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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Abstract

- **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. We aim studying the effects of climatic variability and gradient of herbage utilization by grazing on root production during two years. Root and leaf traits of the communities were determined to detect their capacity to predict above and below-ground production (ANPP, BNPP).
- **Methods**. A long-term field experiment was used to compare after 10 years of treatment application, the effects of abandonment, low (Ca-) and high (Ca+) grazing intensities, respectively 15.2 cm and 7.7cm mean residual plant height, induced by grazing rotation on upland fertile grasslands. Ingrowth-cores and exclusion cages were used to measure, respectively, root and shoot mass production several times each year and at annual scale. Root and leaf traits of the communities were measured close to the vegetation peak growing season.
- **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 the two cattle treatments, possibly due to later canopy green-up induced by lower soil temperature and accumulation of litter. BNPP was slightly lower in abandonment compared to cattle treatments only during the dry year, whereas decline of ANPP in abandonment compared to Ca+ was observed the wet year. In response to drought (case of the second year), the root-to-shoot biomass ratio was stable in cattle treatments but declined in abandonment. The higher allocation to root mass can be beneficial for plant communities under drier conditions.
- **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. The stability of the root-to-shoot biomass ratio during the dry year put into evidence higher resistance to drought of grazed grassland communities compared to abandonment.

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. Root activity (growth, exudation, turnover) contributes to C and N inputs but is also a main determinant of both nutrients and water uptake, essential to fix atmospheric CO_2 and to produce biomass. Intensification of management practices may affect these services as well as climate variability (Conant et al. 2001; Jones and Donnelly 2004; Soussana and Duru 2007). Thus, improving our understanding of grassland roots dynamics under different management and climatic conditions may help to identify management options to maintain forage production and C sequestration abilities of this ecosystem and thus its sustainability.

Different practices of management modify forage production and the amount of soil C and N 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 two meta-analyses emphasized negative effect of grazing intensity on above- and below-ground carbon stocks compared to ungrazed systems (Zhou et al. 2017; Li et al. 2018). In addition, repeated defoliations induced by grazing or mowing of grassland can simultaneously increase soil temperature and soil moisture (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 and by changes of leaf area index after defoliation (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) whereas at the opposite extensive practices (low grazing intensity, absence of fertilization) favour slow growing species (conservative strategy) (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 intensive grazed 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 (Louault et al. 2005).

99 Increased climate variability is another source of response uncertainty in managed
100 ecosystems. As more frequent and longer period of drought associated with heat waves may
101 threaten and shape the long-term dynamics of perennial ecosystems such as grasslands
102 (Brookshire and Weaver 2015), it is important to understand how above- and below-ground
103 compartments respond to climate variability. However, there are few data on above- and below-
104 ground biomass responses to drought for grassland (Byrne et al. 2013; Wilcox et al. 2015; Li et
105 al. 2018), although some evidence shows that the 'slow' trait strategy (resource conservation)
106 is associated with drought tolerance (Pérez-Ramos et al. 2012; Reich 2014). Changes in root
107 morphology and functioning may thus be important determinants in plant adaptive strategies to
108 drought, and have been less studied than above-ground plant responses (Biswell and Weaver
109 1933; Dawson et al. 2000; McInenly et al. 2010). However, there are not enough data to make
110 generalizations about combined impacts of management and climatic conditions variability
111 such as precipitation reduction on root and shoot biomass production and plant traits defining
112 plant strategies related to resource use and grazing intensity.

113 The study was carried out in a long-term field experiment for which controlled grazing
114 intensity had been applied for 10 years. We compared abandonment of grazing and two levels
115 of herbage utilization by grazing based on five rotations per year. In two consecutive years, the
116 ingrowth core method was used to measure monthly root biomass production and calculate
117 annual root production (BNPP); ANPP was measured by grazing exclusion cages and
118 community-weighted mean leaf and root traits were assessed the first year. We tested the
119 following hypotheses: (i) high grazing intensity increases above-ground mass at the expense of
120 root production as a result of the direct negative effect of defoliation on root growth, whatever
121 the climatic conditions, (ii) inter-annual climatic conditions modulate above and below-ground
122 biomass production response to grazing intensity as a consequence of higher presence of
123 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 precipitation 784 mm, Table 1). The site supports mesotrophic multi-specific permanent grassland, dominated by species with high Ellenberg indicator values for N (Schaffers and Sykora 2000), indicating a high level of fertility for the site (Table S1; Louault et al. 2017). The soil is a cambisol with a sandy loam texture, developed on granitic bedrock. Differences in local soil composition and profile led us to consider two blocks characterized respectively by a eutric cambisol (54% sand; 26% silt; 20% clay; 7.0% organic matter; pH: 5.9) and a colluvic cambisol (50% sand; 26% silt; 24% clay; 7.4% organic matter; pH: 6.0) including some volcanic materials.

Management

Prior to the installation of this experiment in 2005, the study area had been used for intensive hay and silage production (combining grazing, mowing and fertilization), with mineral fertilization, and two years preceding the start of the experiment (2003 and 2004), the grassland site was mown three times per year without fertilization. Then, from 2005, the grassland had been managed for 10 years with a gradient of grazing intensity resulting from three treatments: abandonment (Ab), low (Cattle-) and high (Cattle+) level of herbage utilization obtained by modification of stocking density (6.9 and 13.8 LSU ha^{-1}, livestock unit, respectively) with five

grazing rotations each year: mid-April, late May, early July, September and November, lasting on average 9.6, 9.0, 10.7, 8.6, and 2.1 days, respectively. The two cattle treatments corresponded to two levels of herbage utilization by grazing, and had on average 15.2 ± 0.5 cm (mean ± se, Cattle-) and 7.7 ± 0.2 cm (Cattle+) residual plant height at the end of each grazing rotation, respectively. For each treatment, two replicate plots were set up per block, resulting in four replicates per treatment, and a total of 12 plots (2 blocks x 2 plots x 3 treatments). The average distance between the two blocks is about 230 m and all treatments are randomized within each block. The size of the plots differs according to treatments: 2200 m^2 for the two cattle treatments and 400 m^2 for the abandonment.

Climatic and edaphic conditions

Daily precipitation (mm) and air temperature (°C) were measured for the two years, and recorded with a meteorological station located at the site. An aridity index was calculated as precipitation minus potential evapotranspiration (P - PET, mm) with the Penman-Monteith equation. Daily soil temperature (°C) was measured with thermocouple sensors (home-made copper-constantan sensors) inserted at 20 cm depth in each plot and recorded with a HOBO data logger (U12-014, Onset Instruments, MA, USA). Daily soil volumetric water content (SWC, m^3 m^{-3}) of each plot was measured with two probes (ECHO-10, Decagon, USA), inserted horizontally at 20 cm depth, and connected to dataloggers (EM5 and EM50, Decagon, USA). From January 2014 to November 2015 (DOY 132–326), SWC was measured every 30 min and averaged at daily scale. For each plot, average values of the two probes were used. Daily relative soil water content data are shown and calculated as the ratio:

$RSWC = \frac{\mathrm{SWC - SWCmin}}{\mathrm{SWCmax - SWCmin}}$, where SWC is the soil moisture at a given day, SWCmin is the minimum value of soil moisture and SWCmax is the maximum value of soil moisture, both

observed during the two years. For soil temperature and RSWC, values were averaged according to root growth time scale.

Root growth and root mass

Six months beforehand, shallow (0-20 cm) soil was collected on each of the two blocks of the site and sieved (5 mm mesh size) to remove stones and coarse organic matter, and then left unused outside covered under a shelter and protected from direct sunlight. Thereafter, this air-dried soil was used to fill the ingrowth-core each month.

In December 2013 and for each of the 12 plots, soil cores were collected with an auger (8 cm diameter, 0-20 cm depth) at four locations representative of the plant community in the treatment. On average mean distance between locations are 19.8 m ± 0.2, 21.7 m ± 0.1 and 17.2 m ± 0.2 for Ca+, Ca- and Ab (mean ± SD, see Fig S1), respectively. After core harvest, each hole was filled with a plastic net (8 mm mesh size) containing a fixed volume of air-dried sieved soil (ingrowth core), collected six months beforehand. Then, about each month and for two years (2 x 10 times), ingrowth cores, containing soil and the root and rhizome material that had grown therein, were extracted, and then replenished with another fixed volume of dry sieved soil. Thus monthly and annual root production (BNPP, g m^{-2} y^{-1}) were measured from February 2014 to December 2015. Root production period ranged on average 36.5 days, but with longer and shorter periods in winter and spring-summer, respectively (Table 1). In periods with absence of precipitation, a fixed volume of water was added to adjust soil humidity to field conditions. After collection, the ingrowth cores were transported to the laboratory and immediately stored at 4 °C before processing in the next five days. The roots were washed under tap water and with a 200 µm sieve, and then oven-dried (48 h, 60 °C).

In order to measure root mass stock, soil cores were collected three times (December 2013, March and June 2014) with the same auger and near the ingrowth cores locations. These

samples were stored in the freezer (-18°C), and after defrosting, the roots were washed with the same procedure as that used for the ingrowth cores, and then oven-dried (48 h, 60 °C).

Root traits

Subsamples of washed roots collected with the ingrowth cores collected in June 2014, were fresh weighed, and then frozen (-18 °C) before morphology analysis. After defrosting, roots were stained with methylene blue (5 g L^{-1}) for about 5-10 minutes, rinsed in water, spread in a transparent glass box containing a thin layer of water, and covered with a transparent plastic sheet. High resolution images were recorded with a double light scanner (800 dpi, perfection V700, Epson, JA) and analyzed with WinRhizo software (PRO 2012b, Regent Instruments, CA) with the automatic procedure. Two scans per location were recorded and separately analyzed to measure root length (m), root volume (cm^3), root surface area (m^2), average root diameter (mm) and length by class diameter (13 classes: 11 with 0.1 mm interval and 2 with 0.5 mm interval). Specific root length (m g^{-1}), root tissue density (g cm^{-3}) and specific root area (m^2 g^{-1}) were calculated for fine roots as in Picon-Cochard et al. (2012).

Botanical composition

Species contribution (%) was visually observed on a circle (20 cm diameter) around each ingrowth core location in April (cattle treatments) and May (abandonment) 2014. For each zone, a score on a ten-point scale was allocated to species present according to their volume occupancy, and the percentage of each species was calculated at the plot scale by averaging values of the four zones. The list of species and their relative contributions is given in Table S3.

223 Above-ground biomass production

224 On each plot and on each sampling date, four fenced sampling areas (0.6 × 0.6 m) were used to
225 measure accumulation of above-ground biomass after above-ground standing biomass was
226 clipped at 5.5 cm. At each sampling date, biomass was sampled at a height of 5.5 cm, oven-
227 dried and weighed. Measurements were made five times in the course of the year, before each
228 grazing event in Cattle+ and Cattle- plots, and three times (spring, summer, autumn) in
229 abandonment plots. Sampling areas were moved within the plot at each measurement date
230 during the year. Annual above-ground net primary production (ANPP, g m^{-2} y^{-1}) was calculated
231 as the sum of the successive biomass accumulations along the year.

232

233 Leaf traits

234 Community-weighted mean (CWM) trait values of leaf dry matter content (LDMC), specific
235 leaf area (SLA) and reproductive plant height (H) were calculated for each ingrowth core zone
236 using (i) the relative contribution of the dominant species to the community (i.e. species that
237 account for at least 85% of the cumulated species contribution of the community) measured in
238 2014, and (ii) leaf trait measurements made at plot scale in 2006 and 2007. Traits were measured
239 on ten vegetative plants using standard protocols (see methods in Louault et al. 2005).
240 Reproductive plant height was measured on mature plants located in fenced zones to allow full
241 plant development. CWM is expressed with the following equation: CWM = $\sum p_i \times \text{trait}_i$,
242 where p_i is the relative contribution of species i to the community and trait_i the trait of species
243 i.

244

245 Statistical analyses

246 For a given date, root mass and root traits collected at each location (four ingrowth-cores in
247 each plot), averages of data coming from the four locations were used to have a single value for

each of the 12 plots and test for the effect of treatment and dates. Before ANOVA, normality of residuals was inspected with quantile-quantile plots of model residuals, and variance homogeneity was confirmed by checking the plots of model residuals *vs.* fitted values. Data were transformed if they deviated from ANOVA assumptions (square root, ln, reciprocal). Linear mixed effects models as available in the R 'nlme' package (Pinheiro et al. 2015) were used to perform repeated measure ANOVAs to test the effects of treatments, dates and their interactions on values of root growth, soil temperature, RSWC, and root mass stock, with plots nested in block as a random factor accounting for temporal pseudo-replication. For root growth dynamics, soil temperature and RSWC (Fig 1, Table S2), dates correspond to 20 dates and for 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). This statistic approach allows comparing sets of traits and properties relationships in order to detect response and effect traits, but also to analyse multiple dimensions of traits relationships, not possible with pairs of correlation.

All statistical analyses were performed in the R environment (version 3.5.2, R Core team 2012) using RStudio (Version 1.1.463). Scripts are shown in Table S4.

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

Soil temperature was significantly affected by treatment, dates and treatment × dates (Figure 1; Table S2). For most of the dates (February to October), abandonment treatment had lower soil temperature (1.76 °C, on average) than the grazing treatments, whereas the Cattle- treatment showed significant lower soil temperature (-0.64 °C) than the Cattle+ treatment. However, this was significantly observed for a limited number of dates in early summer of both years. Relative soil water content (RSWC) fluctuated from 0.6-0.7 at the beginning of spring to 0.38 in June in the wet year and to 0.2 during the dry year, which is in accordance to variation of the atmospheric aridity index (P-PET). In the case of the dry year, from summer until autumn, RSWC remained lower than 0.4 and the aridity index was negative.

298 Root growth dynamics

299 Root growth was affected by date and treatment × date interaction (Figure 1). Each year, peak
300 of root growth occurred twice, in spring and autumn, and growth was markedly reduced in
301 summer and winter. Only in the second year did growth stop in summer, and it was significantly
302 lower than the first year. Regarding treatment effect, abandonment showed significant lower
303 root growth than the two grazing treatments for the spring period in both years, and for the
304 autumn of the second year. While in autumn 2014, a delay of growth peaks was always
305 observed, which led to a two-fold higher root growth for abandonment *vs.* the two cattle
306 treatments (end of September: date 8). The two grazing treatments had similar root growth
307 across years and seasons.

308

309 Seasonal root mass stock, BNPP, ANPP and root-to-shoot biomass ratio

310 Stock of root mass did not change through season and across treatment (Table 2). BNPP, ANPP
311 and root-to-shoot biomass ratio (R/S) were significantly lower during the second year, with a
312 stronger effect on BNPP (-44% on average) than ANPP (-24%) (Figure 2, Table 3). Only the
313 abandonment treatment maintained their value of ANPP in the second year, which led to a 48%
314 decline in R/S (significant treatment × year, $P < 0.01$, Table 3). Accordingly, treatment effect
315 was only observed for BNPP the second year, with a decline of 24% for abandonment compared
316 to cattle grazing treatments and for ANPP the first year: Cattle+ having 22% and 68% higher
317 values than Cattle- and abandonment, respectively, while Cattle- had 38% higher ANPP than
318 abandonment.

319

320 Species composition, leaf and root traits

321 Abandonment treatment was characterized by the dominance of tall grass species: 76% in all
322 with 27.2% of *Alopecurus pratensis*, 18.8% of *Elytrigia repens*, 11.3% of *Poa pratensis* and

323 10.3% of *Arrhenatherum elatius*, the presence of some forbs (19%) and the absence of legumes
324 (Table S3 and Table 4). The two cattle treatments differed from abandonment treatment by
325 equal presence of *Taraxacum officinale* (18% on average) and *Trifolium repens* (17% on
326 average). Difference also concerns grass species (56% in total) with the dominance of *Dactylis*
327 *glomerata* (22.2%), *A. pratensis* (7.6%) and *Festuca arundinacea* (5.6%) for Cattle- and *Lolium*
328 *perenne* (13.6%), *D. glomerata* (9.1%) and *Poa trivialis* (7.2%) for Cattle+. Thus, the Cattle+
329 treatment had a higher percentage of *L. perenne* than Cattle- (Table S3).
330 Community-weighted mean leaf traits (CWM) were significantly modified by the
331 treatments. Plant height and LDMC were significantly higher ($P < 0.05$ and $P < 0.0001$,
332 respectively; Table 4) in abandonment than in the two cattle grazed treatments, whereas SLA
333 was lower ($P < 0.05$). Unlike leaf traits, root traits were only slightly affected by the treatments.
334 Specific root length (SRL, $P < 0.1$) and specific root area (SRA, $P < 0.05$) were lower in
335 abandonment treatment than in Cattle-, but not Cattle+. For other root traits (diameter, RTD
336 and root length % by class diameter) no between-treatment differences were observed (Table
337 4).
338
339 Co-variation of traits and production
340 The two main axes of the standardized PCA explained 60.1% and 56.8% of the community trait
341 and production variation in 2014 and 2015, respectively (Figure 3). For the first year, the first
342 PCA axis (PC1), accounting for 43.4% of the total variation, was significantly related to leaf
343 and root traits, ANPP and soil temperature. Soil temperature, SRA and ANPP had positive
344 loadings, and diameter, plant height and LDMC had negative loadings (Table 5). The second
345 PCA axis (PC2), accounting for 16.7% of the total variation, was significantly and positively
346 related to root diameter and negatively to SRA. For the second year, the first PCA axis (PC1),
347 accounted for 37.4% of the total variation, and was significantly related to leaf and root traits,

348 ANPP and BNPP. BNPP and SRA had negative loadings, and root diameter, plant height and
349 ANPP had positive loadings (Table 5). The second PCA axis (PC2), accounting for 19.4% of
350 the total variation, was significantly and positively related to RSWC and stock of root mass
351 averaged across three dates. Finally, abandonment treatment was significantly related to PC1s
352 with negative and positive loadings for the first and the second year, respectively.
353
354 **Discussion**
355 Ten years of contrasted management had strongly modified the functional diversity and above-
356 ground production of this fertile upland grassland (Herfurth et al. 2015; Louault et al. 2017).
357 Accordingly, we expected that above-ground biomass patterns would be mirrored below-
358 ground, especially during the periods of grazing. Here we first discuss within-year differences
359 of root growth, followed by inter-annual variation responses to grazing intensity and climatic
360 conditions variability between the two contrasting years, and last we analyze relationships
361 between traits and above- and below-ground production.
362
363 <u>Seasonality of root growth was independent of grazing intensity and climatic conditions</u>
364 As expected, root growth of permanent grassland is affected by seasons and peaks in spring and
365 autumn (Garcia-Pausas et al. 2011; Pilon et al. 2013; Steinaker and Wilson 2008), but
366 unexpectedly, grazing pressure applied by rotations and climatic conditions variability had very
367 limited effects on this seasonality. This means that at below-ground level, plant community
368 behavior was not affected by rotational grazing management nor by climatic conditions
369 variability, although a severe drought occurred in summer of the second year. Only the
370 abandonment treatment showed a delayed root growth peak in spring. This delay is probably
371 the result of slower shoot budburst and reduced capacity to produce new green leaves in dense
372 litter canopy, especially at the beginning of the growing season in spring (data not shown).

373 Moreover, the tall and dense canopy of the abandonment treatment strongly modified soil
374 temperature, with cooler soil conditions as expected in such abandoned vegetation (Picon-
375 Cochard et al. 2006; Zhou et al. 2017; Zhu et al. 2016). As shown in some studies, light or soil
376 water and nutrient availabilities (Edwards et al. 2004; Garcia-Pausas et al. 2011; Steinaker and
377 Wilson 2008) are other abiotic factors determining dynamics of root growth in grasslands, as
378 root peaks were observed before the peak of soil temperature in summer when negative climatic
379 water balance occurred, especially in the second year. Nevertheless, plants growing in
380 abandonment offset their slower root growth by producing similar root biomass at annual scale,
381 especially during the wet year. The presence of tall grass species such as *A. pratensis*, *A. elatius*
382 and *E. repens* with plant trait syndromes related to both disturbance and resource conservation
383 strategies (lower SLA and SRL and higher plant height and root depth; Pagès and Picon-
384 Cochard 2014) might explain their capacity to produce higher root biomass on a shorter-term
385 period before canopy senescence onset. Also pre-existing soil fertility can be maintained in
386 conditions of very low levels of herbage utilization (near-abandonment), because of the absence
387 of biomass exportation and increased internal recycling of N within senescent plants, both
388 contributing to an increase in total N available for plant growth (Loiseau et al. 2005).

389 The similar root growth dynamics of the two cattle treatments was unexpected, considering
390 that infrequent defoliation and moderate excreta returns to the soil might increase root biomass
391 production at the expense of shoot biomass (Klumpp et al. 2009). The absence of effect on root
392 growth and BNPP means that grazing applied on plant communities by rotations was too short
393 but enough to observe effect on ANPP, in wet conditions. Worldwide there are different ways
394 to manage grassland by grazing (Huyghe et al. 2014), rotational or permanent grazing options
395 with different stocking densities, durations, types of herbivores. In general, this management
396 creates high spatial heterogeneity within the plots due to animals' selective defoliation of plant
397 species, and also because returns to soil are spatially heterogeneous. Thus in grazed grassland,

398 disturbance creates patches of vegetation, which should affect locally root growth and below-
399 ground biomass of plant communities if intensity of grazing is sufficient. The complexity of
400 these phenomena in grazed grassland is greater than in mown systems owing (Rossignol et al.
401 2011).

402 Then, again, the confounding effect of soil fertility and defoliation may mask a clear
403 response of the below-ground compartment in grazed grasslands. In view of that, we postulate
404 that root growth in Cattle+ treatment was favored by the higher soil temperature compensating
405 for the negative effects of frequent defoliation on root growth while the cooler soil conditions
406 encountered in Cattle- might have slowed root growth. Soil moisture is a main determinant of
407 plant growth and can be affected by cattle treatments. Some studies showed an increase in
408 grazed compared ungrazed treatment due to lower leaf area index in the grazed conditions
409 (Moretto et al. 2001; Pineiro et al. 2010), or an absence of effects in others (LeCain et al. 2002;
410 Smith et al. 2014). The presence of herbivores can increase soil bulk density and consequently
411 modify soil moisture. In our field conditions and after 10 years of treatments application, the
412 absence of effect on soil moisture can be due to several reasons. A first determinant to consider
413 is the soil density, which is expected to be higher in grazed plots, but in case of rotational
414 grazing this effect is less clear as cattle spend less time than in continuous grazing systems. A
415 second determinant is the functional composition of community regarding both the response to
416 defoliation and their water use strategies. A last determinant of this response can be linked to
417 the temporal scale used (monthly-based) which could buffer shorter-term response.

418 We should also consider the level of soil fertility and species composition as drivers of root
419 growth and trait plasticity (Dawson et al. 2000). The soil fertility of our site, reflected by the
420 nitrogen nutrition index (NNI, Lemaire and Gastal 1997), was very similar along our grazing
421 intensity gradient (Table S1), at least in 2014. Thus in our site we had the opportunity to
422 compare grazing intensity effect at equivalent soil fertility. Knowing that root trait plasticity

423 generally shows larger differences with respect to soil fertility than by cutting or defoliation
424 (Leuschner et al. 2013; Picon-Cochard et al. 2009), we can expect that under similar soil fertility
425 grazing intensity had a less pronounced effect on root growth. Indeed, the higher presence of
426 species tolerating defoliation, with shorter stature and root system (*L. perenne*, *P. trivialis*), but
427 having higher shoot and root growth capacity after defoliation and also higher rhizosphere
428 activity (Dawson et al. 2000), probably compensated for the negative effect of defoliation in
429 the Cattle+ treatment. Also the sampling depth might have had an effect, as we expect that
430 harvesting root systems deeper than 20 cm should give more contrasting root growth response
431 across the two cattle treatments according to the grass species composition due to species-
432 specific differential root depth distribution (Xu et al. 2014). Taken together, we provide
433 evidence that higher soil temperature, high soil fertility and species composition have
434 moderated root growth response along our grazing intensity gradient. The difficulty to assign
435 species composition in root mixtures, however, makes it difficult to draw firm conclusions.
436
437 Climatic conditions variability shaped responses of ANPP, BNPP and root-to-shoot biomass
438 production ratio along the grazing intensity gradient
439 According to meta-analyses and recent results (McSherry and Ritchie 2013; Zeng et al. 2015;
440 Zhou et al. 2017; Li et al. 2018), grazing intensity generally has negative effects on above- and
441 below-ground biomass of grasslands whatever the climatic conditions or vegetation type,
442 although these effects can be modulated by levels of grazing intensity. Our results do not
443 confirm these findings, because ANPP and BNPP increased in response to grazing intensity
444 compared to abandonment, in the wet and the dry year, respectively. Methodology issues for
445 estimating ANPP and BNPP in grazed grasslands should thus be taken into account, as some
446 papers report either biomass stock or fluxes measured once at peak of growth or at several
447 periods (Scurlock et al. 2002), but also estimation of BNPP from indirect measurements (e.g.

448 Zeng et al. 2015). Mass based on stock gives a snapshot of plant functioning, generally
449 including mixtures of living and senescent tissues, thus depending on abiotic factors and plant
450 growth, whereas measurements based on new shoot and root biomass reflect the growth
451 potential of grasslands. We are aware that these methods are very different, but in response to
452 grazing intensity, BNPP measured with ingrowth cores gave similar results as root mass stock
453 assessed at three seasons. Another point to consider is the number of samples used to compare
454 treatments and detect significant differences. In grasslands, the coefficient of variation of root
455 dry weight in auger samples from grassland are generally between 30 and 50% (Bengough et
456 al., 2000). According to these authors, our sampling protocol (with 16 samples) is adapted to
457 detect at least 35% differences between treatments, whereas to detect less than 10% differences,
458 more than 100 replicates should be collected. Thus, we cannot rule out that collecting more
459 samples should have highlighted significant differences across treatments. Nevertheless, we
460 had to find compromise between more frequent samplings (20 dates) to study seasonal
461 dynamics of root growth compared with more samples collected at plot level but less frequently.
462 Climatic aridity (P-PET) had stronger effects on ANPP and BNPP than grazing intensity,
463 because severe drought had a direct negative effect on plant growth. In comparison with another
464 experiments located alongside ours, 80% of canopy senescence was reached for a cumulated
465 aridity of -156 mm (Zwicke et al. 2013). As this index reached -303 mm from March to August,
466 this confirmed that a severe drought occurred in the second year of our experiment, and
467 explained root growth cessation in summer. At annual scale, ANPP of the two cattle treatments
468 showed lower resistance to increased aridity (resistance defined as $ANPP_{year2}$ / $ANPP_{year1}$, being
469 equal to 0.63) than abandonment treatment (ratio=1). For BNPP, results were inversed, leading
470 to a lower resistance of root-to-shoot biomass ratio in abandonment than in the two cattle
471 treatments. The absence of root growth modification by grazing at annual scale the wet year
472 reflects well the change in root-to-shoot biomass allocation, albeit not significant. Other

473 processes such as root turnover (mortality, rhizodeposition) are expected to change in grazed
474 *vs.* ungrazed grassland. For our site Herfurth et al. (2015) observed similar root mass stock
475 along a grazing disturbance gradient as in the present study, but by using a simplified C flux
476 model, these authors showed that the Cattle+ treatment tended to accelerate C cycling in plant
477 communities, resulting in a higher quantity of C allocated to the soil organic matter continuum.
478 Taken together, these results suggest that the slight BNPP increase under grazing may occur
479 with an increase in rhizodeposition, because root turnover calculated as BNPP to root mass
480 stock ratio (data not shown, Lauenroth and Gill 2003) was not different across treatments.
481 Furthermore, our results suggest that grazing treatments slow down the negative effect of
482 aridity on the root-to-shoot biomass ratio, underling that these treatments seem to be better
483 adapted to buffering the negative effect of drought on grassland root production than for
484 abandoned grasslands. This is consistent with previous work showing that moderate grazing
485 could be more beneficial than no grazing for drought resistance and recovery of ANPP and
486 BNPP (Frank 2007; Xu et al. 2012), and that BNPP was more resistant than ANPP to change
487 in precipitation (Yan et al. 2013). Other studies showed no prevalence effects of grazing,
488 drought or fire observed on grassland production in North America and South Africa (Koerner
489 and Collins 2014). Nevertheless, this points to a need for further research to determine whether
490 grazing pressure has additive or combined effects on drought response of grasslands (Ruppert
491 et al. 2015).
492
493 <u>Community-weighted mean leaf and root traits as predictors of ANPP and BNPP</u>
494 As shown by other studies (e.g. Diaz et al. 2007; Laliberté and Tylianakis 2012; Louault et al.
495 2017; Zheng et al. 2015), disturbance induced by grazing pressure has profound effects on plant
496 community and functional traits by selecting tolerant species to defoliation such as *L. perenne*,
497 *P. trivialis* or *T. repens*, with possible cascading effects on multiple ecosystem functions. With

498 the capacity to regrow quickly after defoliation, these species generally exhibited high values
499 of SLA and low values of LDMC and plant height. They contrast with species adapted to fertile
500 soil, but with a slower regrowth capacity after defoliation such as *D. glomerata* or *F.*
501 *arundinacea*, with opposite leaf trait values. In abandonment, competition for light tends to
502 select plants with trait syndromes related to disturbance and conservative strategies (tall plants,
503 low SLA and high LDMC values). Thus, the CWM traits of the community will depend on the
504 balance between these species groups, which are expected to affect ANPP and BNPP (Klumpp
505 et al. 2009; Milchunas and Lauenroth 1993). Although the presence of tolerant and intolerant
506 species to defoliation in both cattle treatments, leaf trait values were similarly and positively
507 related to ANPP, and only differed from traits of species present in the abandonment treatment.
508 This means that cessation of grazing strongly differentiated plant communities, whereas within
509 the two cattle treatments differences were slighter.

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

with fine roots such as *D. glomerata* or *H. lanatus* (Picon-Cochard et al. 2012), because soil fertility approximated by NNI was near comparable across treatments.

Conclusions

Near similar functional traits 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. 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 at larger time scale on the grazing practices allowing higher resilience of grasslands to more frequent and intense climatic events such as drought and heat waves.

Data accessibility

Data are available online: https://zenodo.org/record/4034903#.YA129-fjJPZ

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Conflict of interest disclosure

The authors of this preprint declare that they have no financial conflict of interest with the content of this article

References

Bardgett RD, Wardle DA (2003) Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258-2268

Bengough AG, Castrignano A, Pagès L, van Noordwijk M. 2000. Sampling strategies, scaling, and statistics. In: Smit AL, Bengough AG, Engels C, van Noordwijk M, Pellerin S, van de Geijn SC, eds. *Root Methods.* Berlin, Germany: Springer, 147-173.

Biswell H, Weaver JE (1933) Effect of frequent clipping on the development of roots and tops of grasses in prairie sod. Ecology 14:368-390

Brookshire ENJ, Weaver T (2015) Long-term decline in grassland productivity driven by increasing dryness. Nature Commun 6:7148

Byrne KM, Lauenroth WK, Adler PB (2013) Contrasting Effects of Precipitation Manipulations on Production in Two Sites within the Central Grassland Region, USA. Ecosystems 16:1039-1051

Conant RT, Paustian K, Elliott ET (2001) Grassland management and conversion into grassland: effects on soil carbon. Ecol App 11:343-355

Dawson LA, Grayston SJ, Paterson E (2000) Effects of grazing on the roots and rhizosphere of grasses. In: Lemaire G, Hodgson J, de Moraes A, Nabinger C, De F. Carvalho PC (eds) Grassland ecophysiology and grazing ecology. CABI Publishing, Wallingford, pp 61–84

Diaz S, Lavorel S, McIntyre S, Falczuk V, Casanoves F, et al. (2007) Plant trait responses to grazing-a global synthesis. Glob Chang Biol 13:313-341

Duru M, Balent G, Gibon A, Magda D, Theau JP, Cruz P, Jouany C (1998) Fonctionnement et dynamique des prairies permanentes. Exemple des Pyrénées centrales. Fourrages 153:97-113

Edwards EJ, Benham DG, Marland LA, Fitter AH (2004) Root production is determined by radiation flux in a temperate grassland community. Glob Chang Biol 10**:**209-227

Frank DA (2007) Drought effects on above-and belowground production of a grazed temperate grassland ecosystem. Oecologia 152:131–139

Garcia-Pausas J, Casals P, Romanyà J, Vallecillo S, Sebastià M-T (2011) Seasonal patterns of belowground biomass and productivity in mountain grasslands in the Pyrenees. Plant Soil 340:315–326

Herfurth D, Vassal N, Louault F, Alvarez G, Pottier J, Picon-Cochard C, Bosio I, Carrère P (2015) How does soil particulate organic carbon respond to grazing intensity in permanent grasslands? Plant Soil 394:239-255

Huyghe C, De Vliegher A, van Gils B, Petters A (2014) Grasslands and herbivore production in Europe and effects of common policies. In, Huyghe C, De Vliegher A, van Gils B, Petters A eds, Quæ editions, Versailles, France, 287pp.

Jones MB, Donnelly A (2004) Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO_2. New Phytol 164:423-439

Klumpp K, Fontaine S, Attard E, Le Roux X, Gleixner G, Soussana JF (2009) Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. J Ecol 97:876–885.

596 Klumpp K, Soussana J-F (2009) Using functional traits to predict grassland ecosystem change:
597 a mathematical test of the response-and-effect trait approach. Glob Chang Biol 15:2921-
598 2934
599 Koerner SE, Collins SL (2014) Interactive effects of grazing, drought, and fire on grassland
600 plant communities in North America and South Africa. Ecology 95:98-109
601 Laliberté E, Tylianakis JM (2012) Cascading effects of long-term land-use changes on plant
602 traits and ecosystem functioning. Ecology 93:145–155
603 Lauenroth WK, Gill R (2003) Turnover of root systems. In: de Kroon H, Visser EJW (eds) Root
604 Ecology, Springer-Verlag, Berlin, pp 61-83
605 LeCain DR, Morgan JA, Schuman GE, Reeder JD, Hart RH (2002) Carbon exchange and
606 species composition of grazed pastures and exclosures in the shortgrass steppe of
607 Colorado. Agriculture, Ecosystems & Environment 93(1): 421-435.
608 Lemaire G, Gastal F (1997) N uptake and distribution on plant canopy. In: Lemaire, G (ed)
609 Diagnosis of the nitrogen status in crops, Springer-Verlag, Berlin, pp 3-43
610 Leuschner C, Gebel S, Rose L (2013) Root trait responses of six temperate grassland species to
611 intensive mowing and NPK fertilisation: a field study in a temperate grassland. Plant Soil
612 373:687-698
613 Li, W., X. Li, Y. Zhao, S. Zheng, and Y. Bai. 2018. Ecosystem structure, functioning and
614 stability under climate change and grazing in grasslands: current status and future
615 prospects. Curr Opin Environ Sustain 33:124-135.
616 Loiseau P, Louault F, Le Roux X, Bardy M (2005) Does extensification of rich grasslands alter
617 the C and N cycles, directly or via species composition? Basic App Ecol 6:275-287
618 Louault F, Pillar VD, Aufrere J, Garnier E, Soussana JF (2005) Plant traits and function types
619 in response to reduced disturbance in a semi-natural grassland. J Veg Sci 16:151–160

620 Louault F, Pottier J, Note P, Vile D, Soussana JF, Carrère P (2017) Complex plant community
621 responses to modifications of disturbance and nutrients availability in productive
622 grasslands. J Veg Sci, in press
623 McInenly LE, Merrill EH, Cahill JF, Juma NG (2010) Festuca campestris alters root
624 morphology and growth in response to simulated grazing and nitrogen form. Funct Ecol
625 24:283-292
626 McSherry ME, Ritchie ME (2013) Effects of grazing on grassland soil carbon: a global review.
627 Glob Chang Biol 19:1347–1357
628 Milchunas DG, Lauenroth WK (1993) Quantitative effects of grazing on vegetation and soils
629 over a global range of environments. Ecol Mono 63:327-366
630 Moretto AS, Distel RA, Didoné NG (2001) Decomposition and nutrient dynamic of leaf litter
631 and roots from palatable and unpalatable grasses in a semi-arid grassland. App Soil Ecol
632 18: 31–37
633 Pagès L, Picon-Cochard C (2014) Modelling the root system architecture of Poaceae. Can we
634 simulate integrated traits from morphological parameters of growth and branching? New
635 Phytol 204:149-158
636 Pérez-Ramos IM, Roumet C, Cruz P, Blanchard A, Autran P, Garnier E (2012) Evidence for a
637 'plant community economics spectrum' driven by nutrient and water limitations in a
638 Mediterranean rangeland of southern France. J Ecol 100:1315-1327
639 Picon-Cochard C, Coll L, Balandier P (2006) The role of below-ground competition during
640 early stages of secondary succession: the case of three-year-old Scots pine (*Pinus*
641 *sylvestris* L.) seedlings in an abandoned grassland. Oecologia 148:373-383
642 Picon-Cochard C, Pilon R, Revaillot S (2009) Plasticity of grass root functional traits and root
643 mass in response to cutting frequency and N fertilisation. Proceedings of the 7th ISRR

Symposium, Root Research and Applications (RootRAP), Vienne September 2–4, 2009. 4pp

Picon-Cochard C, Pilon R, Tarroux E, Pagès L, Robertson J, Dawson L (2012) Effects of species, root branching order and season on root traits of 13 perennial grass species. Plant Soil 353:47-57

Pilon R, Picon-Cochard C, Bloor JMG, Revaillot S, Kuhn E, Falcimagne R, Balandier P, Soussana J-F (2013) Grassland root demography responses to multiple climate change drivers depend on root morphology. Plant Soil 364:395-408

Pineiro G, Paruelo JM, Oesterheld M, Jobbagy EG (2010) Pathways of grazing effects on soil organic carbon and nitrogen. Rangeland Ecol Manage 63:109–119

Pinheiro J, Bates D, Debroy S, Sarkar D and R Core Team (2015) nlme: linear and non linear mixed effect models. R Package Version 3.1-119

R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reich PB (2014) The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. J Ecol 102:275-301

Rossignol N, Chadoeuf J, Carrère P, Dumont B (2011) A hierarchical model for analysing the stability of vegetation patterns created by grazing in temperate pastures. App Veg Sci 14:189-199.

Ruppert JC, Harmoney K, Henkin Z, Snyman HA, Sternberg M, Willms W, Linstädter A (2015) Quantifying drylands' drought resistance and recovery: the importance of drought intensity, dominant life history and grazing regime. Glob Chang Biol 21:1258-1270

Schaffers A, Sykora K (2000) Reliability of Ellenberg indicator value for moisture, nitrogen and soil reaction: a comparison with field measurements. J Veg Sci 11:225-244

668 Scurlock JMO, Johnson K, Olson RJ (2002) Estimating net primary productivity from grassland
669 biomass dynamics measurements. Glob Chang Biol 8:736-753
670 Smith SW, Woodin SJ, Pakeman RJ, Johnson D, van der Wal R (2014) Root traits predict
671 decomposition across a landscape-scale grazing experiment. New Phytol 203:851-862.
672 Soussana JF, Loiseau P, Vuichard N, Ceschia E, Balesdent J, Chevallier T, Arrouays D (2004)
673 Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use Manag
674 20:219-230
675 Soussana JF, Duru M (2007) Grassland science in Europe facing new challenges: biodiversity
676 and global environmental change. CAB Reviews: Perspectives in Agriculture, Veterinary
677 Science, Nutrition and Natural Resources 272:1-12
678 Soussana JF, Lemaire G (2014) Coupling carbon and nitrogen cycles for environmentally
679 sustainable intensification of grasslands and crop-livestock systems. Agr Ecosyst Environ
680 190:9-17
681 Steinaker DF, Wilson SD (2008) Phenology of fine roots and leaves in forest and grassland. J
682 Ecol 96:1222-1229
683 Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, Wall DH (2004)
684 Ecological linkages between aboveground and belowground biota. Science 304:1629-
685 1633
686 Wilcox KR, von Fischer JC, Muscha JM, Petersen MK, Knapp AK (2015) Contrasting above-
687 and belowground sensitivity of three Great Plains grasslands to altered rainfall regimes.
688 Glob Chang Biol 21:335-344
689 Xu X, Sherry RA, Niu S, Li D, Luo Y (2012) Net primary productivity and rain-use efficiency
690 as affected by warming, altered precipitation, and clipping in a mixed-grass prairie. Glob
691 Chang Biol 19:2753-2764

692 Xu X, Luo Y, Shi Z, Zhou X, and Li D (2014) Consistent proportional increments in responses
693 of belowground net primary productivity to long-term warming and clipping at various
694 soil depths in a tallgrass prairie. Oecologia 174, 1045-1054
695 Yan L, Zhou G, Zhang F (2013) Effects of different grazing intensities on grassland production
696 in China: A meta-analysis. PLoS ONE 8:e81466
697 Zeng C, Wu J, Zhang X (2015) Effects of grazing on above- vs. below-ground biomass
698 allocation of alpine grasslands on the northern tibetan plateau. PLoS ONE 10:e0135173
699 Zheng S, Li W, Lan Z, Ren H, Wang K (2015) Functional trait responses to grazing are
700 mediated by soil moisture and plant functional group identity. Sci Rep 5:18163
701 Zhou G, Zhou X, He Y, Shao J, Hu Z, Liu R, Zhou H, Hosseinibai S (2017) Grazing intensity
702 significantly affects belowground carbon and nitrogen cycling in grassland ecosystems:
703 a meta-analysis. Glob Chang Biol 23:1167-1179
704 Zhu J, Zhang Y, Liu Y (2016) Effects of short-term grazing exclusion on plant phenology and
705 reproductive succession in a Tibetan alpine meadow. Sci Rep 6:27781
706 Ziter C, MacDougall AS (2013) Nutrients and defoliation increase soil carbon inputs in
707 grassland. Ecology 94:106-116
708 Zwicke M, Alessio GA, Thiery L, Falcimagne R, Baumont R, Rossignol N, Soussana J-F,
709 Picon-Cochard C (2013) Lasting effects of climate disturbance on perennial grassland
710 above-ground biomass production under two cutting frequencies. Glob Chang Biol
711 19:3435–3448.
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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**

720 Table 2. a) Repeated measure ANOVA is shown for treatment, date (December 2013, March
721 2014, June 2014) and interaction effects on root mass (g m^{-2}). Numerator (num), denominator
722 (den) of degree of freedom (DF) and *F* values are shown. b) Root mass (g m^{-2}) of abandonment,
723 low (Cattle-) and high (Cattle+) stocking density treatments measured in winter (December 12
724 2013), spring (March 20 2014), summer (June 20 2014) and averaged across the three dates.
725 Means ± se are 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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Table 3. Repeated measure ANOVA is shown for treatment, year and interaction effects on annual root production (BNPP, g m^{-2} y^{-1}), annual above-ground production (ANPP, g m^{-2} y^{-1}) and root to shoot ratio (R/S). Numerator (num), denominator (den) of degree of freedom (DF), *F* values are shown. Superscripts $^{ns, *, **, ***}$ correspond to $P > 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

		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^{ns}	22.21^{**}	9.52^{**}

766 Table 4. Root traits measured from ingrowth core collected in June 2014 and leaf traits
767 measured from botanical observation in abandonment (May 2014), Cattle- and Cattle+ (April
768 2014) treatments. Diameter: root diameter (mm); SRL: specific root length (m g^{-1}); RTD: root
769 tissue density (g cm^{-3}); SRA: specific root area (m^2 g^{-1}); % 0-0.1 mm: percentage of length in
770 the class diameter 0-0.1 mm; % 0.1-0.2 mm: percentage of length in the class diameter 0.1-0.2
771 mm; % 0.2-0.3 mm: percentage of length in the class diameter 0.2-0.3 mm; % > 0.3 mm:
772 percentage of length in the class diameter > 0.3 mm; Community-weighted mean (CWM)
773 Height: plant height (cm); SLA: specific leaf area (cm^2 g^{-1}); LDMC: leaf dry matter content (g
774 g^{-1}). Means ± se are shown (n = 4). num/den DF: numerator and denominator of degree of
775 freedom. Superscripts $^{ns, +, *, **, ***}$ correspond to P > 0.1, P ≤ 0.1, P < 0.05, P < 0.01, P < 0.001,
776 respectively. For SRL and SRA, different letters correspond to significant differences between
777 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+) grazing intensiy 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

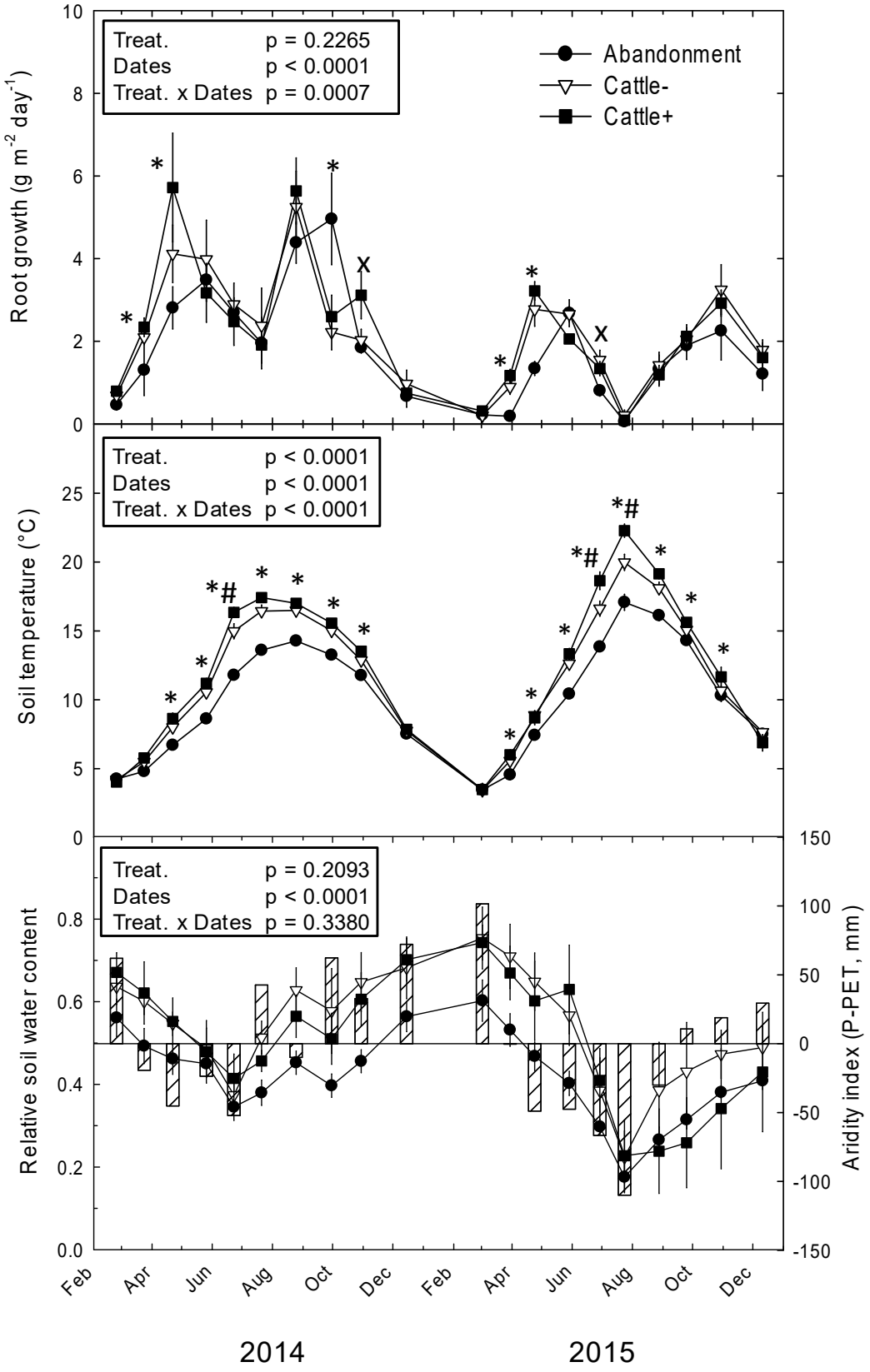
Treat. p = 0.2265
Dates p < 0.0001
Treat. x Dates p = 0.0007
Abandonment
Cattle-
Cattle+
Root growth (g m-2 day-1)
Treat. p < 0.0001
Dates p < 0.0001
Treat. x Dates p < 0.0001
Soil temperature (°C)
Treat. p = 0.2093
Dates p < 0.0001
Treat. x Dates p = 0.3380
Relative soil water content
Aridity index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

Figure 2

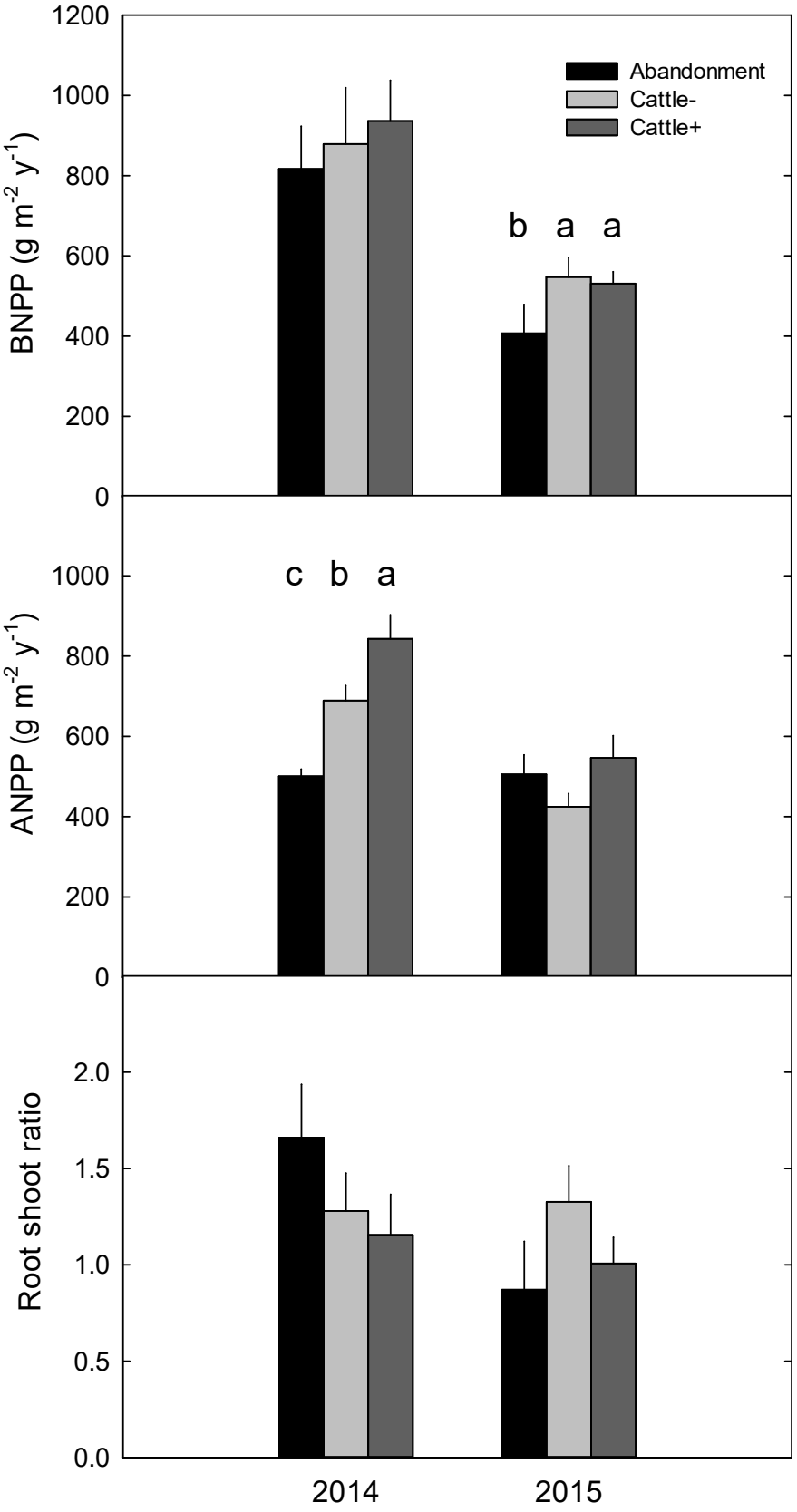
Abandonment
Cattle-
Cattle+
BNPP (g m-2 y-1)
1200
1000
800
600
400
200
0
b a a
ANPP (g m-2 y-1)
1000
800
600
400
200
0
c b a
Root shoot ratio
2.0
1.5
1.0
0.5
0.0
2014
2015

Figure 3

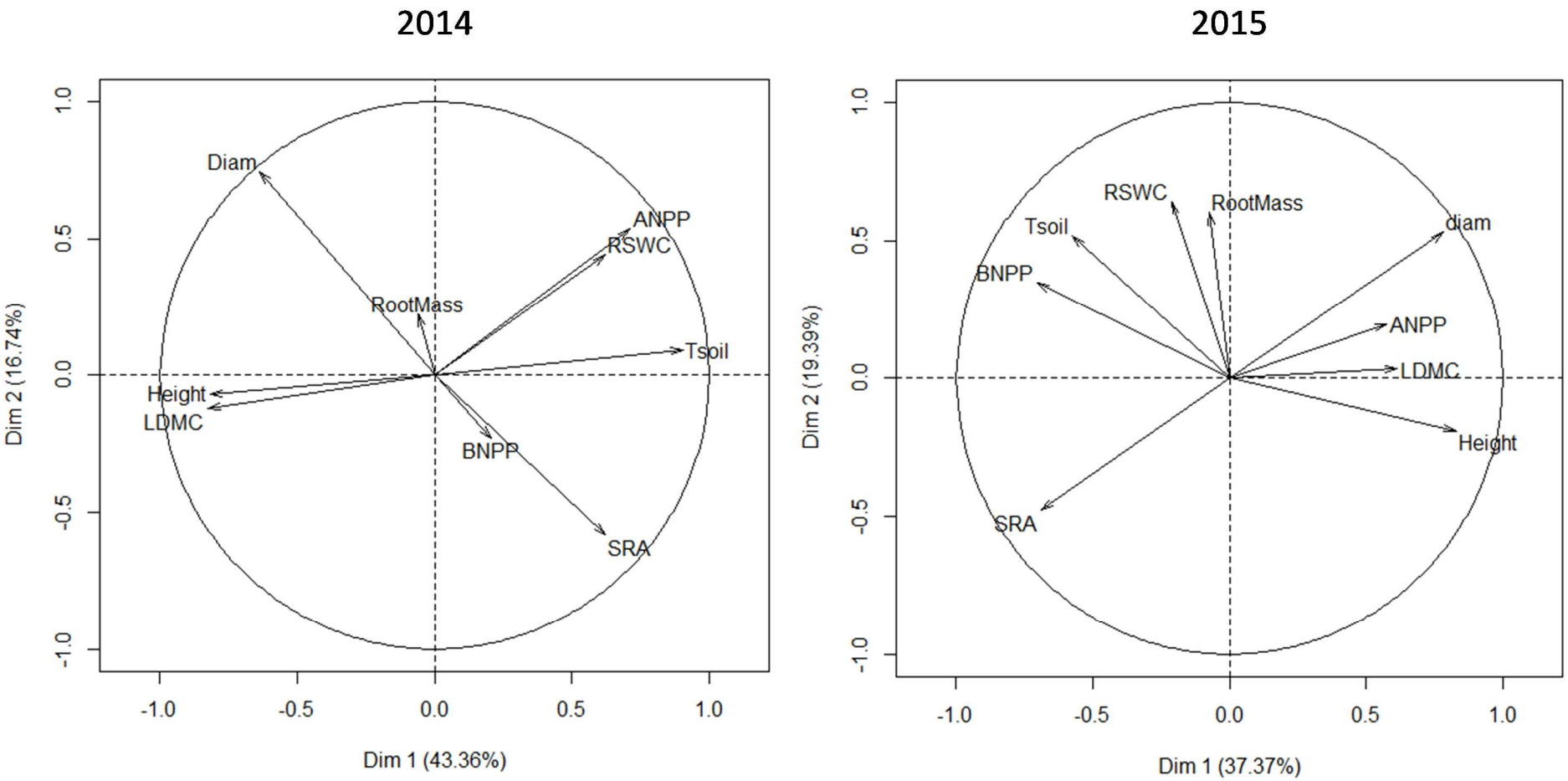
2014
Diam
ANPP
RSWC
RootMass
Tsoil
Height
LDMC
BNPP
SRA
Dim 2 (16.74%)
Dim 1 (43.36%)
2015
RSWC
RootMass
Tsoil
diam
BNPP
ANPP
LDMC
Height
SRA
Dim 2 (19.39%)
Dim 1 (37.37%)

Table S1. 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 S2. Repeated measure ANOVA is shown for root growth (g m^{-2} day^{-1}), soil temperature (Tsoil, °C) and relative soil water content (RSWC) responses to treatment, dates (d1 to d20) and interaction effects. Numerator (num), denominator (den) of degree of freedom (DF) and *F* values are shown. Superscripts ^{ns}, **, *** correspond to $P > 0.05$, $P < 0.001$, $P < 0.0001$, respectively.

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}

874 Table S3. Species contribution (%) in the community present around the ingrowth core
875 measured in April and May 2014 for Cattle-, Cattle+ and Abandonment, respectively. Mean ±
876 se is shown (n = 4). For each species, different letters correspond to significant differences at
877 *: 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
	Festuca arundinacea[x]	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

878 [x]: new species name: *Schedonorus arundinaceus*

S4: R scripts used in the paper

1) Root growth, soil temperature and relative soil water content

```
> lme1 <- lme (variable ~ trait*date, random = ~ 1 | bloc/ID,
+                    data=IGC_ID, method="ML")
> anova(lme1)

plot(lme1)
> par(mfrow=c(2,2))
> plot(residuals(lme1))
> qqPlot(residuals(lme1))
> hist(residuals(lme1))

> lme1.lsmeans <-lsmeans(lme1, pairwise ~  trait | date)
> print(lme1.lsmeans[[2]])
```

2) Root mass stock measured at three dates

```
> lme1 <- lme (StockRac ~ trait*date, random = ~ 1 | bloc/ID,
+                data=Stock_ID, method="ML")
> anova(lme1)

plot(lme1)
> par(mfrow=c(2,2))
> plot(residuals(lme1))
> qqPlot(residuals(lme1))
> hist(residuals(lme1))

> lme1.lsmeans <-lsmeans(lme1, pairwise ~  trait | date)
> print(lme1.lsmeans[[2]])
```

3) Root mass stock: averaged of the three dates, root and leaf traits

```
> lme1 <- lme (variable ~ trait, random = ~ 1 | bloc/ID,
+                    data=Stock3, method="ML")
> anova(lme1)

plot(lme1)
> par(mfrow=c(2,2))
> plot(residuals(lme1))
> qqPlot(residuals(lme1))
> hist(residuals(lme1))

> lme1.lsmeans <-lsmeans(lme1, pairwise ~  trait)
> print(lme1.lsmeans[[2]])
```

4) BNPP, ANPP, Root shoot mass ratio, NNI

```
> lme1 <- lme (variable ~ trait*year, random = ~ 1 | bloc/ID,
+                    data=BNPP, method="ML")
> anova(lme1)

plot(lme1)
> par(mfrow=c(2,2))
> plot(residuals(lme1))
> qqPlot(residuals(lme1))
> hist(residuals(lme1))

> lme1.lsmeans <-lsmeans(lme1, pairwise ~  trait | year)
> print(lme1.lsmeans[[2]])
```
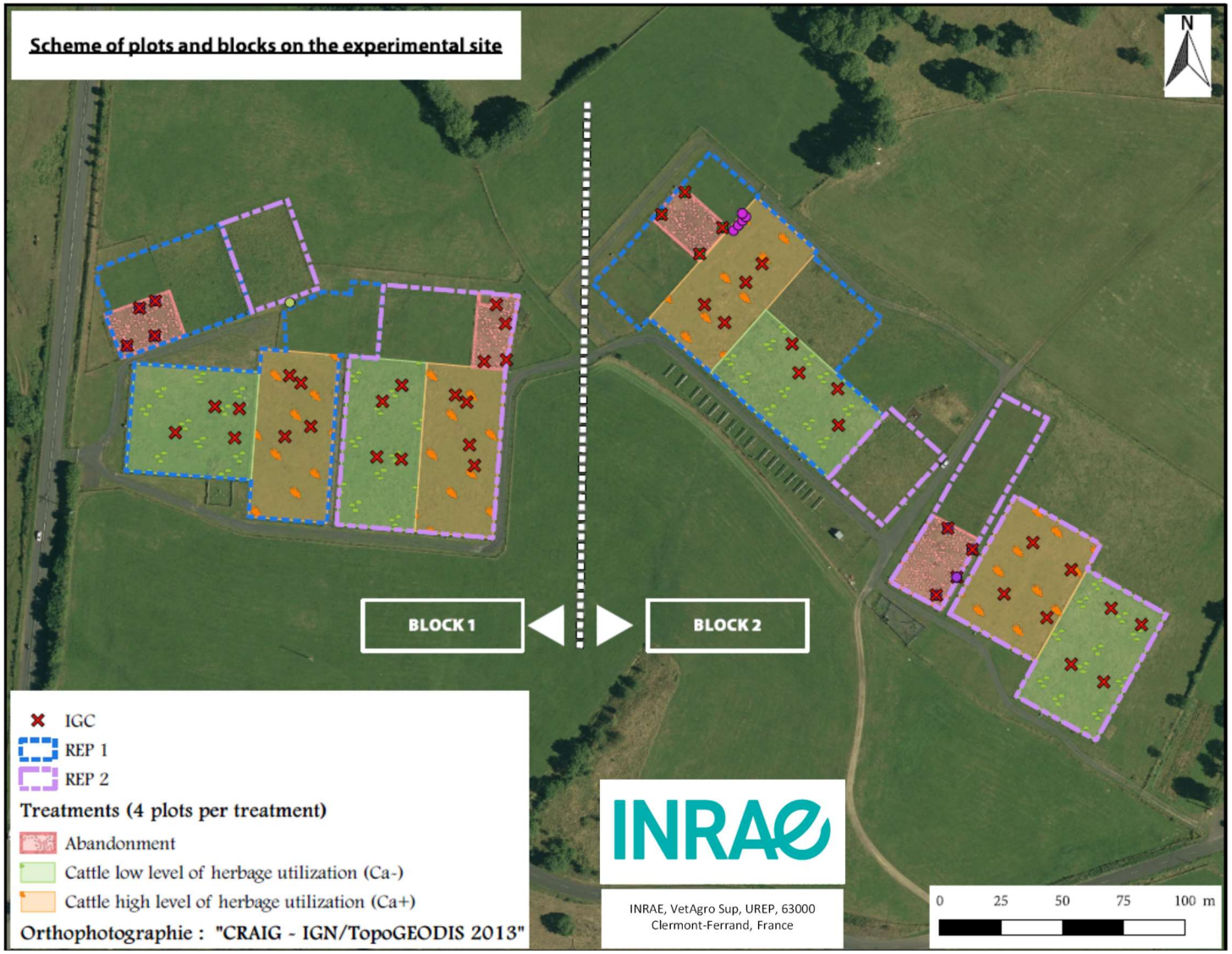
Fig S1 : Scheme of the plots and blocks on the experimental site