How does soil particulate organic carbon respond to grazing intensity in permanent grasslands?

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Abstract

Background and aims Modification in grazing intensity causes functional changes in permanent grasslands, e.g. in carbon (C) cycling. However, we still know little about how the soil organic C of permanent grasslands responds to grazing intensity.

Methods In a grassland experiment with three levels of grazing intensity, we monitored root and rhizome C stocks, particulate organic C stocks, total soil C stocks, above-ground net primary production and plant species groups abundance over 7 years. A simple model was used to estimate the mortality of roots and rhizomes, decomposition rates of particulate organic C, and C fluxes under different grazing intensities.

Results After 7 years, low grazing intensity and no grazing led to a modification in above-ground vegetation (production, plant species composition, nitrogen content) and a reduction in C transferred between roots and particulate organic matter fractions, while the C stocks of root and rhizomes, particulate organic matter and total soil were not significantly affected by grazing intensity. However, particulate organic C showed a strong interannual variability.

Conclusion Particulate organic C could have reacted more slowly than expected to changes in grazing intensity, or a marked interannual variability of particulate organic C stocks, through an increase in decomposition rates in all the grazing treatments, could have slowed down the accumulation of particulate organic C and masked the effect of the grazing intensity treatments.

Keywords Carbon cycling · Decomposition rates · Grassland ecosystem · Grazing intensity · Particulate organic matter

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Abbreviations

AB	Abandonment treatment
ANPP	Above-ground net primary production
BNPP	Below-ground net primary production
AOM	Aggregated organic matter
AOC	Aggregated organic carbon
Cat−	Low cattle grazing intensity treatment
Cat+	High cattle grazing intensity treatment
cPOM	Coarse particulate organic matter
cPOC	Coarse particulate organic carbon
DMD	Dry matter digestibility
fPOM	Fine particulate organic matter
fPOC	Fine particulate organic carbon
LSU	Livestock unit
MRT	Mean residence time
NC	Nitrogen content
NIRS	Near infrared spectroscopy

Introduction

Grazing has been reported to be a major driver of terrestrial biogeochemical cycles. Large herbivores regulate carbon (C) and nutrient fluxes linking the producer and decomposer systems (McNaughton et al. 1997; De Mazancourt et al. 1998; Bardgett and Wardle 2003; Semmartin et al. 2008). Grazing intensity impacts C cycling, and is thought to have effects on C storage across grasslands (Derner and Schuman 2007; Pineiro et al. 2010; McSherry and Ritchie 2013), although these effects are often inconsistent and difficult to predict (Milchunas and Lauenroth 1993; Schuman et al. 2001; Reeder and Schuman 2002). Average soil organic C stocks in French grasslands are estimated to be 70 t C ha^{-1} at depth 0–30 cm (Arrouays et al. 2001), with an average sequestration rate of 0.5 t ha^{-1} year^{-1}, which represents less than 1 % of soil organic C stock change per year (Vleeshouwers and Verhagen 2002). There is thus a need to understand the influence of grazing intensity on C cycling, and ultimately on soil C storage in permanent grassland ecosystems.

In theory, grazing intensity can have an effect on C stocks and C sequestration rate through modification of net C flows from atmosphere to vegetation and soil, by modifying amount and decomposition rates of residual plant material (Shariff et al. 1994; Soussana and Lemaire 2014), and by providing an alternative pathway of C cycling via manure and urine deposition (Bardgett and Wardle 2003). It has been reported that these modifications are mediated by changes in functional diversity of above- and below-ground biota and primary productivity (Klumpp et al. 2009), and in the case of frequent herbage use and fertilisation, decoupling of C and nitrogen (N) cycling (Soussana and Lemaire 2014).

In the short term (hours to days) defoliation increases the amount of C input in soils by increasing root exudation (Paterson and Sim 1999; Murray et al. 2004; Paterson et al. 2005). This stimulates the growth and activity of heterotrophic microbes in the rhizosphere (Hamilton et al. 2008). These effects have been shown to feedback positively on N availability, plant N acquisition, leaf N content and photosynthesis, leading in the longer term (within a year) to an increase in the quantity of litter returned to soil, followed by a shift in ecosystem net primary productivity. In the long run, grazing intensity also leads to significant changes in the botanical and functional composition of the plant community (Louault et al. 2005). For example, it has been reported that

different management of fertile temperate grassland, with low versus moderate or high level of herbage use, leads to modified plant type composition: the proportion of tall species with high leaf dry matter content, probably better able to compete for light, increases at the expense of short plant height types. It has also been shown that legumes and forbs are strongly disadvantaged by biomass accumulation, owing to a decrease in stocking density (Dumont et al. 2011). This impacts not only on the litter quantity, but also litter quality, thereby affecting soil biota and nutrient cycling (Wardle et al. 2004; Loiseau et al. 2005).

Dung and urine produced by herbivores have been shown to facilitate the effect on soil biological processes. Excreta return to soil is commonly underlined as one of the main driving mechanisms for grazers, stimulating N availability and plant nutrient uptake at the plant community scale, especially in fertile grasslands that carry large numbers of grazing animals (e.g. McNaughton et al. 1997; Frank and Groffman 1998; Bardgett and Wardle 2003). However, only a small proportion of dung C is retained in grassland soils as the major part is lost in the environment (microorganism's respiration and leaching) (Bol et al. 2000).

Soil C is stored in different fractions, which can be described as a continuum of pools with decreasing degree of organic matter degradation: coarse (>1 mm) and fine (>0.2 mm) particulate organic matter (cPOM and fPOM), aggregate organo-mineral fraction (AOM) and humified C (Cambardella and Elliott 1992; Jastrow and Miller 1997; Personeni and Loiseau 2004). POM fractions have faster turnover rates (less than 1–10 years) than AOM and humified C associated with mineral particles (10–1000 years) (Christensen 1996). POM fractions are highly degradable and supply microorganism respiration and C fluxes to stable soil organic matter fractions (AOM, humified C). Hence POM fractions are considered as the "active" pools of C and N in soil (Gosling et al. 2013), and are expected to be highly responsive to grazing intensity modifications, as they are strongly linked to vegetation composition and functioning (Alvarez et al. 1998; Personeni and Loiseau 2004). POM stocks and dynamics could therefore be good predictors of long-term changes in soil organic matter following changes in grazing intensity (Gosling et al. 2013) as they supply the most stable C stock in soil (AOM and humified C). Turnover rates and C mean residence time (MRT) of POM fractions are a function of the quantity of C entering the soil, and rates of

decomposition induced by micro-organism activity. According to these theoretical expectations, Klumpp et al. (2009) showed in a mesocosm experiment that decomposition of POM is fostered in more productive and disturbed grasslands (i.e. lower C MRT), leading to higher respiration rates and increasing C fluxes to more stable fractions. While C entering the soil depends mainly on below-ground net primary productivity (BNPP) in grasslands (Robinson 2007; Freschet et al. 2013), decomposition rates of POM are determined by (i) the C:N ratio of plant litter (Bardgett et al. 1998), (ii) accessibility of POM to decomposer or catalytic enzymes (Dungait et al. 2012), soil pH (Leifeld et al. 2008) and (iii) constraint imposed on decomposition by environmental conditions, including soil moisture and temperature (Vaieretti et al. 2013).

Assessing the effects of grazing intensity on C storage involves considering particulate organic carbon (POC) dynamics and C fluxes between soil organic matter fractions and their links to changes in plant productivity, the proportion of the vegetation consumed by herbivore or cycling through senescence, and abundance of plant functional types. However, the few studies that have reported such analyses used litter bag experiments (Olofsson and Oksanen 2002; Raiesi and Asadi 2006), mesocosms (Klumpp et al. 2007, 2009), or comparison of semi-natural grasslands close to steady-state or along chronosequences, where several factors were likely to confound the grazing intensity effect (e.g. soil mineralogy, soil depth, climate) (Bardgett and McAlistair 1999; Wardle et al. 2004; Bardgett et al. 2005; Patra et al. 2006; Klumpp et al. 2009). In the Swiss Alps, Leifeld and Fuhrer (2009) found slightly more POM in the uppermost strata of a pasture soil under cattle grazing than in an ungrazed meadow. Conversely, at a lower alpine site in southern Norway, Martinsen et al. (2011) detected a decrease in POC under intensive sheep grazing compared with no grazing. Findings from these studies provide mixed support to theory, and offer a poor understanding of POM dynamics and ultimately C storage. Analyses from in situ experiments under comparable climate and soil parameters are therefore required.

The aim of this study was to quantify changes in POC stocks and C fluxes between roots and rhizomes and POC fractions following modification of grazing intensity associated with changes in vegetation composition and productivity. Previous findings led us to expect, in the years subsequent to a decrease in grazing intensity,

(i) a modification of plant species composition, tissue quality and above-ground production, (ii) an increase in MRT of roots and rhizomes and POM fractions related to lowered decomposition rates, and (iii) a modification of POC stock mediated by a change in C fluxes between fractions. Alternatively, these soil organic matter fractions might take longer to react to a modification in grazing intensity, so that its effect on POC dynamics in the short to medium term (<10 years) might be weak compared with the effect of interannual variation of climate and local soil environment.

In 2005 we designed an experiment to test these hypotheses on an in situ upland permanent grassland with three levels of grazing intensity (high, moderate, and zero). In 2005, 2008 and 2012 we monitored roots and rhizomes, coarse and fine particulate organic C (cPOC and fPOC, respectively) and total C stocks for each grazing treatment. A simple C flux model was used to estimate root and rhizome mortality, decomposition rates of cPOC and fPOC, and C fluxes between roots and rhizomes and POC fractions.

Materials and methods

Site, experimental design and treatments

This study was carried out at the upland grassland site of the SOERE ACBB (Système d'Observation et d'Expérimentation pour la Recherche en Environnement, Agro-écosystèmes, Cycles Biogéochimiques et Biodiversité) long-term ecological research program (Theix, France, 45°43′N, 03°01′E, 880 m a.s.l.). The climate is semi-continental with oceanic influences (mean annual temperature 8.7 °C, mean annual precipitation 780 mm). The site supports a mesotrophic multispecific permanent grassland. Dominant plant species display high Ellenberg indicator values for N (Schaffers and Sykora 2000), indicating a high level of fertility for the site (Dumont et al. 2011).

Before this experiment was set up in 2005, the study area had been used for intensive hay and silage production (combining grazing, mowing and mineral fertilisation). In the 2 years preceding the start of the experiment (2003 and 2004), the grassland site was mown three times per year with no fertilisation.

The soil of the site is a Cambisol with a sandy loam texture, developed on a granitic bedrock. Differences in local soil composition and profile led to consideration of

two blocks characterized respectively by an eutric cambisol and a colluvic cambisol as including some volcanic materials.

The study was conducted on a grazing intensity gradient comprising three levels (i.e. three treatments). For each treatment, two replicates were set up per block, resulting in four replicates per treatment and a total of 12 plots. To provide three levels of grazing intensity, plots were either grazed by cattle at a high stocking density (Cat+: four heifers grazing on 2200 m^2 plots, i.e. 13.8 LSU ha^{-1} with 1 LSU= 600 kg liveweight) or a low stocking density (Cat−: two heifers on 2200 m^2 plots, i.e. 6.9 LSU ha^{-1}) or left ungrazed (AB). The rotational grazing method was applied to Cat+and Cat−plots with five grazing periods per year: mid-April, end May, early July, September and November, named R1, R2, R3, R4, and R5 respectively. Animals were removed from the pastures in all grazed treatments when the median green plant height in Cat+plots dropped to 7 cm, so that rotations lasted 9.6, 9.0, 10.7, 8.6, and 2.1 days on average for R1–5, respectively, over the whole study period. From 2006 to 2012, the median green sward surface height at the end of each grazing period was significantly lower in Cat+plots compared to Cat-plots (7.7 ± 0.23 cm vs 15.2 ± 0.54 cm, $n=280$, $p<0.001$). This is consistent with data obtained the first 2 years of experiment reported by Dumont et al. (2011) and the annual stocking rate reach 1.46 LSU ha^{-1} in Cat+treatment and 0.73 LSU ha^{-1} in Cat− treatment (mean over the 2006 to 2012 period). When off the experimental plots, the animals grazed an adjacent pasture presenting the same plant community. For comparison, the average stocking rate was about 1.4 LSU ha^{-1} in Europe with 1.2 LSU ha^{-1} in France, for the grazing livestock of the permanent grassland area in 2007 (Huyghe et al. 2014).

Precipitation and temperature data were obtained for the period 2005–2012 from a meteorological station located on the SOERE site. Daily data were averaged per month to characterise annual patterns of rainfall and temperature.

Vegetation measurements

Two measurements of above-ground plant biomass were made each year in each plot. First, standing biomass up to 5.5 cm, hereafter termed above-ground standing biomass, was sampled, at the end of winter,

before the start of the growing season, and then at the beginning of each grazing period in grazed plots, and in spring, summer and autumn in AB plots. At each date, above-ground standing biomass was clipped on four sampling areas per plot, measuring 0.6×0.6 m in grazed plots and 0.6×0.3 m in AB plots. Samples were dried at 60 °C for 48 h and weighed.

Secondly, the above-ground net primary production (i.e. ANPP, above-ground biomass accumulation) was estimated each year. On each plot and at each sampling date, the four sampling areas where above-ground standing biomass was clipped at 5.5 cm were fenced off. At each sampling date, biomass, i.e. the accumulation of above-ground biomass inside the fence from the previous date, was sampled at a height of 5.5 cm, dried and weighed. Measurements were made throughout the year, before each grazing event in Cat+and Cat−plots, and in spring, summer and autumn in AB plots. Sampling areas were moved within the plot at each measurement date throughout the year. Annual above-ground net primary production (ANPP, g DM m^{-2} year^{-1}) was calculated as the sum of the successive biomass accumulation along the year.

Nitrogen content (NC, %) and dry matter digestibility (DMD, %) in the Cat+and Cat−treatments were determined on the above-ground standing biomass, using near infrared reflectance spectroscopy (NIRS, Foss) method, (see Pontes et al. 2007).

Botanical composition was determined in May 2004, before application of the treatments, and in 2006, 2007, 2008, 2010, 2011 and 2012 by a point-quadrat method. Mean relative abundance of grasses, legumes and forbs within each plot was calculated as a ratio of the total score for species in each of these three plant species groups divided by the sum of scores for all species and expressed as a percentage (see Dumont et al. 2011).

Soil sampling

Soil inventories were made during winter 2005, 2008 and 2012, using a soil corer of inside diameter 8.3 cm (Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands). In each plot, four soil cores of depth 20 cm were sampled using a motor hammer (Cobra Combi, Atlas Copco AB, Nacka, Sweden) to drive the corer. The depth of the borehole and the length of the extracted core were measured and compared to estimate

soil compaction during coring. A depth of 20 cm was chosen, because roots are still abundant at that depth and produce POM.

After air drying, soil cores were weighed and then pooled in pairs to reduce the number of subsequent analyses. Roots, rhizomes and POM were separated by wet sieving (5.0, 1.0 and 0.2 mm). The remaining material was separated into organic and mineral fractions by density flotation in water. Organic fractions containing roots, rhizomes, and coarse (>1 mm) and fine (0.2–1 mm) POM were oven-dried, weighed and analysed for C and N content by elemental analysis. This included oxidation of C to CO_2 and N to NO_x in an O_2 stream and subsequent reduction of NO_x to N_2 by a copper-tungsten granule, separation of CO_2 and N_2 by GC-TCD and quantification using acetanilide as an external standard (Hekatech Euro EA 3000, Wegberg, Germany). C stocks (kg ha^{-1}) of each organic fraction were calculated. Stone fractions (>2 mm) were weighed, and densities were determined by water displacement to calculate bulk densities of the fine soil (<2 mm).

In 2012, prior to wet sievings, a 50 g soil sample was taken to measure total soil C contents (on soil<2 mm).

Model description

The model used is a simplified C flux model (Klumpp et al. 2009, Fig. 1) that describes three pools of soil C (expressed in kg C ha^{-1}): roots and rhizomes considered in the same pool (R), coarse particulate organic C (cPOC) and fine particulate organic C (fPOC). The R pool was supplied by a flux (S) representing a fraction of the daily net plant photosynthesis (kg C ha^{-1} day^{-1}). R mortality (m) leads to release of POC by supplying C to the fPOC, with a fraction (β), and a fraction ($1-\beta$) to the cPOC pool. The cPOC and fPOC

pools have distinct decomposition rate constants, (k_c and k_f respectively). The decomposition flux of cPOC and fPOC is split into two fluxes: a fraction ($1-\alpha$) is mineralised in CO_2, and the remainder supplies the next pool with a fraction (α). Therefore, the model comprises three equations representing the dynamics of each organic C pool and five parameters (α, β, m, k_c and k_f):

$$\frac{d(R)}{dt} = S - m \times R \qquad (1)$$

$$\frac{d(cPOC)}{dt} = m \times R \times (1-\beta) - k_c \times cPOC \qquad (2)$$

$$\frac{d(fPOC)}{dt} = m \times R \times \beta + \alpha \times k_c \times cPOC - k_f \times fPOC \qquad (3)$$

α and β were set respectively at 0.59 and 0.22 according to the modelling work done by Klumpp et al. (2009) on a mesocosm study.

The flux S of C supplied to the R pool was calculated according to the measured mean annual above-ground net primary production (ANPP, kg ha^{-1} year^{-1}), roots and rhizomes C content (%) and ratio of below-ground net primary production (i.e. roots and rhizomes) to above-ground net primary production (i.e. RootShoot, see below). Thus for each day, S is written:

$$S = \frac{RootShoot \times ANPP \times \%C}{365} \qquad (4)$$

Mortality of root and rhizome fractions and decomposition rates of POC were estimated by constraining the model with measured C in root and rhizome biomass and POC in the topmost 0–20 cm soil layer during the two periods 2005–2008 and 2008–2012.
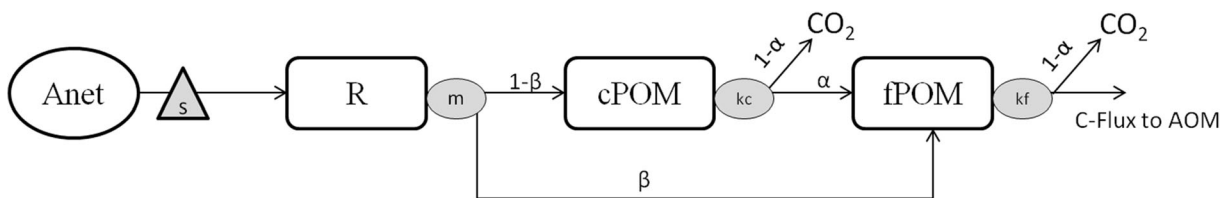

Fig. 1 Model flowchart (adapted and modified from Klumpp et al. 2009). S is the fraction of daily net plant photosynthesis (Anet) going to roots and rhizomes (R); m is the root and rhizome mortality; k_c and k_f are the decomposition rates of cPOC and fPOC compartments; $1-\beta$ and β the fraction of decomposed roots going to cPOC and fPOC; α and $1-\alpha$; fraction of decomposed cPOC going to fPOC and released as CO_2

The model was implemented in Vensim DSS (Ventana Systems Inc. 2013).

The three unknown parameters (m, k_c and k_f) were then estimated for each plot by adjusting the stock dynamics predicted by the model to the measured values of each compartment (R, cPOC and fPOC) at a daily time step.

We assume that C MRT (MRT=$1/m$, Tc=$1/k_c$, Tf=$1/k_f$) of each C compartment (R, cPOC, fPOC) may change from the initial state according to the effect of management practices and climate. In order to evaluate treatment effects on m, k_c and k_f, the model was run for the two periods, 2005–2008 and 2008–2012.

Initial state was calculated for each plot and each C pool according to the following three equations, assuming the ecosystem was at steady state before the management change in 2005. The estimation of m, k_c and k_f at steady state was then given by:

$$m = \frac{S}{R} \tag{5}$$

$$k_g = \frac{m \times R \times (1-\beta)}{cPOC} \tag{6}$$

$$k_f = \frac{m \times R \times \beta + \alpha \times k_c \times cPOC}{fPOC} \tag{7}$$

C fluxes exiting R, cPOC and fPOC compartments were calculated as the product of estimated mortality of R (m) and decomposition rates of cPOC and fPOC (k_c and k_f) and C stock measured for each period (initial state, 2005–2008 and 2008–2012).

Measurement of the RootShoot ratio used in the model

To estimate S, the calculation of a RootShoot ratio representing the quantity of dry matter allocated to roots and rhizomes for a quantity of dry matter allocated to shoot was needed in the three treatments. From December 2013 to December 2014, four ingrowth cores (mesh size 8 mm) per plots, filled with dry and sieved (10 mm) soil previously collected on the site, were placed in holes 20 cm deep (8 cm in diameter) and left for around 4 weeks. At each sampling date (10 throughout the year), 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. In the laboratory, roots and rhizomes were gently washed from soil with a sieve of

200 μm, manually separated, dried at 60 °C for 48 h and weighed. Annual below-ground net primary production (BNPP, g DM m^{-2} year^{-1}) was then calculated as the cumulative root and rhizome mass of all dates over 1 year. Above-ground net primary production was also estimated during 2014 (ANPP, g DM m^{-2} year^{-1}, see above). The RootShoot ratio was then estimated as

$$RootShoot = \frac{BNPP \ (g \ DM \ m^{-2}y^{-1})}{ANPP \ (g \ DM \ m^{-2}y^{-1})} \tag{8}$$

Statistical analyses

All the variables were characterised by descriptive statistics (means of the four plots for each treatments and standard error of the mean). Data collected within each plot were averaged and analysed using a mixed model procedure. Treatments and years were used as fixed factor with a constant random block effect with a first autoregressive repeated covariance structure (AR1). The explanatory variables were treatment, year, and the interaction treatment*year (T*Y). The structure of the linear mixed model was determined by first including year, treatment and the interaction Y*T, and then stepwise excluding unnecessary factors based on model performance (reflected by the Akaike information criterion). Differences between treatments Cat+, Cat− and AB were tested using contrast statement. Variance homogeneity was confirmed by checking the plots of model residuals vs. fitted values. Normality of residuals was inspected with quantile-quantile plots of model residuals. Statistical analyses were performed under the R environment (Rstudio 2013). Integrated development environment for R (Version 0.98.501), Boston MA.,USA) using "nlme" (Pinheiro et al. 2015) and "lsmeans" packages.

Results

Above-ground net primary production, above-ground standing biomass and botanical composition

Low grazing intensity (Cat−) and ungrazed (AB) treatments significantly decreased ANPP compared with high grazing intensity treatment (Fig. 2a; Table 1). In 2005, the first year of application of treatments, the ANPP did not show any significant differences.
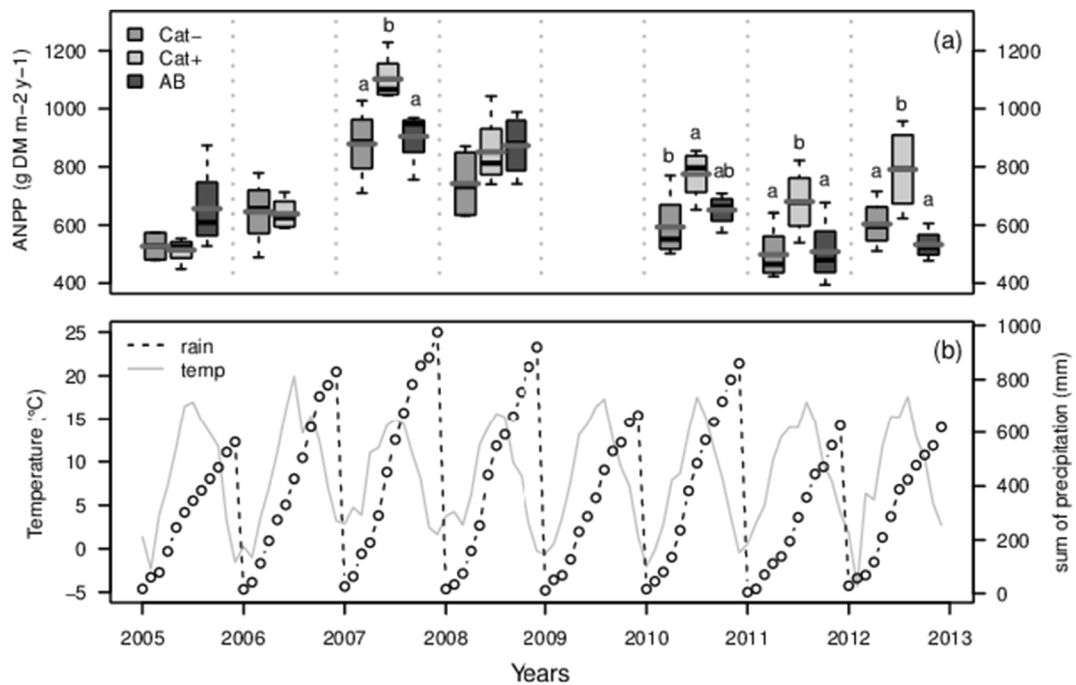
Fig. 2 **a**. Annual above-ground net primary production (ANPP, g DM m^{-2} years^{-1}) of AB, Cat−and Cat+from 2005 to 2012. Means are by treatments (*n*=4) (*grey bars*). Boxplots show median, interquartile range, and range of data. Letters are the result of contrast statement and show the differences between treatments for each year. **b**. Temperature (°C) (*grey line*) and sum of precipitation (mm) (*black dotted line*) between 2005 and 2012, averaged by month

During the 7 years of treatment application, mean annual ANPP was significantly lower in Cat−and AB than in Cat+, with means of 764±37, 619±36 and 660±44 g m^{-2} years^{-1} respectively for Cat+, Cat−and AB. Cat−treatment was significantly less productive in 2007, 2010, 2011 and 2012 than Cat+, and AB was significantly less productive during 2007, 2011 and 2012 than Cat+. There was no significant difference between Cat−and AB. While ANPP was modified by grazing intensity treatments, there was a strong interannual variability. Mean annual ANPP was significantly higher in 2007, followed by 2008. Both years were significantly different from 2005, 2010, 2011, and 2012.

Grazing intensity treatments led to a differentiation of plant communities. Plant species groups showed marked changes between treatments (Fig. 3). After 2 years of treatment application, legumes had nearly disappeared in AB treatments, while increasing significantly in Cat+treatments (*p*<0.001). Legumes in Cat− remained stable. Grasses increased significantly (*p*<0.001) in AB and Ca−, while decreasing slightly in Cat+treatments. Treatment also significantly affected forbs, with a slight decrease in Cat−and AB compared with Cat+treatment. The change in plant community

was also characterised by a significant decrease in above-ground standing biomass NC in Cat−for the whole study period compared with Cat+(Table 1) and that from the first year of treatments application. This decrease in NC was associated with a lower dry matter digestibility in Cat−than in Cat+(Table 1). However, measurements were made only in the Cat+and Cat− treatments.

Below-ground C stock

In 2005, before the start of the experiment, future treatments showed no significant differences between the C stocks of any measured below-ground fractions (C-Roots and rhizomes, cPOC, fPOC). The C:N ratio decreased with fraction size (Fig. 4). The average C:N ratios were 39.2±1.3 for roots and rhizomes, 24.2±0.5 for cPOM and 18.4±0.41 for fPOM. The amount of C accumulated in fPOC was twice that of cPOC, with means of 2554±113.1 kg C ha^{-1} and 1284±43.94 kg C ha^{-1} respectively.

C stock measured in roots and rhizomes (Fig. 4a) was not significantly affected by the different levels of grazing intensity, and showed no differences between years

Table 1 Results of ANOVA on the mixed model procedure for above-ground and below-ground measurements for ungrazed (AB), low grazing intensity (Cat−), and high grazing intensity (Cat+), mean value (±SE) of the four plots for each treatment and each year are presented

	Year	AB	Cat−	Cat+	Treatment	Year	T*Y	Cat+ vs. Cat−	Cat+ vs. AB	Cat− vs. AB
Mean annual above-ground net primary production (g DM m⁻² y⁻¹)	All years	660 (44)	619 (36)	764 (37)	*	***	**			
Mean Nitrogen Content (%) of standing biomass	All years	–	2.05 (0.04)	2.81 (0.05)	***	***	**			
Dry matter digestibility of standing biomass (%)	All years	–	53.3 (0.74)	66.5 (0.70)	***	**	**			
Roots & rhizomes (kg C ha⁻¹)	All years	2293 (282)	2241 (219)	2076 (183)	ns	ns	ns			
	2005	2129 (255)	2574 (496)	2304 (310)				ns	ns	ns
	2008	1850 (269)	2132 (362)	2212 (344)				ns	ns	ns
	2012	2899 (736)	2017 (303)	1713 (294)				ns	ns	ns
cPOC (kg C ha⁻¹)	All years	1174 (81)	1379 (77)	1298 (61)	ns	***	ns			
	2005	1357 (110)	1468 (61)	1450 (75)				ns	ns	ns
	2008	858 (63)	1232 (59)	1111 (78)				ns	ns	ns
	2012	1306 (81)	1437 (218)	1334 (99)				ns	ns	ns
fPOC (kg C ha⁻¹)	All years	2362 (219)	2804 (171)	2497 (188)	ns	***	ns			
	2005	2556 (181)	3489 (357)	2598 (140)				ns	ns	ns
	2008	1601 (47)	2518 (205)	1817 (107)				ns	ns	*
	2012	2930 (425)	2625 (211)	3077 (296)				ns	ns	ns
C:N ratio roots & rhizomes	All years	41 (3.0)	37 (1.0)	38 (2.6)	ns	***	*			
	2005	52 (5.1)	39 (2.3)	47 (4.3)				ns	ns	**
	2008	39 (1.9)	37 (1.5)	39 (2.6)				ns	ns	ns
	2012	32 (1.1)	36 (1.6)	29 (0.5)				ns	ns	ns
C:N ratio cPOM	All years	25 (0.8)	22 (0.5)	24 (1.3)	ns	***	***			
	2005	26 (1.1)	22 (1.2)	27 (1.2)				**	ns	**
	2008	27 (1.1)	21 (0.4)	27 (1.6)				***	ns	***
	2012	22 (0.5)	23 (0.5)	19 (0.7)				*	ns	ns
C:N ratio fPOM	All years	19 (0.5)	16 (0.2)	19 (1.0)	*	***	***			
	2005	20 (0.7)	16 (0.1)	20 (1.0)				ns	ns	***
	2008	20 (0.1)	16 (0.1)	21 (1.1)				ns	ns	***
	2012	16 (0.5)	17 (0.2)	15 (0.5)				*	*	ns
C content roots & rhizomes (%)	All years	43.6 (0.2)	42.7 (0.3)	43.6 (0.4)	**	ns	***			
	2005	43.9 (0.4)	42.7 (0.6)	44.1 (0.7)				ns	ns	*
	2008	44.0 (0.6)	41.8 (0.5)	44.4 (0.3)				ns	ns	***
	2012	43.0 (0.3)	43.5 (0.3)	42.2 (0.5)				ns	ns	ns

Table 1 (continued)

	Year	AB	Cat−	Cat+	Treatment	Year	T*Y	Cat+ vs. Cat−	Cat+ vs. AB	Cat− vs. AB
C content cPOM (%)	All years	38.8 (1.0)	34.5 (0.8)	38.6 (1.0)	***	***	**			
	2005	40.1 (0.7)	35.8 (1.4)	41.3 (0.6)				ns	ns	*
	2008	41.4 (0.7)	33.0 (1.1)	40.3 (0.7)				ns	ns	***
	2012	34.7 (1.6)	34.8 (1.6)	34.0 (0.8)				ns	ns	ns
C content fPOM (%)	All years	28.7 (1.3)	24.9 (0.5)	30.1 (1.8)	**	***	***			
	2005	29.7 (1.3)	25.2 (0.7)	33.5 (1.3)				ns	ns	*
	2008	32.7 (1.2)	24.7 (1.2)	34.4 (0.7)				ns	ns	***
	2012	23.6 (1.9)	24.7 (1.0)	22.5 (1.9)				ns	ns	ns
Total C stock (kg C m^{-2}, <2 mm)	2012	6.92 (0.267)	6.95 (0.730)	6.57 (0.154)	ns	−	−	ns	ns	ns
Total soil C:N ratio	2012	10.27 (0.302)	10.29 (0.307)	10.18 (0.277)	ns	−	−	ns	ns	ns
Total soil C content (%)	2012	4.96 (0.211)	4.64 (0.656)	4.33 (0.286)	ns	−	−	*	ns	ns

(−) no measurements were made. Significance levels $p<0.05$ *, $p<0.01$ ** and $p<0.001$ ***

(Table 1). However, taken apart, C-rhizomes in 2012 were significantly higher ($p<0.01$) in the AB treatment than in Cat+ due to an increase in rhizome masses in AB and a slight decrease in Cat+ (data not shown). No treatment effect could be highlighted on cPOC and fPOC (Table 1). However, there was a strong significant effect of period variability on both cPOC ($p<0.001$) and fPOC ($p<0.001$) (Fig. 4a and b). In 2008 all the stocks of cPOC and fPOC significantly decreased and reached the same value as the initial state in 2012.

The C:N ratio of soil fractions (Fig. 4d, e and f; Table 1) changed during the experiment. At the initial state, Cat− showed a lower C:N ratio value in roots and rhizomes, cPOM and fPOM than Cat+ and AB. C:N ratio of roots and rhizomes showed a strong decrease in Cat+ and AB between 2005 and 2012, while C:N ratio in Cat− decreased only slightly. However, in 2012 there was no difference in C:N ratio between roots and rhizomes in any of the treatments. C:N ratio of cPOM showed a decrease in 2012 for Cat+ and AB compared with 2005 and 2008, while there was no change in Cat−. In 2012 C:N ratio of cPOM was higher in Cat−. C:N ratio of fPOM also showed a decrease in 2012 for Cat+ and AB compared with 2005 and 2008, while there was a slight increase in Cat−. However, they all reached the same value in 2012.

After 7 years of differentiated management, total C stock and C:N measured in total soil were not significantly different between treatments (Table 1). However, C content was significantly higher ($p<0.05$) in AB than in Cat+. C content was significantly different between treatments AB and Cat+.

RootShoot ratio and estimation of S

The RootShoot ratio (annual BNPP on annual ANPP) measured in 2014 showed no significant differences between treatments. However, there was a tendency towards a higher value in the AB treatment (AB: 1.7± 0.27; Cat−: 1.3±0.19 in Cat+:1.2±0.21; ANOVA: $p=$ 0.2). We thus assume a mean ratio of 1.4 in all treatments and all years to estimate the value of S for the model.

Figure 5 shows the mean value of S (estimation of the allocation of C to the roots and rhizome pool in the model) for each period (2005, 2005–2008 and 2008–2012) and each treatment (AB, Cat− and Cat+). The estimation of S in AB showed significant higher value at initial state in 2005. During 2005–2008 there were no

Fig. 3 Plant species groups measured from 2004 to 2012 (no measurements were made in 2009). Forbs in grey, legumes in black and grasses in light grey. Results were obtained by averaging the four replicates (two replicates on two blocks) for each treatment
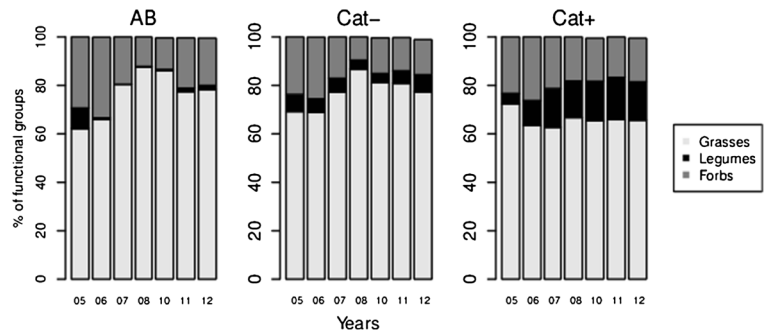
significant differences in S between treatments. However, during the last period, Cat−showed a significant lower value of C allocated to R than Cat+, while there were no significant differences between AB and Cat−or AB and Cat+.

Carbon MRT and decomposition rates

Although it is difficult to see any pattern in measured POC stock, estimated decomposition rates and C fluxes between soil fractions (C-Roots and rhizomes to cPOC,

cPOC to fPOC, and fPOC to AOC) show differences in ecosystem dynamics between treatments.

At the initial state, m (root mortality) and k_g and k_f, (decomposition rates), showed no differences between future treatments or for C fluxes of any below-ground fractions (Fig. 6; Table 2).

Mortality (m) and decomposition rates of cPOC and fPOC (k_c and k_f) and C fluxes from roots to POC; cPOC to fPOC and fPOC to AOC showed a strong significant ($p<0.001$) year effect, and a significant year*treatment interaction (Fig. 6b and c; Table 2).
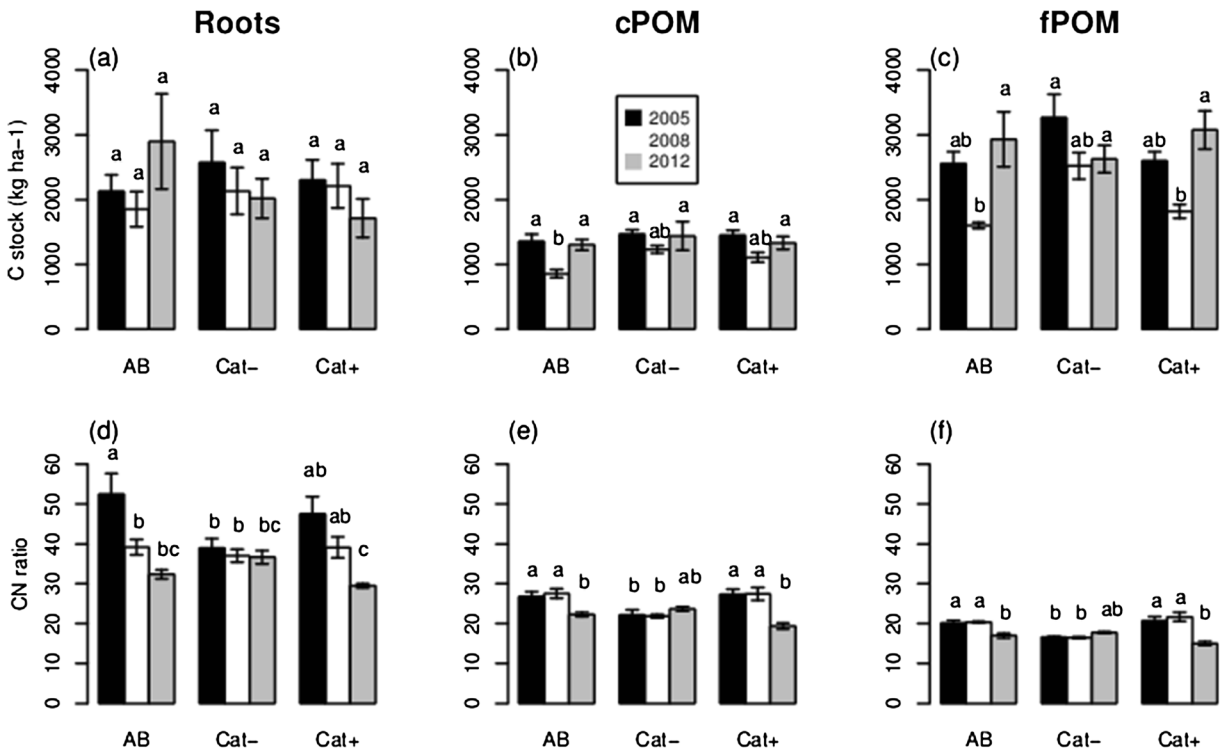

Fig. 4 Measured C stock (kg C ha^{-1}) and C:N ratio with standard error of below-ground fractions in 2005 (*black*), 2008 (*white*) and 2012 (*grey*) for AB, Cat−, Cat+. Results were obtained by averaging the four treatment replicates (two replicates on two blocs) for each treatment. Letters are the result of the contrast statement and show differences between each fraction comparing years and treatments
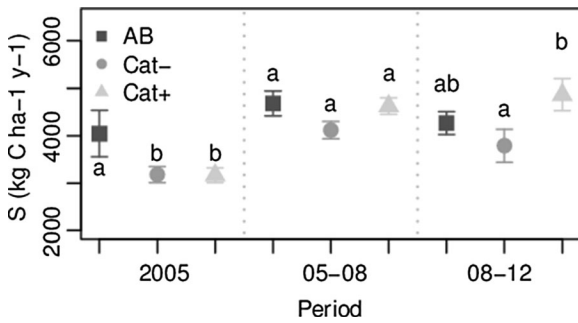
Fig. 5 Mean estimation of S (kg C ha^{-1} year^{-1}) for each period (2005, 2005–2008 and 2008–2012) of the study and for each treatments (AB, Cat− and Cat+). Letters are the result of contrast statement on the mixed model procedure and show the differences between treatments for each year. Error bars indicate standard error ($n=4$)

During 2005–2008, k_c and k_f showed a significant lower value in Cat− than in AB and Cat+.

The C flux allocated from roots and rhizomes to POC was significantly higher in Cat+than in Cat−and AB (Fig. 6d). The C flux from cPOC to fPOC was significantly higher in Cat+than in AB and Cat−, and the C allocated from fPOC to AOC was significantly higher Cat+than in Cat−, showing no differences with AB.

During the second period (2008–2012), root and rhizome mortality increased in Cat+compared with AB (Fig. 6a). This led to a significant modification of root and rhizome C MRT, with a higher value in AB than in Cat+(Table 2). C MRT of roots and rhizomes in AB increased to reach 305±67 days and 146±21 days in Cat+, with Cat−in between at 235±35 days. k_c and k_f showed no significant differences between treatments. During this period, C fluxes from roots and rhizomes to POC was higher in Cat+than in Cat−, showing no significant differences from AB. Treatments also significantly modified C fluxes from cPOC to fPOC (Fig. 6e; Table 2) and from fPOC to AOC. This led to more C transferred from cPOC to fPOC in Cat+than in Cat−, showing no significant differences with AB, and more the C transferred from fPOC to AOC with a significant higher value in Cat+than in AB and Cat−.
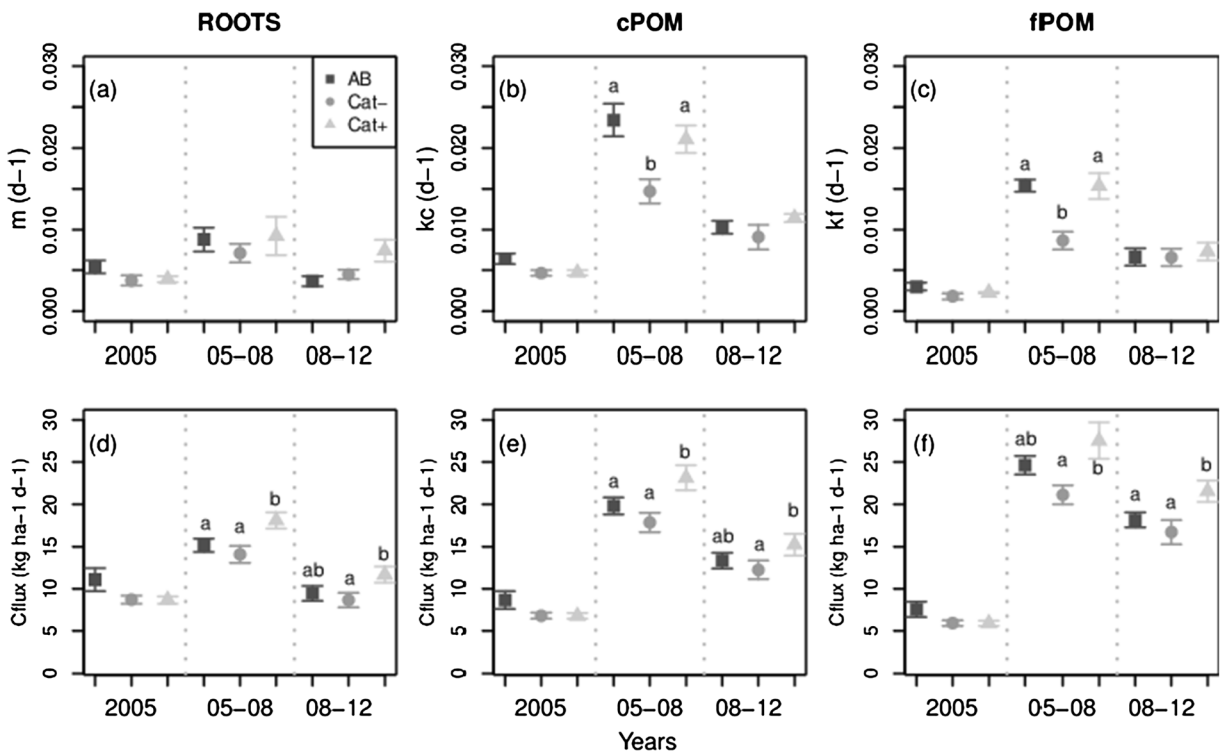

Fig. 6 Modelled root mortality (m) and decomposition rates (k_c and k_f) (day^{-1}) and exiting C fluxes (kg C ha^{-1} day^{-1}) from roots and rhizomes, cPOC and fPOC (as the product of C stock measured and estimated m, k_c or k_f) with standard error at initial state in 2005, during the periods 2005–2008 and 2008–2012 for treatments AB, Cat−and Cat+. Each point was obtained with the mean value of four replicates. Letters are the result of the contrast statement

Table 2 Results of ANOVA on the mixed model procedure for the model simulation for ungrazed (AB), light cattle grazing intensity (Cat−), and high grazing intensity (Cat+), mean value (±SE) of the four plots for each treatment, at equilibrium (2005), and during the period 2005–2008 and 2008–2012 are presented

	Year	AB	Cat−	Cat+	Tr	Year	Tr*Y	Cat+vs. Cat−	Cat+vs. AB	Cat−vs. AB
m (d^{-1})	All years	0.0059 (0.0008)	0.0051 (0.0006)	0.0068 (0.0010)	ns	**	*			
	2005	0.0054 (0.0007)	0.0037 (0.0006)	0.0039 (0.0003)				ns	ns	ns
	05–08	0.0087 (0.0014)	0.0071 (0.0011)	0.0092 (0.0023)				ns	ns	ns
	08–12	0.0036 (0.0006)	0.0044 (0.0005)	0.0074 (0.0013)				ns	ns	ns
Kc (d^{-1})	All years	0.0133 (0.0022)	0.0094 (0.0013)	0.0124 (0.0020)	*	***	*			
	2005	0.0064 (0.0006)	0.0046 (0.0003)	0.0047 (0.0003)				ns	ns	ns
	05–08	0.0234 (0.0019)	0.0146 (0.0001)	0.0210 (0.0016)				**	ns	***
	08–12	0.0102 (0.0008)	0.0090 (0.0015)	0.0114 (0.0005)				ns	ns	ns
Kf (d^{-1})	All years	0.0083 (0.0016)	0.0056 (0.0009)	0.0083 (0.0017)	*	***	**			
	2005	0.0030 (0.0004)	0.0018 (0.0003)	0.0022 (0.0001)				ns	ns	ns
	05–08	0.0153 (0.0007)	0.0086 (0.0010)	0.0153 (0.0015)				***	*	***
	08–12	0.0066 (0.0010)	0.0065 (0.0010)	0.0073 (0.0010)				ns	ns	ns
C flux from roots & rhiz to POC (kg ha^{-1} d^{-1})	All years	11.88 (0.90)	10.48 (0.87)	12.81 (1.26)	*	***	**			
	2005	11.07 (1.34)	8.70 (0.46)	8.67 (0.43)				ns	ns	ns
	05–08	15.14 (0.78)	14.07 (1.01)	18.09 (0.97)				***	*	ns
	08–12	9.45 (0.87)	8.65 (0.84)	11.67 (0.96)				*	ns	ns
C flux from cPOM to fPOC (kg ha^{-1} d^{-1})	All years	13.92 (1.47)	12.29 (1.44)	15.04 (2.10)	ns	***	*			
	2005	8.63 (1.04)	6.79 (0.36)	6.76 (0.33)				ns	ns	ns
	05–08	19.81 (1.00)	17.86 (1.15)	23.13 (1.45)				***	*	ns
	08–12	13.32 (0.92)	12.23 (1.10)	15.22 (1.28)				*	ns	ns
C flux from fPOM to AOC (kg ha^{-1} d^{-1})	All years	16.76 (2.18)	14.58 (2.00)	18.32 (2.85)	*	***	*			
	2005	7.53 (0.91)	5.92 (0.31)	5.89 (0.29)				ns	ns	ns
	05–08	24.59 (1.11)	21.13 (1.14)	27.51 (2.15)				***	ns	*
	08–12	18.15 (0.87)	16.71 (1.43)	21.56 (1.25)				**	*	ns
C MRT Roots & rhizomes (days)	All years	209 (32)	228 (28)	178 (21)	ns	**	*			
	2005	197 (28)	297 (62)	263 (25)				ns	ns	ns
	05–08	124 (20)	150 (20)	125 (23)				ns	ns	ns
	08–12	305 (67)	235 (35)	146 (21)				***	**	***
C MRT cPOM (days)	All years	101 (15)	136 (20)	117 (22)	ns	***	*			
	2005	161 (16)	218 (15)	215 (15)				ns	**	**
	05–08	43 (3)	70 (7)	48 (4)				ns	ns	ns

Table 2 (continued)

	Year	AB	Cat−	Cat+	Tr	Year	Tr*Y	Cat+ vs. Cat−	Cat+ vs. AB	Cat− vs. AB
C MRT fPOM (days)	08–12	98 (6)	120 (20)	88 (4)				ns	ns	ns
	All years	195 (40)	294 (72)	218 (49)	*	***			ns	***
	2005	354 (50)	601 (102)	441 (18)			*	**	ns	ns
	05–08	65 (3)	121 (15)	67 (7)				ns	ns	ns
	08–12	165 (31)	161 (20)	146 (21)				ns	ns	ns

Significance levels $p < 0.05$ *, $p < 0.01$ ** and $p < 0.001$ ***

Discussion

This experiment was designed to determine the impact of three grazing intensity treatments (AB, Cat− and Cat+) on permanent grassland soil C stock fractions and C cycling. We studied C fluxes between the plant-soil continuums on an in situ grassland ecosystem.

After 7 years of differentiated treatments, the ecosystems turned to contrasting functioning. While above-ground vegetation evolved markedly during the period of the study, C stock measured in roots, POC and total soil were not significantly affected by the grazing intensity treatments. However, estimated decomposition rates and C fluxes between plant and soil organic C fractions showed that the ecosystems had changed in functioning and C cycling.

Did grazing treatment lead to vegetation modification?

From 2008 and during the rest of the experiment, plant species group composition, NC, dry matter digestibility and above-ground net primary production already showed an evolution between treatments. Grassland vegetation exposed to low grazing intensity was characterised by a lower above-ground net primary production, NC and dry matter digestibility. By contrast, the vegetation in the high grazing intensity treatments had high NC, high dry matter digestibility and displayed a higher above-ground net primary production. These results were consistent with commonly observed patterns reporting the impact of grazing intensity on vegetation (McNaughton 1985; McNaughton et al. 1997; Baron et al. 2002; Wardle et al. 2004; Bardgett et al. 2005; Klumpp et al. 2007, 2009; De Deyn et al. 2008). These studies suggest that grazing intensity induces a change in plant strategies. Low grazing intensity systems are dominated by long-lived high-stature plant species with lower NC and dry matter digestibility, indicating slow growth, corresponding to a conservative strategy. By contrast, high grazing intensity systems are dominated by low-stature plant species with high NC and dry matter digestibility that tolerate frequent defoliations, indicating fast growth, corresponding to an exploitative strategy. The modification in ecosystem functioning was also supported by the change in vegetation species groups. For example, high grazing intensity systems were characterised by the presence of legume species, whereas legumes disappeared in AB from the second

year of the experiment. The symbiotic interaction between legume plants and rhizobium bacteria uniquely enables the host plant to access a large N source from the atmosphere (Herridge et al. 2008). It has also been shown that a part of this N is transferred to associated grasses (Ledgard and Steele 1992), inducing a higher production of biomass in the field. Dung and urine return is also known to stimulate N availability and plant nutrient uptake at the plant community scale by providing highly decomposable resources, rich in labile nutrients, and which can stimulate microbial biomass and activity, net C and N mineralisation and ultimately plant nutrient acquisition and growth in grazed treatments (Bardgett and Wardle 2003; Wardle et al. 2004).

While above-ground components of the vegetation showed a fast evolution, no changes could be evidenced in root C stock during the period of the study. However, model outputs show an increase in root and rhizome C MRT in low-disturbed ecosystem mainly during the last period of the study, inducing a lower mortality and a significant decrease in fresh C inputs to soil. This had already been reported in other studies. For example, Pucheta et al. (2004), in a temperate permanent grassland, showed no differences between remaining root mass on an intensively grazed site and ungrazed grassland, while below-ground net primary productivity was doubled in the grazed site, leading to doubled turnover rates of root fractions compared with the ungrazed site. Hence our study suggests that grazing intensity induced a change in plant C cycling at the field scale. Low-grazing intensity systems tend to slow down C cycling in plant communities, resulting in a smaller quantity of C allocated to the soil organic matter continuum.

Grazing effects on soil organic matter fractions and the C fluxes between them?

The decreasing C:N ratio between roots, cPOM and fPOM supports the hypothesis of a continuum of litter decomposition (Personeni and Loiseau 2004). However, C stocks in POC and in total soil were not significantly different after 3 or 7 years of differentiated grazing intensity treatments. There was a strong significant year effect due to a decrease in POC (cPOC and fPOC) in all treatments in 2008, followed by an increase in all treatments during the last period of the study (2008–2012) to reach a similar

level to that at the beginning of the experiment. The strong decrease in POC during 2005–2008 was most probably the consequence of 2007 being a wet year during spring, summer and autumn, leading to higher decomposition rates as supported by the model outputs and by other studies highlighting the effect of watering treatments on POM in permanent grasslands (Garten et al. 2008; Condron et al. 2014). While 2007 was the most productive year, we might have expected an increase in POC due to higher inputs of litter in soil. However, in January 2008 the POC (cPOC and fPOC) stock had strongly decreased in all treatments. This shows that C inputs did not compensate for the high decomposition rates of POC during this period, leading to a decrease in POC stock. This pronounced interannual variability may have masked treatments effects on POC stocks.

The time was too short to induce a modification of POC stocks, but model outputs showed that the ecosystem exhibited different C cycling. During 2005–2008, model outputs showed that the increase in decomposition rates was higher in the AB and Cat+treatment than in Cat−for both cPOC and fPOC, indicating different responses between treatments. This led to significant differences in C transferred between POC fractions, with faster C cycling in the Cat+treatment. During 2008–2012, high grazing intensity ecosystem tended to have higher decomposition rates for both cPOC and fPOC than low grazing intensity treatments, inducing a significant modification of the C transferring from cPOC to fPOC and from fPOC to AOC. This led to less C transferred in soil organic matter fractions in low grazing intensity systems. This had already been observed in other studies. For example, Medina-Roldan et al. (2012) showed in a 7 years in situ experiment that decreasing intensity of perturbation led to decreased microbial biomass and activity in soil, leading to lower C and N turnover rates, while total C stock was not affected by treatments. Also, while no significant change in total C stock could be highlighted, recent studies suggest than management-related change in soil C stocks are difficult to capture fully by only sampling the top and medium soil layer (0–30 cm) (Leifeld et al. 2011).

Our results thus suggest that low-grazing intensity ecosystems decrease C cycling turnover, leading to higher C MRT in roots and rhizomes, causing less C to enter the continuum of litter decomposition. The

decrease in fresh C entry in soil and decomposition rates of POM suggest a lower micro-organism activity, as reported in other studies (Smith and Paul 1990; Tracy and Frank 1998). After 7 years of low grazing intensity, this has led to a decrease in the C transferred between soil fractions, also reducing the C released by respiration to the atmosphere. By contrast, high grazing intensity systems took on a higher C cycling speed, as suggested by a higher above-ground production, leading to more C entering the soil. This increased decomposition rates of POM, suggesting an increase in micro-organism activity. This resulted in a greater amount of C transferred between soil fractions, leading to lower C MRT in POM, suggesting a faster C cycle between plant and soil organic matter fractions and the atmosphere. This agrees with the general pattern found in other studies (Shariff et al. 1994; Bardgett and Wardle 2003; Wardle et al. 2004; Klumpp et al. 2007, 2009).

However, while model outputs indicated that grazing intensity treatments induced modifications of C cycling, these results are mainly based on the estimation of the quantity of C entering the R pool (S) and variations in C-roots, cPOC and fPOC stock between treatments. For example, during the period 2005–2008, no significant differences in S, R and POC stocks could be highlighted between treatments, when model outputs showed higher C fluxes exiting R, cPOC and fPOC in Cat+compared with Cat−and AB. This highlights the sensitivity of the model to S and C stock modification in the model pools (R, cPOM and fPOM). However, as S was calculated with a constant RootShoot ratio estimated during 2014 and above-ground net primary production measured on the site, a decoupling of above- and below-ground components of the vegetation would lead to an inapt use of the RootShoot ratio and thereby a misestimation of S. Another hypothesis is that above- and below-ground components of the vegetation react at different time steps, and that grazing intensity treatments did not modify roots and POM functioning, suggesting that above-ground components tend to react faster than below-ground components. However, this hypothesis is less likely given that Klumpp et al. (2009), in a mesocosm experiment, showed a fast (less than 2 years) evolution of below-ground components of the vegetation following change in disturbance intensity leading to an increase in C cycling turnover in C roots and rhizomes and POC in high-disturbed treatments. Also, the increased root turnover in high grazing intensity treatment compared with ungrazed was also confirmed by Pucheta et al. (2004) in an in situ permanent temperate grassland experiment.

In conclusion, this study confirms that grazing management has an impact on plant and soil organic matter C cycling. While vegetation shows fast modifications between treatments, 7 years of differentiated grazing intensity treatments were not enough to induce modifications of C stock in POC and total soil. It has been argued that POC stock could be a good indicator/predictor of C cycling modification after a management change. However, while these fractions seem highly reactive, marked interannual variability due to an increase in decomposition rates of POC in all treatments during 2005–2008 could have slowed down the accumulation of POC and masked the effect of grazing intensity treatments on POC stock. Then in the short to medium term (<10 years), the effect of precipitation on POC stocks may have been stronger than grazing intensity treatments. Modification of C stock in POM fractions according to grazing intensity may be slower than previously thought. Our study thus highlights the importance of taking into account C fluxes between soil organic matter fractions (cPOC, fPOC, AOC) to characterise evolution between grassland ecosystems under different management in short- to medium-term experiments. The coupling of modelling and POC fraction time sequences may help us understand C dynamics under grazing treatments. However, a fuller understanding and better estimation of the quantity of C allocated to below-ground components of the vegetation and to the POC fractions according to grazing intensity treatments would help us elucidate POM dynamics.

In 7 years of differentiated management, treatments have most probably not yet reached their equilibrium state. This experiment therefore needs to be continued to find the equilibrium state reached under three grazing intensity levels, and determine whether in the longer term this might lead to changes in C stock in soil organic matter fractions.

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