Title: Intra and inter-annual climatic conditions have stronger effect than grazing intensity on root growth of permanent grasslands

Running head: Root production in grazed grasslands

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Keywords: annual root and above-ground production; ingrowth core; leaf and root traits; root dynamics; soil moisture; soil temperature

Type of paper: Regular article

Abstract (200 words)

- **Background and Aims**. Understanding how direct and indirect changes in climatic conditions, management, and species composition affect root production and root traits is of prime importance for grassland C sequestration service delivery.
- **Methods**. We studied during two years the dynamics of root mass production with ingrowth-cores and annual above- and below-ground biomass (ANPP, BNPP) of upland fertile grasslands subjected for 10 years to a gradient of herbage utilization by grazing.
- **Results**. We observed strong seasonal root production across treatments in both a wet and a dry year but response to grazing intensity was hardly observed within growing seasons. In abandonment, spring and autumn peaks of root growth were delayed by about one month compared to cattle treatments, possibly due to later canopy green-up and lower soil temperature. BNPP was slightly lower in abandonment compared to cattle treatments only during the dry year, whereas this effect on ANPP was observed the wet year. In response to drought, the root-to-shoot biomass ratio declined in the abandonment but not in the cattle treatment, underlining higher resistance to drought of grazed grassland communities.
- **Conclusions**. Rotational grazing pressure and climatic conditions variability had very limited effects on root growth seasonality although drought had stronger effects on BNPP than on ANPP.

Introduction

Permanent grasslands provide many services that tie in to human activities through livestock products, but also contribute to regulate greenhouse gas emission, because their soils accumulate large amounts of carbon in organic matter fractions. Intensification of management practices through changes in mowing, fertilization and grazing intensity may affect these services as well as climate variability through increased drought intensity and frequency (Conant et al. 2001; Jones and Donnelly 2004; Soussana and Duru 2007). As root activity (growth, exudation, turnover) is a major input of C and N compounds into grassland soils, improving our understanding of plant roots dynamics under different management and climatic conditions may help to identify management options to maintain the C sequestration abilities of this ecosystem and thus its sustainability.

Different practices of management, such as mowing and grazing, modify the amount of soil C and N fluxes through direct effects of defoliation, fertilization or returns of excreta to soil on root growth and soil abiotic factors and indirect effects through species composition changes (Bardgett and Wardle 2003; Dawson et al. 2000; Soussana et al. 2004). In mown grasslands it has been shown that root mass production is generally lower when grass is frequently mown and fertilised (Leuschner et al. 2013; Picon-Cochard et al. 2009). This may be explained by changes in root-to-shoot allocation, with increase of above-ground growth in order to maximize light capture. The complexity of these phenomena in grazed grassland is greater than in mown systems owing to animals' selective defoliation of plant species, and also because returns to soil are spatially heterogeneous (Rossignol et al. 2011). In addition, level of soil fertility may buffer the degree of root response to defoliation in grazed grasslands as plants exhibit specific responses to defoliation in fertile and unfertile grasslands (Duru et al. 1998). Overall this can explain why no clear trend is found for the effects of grazing on above- and below-ground production (e.g. see syntheses of Milchunas and Lauenroth (1993) and McSherry and Ritchie

(2013)), although a meta-analysis emphasizes a negative effect of grazing intensity on above- and below-ground carbon stocks compared to ungrazed systems (Zhou et al. 2017). In addition, repeated defoliations induced by grazing and mowing of grassland can simultaneously increase (i) soil temperature by increasing solar radiation reaching the soil and (ii) soil moisture due to lower leaf area index and reduction of vegetation transpiration (Moretto et al. 2001; Pineiro et al. 2010; Smith et al. 2014). Soil moisture can also be modified by high stocking rate through changes of soil bulk density due to soil compaction (Pineiro et al. 2010). These direct effects of grazing on soil abiotic factors should affect root growth of grazed grassland, although all these phenomena are not very well documented in field conditions.

Species composition change induced by management is also an important determinant of above- and below-ground response in grazed grassland; intensive practices (high grazing intensity, fertilization) generally favour the development of fast growing species (exploitative strategy) with highly digestible shoot and root tissues, low C/N and tissue density whereas at the opposite extensive practices (low grazing intensity, absence of fertilization) favour slow growing species (conservative strategy) with poorly digestible organs and high tissue density (Klumpp et al. 2009; Louault et al. 2005; Soussana and Lemaire 2014; Wardle et al. 2004). Root-to-shoot biomass allocation, but also functional traits (used as proxies of ecosystems properties like ANPP or BNPP, e.g. Laliberté and Tylianakis 2012), are thus likely to change in response to intensification of practices, e.g. from ungrazed to intensely grazed in temperate grassland (Klumpp and Soussana 2009) or in alpine meadows, steppes and desert-steppes (Zeng et al. 2015). Overall, according to Ziter and MacDougall (2013), the uncertainty surrounding nutrient-defoliation responses makes it difficult to predict whether C storage will be higher in managed compared to unmanaged grasslands. Thus soil fertility should be considered when comparing different grazing intensities in grassland, as species adapted to fertile conditions will

exhibit either trait related to avoidance or to tolerance strategies toward defoliation, both having similar exploitative resource-use strategy (Louault et al. 2005).

Increased climate variability is another source of response uncertainty in managed ecosystems. As more frequent and longer period of drought associated with heat waves may threaten and shape the long-term dynamics of perennial ecosystems such as grasslands (Brookshire and Weaver 2015), it is important to understand how above- and below-ground compartments respond to climatic conditions variability. However, there are few data on above- and below-ground biomass responses to drought for grassland (Byrne et al. 2013; Wilcox et al. 2015), although some evidence shows that the 'slow' trait strategy (resource conservation) is associated with drought tolerance (Pérez-Ramos et al. 2012; Reich 2014). It has also been shown that the timing of drought has more influence on the below- than on the above-ground compartment especially in grazed *vs*. ungrazed grassland, as peak of shoot biomass can occur before the drought period (Frank 2007). In addition, comparing two contrasting grazed grasslands, Klumpp et al. (2011) showed that during wet years extensive managed grassland (low stocking density combined with low soil fertility) had a higher storage capacity than intensive managed grassland (moderate stocking density combined with N fertilization), whereas the reverse was observed during dry years, as a result of higher canopy senescence in extensive *vs.* intensive management. Changes in root morphology and functioning may thus be an important mechanism in plant adaptive strategies to drought, and have been less well studied than above-ground plant responses (Biswell and Weaver 1933; Dawson et al. 2000; McInenly et al. 2010). However, there are not enough data to make generalizations about combined impacts of management and climatic conditions variability such as precipitation reduction on root and shoot biomass production and plant traits defining plant strategies related to resource use and grazing intensity.

In the present experiment, we sought to assess whether grazing intensity affected root growth dynamics, root and leaf functional traits and annual below-ground biomass (BNPP) in a fertile and productive grassland and whether root response is mirrored by the annual above-ground biomass production (ANPP) and leaf traits and by changes of climatic conditions. These responses could be modulated by direct effects of grazing intensity on soil microclimate. Root and leaf traits were studied as response traits to grazing intensity and as effect traits of BNPP and ANPP, respectively. The study was carried out in a long-term field experiment for which controlled grazing intensity had been applied for 10 years. We compared abandonment of grazing and two levels of herbage utilization by grazing based on five rotations per year. In two consecutive years, the ingrowth core method was used to measure monthly root biomass production and calculate annual root production (BNPP); ANPP was measured by grazing exclusion cages and community-weighted mean leaf and root traits were assessed the first year. We tested the following hypotheses: (i) high grazing intensity increases above-ground mass at the expense of root production as a result of the direct negative effect of defoliation on root growth, whatever the climatic conditions, (ii) inter-annual climatic conditions modulate above and below-ground biomass production response to grazing intensity as a consequence of higher presence of defoliation tolerant and drought-sensitive species (*Lolium perenne* or *Trifolium repens*) in the high grazing intensity treatment; (iii) root traits respond to treatment and is a determinant of BNPP, as observed for leaf traits for ANPP.

Materials and methods

Site characteristics

The experiment took place in the long-term observatory network (ACBB-SOERE) located at St-Genès-Champanelle, France (45°43′N, 03°01′E, 880 m a.s.l.). The local climate is semi-continental with oceanic influences (mean annual temperature 8.5 °C, mean annual

148 precipitation 784 mm, Table 1). The site supports mesotrophic multi-specific permanent
149 grassland, dominated by species with high Ellenberg indicator values for N (Schaffers and
150 Sykora 2000), indicating a high level of fertility for the site (Table S1; Louault et al. 2017). The
151 soil is a cambisol with a sandy loam texture, developed on granitic bedrock. Differences in local
152 soil composition and profile led us to consider two blocks characterized respectively by a eutric
153 cambisol (54% sand; 26% silt; 20% clay; 7.0% organic matter; pH: 5.9) and a colluvic cambisol
154 (50% sand; 26% silt; 24% clay; 7.4% organic matter; pH: 6.0) including some volcanic
155 materials.
156
157 Management
158 Prior to the installation of this experiment in 2005, the study area had been used for intensive
159 hay and silage production (combining grazing, mowing and fertilization), with mineral
160 fertilization, and two years preceding the start of the experiment (2003 and 2004), the grassland
161 site was mown three times per year without fertilization. Then, from 2005, the grassland had
162 been managed for 10 years with a gradient of grazing intensity resulting from three treatments:
163 abandonment (Ab), low (Cattle-) and high (Cattle+) level of herbage utilization obtained by
164 modification of stocking density (0, 6.9 and 13.8 LSU ha^{-1}, livestock unit, respectively) with
165 five grazing rotations each year: mid-April, late May, early July, September and November,
166 lasting on average 9.6, 9.0, 10.7, 8.6, and 2.1 days, respectively. The two cattle treatments
167 corresponded to two levels of herbage utilization by grazing, and had on average 15.2 ± 0.5 cm
168 (mean ± se, Cattle-) and 7.7 ± 0.2 cm (Cattle+) residual plant height at the end of each grazing
169 rotation, respectively. For each treatment, two replicate plots were set up per block, resulting in
170 four replicates per treatment, and a total of 12 plots (2 blocks x 2 plots x 3 treatments). The
171 average distance between the two blocks is about 230 m and all treatments are randomized

172 within each block. The size of the plots differs according to treatments: 2200 m^2 for the two
173 cattle treatments and 400 m^2 for the abandonment.
174
175 Climatic and edaphic conditions
176 Daily precipitation (mm) and air temperature (°C) were measured for the two years, and
177 recorded with a meteorological station located at the site. An aridity index was calculated as
178 precipitation minus potential evapotranspiration (P - PET, mm) with the Penman-Monteith
179 equation. Daily soil temperature (°C) was measured with thermocouple sensors (home-made
180 copper-constantan sensors) inserted at 20 cm depth in each plot and recorded with a HOBO
181 data logger (U12-014, Onset Instruments, MA, USA). Daily soil volumetric water content
182 (SWC, m^3 m^{-3}) of each plot was measured with two probes (ECHO-10, Decagon, USA),
183 inserted horizontally at 20 cm depth, and connected to dataloggers (EM5 and EM50, Decagon,
184 USA). From January 2014 to November 2015 (DOY 132–326), SWC was measured every 30
185 min and averaged at daily scale. For each plot, average values of the two probes were used.
186 Daily relative soil water content data are shown and calculated as the ratio:
187 $RSWC = \frac{\mathrm{SWC - SWCmin}}{\mathrm{SWCmax - SWCmin}}$, where SWC is the soil moisture at a given day, SWCmin is the
188 minimum value of soil moisture and SWCmax is the maximum value of soil moisture, both
189 observed during the two years. For soil temperature and RSWC, values were averaged
190 according to root growth time scale.
191
192 Root growth and root mass
193 Six months beforehand, shallow (0-20 cm) soil was collected on each of the two blocks of the
194 site and sieved (5 mm mesh size) to remove stones and coarse organic matter, and then left
195 unused outside covered under a shelter and protected from direct sunlight. Thereafter, this air-
196 dried soil was used to fill the ingrowth-core each month.

197 In December 2013 and for each of the 12 plots, soil cores were collected with an auger (8 cm
198 diameter, 0-20 cm depth) at four locations representative of the plant community in the
199 treatment. On average mean distance between locations are 19.8 m ± 0.2, 21.7 m ± 0.1 and 17.2
200 m ± 0.2 for Ca+, Ca- and Ab (mean ± SD, see Fig S1), respectively. After core harvest, each
201 hole was filled with a plastic net (8 mm mesh size) containing a fixed volume of air-dried sieved
202 soil (ingrowth core), collected six months beforehand. Then, about each month and for two
203 years (2 x 10 times), ingrowth cores, containing soil and the root and rhizome material that had
204 grown therein, were extracted, and then replenished with another fixed volume of dry sieved
205 soil. Thus monthly and annual root production (BNPP, g m^{-2} y^{-1}) were measured from February
206 2014 to December 2015. Root production period ranged on average 36.5 days, but with longer
207 and shorter periods in winter and spring-summer, respectively (Table 1). In periods with
208 absence of precipitation, a fixed volume of water was added to adjust soil humidity to field
209 conditions. After collection, the ingrowth cores were transported to the laboratory and
210 immediately stored at 4 °C before processing in the next five days. The roots were washed
211 under tap water and with a 200 µm sieve, and then oven-dried (48 h, 60 °C).
212 In order to measure root mass stock, soil cores were collected three times (December 2013,
213 March and June 2014) with the same auger and near the ingrowth cores locations. These
214 samples were stored in the freezer (-18°C), and after defrosting, the roots were washed with the
215 same procedure as that used for the ingrowth cores, and then oven-dried (48 h, 60 °C).
216
217 Root traits
218 Subsamples of washed roots collected with the ingrowth cores collected in June 2014, were
219 fresh weighed, and then frozen (-18 °C) before morphology analysis. After defrosting, roots
220 were stained with methylene blue (5 g L^{-1}) for about 5-10 minutes, rinsed in water, spread in a
221 transparent glass box containing a thin layer of water, and covered with a transparent plastic

222 sheet. High resolution images were recorded with a double light scanner (800 dpi, perfection
223 V700, Epson, JA) and analyzed with WinRhizo software (PRO 2012b, Regent Instruments,
224 CA) with the automatic procedure. Two scans per location were recorded and separately
225 analyzed to measure root length (m), root volume (cm^3), root surface area (m^2), average root
226 diameter (mm) and length by class diameter (13 classes: 11 with 0.1 mm interval and 2 with
227 0.5 mm interval). Specific root length (m g^{-1}), root tissue density (g cm^{-3}) and specific root area
228 (m^2 g^{-1}) were calculated for fine roots as in Picon-Cochard et al. (2012).
229
230 Botanical composition
231 Species contribution (%) was visually observed on a circle (20 cm diameter) around each
232 ingrowth core location in April (cattle treatments) and May (abandonment) 2014. For each
233 zone, a score on a ten-point scale was allocated to species present according to their volume
234 occupancy, and the percentage of each species was calculated at the plot scale by averaging
235 values of the four zones. The list of species and their relative contributions is given in Table
236 S2.
237
238 Above-ground biomass production
239 On each plot and on each sampling date, four fenced sampling areas (0.6 × 0.6 m) were used to
240 measure accumulation of above-ground biomass after above-ground standing biomass was
241 clipped at 5.5 cm. At each sampling date, biomass was sampled at a height of 5.5 cm, oven-
242 dried and weighed. Measurements were made five times in the course of the year, before each
243 grazing event in Cattle+ and Cattle- plots, and three times (spring, summer, autumn) in
244 abandonment plots. Sampling areas were moved within the plot at each measurement date
245 during the year. Annual above-ground net primary production (ANPP, g m^{-2} y^{-1}) was calculated
246 as the sum of the successive biomass accumulations along the year.

247 Leaf traits

248 Community-weighted mean (CWM) trait values of leaf dry matter content (LDMC), specific
249 leaf area (SLA) and reproductive plant height (H) were calculated for each ingrowth core zone
250 using (i) the relative contribution of the dominant species to the community (i.e. species that
251 account for at least 85% of the cumulated species contribution of the community) measured in
252 2014, and (ii) leaf trait measurements made at plot scale in 2006 and 2007. Traits were measured
253 on ten vegetative plants using standard protocols (see methods in Louault et al. 2005).
254 Reproductive plant height was measured on mature plants located in fenced zones to allow full
255 plant development. CWM is expressed with the following equation: CWM = $\sum p_i \times \text{trait}_i$,
256 where p_i is the relative contribution of species i to the community and trait_i the trait of species
257 i.

258

259 Statistical analyses

260 For a given date, root mass and root traits collected at each location (four ingrowth-cores in
261 each plot), averages of data coming from the four locations were used to have a single value for
262 each of the 12 plots and test for the effect of treatment and dates. Before ANOVA, normality
263 of residuals was inspected with quantile-quantile plots of model residuals, and variance
264 homogeneity was confirmed by checking the plots of model residuals *vs.* fitted values. Data
265 were transformed if they deviated from ANOVA assumptions (square root, ln, reciprocal).
266 Linear mixed effects models as available in the R 'nlme' package (Pinheiro et al. 2015) were
267 used to perform repeated measure ANOVAs to test the effects of treatments, dates and their
268 interactions on values of root growth, soil temperature, RSWC, and root mass stock, with plots
269 nested in block as a random factor accounting for temporal pseudo-replication. For root growth
270 dynamics, soil temperature and RSWC (Fig 1, Table S1), dates correspond to 20 dates and for
271 root mass stock, dates correspond to three harvest dates (Table 2). For BNPP, ANPP and root

to shoot ratio (BNPP/ANPP), data were analyzed using a nested mixed model procedure, with treatments and year used as fixed factors with plot nested in block as random factors. For leaf and root traits data, treatments were used as fixed factors with plots nested in block as a random factor. *Post hoc* tests were performed to compare significance levels across fixed factors with a Tukey test ('lsmeans' package). Principal component analyses (PCA) were performed for each year to analyze relationships between leaf and root traits, soil temperature, RSWC, root mass stock, ANPP and BNPP measured at plot level; treatments were considered as supplementary categories ('FactoMineR' package). All statistical analyses were performed in the R environment (version 3.5.2, R Core team 2012) using RStudio (Version 1.1.463).

Results

Climatic conditions during the experiment

Compared with average long-term climatic data for the site, the first and second years of the experiment had higher (+92 mm) and lower (-199 mm) precipitation, respectively (Table 1). Potential evapotranspiration (PET) in the second year was also higher than the long-term average (difference of 73 mm), leading to a negative annual climatic water balance (P - PET = -181 mm and a deficit of 271 mm compared to the long-term average). Annual temperature in the two experimental years was similar and about 0.8°C higher than the long-term average for the site (Table 1). At monthly time scale and during part of the growing season (March to September), in comparison with the first year, the second year had a cumulated water deficit difference of -266 mm and a temperature warmer by +1.9 °C than the first year. Larger differences between the two years occurred in June-July with higher temperature (+6 °C), higher water deficit (P-PET = -152.6 mm) and less precipitation (-81%) in the second year.

Dynamics of soil temperature and relative soil water content

297 Soil temperature was significantly affected by treatment, dates and treatment × dates (Figure 1;
298 Table S1). For most of the dates (February to October), abandonment treatment had lower soil
299 temperature (1.76 °C, on average) than the grazing treatments, whereas the Cattle- treatment
300 showed significant lower soil temperature (-0.64 °C) than the Cattle+ treatment. However, this
301 was significantly observed for a limited number of dates in early summer of both years. Relative
302 soil water content (RSWC) fluctuated from 0.6-0.7 at the beginning of spring to 0.38 in June in
303 the wet year and to 0.2 during the dry year, which is in accordance to variation of the
304 atmospheric aridity index (P-PET). In the case of the dry year, from summer until autumn,
305 RSWC remained lower than 0.4 and the aridity index was negative.
306
307 Root growth dynamics
308 Root growth was affected by date and treatment × date interaction (Figure 1). Each year, peak
309 of root growth occurred twice, in spring and autumn, and growth was markedly reduced in
310 summer and winter. Only in the second year did growth stop in summer, and it was significantly
311 lower than the first year. Regarding treatment effect, abandonment showed significant lower
312 root growth than the two grazing treatments for the spring period in both years, and for the
313 autumn of the second year. While in autumn 2014, a delay of growth peaks was always
314 observed, which led to a two-fold higher root growth for abandonment *vs.* the two cattle
315 treatments (end of September: date 8). The two grazing treatments had similar root growth
316 across years and seasons.
317
318 Seasonal root mass stock, BNPP, ANPP and root-to-shoot biomass ratio
319 Stock of root mass did not change through season and across treatment (Table 2). BNPP, ANPP
320 and root-to-shoot biomass ratio (R/S) were significantly lower during the second year, with a
321 stronger effect on BNPP (-44% on average) than ANPP (-24%) (Figure 2, Table 3). Only the

322 abandonment treatment maintained their value of ANPP in the second year, which led to a 48%
323 decline in R/S (significant treatment × year, $P < 0.01$, Table 3). Accordingly, treatment effect
324 was only observed for BNPP the second year, with a decline of 24% for abandonment compared
325 to cattle treatments and for ANPP the first year: Cattle+ having 22% and 68% higher values
326 than Cattle- and abandonment, respectively, while Cattle- had 38% higher ANPP than
327 abandonment.
328
329 Species composition, leaf and root traits
330 Abandonment treatment was characterized by the dominance of tall grass species: 76% in all
331 with 27.2% of *Alopecurus pratensis*, 18.8% of *Elytrigia repens*, 11.3% of *Poa pratensis* and
332 10.3% of *Arrhenatherum elatius*, the presence of some forbs (19%) and the absence of legumes
333 (Table S2 and Table 4). The two cattle treatments differed from abandonment treatment by
334 equal presence of *Taraxacum officinale* (18% on average) and *Trifolium repens* (17% on
335 average). Difference also concerns grass species (56% in total) with the dominance of *Dactylis*
336 *glomerata* (22.2%), *A. pratensis* (7.6%) and *Schedurus arundinaceus* (5.6%) for Cattle- and
337 *Lolium perenne* (13.6%), *D. glomerata* (9.1%) and *Poa trivialis* (7.2%) for Cattle+. Thus, the
338 Cattle+ treatment had a higher percentage of *L. perenne* than Cattle- (Table S2).
339 Community-weighted mean leaf traits (CWM) were significantly modified by the
340 treatments. Plant height and LDMC were significantly higher ($P < 0.05$ and $P < 0.0001$,
341 respectively; Table 4) in abandonment than in the two cattle grazed treatments, whereas SLA
342 was lower ($P < 0.05$). Unlike leaf traits, root traits were only slightly affected by the treatments.
343 Specific root length (SRL, $P < 0.1$) and specific root area (SRA, $P < 0.05$) were lower in
344 abandonment treatment than in Cattle-, but not Cattle+. For other root traits (diameter, RTD
345 and root length % by class diameter) no between-treatment differences were observed (Table
346 4).

347 Co-variation of traits and production

348 The two main axes of the standardized PCA explained 60.1% and 56.8% of the community trait
349 and production variation in 2014 and 2015, respectively (Figure 3). For the first year, the first
350 PCA axis (PC1), accounting for 43.4% of the total variation, was significantly related to leaf
351 and root traits, ANPP and soil temperature. Soil temperature, SRA and ANPP had positive
352 loadings, and diameter, plant height and LDMC had negative loadings (Table 5). The second
353 PCA axis (PC2), accounting for 16.7% of the total variation, was significantly and positively
354 related to root diameter and negatively to SRA. For the second year, the first PCA axis (PC1),
355 accounted for 37.4% of the total variation, and was significantly related to leaf and root traits,
356 ANPP and BNPP. BNPP and SRA had negative loadings, and root diameter, plant height and
357 ANPP had positive loadings (Table 5). The second PCA axis (PC2), accounting for 19.4% of
358 the total variation, was significantly and positively related to RSWC and stock of root mass
359 averaged across three dates. Finally, abandonment treatment was significantly related to PC1s
360 with negative and positive loading s for the first and the second year, respectively.

361

362 **Discussion**

363 Ten years of contrasted management had strongly modified the functional diversity and above-
364 ground production of this fertile upland grassland (Herfurth et al. 2015; Louault et al. 2017).
365 Accordingly, we expected that above-ground biomass patterns would be mirrored below-
366 ground, especially during the periods of grazing. Here we first discuss within-year differences
367 of root growth, followed by inter-annual variation responses to grazing intensity and climatic
368 conditions variability between the two contrasting years, and last we analyze relationships
369 between traits and above- and below-ground production.

370

371 Seasonality of root growth was independent of grazing intensity and climatic conditions

372 As expected, root growth of permanent grassland is affected by seasons and peaks in spring and
373 autumn (Garcia-Pausas et al. 2011; Pilon et al. 2013; Steinaker and Wilson 2008), but
374 unexpectedly, grazing pressure applied by rotations and climatic conditions variability had very
375 limited effects on this seasonality. This means that at below-ground level, plant community
376 behavior was not affected by rotational grazing management nor by climatic conditions
377 variability, although a severe drought occurred in summer of the second year. Only the
378 abandonment treatment showed a delayed root growth peak in spring. This delay is probably
379 the result of slower shoot budburst and reduced capacity to produce new green leaves in dense
380 litter canopy, especially at the beginning of the growing season in spring (data not shown).
381 Moreover, the tall and dense canopy of the abandonment treatment strongly modified soil
382 temperature, with cooler soil conditions as expected in such abandoned vegetation (Picon-
383 Cochard et al. 2006; Zhou et al. 2017; Zhu et al. 2016). As shown in some studies, light or soil
384 water and nutrient availabilities (Edwards et al. 2004; Garcia-Pausas et al. 2011; Steinaker and
385 Wilson 2008) are other abiotic factors determining dynamics of root growth in grasslands, as
386 root peaks were observed before the peak of soil temperature in summer when negative climatic
387 water balance occurred, especially in the second year. Nevertheless, plants growing in
388 abandonment offset their slower root growth by producing similar root biomass at annual scale,
389 especially during the wet year. The presence of tall grass species such as *A. pratensis*, *A. elatius*
390 and *E. repens* with plant trait syndromes related to resource conservation strategy (lower SLA
391 and SRL and higher plant height and root depth; Pagès and Picon-Cochard 2014) might explain
392 their capacity to produce higher root biomass on a shorter-term period before canopy
393 senescence onset. Also pre-existing soil fertility can be maintained in conditions of very low
394 levels of herbage utilization (near-abandonment), because of the absence of biomass
395 exportation and increased internal recycling of N within senescent plants, both contributing to
396 an increase in total N available for plant growth (Loiseau et al. 2005).

397 The similar root growth dynamics of the two cattle treatments was unexpected, considering
398 that infrequent defoliation and moderate excreta returns to the soil might increase root biomass
399 production at the expense of shoot biomass (Klumpp et al. 2009). The absence of effect on root
400 growth and BNPP means that grazing pressure applied on plant communities by rotations (5
401 rotations of 9 days each on average) was too short but enough to observe effect on ANPP, in
402 wet conditions. Worldwide there are different ways to manage grassland by grazing (Huyghe
403 et al. 2014), rotational or permanent grazing options with different stocking rates, durations,
404 types of herbivores. In general, this management creates high spatial heterogeneity within the
405 plots due to animals' selective defoliation of plant species, and also because returns to soil are
406 spatially heterogeneous. Thus in grazed grassland, disturbance induced by grazing creates
407 patches of vegetation, which should affect locally root growth and below-ground biomass of
408 plant communities if duration of grazing is sufficient. The complexity of these phenomena in
409 grazed grassland is greater than in mown systems owing (Rossignol et al. 2011).
410 Then, again, the confounding effect of soil fertility and defoliation may mask a clear
411 response of the below-ground compartment in grazed grasslands. In view of that, we postulate
412 that root growth in Cattle+ treatment was favored by the higher soil temperature compensating
413 for the negative effects of frequent defoliation on root growth while the cooler soil conditions
414 encountered in Cattle- might have slowed root growth. Soil moisture is a main determinant of
415 plant growth and can be affected by cattle treatments. Some studies showed an increase of soil
416 moisture in grazed compared ungrazed treatment due to lower leaf area index in the grazed
417 conditions (Moretto et al. 2001; Pineiro et al. 2010), or an absence of effects in others (LeCain
418 et al. 2002; Smith et al. 2014). The presence of herbivores can increase soil bulk density and
419 consequently modify soil moisture. However, in our field conditions and after 10 years of
420 treatments application, soil moisture was not affected by the rotational grazing, probably
421 because the temporal scale used buffer shorter-term response.

422 We should also consider the level of soil fertility and species composition as drivers of root
423 growth and trait plasticity (Dawson et al. 2000). The soil fertility of our site, reflected by the
424 nitrogen nutrition index (NNI, Lemaire and Gastal 1997), was very similar along our grazing
425 intensity gradient (Table S1), at least in 2014. Thus in our site we had the opportunity to
426 compare grazing intensity effect at equivalent soil fertility. Knowing that root trait plasticity
427 generally shows larger differences with respect to soil fertility than by cutting or defoliation
428 (Leuschner et al. 2013; Picon-Cochard et al. 2009), we can expect that under similar soil fertility
429 grazing intensity had a less pronounced effect on root growth. Indeed, the higher presence of
430 species tolerating defoliation, with shorter stature and root system (*L. perenne*, *P. trivialis*), but
431 having higher shoot and root growth capacity after defoliation and also higher rhizosphere
432 activity (Dawson et al. 2000), probably compensated for the negative effect of defoliation in
433 the Cattle+ treatment. Also the sampling depth might have had an effect, as we expect that
434 harvesting root systems deeper than 20 cm should give more contrasting root growth response
435 across the two cattle treatments according to the grass species composition due to species-
436 specific differential root depth distribution (Xu et al. 2014). Taken together, we provide
437 evidence that higher soil temperature, high soil fertility and species composition have
438 moderated root growth response along our grazing intensity gradient. The difficulty to assign
439 species composition in root mixtures, however, makes it difficult to draw firm conclusions.
440
441 <u>Climatic conditions variability shaped responses of ANPP, BNPP and root-to-shoot biomass</u>
442 <u>production ratio along the grazing intensity gradient</u>
443 According to meta-analyses and recent results (McSherry and Ritchie 2013; Zeng et al. 2015;
444 Zhou et al. 2017), grazing intensity generally has negative effects on above- and below-ground
445 biomass of grasslands whatever the climatic conditions or vegetation type, although these
446 effects can be modulated by levels of grazing intensity. Our results do not confirm these

447 findings, because ANPP and BNPP increased in response to grazing intensity compared to
448 abandonment, in the wet and the dry year, respectively. Methodology issues for estimating
449 ANPP and BNPP in grazed grasslands should thus be taken into account, as some papers report
450 either biomass stock or fluxes measured once at peak of growth or at several periods (Scurlock
451 et al. 2002), but also estimation of BNPP from indirect measurements (e.g. Zeng et al. 2015).
452 Mass based on stock gives a snapshot of plant functioning, generally including mixtures of
453 living and senescent tissues, thus depending on abiotic factors and plant growth, whereas
454 measurements based on new shoot and root biomass reflect the growth potential of grasslands.
455 We are aware that these methods are very different, but in response to grazing intensity, BNPP
456 measured with ingrowth cores gave similar results as root mass stock assessed at three seasons.
457 Nevertheless, climatic aridity index (P - PET) had stronger effects on ANPP and BNPP than
458 grazing intensity, because severe drought had a direct negative effect on plant growth. In
459 comparison with another experiments located alongside ours, 80% of canopy senescence was
460 reached for a cumulated aridity index of -156 mm (Zwicke et al. 2013). As this index reached
461 -303 mm from March to August, this confirmed that a severe drought occurred in the second
462 year of our experiment, and explained root growth cessation in summer. At annual scale, ANPP
463 of the two cattle treatments showed lower resistance to increased aridity (resistance defined as
464 $ANPP_{year2}$ / $ANPP_{year1}$, being equal to 0.63) than abandonment treatment (ratio=1). For BNPP,
465 results were inversed, leading to a lower resistance of root-to-shoot biomass ratio in
466 abandonment than in the two cattle treatments. The absence of root growth modification by
467 grazing at annual scale the wet year reflects well the change in root-to-shoot biomass allocation,
468 albeit not significant. Other processes such as root turnover (mortality, rhizodeposition) are
469 expected to change in grazed *vs.* ungrazed grassland. For our site Herfurth et al. (2015) observed
470 similar root mass stock along a grazing disturbance gradient as in the present study, but by using
471 a simplified C flux model, these authors showed that the Cattle+ treatment tended to accelerate

472 C cycling in plant communities, resulting in a higher quantity of C allocated to the soil organic
473 matter continuum. Taken together, these results suggest that the slight BNPP increase under
474 grazing may occur with an increase in rhizodeposition, because root turnover calculated as
475 BNPP to root mass stock ratio (data not shown, Lauenroth and Gill 2003) was not different
476 across treatments.

477 Furthermore, our results suggest that grazing treatments slow down the negative effect of
478 aridity on root-to-shoot biomass ratio, and seem to be better adapted to buffering the negative
479 effect of drought on grassland production than for abandoned grasslands. This is consistent with
480 previous work showing that moderate grazing could be more beneficial than no grazing for
481 drought resistance and recovery of ANPP and BNPP (Frank 2007; Xu et al. 2012), and that
482 BNPP was more resistant than ANPP to change in precipitation (Yan et al. 2013). Other studies
483 showed no prevalence effects of grazing, drought or fire observed on grassland production in
484 North America and South Africa (Koerner and Collins 2014). Nevertheless, this points to a need
485 for further research to determine whether grazing pressure has additive or combined effects on
486 drought response of grasslands (Ruppert et al. 2015).

487

488 <u>Community-weighted mean leaf and root traits as predictors of ANPP and BNPP</u>

489 As shown by other studies (e.g. Diaz et al. 2007; Laliberté and Tylianakis 2012; Louault et al.
490 2017; Zheng et al. 2015), disturbance induced by grazing pressure has profound effects on plant
491 community and functional traits by selecting tolerant species to defoliation such as *L. perenne*,
492 *P. trivialis* or *T. repens*, with possible cascading effects on multiple ecosystem functions. With
493 the capacity to regrow quickly after defoliation, these species generally exhibited high values
494 of SLA and low values of LDMC and plant height. They contrast with species adapted to fertile
495 soil, but with a slower regrowth capacity after defoliation such as *D. glomerata* or *F.*
496 *arundinacea*, with opposite leaf trait values. In abandonment, competition for light tends to

497 select plants with trait syndromes related to conservative strategy (tall plants, low SLA and high
498 LDMC values). Thus, the CWM traits of the community will depend on the balance between
499 these species groups, which are expected to affect ANPP and BNPP (Klumpp et al. 2009;
500 Milchunas and Lauenroth 1993). Although the presence of tolerant and intolerant species to
501 defoliation in both cattle treatments, leaf trait values were similarly and positively related to
502 ANPP, and only differed from traits of species present in the abandonment treatment. This
503 means that cessation of grazing strongly differentiated plant communities, whereas within the
504 two cattle treatments differences were slighter.

505 For the below-ground compartment, we expected that above-ground differences were
506 mirrored by the root growth and traits, assuming that higher root diameter values, and lower
507 SRL and SRA values are associated with lower BNPP in abandonment compared with the two
508 cattle treatments. Although root response to grazing (mainly through defoliation) generally
509 reported reduction of root mass or root length (Dawson et al. 2000) our study did not confirm
510 these assumptions. The contrasting results are possibly due to variable abundance of tolerant
511 species to defoliation or with confounding effects of both defoliation and level of soil fertility
512 on roots of grazed grasslands (Leuschner et al. 2013; Picon-Cochard et al. 2009; Yan et al.
513 2013; Ziter and McDougall 2013). Thus, root growth reductions associated with grazing may
514 have a greater impact in locations where grazer-mediated nitrogen return is spatially decoupled
515 from defoliation (McInenly et al. 2010). Further, higher specific root area (SRA) observed in
516 Cattle- than in abandonment and Cattle+ treatments should reflect higher presence of species
517 with fine roots such as *D. glomerata* or *H. lanatus* (Picon-Cochard et al. 2012), because soil
518 fertility approximated by NNI was near comparable across treatments.

519

520 **Conclusions**

Similar functional diversity of the plant communities and similar soil fertility across the two cattle treatments explained the absence of changes in root mass production for these treatments. Our site disentangled confounding effects of fertility and defoliation on root production, which is not generally the case for other studies. Thus, our results suggest the prevalence of a soil fertility effect on root production response rather than a defoliation effect. However, we cannot rule out the possibility that continuous rather than rotational grazing practice would give similar results. In view of that, grazing practices information should be considered in order to give some generalizations about below-ground compartment response of fertile grassland with respect to grazing intensity. Besides, the strong effect of climatic conditions variability on ANPP and BNPP observed at short term could increase in the future as more frequent climatic extremes are expected. It is thus necessary to improve our knowledge of grazing practices that allow higher resilience of grasslands to more frequent and intense climatic events such as drought and heat waves.

Acknowledgments

We thank staff from INRAE-UMR0874: V. Guillot and E. Viallard for their technical expertise in field measurements, D. Colosse and S. Toillon for the soil temperature database, and S. Revaillot, A. Bartout, L. Bulon and S. Sauvat and M Mattei (VetAgro Sup) for their help in root sample measurements, and the staff of INRA-UE1414 Herbipôle. The experiment is part of the SOERE-ACBB project (http://www.soere-acbb.com/) funded by Allenvi and the French National Infrastructure AnaEE-F through ANR-11-INBS-0001. Data of the weather station are coming from the platform INRA CLIMATIK. DH received a doctoral fellowship from VetAgro Sup and DGER pole “ESTIVE”. The present work falls within the thematic area of the French government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25).

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Table 1. Air temperature (°C), precipitation (P, mm), potential evapotranspiration (PET) and climatic water balance: cumulated (P - PET, mm) and calculated for the 28 y period 1986-2013, mean values ± SD) and measured for the 10 dates in 2014 and 2015 corresponding to measurements of root growth and averaged (temperature) or summed (P, PET, P PET) at annual scale.

Year	Dates	Air temperature	Precipitation	PET	P - PET
	Annual long-term average	**8.5 ± 0.6**	**784 ± 1376**	**693 ± 96**	**91 ± 195**
2014	December 12 – February 23	3.7	98	37.5	60.5
	February 24 – March 23	5.3	27	46.3	-19.3
	March 24 – April 21	7.2	23.5	68.7	-45.2
	April 22 – May 25	9.2	79.5	103.1	-23.6
	May 26 – June 22	14.2	58	110.2	-52.2
	June 23 –July 20	15.1	136.5	93.9	42.6
	July 21 – August 24	14.4	90.5	100.5	-10
	August 25 – September 29	13.7	141.8	79.5	62.3
	September 30 – October 29	11.7	69	36.3	32.7
	October 30 – December 14	5.3	111	10.9	72.1
	Annual	**9.2**	**876**	**691**	**157.7**
2015	December 15 – March 1	1.3	132.5	31	101.5
	March 2 – March 29	4.5	36.5	36.8	-0.3
	March 30 – April 23	8.5	17.5	66.4	-48.9
	April 24 – May 28	11.0	66	113.6	-47.6
	May 29 – June 28	15.5	62.5	129.1	-66.6
	June 29 –July 23	21.1	26	136	-110
	July 24 – August 27	16.4	94.5	124.6	-30.1
	August 28 – September 24	12.8	77	66.3	10.7
	September 25 – October 29	7.8	55	36.1	18.9
	October 30 – December 11	7.0	54.5	25.1	29.4
	Annual	**9.4**	**585**	**766**	**-180.9**

706 Table 2. a) Repeated measure ANOVA is shown for treatment, date (December 2013, March
707 2014, June 2014) and interaction effects. Numerator (num), denominator (den) of degree of
708 freedom (DF) and *F* values are shown. b) Root mass (g m^{-2}) of abandonment, low (Cattle-) and
709 high (Cattle+) stocking density treatments measured in winter (December 12 2013), spring
710 (March 20 2014), summer (June 20 2014) and averaged across the three dates. Means ± se are
711 shown, n = 4. Superscripts ^{ns} correspond to P > 0.05.

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a)	num/den DF	*F-value*
Treatment	2/8	1.151^{ns}
Date	2/18	2.027^{ns}
Treatment × date	4/18	1.340^{ns}

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b) Date	Abandonment	Cattle-	Cattle+
December 2013	636.4 ± 133.1	403.3 ± 66.4	496.5 ± 20.6
March 2014	559.1 ± 166.2	609.2 ± 45.3	719.8 ± 47.5
June 2014	574.2 ± 84.8	482.2 ± 38.6	591.2 ± 101.7
3 dates average	589.9 ± 99.9	498.2 ± 43.6	602.5 ± 44.4

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730 Table 3. Repeated measure ANOVA is shown for treatment, year and interaction effects on
731 annual root production (BNPP, g m^{-2} y^{-1}), annual above-ground production (ANPP, g m^{-2} y^{-1})
732 and root to shoot ratio (R/S). Numerator (num), denominator (den) of degree of freedom (DF),
733 *F* values are shown. Superscripts $^{ns, +, *, **, ***}$ correspond to P > 0.05, P < 0.10, P < 0.05, P <
734 0.01, P < 0.001, respectively.
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		BNPP	ANPP	R/S
	num/den DF	*F-value*	*F-value*	*F-value*
Treatment	2/8	2.51^{ns}	8.10^{*}	0.46^{ns}
Year	1/9	70.72^{***}	83.77^{***}	13.09^{**}
Treatment × Year	2/9	3.83^{+}	22.21^{**}	9.52^{**}

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752 Table 4. Root traits measured from ingrowth core collected in June 2014 and leaf traits
753 measured from botanical observation in abandonment (May 2014), Cattle- and Cattle+ (April
754 2014) treatments. Diameter: root diameter (mm); SRL: specific root length (m g^{-1}); RTD: root
755 tissue density (g cm^{-3}); SRA: specific root area (m^2 g^{-1}); % 0-0.1 mm: percentage of length in
756 the class diameter 0-0.1 mm; % 0-0.1 mm: percentage of length in the class diameter 0-0.1 mm;
757 % 0-0.1 mm: percentage of length in the class diameter 0-0.1 mm; % 0-0.1 mm: percentage of
758 length in the class diameter 0-0.1 mm; Community-weighted mean (CWM) Height: plant height
759 (cm); SLA: specific leaf area (cm^2 g^{-1}); LDMC: leaf dry matter content (g g^{-1}); Ellenberg value
760 for N. Means ± se are shown (n = 4). num/den DF: numerator and denominator of degree of
761 freedom. Superscripts $^{ns, +, *, **, ***}$ correspond to P > 0.1, P ≤ 0.1, P < 0.05, P < 0.01, P < 0.001,
762 respectively. For SRL and SRA, different letters correspond to significant differences between
763 treatments.

	num/den DF	F-value	Abandonment	Cattle-	Cattle+
Root traits					
Diameter	2/8	1.61^{ns}	0.240 ± 0.015	0.210 ± 0.006	0.222 ± 0.015
SRL	2/8	3.71^{+}	237.2 ± 26.3 b	332.7 ± 30.4 a	277.8 ± 23.8 ab
RTD	2/8	0.55^{ns}	0.099 ± 0.007	0.095 ± 0.003	0.102 ± 0.007
SRA	2/8	4.96*	0.137 ± 0.011 b	0.182 ± 0.008 a	0.155 ± 0.01 ab
% 0-0.1 mm	2/8	1.28^{ns}	28.5 ± 1.1	32.9 ± 5.5	28.8 ± 2.6
% 0.1-0.2 mm	2/8	0.46^{ns}	37.7 ± 4.4	37.7 ± 2.2	39.1 ± 1.8
% 0.2-0.3 mm	2/8	0.30^{ns}	16.6 ± 1.2	16.2 ± 2.4	17.1 ± 1.9
% > 0.3 mm	2/8	1.22^{ns}	17.2 ± 5.0	13.2 ± 1.3	15.1 ± 2.1
Leaf traits					
CWM_Height	2/8	8.45^{*}	93.0 ± 3.5 a	72.8 ± 7.0 b	68.6 ± 3.8 b
CWM_SLA	2/8	5.30^{*}	205.1 ± 5.7 b	231.8 ± 7.3 a	225.5 ± 7.1 ab
CWM_LDMC	2/8	11.22^{*}	0.261 ± 0.008 a	0.227 ± 0.007 b	0.213 ± 0.010 b

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Table 5. Contribution of the different variables to the first two axes of the principal component analysis (PCA) calculated for 2014 and 2015. Variables used in the PCA were annual relative soil water content (RSWC), annual soil temperature (Tsoil, °C), root diameter (Diam, mm), specific root area (SRA, m^2 g^{-1}), root mass averaged over three dates (RootMass, g m^{-2}), annual root production (BNPP, g m^{-2} y^{-1}), plant height (Height, cm), leaf dry matter content (LDMC, g g^{-1}), annual above-ground production (ANPP, g m^{-2} y^{-1}). Treatments were added as supplementary categories.

	2014		2015	
Variable	Axis 1 (43.4 %)	Axis 2 (16.7 %)	Axis 1 (37.4 %)	Axis 2 (19.4 %)
RSWC	0.62	0.44	-0.21	**0.64**
Tsoil	**0.91**	0.09	-0.58	0.52
Diam	**-0.64**	**0.75**	**0.78**	0.53
SRA	**0.62**	**-0.58**	**-0.69**	-0.48
RootMass	-0.06	0.22	-0.07	**0.60**
BNPP	0.21	-0.23	**-0.71**	0.35
Height	**-0.82**	-0.07	**0.83**	-0.19
LDMC	**-0.83**	-0.12	**0.61**	0.03
ANPP	**0.71**	0.54	**0.57**	0.20
Suppl. Categories				
Abandonment	**-2.62**	-0.24	**2.04**	-0.27
Cattle-	1.07	-0.55	-1.21	-0.62
Cattle+	0.70	0.18	-0.83	0.90

Contribution in bold indicates significant correlation of the variables on the PCA axis ($P < 0.05$).

Figure captions

Figure 1. Dynamics of root growth (g m^{-2} day^{-1}), soil temperature (°C), relative soil water content and an aridity index (P-PET, mm) (hashed bars), measured over two years for abandonment, low (Cattle-) and high (Cattle+) stocking density treatments. Vertical bars correspond to 1 se (n = 4). Insets indicate P values from repeated measure two-tailed ANOVA (Treat: treatment, dates and interaction for main treatments). *: $P < 0.05$; x: $P \leq 0.1$. For soil temperature, *# corresponds to significant differences between all treatments (Abandonment < Cattle- < Cattle+).

Figure 2. Annual root biomass production (BNPP, g m^{-2} y^{-1}), annual above-ground biomass production (ANPP, g m^{-2} y^{-1}) and root-to-shoot biomass ratio measured in 2014 and 2015 for abandonment, low (Cattle-) and high (Cattle+) stocking density treatments. Vertical bars correspond to 1 se (n = 4). Within a year, different letters correspond to significant differences at $P < 0.05$.

Figure 3. Principal component analysis (PCA) combining leaf and root traits, above- and below-ground net primary production, root mass stock, relative soil water content and soil temperature measured in 2014 (a) and 2015 (b) for abandonment, low (Cattle-) and high (Cattle+) stocking density treatments. Data of each plot were used in each PCA. The first two axes are shown. Arrows show projections of the variables within the PCA. RSWC: relative soil water content; Tsoil: soil temperature (°C), Diam: root diameter (mm), SRA: specific root area (m^2 g^{-1}), RootMass: root mass averaged over 3 dates (g m^{-2}), BNPP: annual root production (g m^{-2} y^{-1}), Height: plant height (cm), LDMC: leaf dry matter content (g g^{-1}) and ANPP: annual above-ground production (g m^{-2} y^{-1}).

Figure 1

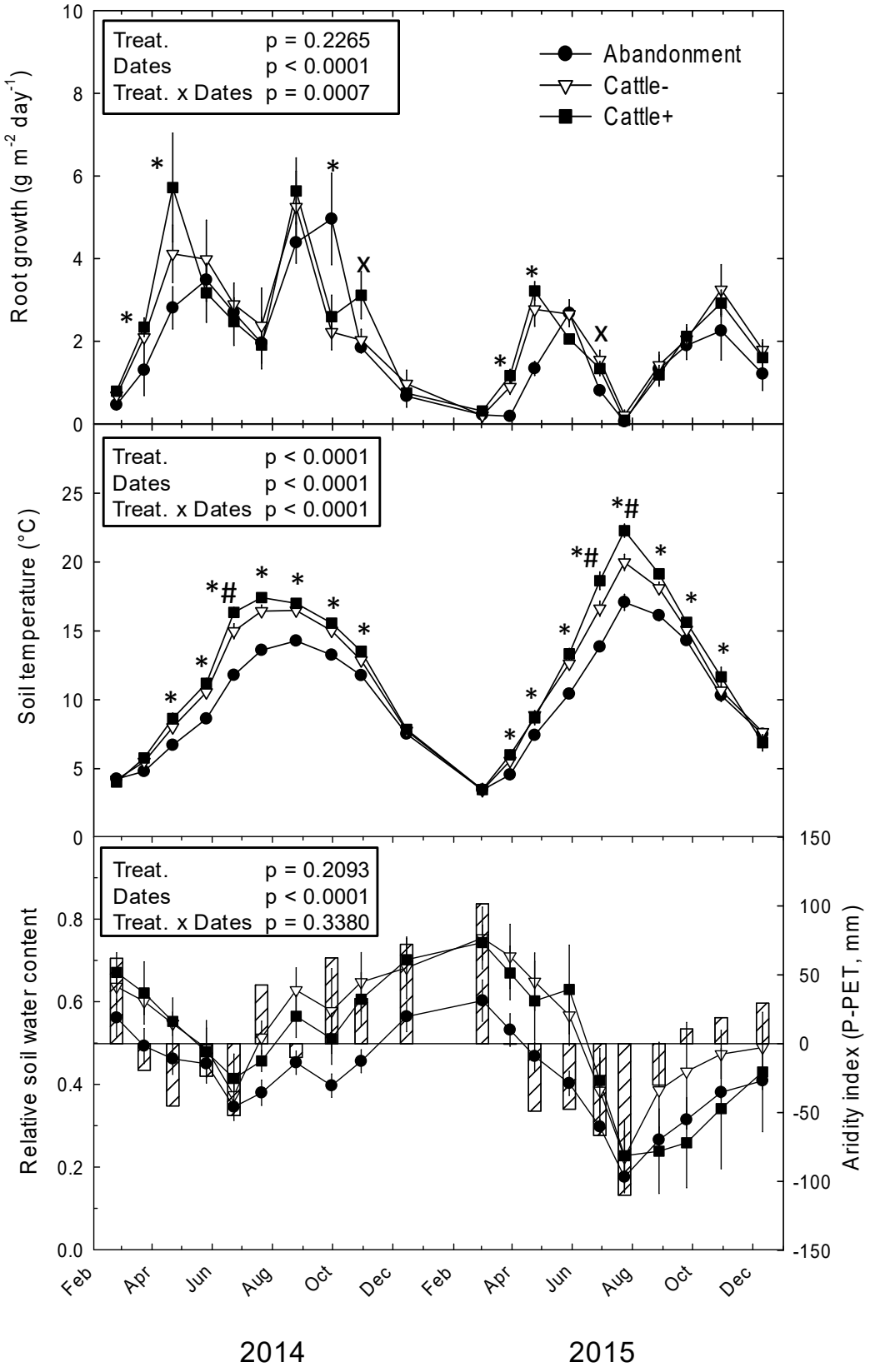

Treat. p = 0.2265
Dates p < 0.0001
Treat. x Dates p = 0.0007
Abandonment
Cattle-
Cattle+
Root growth (g m-2 day-1)
Treat. p < 0.0001
Dates p < 0.0001
Treat. x Dates p < 0.0001
Soil temperature (°C)
Treat. p = 0.2093
Dates p < 0.0001
Treat. x Dates p = 0.3380
Relative soil water content
Aridity index (P-PET, mm)
Feb
Apr
Jun
Aug
Oct
Dec
2014
2015

Figure 2

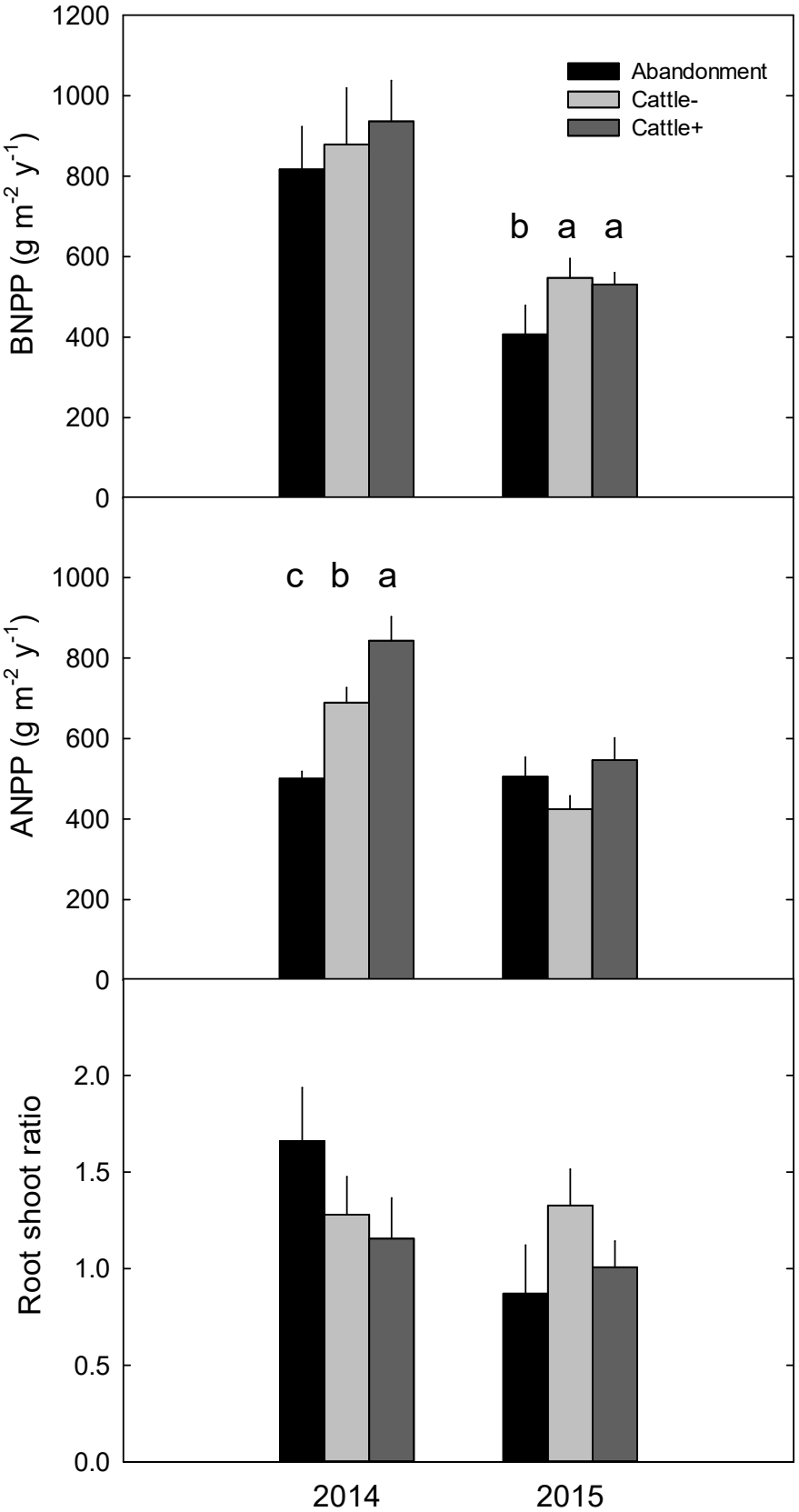
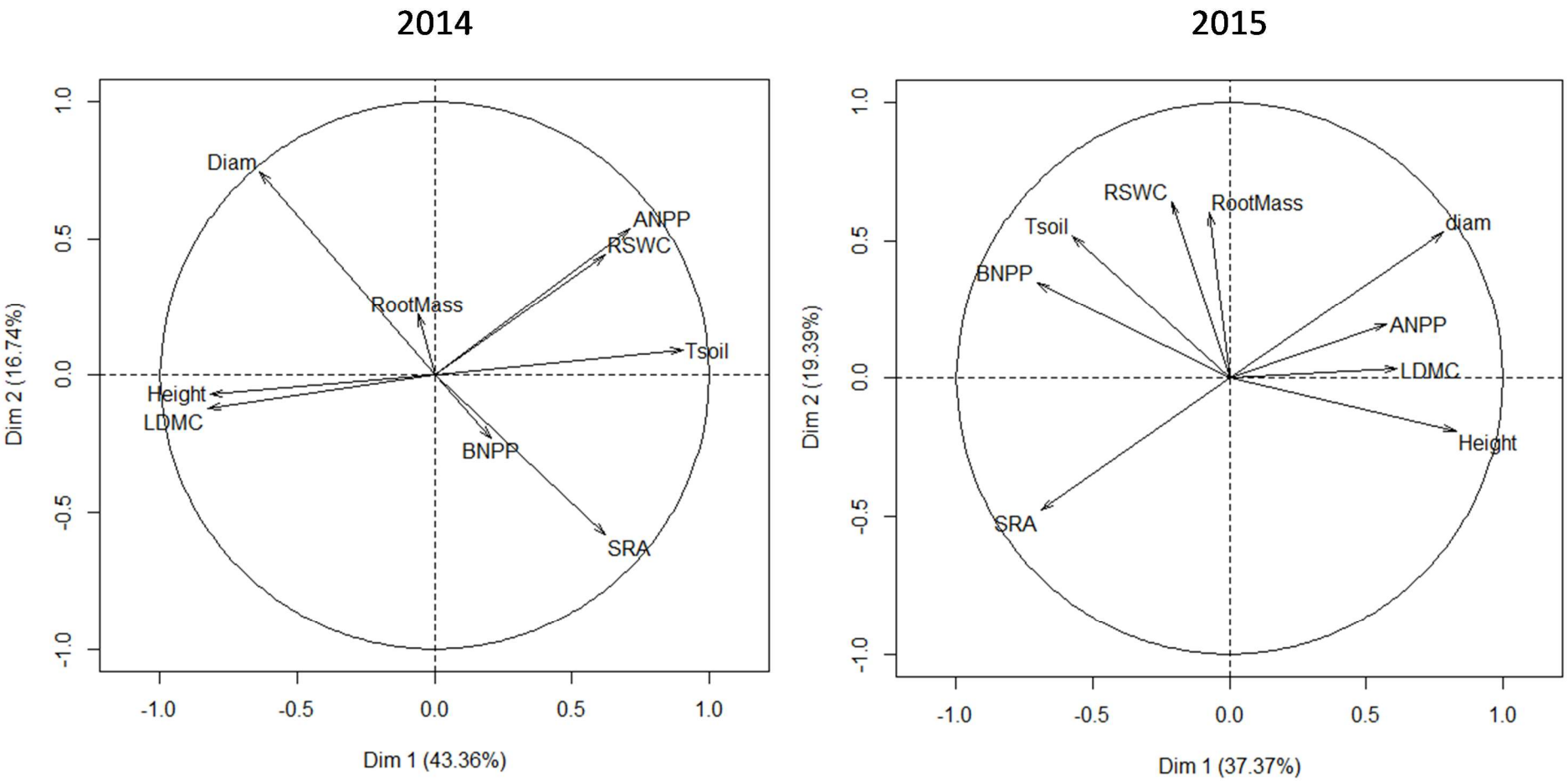

Abandonment
Cattle-
Cattle+
BNPP (g m-2 y-1)
1200
1000
800
600
400
200
0
b a a
ANPP (g m-2 y-1)
1000
800
600
400
200
0
c b a
Root shoot ratio
2.0
1.5
1.0
0.5
0.0
2014
2015

Figure 3


2014
Diam
ANPP
RSWC
RootMass
Tsoil
Height
LDMC
BNPP
SRA
Dim 2 (16.74%)
Dim 1 (43.36%)
2015
RSWC
RootMass
Tsoil
diam
BNPP
ANPP
LDMC
Height
SRA
Dim 2 (19.39%)
Dim 1 (37.37%)

826 Table S1. Repeated measure ANOVA is shown for root growth (g m^{-2} day^{-1}), soil temperature
827 (Tsoil, °C) and relative soil water content (RSWC) responses to treatment, dates (d1 to d20)
828 and interaction effects. Numerator (num), denominator (den) of degree of freedom (DF) and *F*
829 values are shown. Superscripts ^{ns}, **, *** correspond to P > 0.05, P < 0.001, P < 0.0001,
830 respectively.

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Variables	Treatment		Dates		Treat. x Dates	
	num/den DF	F-value	num/den DF	F-value	num/den DF	F-value
Root growth	2/8	1.80^{ns}	19/171	50.40^{***}	38/171	2.096^{**}
Tsoil	2/8	33.93^{***}	19/166	944.83^{***}	38/166	9.75^{***}
RSWC	2/8	1.914^{ns}	19/163	25.287^{***}	38/163	1.097^{ns}

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Table S2. Nitrogen nutrition index (NNI %, Lemaire and Gastal 1997, Cruz et al. 2006) measured on forage regrowth of May in 2014 and 2015 on the non-leguminous part to assess the effect of treatments on N availability according to grazing intensity. When legumes were below 4.5% in the herbage mass, NNI was assessed using the procedure defined by Cruz et al (2006) based on the total forage and the legume contribution. The P-values are associated with a nested mixed model: treatment used as fixed factor with plots nested in blocks as random factors. Mean ± se is shown (n = 4). For each year, different letters correspond to significant differences at P < 0.05.

Year	*P-value*	Abandonment	Cattle-	Cattle+
2014	0.146	65.64 ± 3.10 a	59.54 ± 1.78 a	63.72 ± 2.86 a
2015	0.018	69.72 ± 1.19 a	61.71 ± 1.53 b	69.25 ± 2.09 a

For each year, different letters correspond to significant differences at *: P < 0.05; **: P < 0.01; ***: P < 0.001; ns: P > 0.05.

References

Lemaire G, Gastal F (1997) N uptake and distribution on plant canopy. In: Lemaire, G (ed.) Diagnosis of the nitrogen status in crops, pp. 3-43. Springer-Verlag, Berlin, DE.

Cruz P, Jouany C, Theau J-P, Petibon P, Lecloux E, Duru M (2006) L'utilisation de l'indice de nutrition azotée en prairies naturelles avec présence de légumineuses. Fourrages 187:369-376.

Table S3. Species contribution (%) in the community present around the ingrowth core measured in April and May 2014 for Cattle-, Cattle+ and Abandonment, respectively. Mean ± se is shown (n = 4). For each species, different letters correspond to significant differences at *: P < 0.05; **: P < 0.01; ***: P < 0.001; ns: P > 0.05.

Group	Species	*P-value*	Abandonment	Cattle-	Cattle+
Grasses	*Agrostis capillaris*	ns	0.0 ± 0.0	0.6 ± 0.6	1.7 ± 1.2
	Arrhenatherum elatius	ns	10.3 ± 6.8	2.2 ± 2.2	2.5 ± 2.5
	Alopecurus pratensis	**	27.2 ± 7.9 a	7.8 ± 3.3 b	3.3 ± 1.7 b
	Dactylis glomerata	*	3.1 ± 2.7 b	22.2 ± 9.8 a	9.1 ± 3.8 ab
	Elytrigia repens	*	18.8 ± 9.9 a	2.8 ± 1.8 b	3.8 ± 2.7 b
	Schedurus arundinaceus	ns	5.0 ± 2.3	5.6 ± 2.1	6.3 ± 2.2
	Holcus lanatus	*	0.0 ± 0.0 b	4.7 ± 1.6 a	3.4 ± 1.9 a
	Lolium perenne	***	0.0 ± 0.0 b	0.9 ± 0.9 b	13.6 ± 3.8 a
	Poa pratensis	ns	11.3 ± 2.2	3.1 ± 1.5	3.4 ± 2.5
	Poa trivialis	*	0.0 ± 0.0 b	5.0 ± 2.5 a	7.2 ± 2.4 a
	Trisetum flavescens	ns	0.0 ± 0.0	2.2 ± 1.3	0.6 ± 0.4
Forbs	*Achillea millefolium*	ns	1.3 ± 0.9	3.8 ± 2.4	3.1 ± 2.3
	Anthriscus sylvestris	ns	2.5 ± 2.1	0.0 ± 0.0	0.0 ± 0.0
	Cerastium fontanum	ns	0.0 ± 0.0	1.3 ± 0.9	0.0 ± 0.0
	Cerastium glomeratum	ns	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
	Cirsium arvense	ns	5.0 ± 3.5	0.0 ± 0.0	0.0 ± 0.0
	Hypocheris radicata	ns	0.0 ± 0.0	0.9 ± 0.9	0.0 ± 0.0
	Ranunculus acris	ns	0.0 ± 0.0	0.0 ± 0.0	3.8 ± 3.8
	Stellaria graminea	ns	0.6 ± 0.6	0.6 ± 0.4	0.0 ± 0.0
	Taraxacum officinale agg.	**	0.0 ± 0.0 b	17.5 ± 1.8 a	19.1 ± 6.0 a
	Urtica dioïca	*	9.7 ± 4.9 a	0.0 ± 0.0 b	0.0 ± 0.0 b
	Veronica serpyllifolia	ns	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0
Legumes	*Lathyrus pratensis*	ns	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.3
	Trifolium pratense	ns	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
	Trifolium repens	***	0.0 ± 0.0 b	16.3 ± 4.0 a	17.7 ± 2.5 a
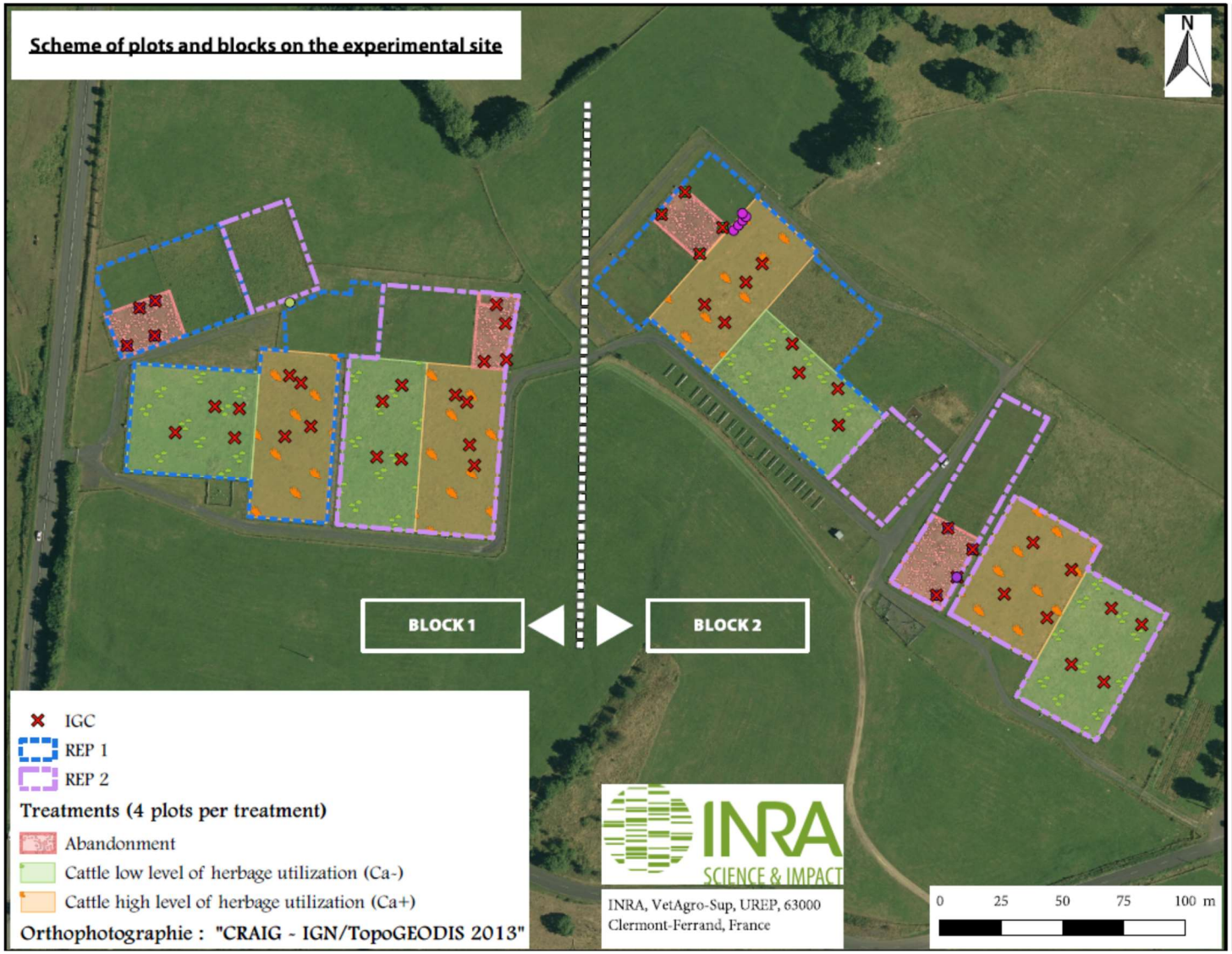
Fig S1 : Scheme of the plots and blocks on the experimental site