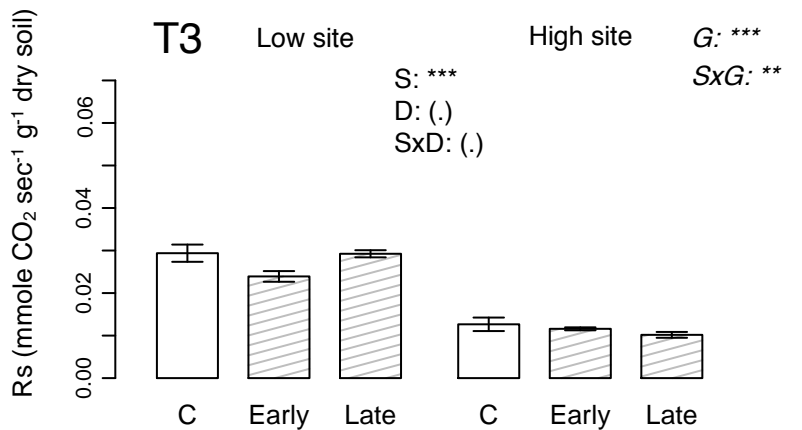
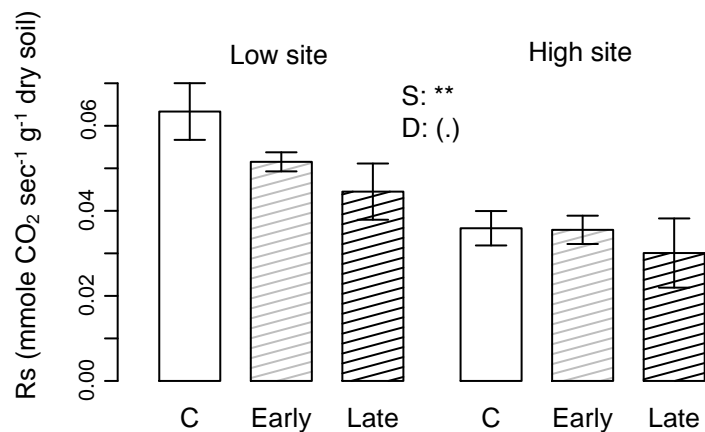
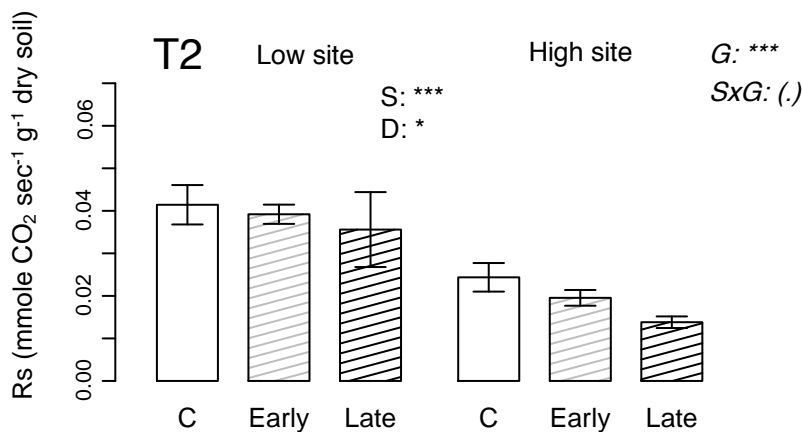
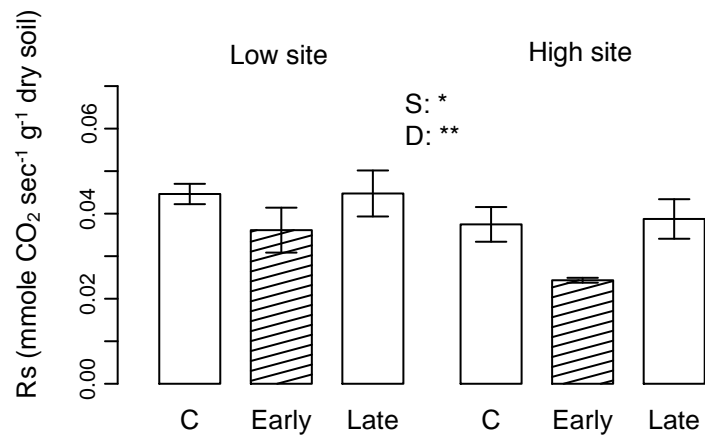
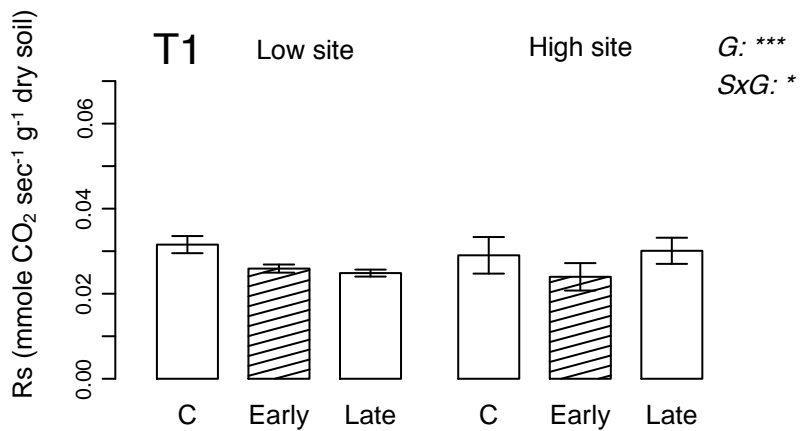


Clay-loam high-organic soil (HOM)

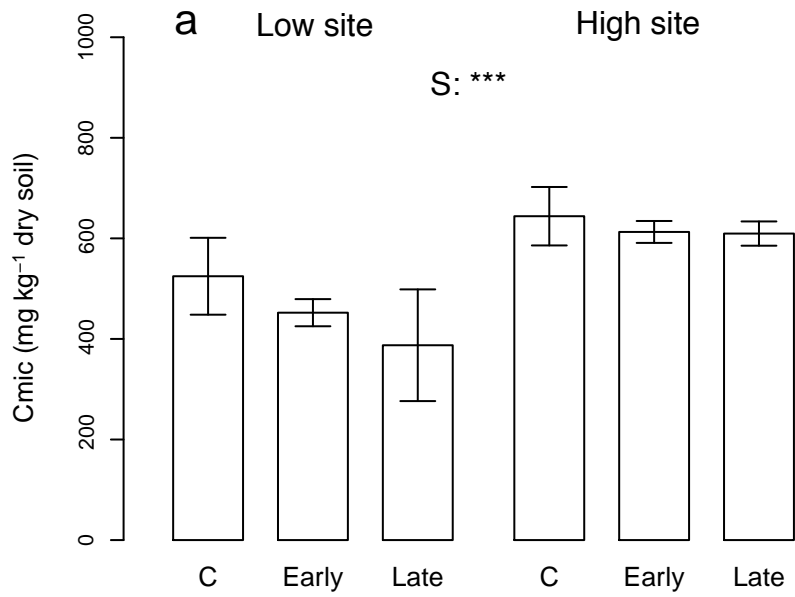


Loamy low-organic soil (LOM)

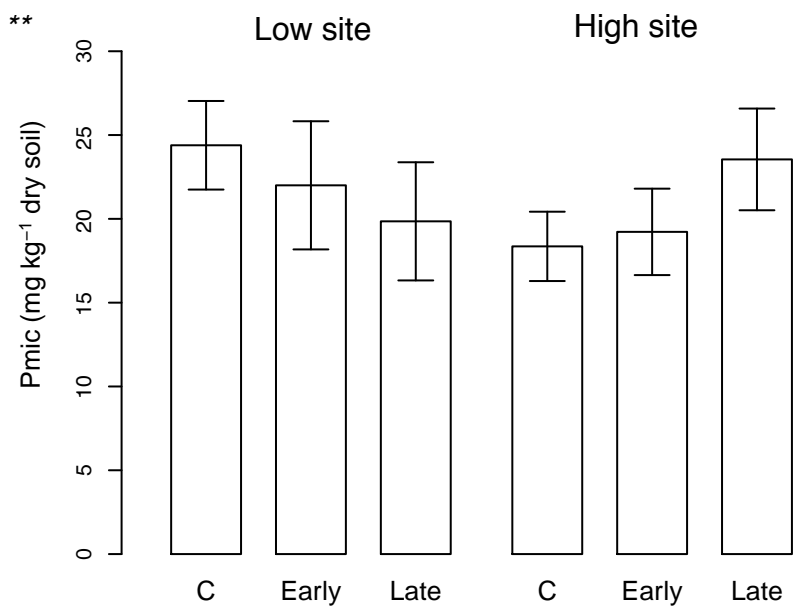
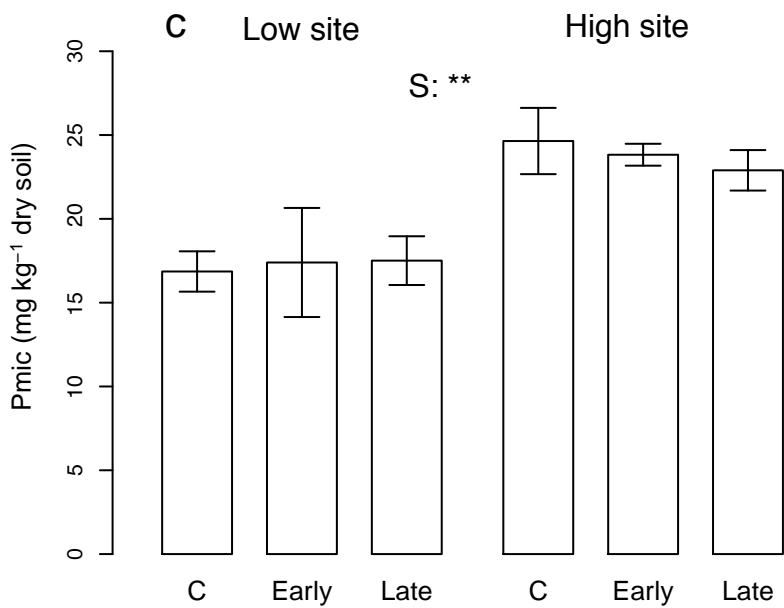
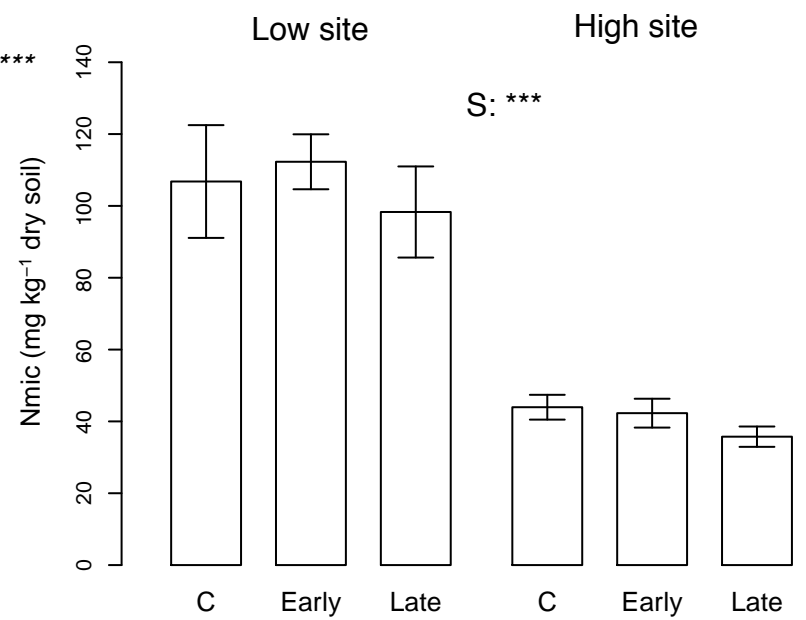
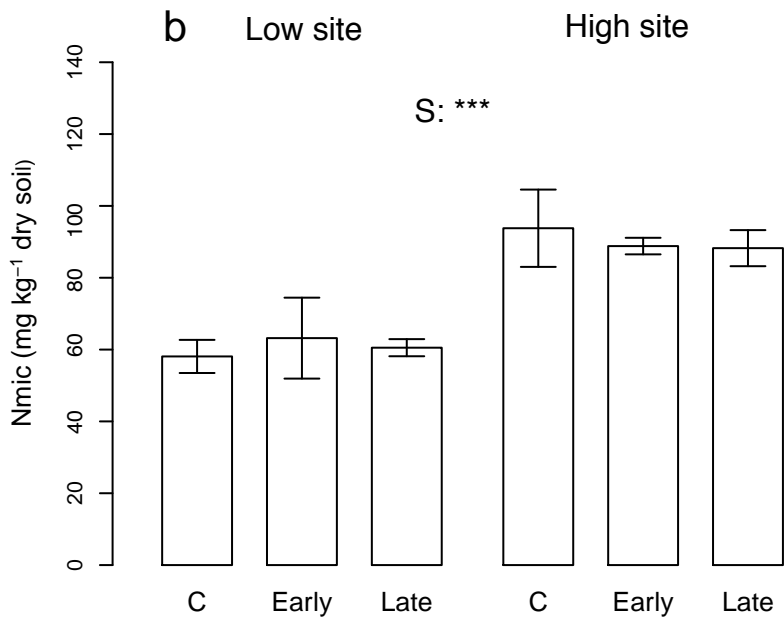
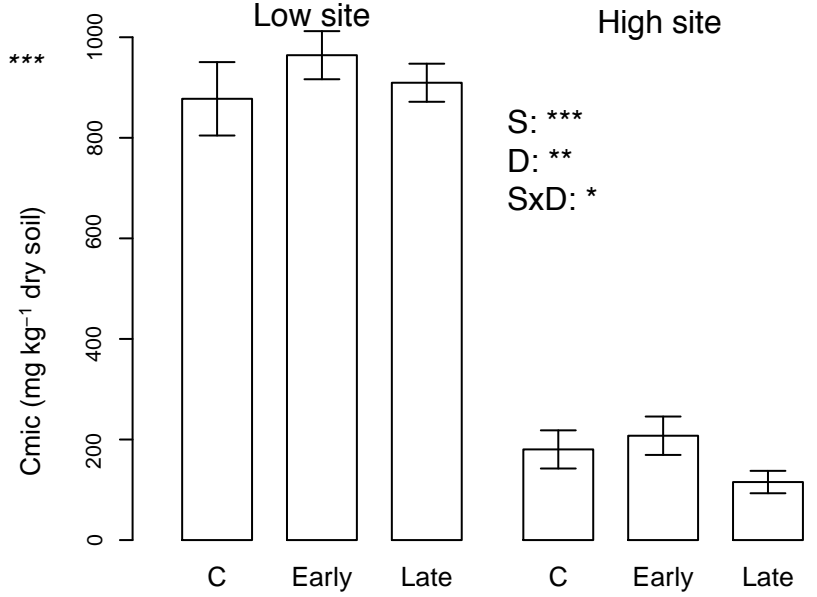
Clay-loam high-organic soil (HOM)



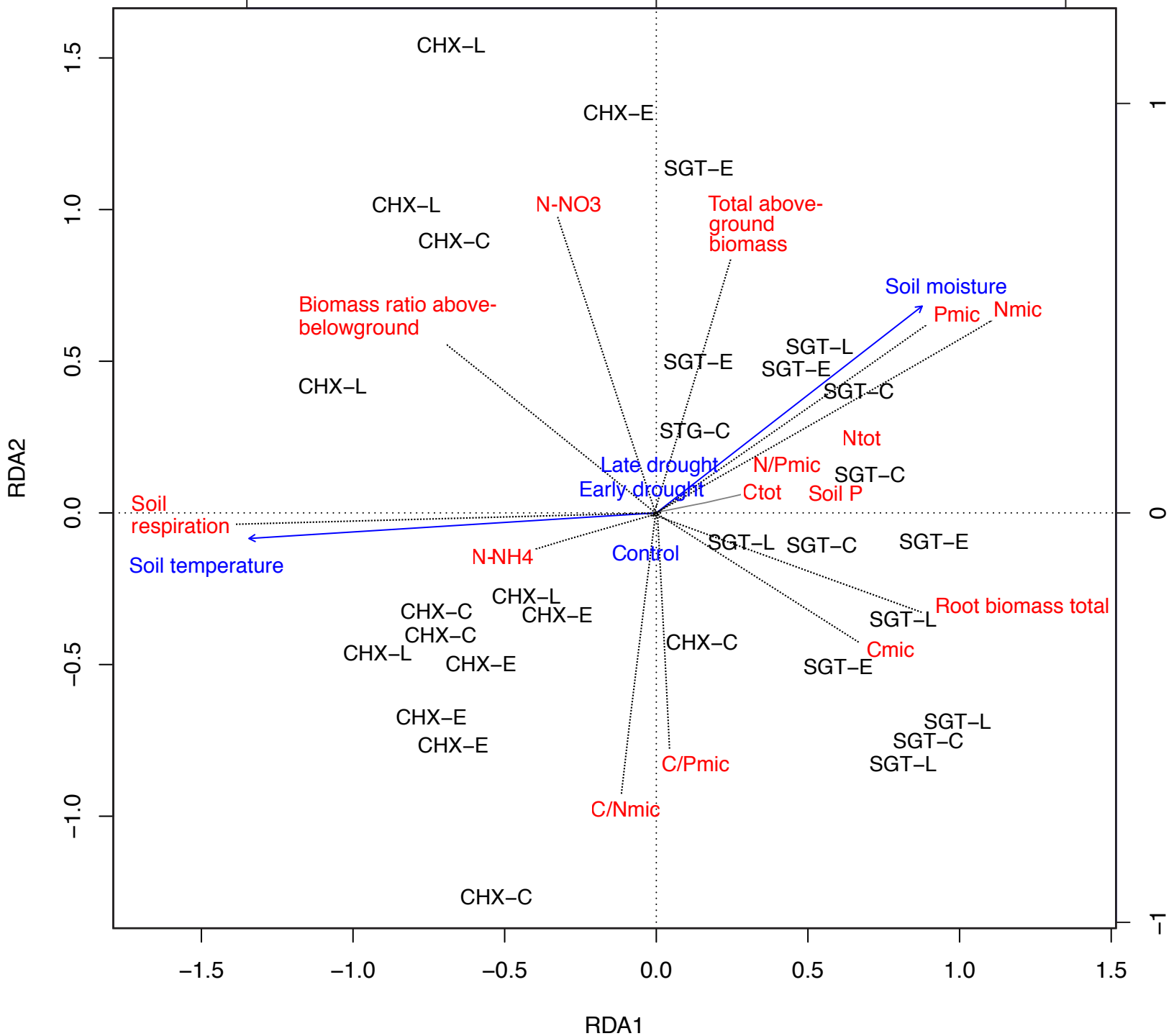
Loamy low-organic soil (LOM)



Clay-loam high-organic soil (HOM)



Loamy low-organic soil (LOM)



Clay-loam high organic soil (HOM)

