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REGULAR ARTICLE

# Seasonal patterns of belowground biomass and productivity in mountain grasslands in the Pyrenees

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**Abstract** Given the importance of root dynamics for soil C storage, the aim of this study was to analyze first the seasonal dynamics of belowground productivity and then the short-term effects of grazing exclosure on root dynamics in mountain grasslands. Soil coring and root ingrowth cores were used to assess belowground biomass (BGB) and productivity in grazed and ungrazed (grazing exclosures) plots in two mountain grasslands. Annual belowground production ranged from 472 to 590 $gm^{-2}$, representing from 14 to 22% of the maximum root biomass measured over the year. Spring was the most productive season, accounting for more than 50% of total annual production, indicating that factors besides temperature may affect seasonal root dynamics. Although belowground production was much higher in the top 5 cm compared to deeper, the relative productivity rate (production-to-BGB ratio) and renewal time was higher at the subsurface (5–15 cm) layer. The contribution of the subsurface layer to total belowground production increased in spring, possibly due to occasional freezing events at the uppermost layer in the early growing season. The stronger seasonality in subsurface relative productivity rates may reflect depth-dependent changes in root characteristics and lifespan. Excluding grazing increased belowground productivity in summer, but its effects on BGB showed great variability between sites.

**Keywords** Belowground biomass · Grazing exclusion · Mountain grasslands · Relative productivity rates · Root dynamics · Surface and subsurface layers

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## Introduction

Soil carbon is mostly derived from root tissues (Loya et al. 2004; Rasse et al. 2005), making root dynamics an essential factor in understanding soil fertility and functioning. In grasslands, root dynamics is an especially relevant component of soil C inputs (Hitz et al. 2001), since aboveground C inputs are lower than in other ecosystems (Steinaker and Wilson 2005). For this reason, the processes

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driving belowground production and turnover are critical to understanding C storage in grassland ecosystems.

Belowground biomass (BGB), production and turnover have been related to local climate conditions (Kaspar and Bland 1992), soil characteristics and land use management (Guo et al. 2007). Microclimate is particularly relevant in mountain ecosystems, where spatial climatic heterogeneity could determine how long mountain soils remain frozen or snow-covered and, consequently, the length of the growing season (Körner 2003). For example, in Alpine grasslands, root production has been recorded to decrease with altitude (Hitz et al. 2001), probably due to the shorter growing seasons at high altitudes. Further, not only production but also root lifespan has been hypothesized to be related to the length of the favourable growing season (Eissenstat and Yanai 1997). But soil environment in mountain areas is characterised by steep thermal gradient (Körner 2003). Typically in mountain areas, the uppermost layer is commonly exposed to diurnal freeze-thaw cycles at the beginning and the end of the growing season (Körner 2003, p. 65) that could reduce fine roots growth. Hence, we hypothesized that this phenomena may constrain fine root production at the uppermost layer and, therefore that the relative contribution of surface layer to root production would be reduced in spring and autumn.

The reduction of stocking rates together with abandonment of extensive seminatural montane and subalpine pastures has resulted in profound changes to mountain landscapes in temperate areas, with grassland converted into shrubland and even forest (Roura-Pascual et al. 2005). These changes in land use are expected to continue in the years to come in most of Europe's rural areas (Roundsevell et al. 2006) and likely impact on ecosystem services provided by mountain grasslands (Quétier et al. 2007) such as fodder quality and production, species diversity, cultural heritage or C sequestration. In the short term, grazing abandonment increase aboveground biomass and necromass, but the effects on belowground biomass remains controversial. In the long term, grazing abandonment of subalpine grasslands entails a change in functional groups and an increase of woody plants. Consequences of shrub encroachment in to mesic grasslands on C storage are uncertain, with studies indicating a promotion (Montané et al. 2007) or decrease (Jackson et al. 2002) of C accumulation with shrub proliferation. In any case, there is a general agreement that the effects of grazing regime on C cycle is in part due to its effect on root systems (Klumpp et al. 2009). Hence, improving our knowledge of the effects of reduced grazing activity on root dynamics is an essential step towards assessing the consequences on C storage in mountain areas (Klumpp et al. 2009).

Aboveground grazing by large herbivores determines vegetation composition and structure (Sebastià et al. 2008) and ecosystem functioning (Bardgett and Wardle 2003). Most studies analyzing the effect of grazing highlighted that changes in biomass and productivity could be mediated by changes in plant species composition and life forms, which may occur at a mid-to-long-term. However, studies on a shorter-term timeframe (a few years) have recorded physiological responses leading to changes in C allocation and productivity when plants are defoliated, particularly in grazing-tolerant species (Holland et al. 1992; Guitian and Bardgett 2000). Grazing or experimental defoliation have been reported to increase aboveground productivity (Ferraro and Oesterheld 2002). However, the effect of defoliation on belowground components remains less clear (Ferraro and Oesterheld 2002). In field experiments, the effect of grazing and defoliation on belowground productivity is usually found to be negative (Biondini et al. 1998; Ruess et al. 1998; Smit and Kooijman 2001), but some studies did not find significant effects (McNaughton et al. 1998; Bazot et al. 2005) or even found positive effects (Frank et al. 2002; Pucheta et al. 2004). These controversial results suggest that the effect of changing the grazing regime on belowground production and biomass may depend on the plant species (e.g. their tolerance to grazing, Guitian and Bardgett 2000) or environmental conditions (Piñeiro et al. 2010).

In this context, this study was given a dual objective: (1) to analyze seasonal patterns of belowground productivity in mountain grasslands and determine whether these patterns differ with depth; and (2) to assess the short-term effects excluding grazing on belowground biomass and productivity under field conditions.

## Material and methods

### Study sites

This study was carried out at two sites (Alinyà and Prat Llong) located on the southern face of the eastern Pyrenees.

Alinyà (42°10′N; 1°28′E) is a seminatural subalpine grassland at 1,848 ma.s.l. The experimental plots were located in a wide pasture area formerly occupied by crops (mainly potatoes and forage legumes) that were growing on the site until about 35 y ago. Since then it has not been ploughed or fertilised and has only extensively grazed (~0.15–0.20 livestock units [LU] $ha^{-1}$ $y^{-1}$) by cattle each summer, from middle to end June up to October. According to the Digital Climatic Atlas of Catalonia (http://www.opengis.uab.cat/acdc/en_index.htm; Ninyerola et al. 2000), this site has a mean annual temperature of 5.8°C and a mean annual precipitation of 1,062 mm. Mean monthly temperatures range between -0.8°C (February) and 14.4°C (July). This grassland is mainly dominated by *Festuca rubra* L. *ssp. commutata* Gaud., but other species like *Phleum pratense* L., *Poa alpina* L., *Taraxacum dissectum* (Ledeb.) Ledeb., *Achillea millefolium* L. and *Medicago lupulina* L. are also abundant. Soils are thin (35–45 cm) and developed on calcareous bedrock, presenting a pHw of 7.1±0.08 and a clay loam texture. Soil organic C content is 6.6±0.81% at the top 5 cm and 3.7±0.04% at the 5–15 cm layer.

Prat Llong (42°12′N; 1°31′E) is a seminatural subalpine grassland at 2,140 ma.s.l. The plots were located in a pasture area that was formerly occupied by *Pinus mugo* Turra ssp *uncinata* (Mill. ex Mirb.) Domin forests, but during at least one century had been only used for extensive grazing (~ 0.10–0.15 LU$ha^{-1}$ $y^{-1}$) by cattle, from middle to end June up to October. This site has a colder and wetter climate (Fig. 1) than Alinyà. Mean annual temperature is 4.4°C ranging from -1.8°C in February to 12.4°C in July. Mean annual precipitation is 1,155 mm (http://www.opengis.uab.cat/acdc/en_index.htm; Ninyerola et al. 2000). Vegetation is co-dominated by *Nardus stricta* L. and *Festuca rubra* L. *ssp commutata* Gaud. Other species like *Carex caryophyllea* Latourr., *Agrostis capillaris* L., *Poa alpina* L., *Thymus serpyllum* L., *Trifolium repens* L., *Euphorbia cyparissias* L. and *Antennaria dioica* (L.) Gaertn. are also abundant. This site also has thin soils (40–45 cm) with a loamy texture, developed on calcareous bedrock. The Prat Llong soil is more acidified (pHw 4.8±0.04) than the Alinyà soil, and its organic C content is 7.1±0.15% at the top 5 cm and 4.2±0.05% at the 5–15 cm layer.

### Experimental design

Four square plots of 625 $m^2$ were established and fenced off at each site. The location of plots was chosen for the apparent homogeneity of plant species composition and biomass. At each site the four plots (two replicates per treatment) were arranged in a randomized block design with subsampling, with two blocks of two treatments (a grazed and an ungrazed plot at each block). As plots were considered our experimental units, there were two replicates per treatment at each site. Four subsamples at each time were taken in each plot within a block. Sampling areas were established at the corner of each plot, at least at 1 m from the edge of the plot in an area of 4 $m^2$ in which we obtained the samples at each sampling time. Ungrazed plots were excluded from grazing for the entire experimental period, from July 2003 to July 2005. At each grazed plot, three cows were introduced for 3 days in each growing season, at the time corresponding to maximum stocking rates in the area (July). This design try to mimic grazing behaviour in the Pyrenees, where cattle graze in herds of about 20–50 animals (cows and calves) that freely move from one to another grassland and do not return to the grazed patch after some months. Livestock grass consumption, which was calculated as the mean difference between before and after grazing events, was 82% and 66% of aboveground biomass at the first year and 26% and 38% at the second year in Alinyà and Prat Llong respectively.

### Belowground biomass and productivity

We used soil cores to estimate BGB and root ingrowth cores to estimate belowground production (Vogt et al. 1998). Six BGB samplings were carried out during two growing seasons (Table 1). Sampling dates of BGB were also the beginning and the end of the periods in which ingrowth cores were in-field (six periods).

BGB was measured using 5 cm-diameter soil cores. At each sampling date, four cores (15 cm

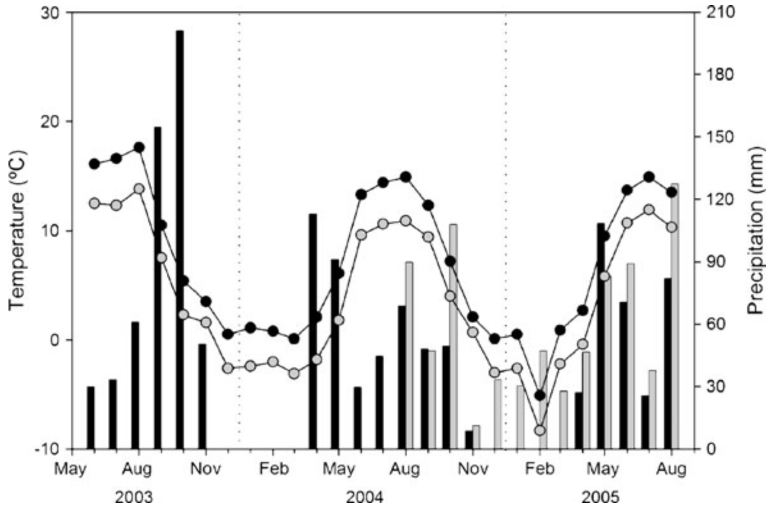



**Fig. 1** Temperature and precipitation values recorded during the experimental period in Alinyà (black) (at 2.4 km from the plots) and Port del Comte meteorological station (grey) (at 1.2 km from the plots in Prat Llong). Precipitation data in Alinyà is not available during winter periods (November–March). In Port del Comte it is not available until August 2004

depth) were obtained at each plot (8 cores per site and per treatment) from the four corners of each plot, always excluding the first meter from the fence to avoid the border effect. At each sampling occasion the samples were taken at 20–50 cm from any previous sampling site in the plot. In the field, each sample was divided into two layers (0–5 and 5–15 cm depth), then frozen at -20°C until lab processing. To determine BGB, each sample was first packed with a fine cloth and shaken by hand under a continuous water flow to eliminate most of the fine soil particles. BGB was then obtained by flotation. This process was repeated to eliminate the remaining particles adhered to the biomass, and then all the obtained BGB was oven-dried at 60°C until constant weight.

At each sampling occasion, the holes from which the soil cores were taken were used to insert the ingrowth cores to assess the subsequent belowground production. Ingrowth cores consisted of fibreglass 1.5 mm-mesh cylinders filled with root-free soil to assess in-hole BGB growth after a given period of time. Soil cores were 15 cm deep and 5 cm in diameter. Meshes were filled with soil that had been previously obtained at each site adjacently to the experimental plots and sieved to 4 mm to retrieve large stones and roots. Root-free soils were introduced into the meshes and compacted to an average density comparable to the mean bulk density of the site, which had been measured previously. After 1.5–2.5 months (except for the winter period, when they were incubated for up to 7 months because of the snow cover), they were pulled out and another ingrowth core was installed in the adjacent area (where soil core for BGB had been taken, see above). Ingrowth cores were also divided into two layers (0–5 and 5–15 cm) in the laboratory, and roots from each layer were first sorted by hand, then washed with water and obtained by flotation. These root samples were then oven-dried at 60°C until constant weight.

**Table 1** Initial and final dates of ingrowth cores in the study sites (Alinyà and Prat Llong) and number of growing days within each period. The initial date of each growing period coincides with the sampling date of root cores to obtain BGB

| | Alinyà | | Prat Llong | |
|---|---|---|---|---|
| | Growing period | nº days | Growing period | nº days |
| Summer 2003 | 24/07/03–25/09/03 | 63 | 25/07/03–02/10/03 | 69 |
| Autumn 2003 | 25/09/03–28/04/04 | 78 | 02/10/03–24/05/04 | 30 |
| Spring 2004 | 28/04/04–05/07/04 | 68 | 24/05/04–05/07/04 | 42 |
| Summer 2004 | 05/07/04–06/09/04 | 63 | 05/07/04–17/09/04 | 74 |
| Autumn 2004 | 06/09/04–25/11/04 | 72 | 17/09/04–23/11/04 | 32 |
| Spring 2005 | 25/11/04–13/06/05 | 57 | 23/11/04–19/07/05 | 84 |

### Calculations and data analyses

To calculate belowground annual production ($gm^{-2}$) the production of each plot at each period was first calculated as the mean production of the ingrowth cores ($n$=4). The sum of all periods of a given year was used as estimation of annual production at each plot, which was then expressed as the mean of two plots. Relative productivity rate ($g\ kg_{BGB}^{-1}\ d^{-1}$) at each period was calculated as the ratio of belowground production per day during that period ($gm^{-2}\ d^{-1}$) to the BGB at the beginning of the growth period ($kgm^{-2}$). Root renewal time (yr) was calculated as the ratio between mean belowground biomass and yearly production.

Since the second and last ingrowth periods included the winter season, we calculated root production only taking into account the estimated length of the growing season, assuming no growth during the winter period. According to Körner and Paulsen (2004), the growing season at the treelines of many mountain areas worldwide starts when mean soil temperature at 10 cm depth exceeds 3.2°C, and corresponds to a mean weekly air temperature above 0°C. Although our study sites were below the treeline, we followed their criterion and considered that the growing season started the last day in spring in which mean air temperature over the previous 7-day week was 0°C or lower. Similarly, we considered that the growing season finished the first day in autumn in which mean weekly air temperature dropped to 0°C. These estimations used air temperature data from the meteorological stations of Port del Comte (1.2 km from the plots in Prat Llong) and Alinyà (2.4 km from the plots).

Differences in belowground biomass and in relative productivity rates between sites, treatments and sampling dates were tested by a randomized complete block design with subsampling in which the grazing treatment interacted with the block effect, both factors tested with one degree of freedom. Block factor was nested to site when the site factor was included in the analysis. Differences between sampling dates were tested by an *a posteriori* Duncan test when ANOVA was significant. Normality of each variable was previously tested by the non-parametric Kolmogorov-Smirnov test. Relative productivity rate ($g\ kg_{BGB}^{-1}\ d^{-1}$) of belowground biomass was log-transformed to achieve normality. All analyses were performed using the SPSS v.17 statistical package.

## Results

### Belowground biomass

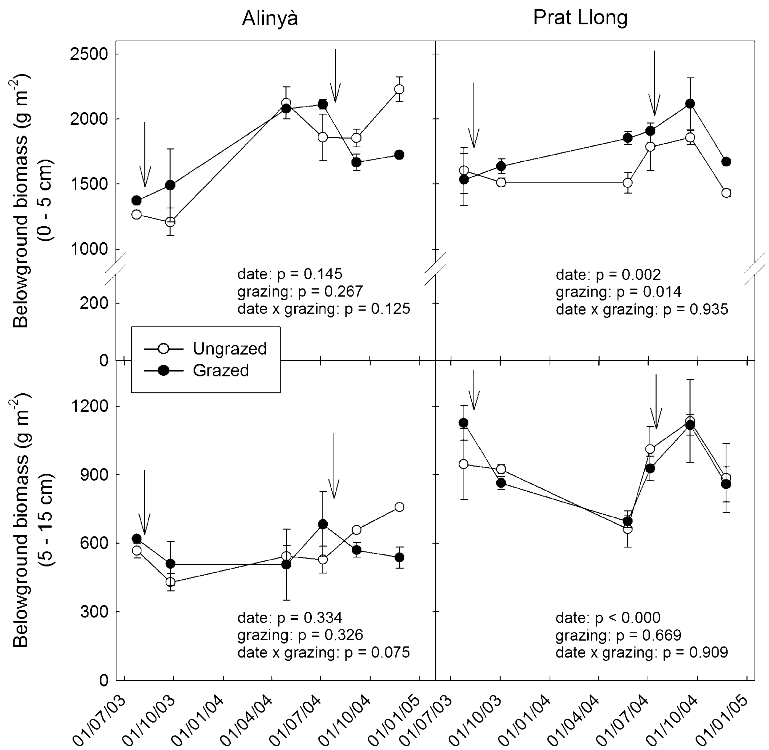
During the study period, BGB to 15 cm depth was in average 2,312 $gm^{-2}$ (ranging from 1,639±144 to 2,900±12 $gm^{-2}$) in Alinyà and 2,639 $gm^{-2}$ (from 2,527±86 to 3,269±186 $gm^{-2}$) in Prat Llong. At both sites, BGB was much higher in surface than subsurface layers (Fig. 2), but the relative distribution of BGB by layers varied between sites. Across the whole study period, the average BGB at 5–15 cm depth of all samplings represented 24.5±1.7% ($n$=6) of total BGB in Alinyà, being significantly lower ($p$<0.001) than in Prat Llong (34.6±2.2%, $n$=6). Conversely, there were no between-site differences in BGB at the top 5 cm. Biomass concentration was much higher ($p$<0.001) at the topsoil than at subsurface, being the average of all samplings ($n$=6) 34.8±2.7 and 33.8±1.3 $mgcm^{-3}$ at the uppermost 5 cm and 5.7±0.3 and 9.3±0.6 $mgcm^{-3}$ at the 5–15 cm layer in Alinyà and Prat Llong respectively.

Seasonal variations in BGB differed between Alinyà and Prat Llong (Fig. 2). In 2003, there was no difference in BGB between sampling dates (July and September) or between grazing treatments in either Alinyà or Prat Llong. In contrast, in 2004, each site showed different seasonal patterns (Fig. 2). In Prat Llong, despite BGB being higher in grazed plots than ungrazed plots, both treatments followed the same temporal pattern, with peak BGB in September and lowest BGB at the beginning and the end of the growing season (May and November) (Fig. 2). The BGB increase in the grazed plots mainly occurred at the top 5 cm ($p$=0.014), while there were no significant differences in the 5–15 cm layer. In contrast, in Alinyà, grazed and ungrazed plots followed different patterns (time × grazing, $p$=0.016), with a sharp decrease in BGB in grazed plots just after the grazing treatment in 2004. At the Alinyà site, while in grazed plots peak BGB occurred just before the grazing treatment (July), in ungrazed plots it occurred at the end of the growing season (November) (Fig. 2).

### Belowground production

Belowground production to 15 cm depth during the first year (July 2003–July 2004) (Table 2) accounted for 21% and 18% of the highest BGB value recorded in that year in Alinyà and Prat Llong, respectively.

**Fig. 2** Belowground biomass ($gm^{-2}$) in grazed and ungrazed plots by site and depth (0–5 and 5–15 cm). Arrows indicate grazing events. Vertical bars show the standard error of the mean ($n$=2). ANOVA results only refer to BGB values in 2004. There were no significant effects in 2003




About 49% of belowground production in Alinyà and 44% of belowground production in Prat Llong occurred within the top 5 cm. There were no significant differences between sites or grazing treatments in annual belowground production. Similar results were obtained at the second year (Table 2).

The contribution of each layer to total belowground production at 0–15 cm depth changed along the growing season. The contribution of the 5–15 cm layer, was significantly higher at the beginning of the growing season (spring), but decreased thereafter (Fig. 3). This decrease was especially strong in Alinyà, where belowground production at the 5–15 cm layer in spring 2004 was about 57% of total (0–15 cm) production and decreased to about 38–40% in summer and autumn before increasing again to 48% the following spring (Fig. 3a). In Prat Llong, after summer 2003, relative belowground production at 5–15 cm layer also increased significantly (from 37% in summer to 53% in autumn 2003 and 61% in spring 2004), but in contrast with the Alinyà site, it did not decrease significantly during the following (2004) summer (Fig. 3b).

Although belowground production was proportionally higher at the top 5 cm at both sites, mean relative productivity rate (g $kg_{BGB}^{-1}$ $d^{-1}$) was much higher ($p$<0.001) in the subsurface layer (Fig. 4). It results in renewal times that were 2–3 times longer at the top 5 cm than at the 5–15 cm layer at both sites (Table 2). Relative productivity rate of belowground biomass also showed a clear seasonality at both sites (Fig. 4). Spring (April/May–June) was the most productive season, accounting for about 55–62% of the whole-year production at both sites. Then, in summer (July–September) and autumn (September–November), relative productivity rates fell to much lower values (Fig. 4). This pattern occurred at both layers, but the temporal variability estimated as the coefficient of variation across samplings, was higher in the subsurface horizon than at the uppermost one (0.8 and 1.1 at 5–15 cm depth compared to 0.4 and 0.6 at the 0–5 cm layer for Alinyà and Prat Llong respectively).

Grazing treatment reduced belowground relative productivity rate in summer 2004 ($p$<0.05), just after the grazing treatments were applied, at both sites (Fig. 4, Table 3). This reduction was actually only significant after the second grazing event (2004), but in 2003 there existed already a non-significant tendency towards a reduction of the relative productivity rates at the 5–15 cm layer ($p$=0.072, Table 3).

**Table 2** Mean belowground biomass, belowground production and renewal times by treatments, sites and depth layers in Alinyà and Prat Llong for each year. Total values for 0–15 cm are also given

| | Alinyà | | | | Prat Llong | | | |
|---|---|---|---|---|---|---|---|---|
| | July 03–July 04 | | July 04–July 05 | | July 03–July 04 | | July 04–July 05 | |
| | Ungrazed | Grazed | Ungrazed | Grazed | Ungrazed | Grazed | Ungrazed | Grazed |
| Mean BGB ($gm^{-2}$)[a] | | | | | | | | |
| 0–5 cm | 1695±429 | 1726±353 | 1981±124 | 1834±140 | 1557±47 | 1695±160 | 1693±132 | 1900±129 |
| 5–15 cm | 556±12 | 563±57 | 649±67 | 597±44 | 804±142 | 911±216 | 1010±72 | 968±78 |
| Total 0–15 cm | 2029±297 | 2204±198 | 2578±171 | 2438±188 | 2387±112 | 2587±48 | 2703±200 | 2879±215 |
| Production ($gm^{-2}$) | | | | | | | | |
| 0–5 cm | 277.2±25.6 | 273.6±20.8 | 293.0±51.2 | 291.8±7.0 | 203.9±11.7 | 234.7±8.7 | 261.2±19.2 | 232.8±3.1 |
| 5–15 cm | 257.2±29.0 | 321.0±1.5 | 265.1±83.4 | 261.7±4.9 | 293.9±82.7 | 259.5±26.0 | 321.4±27.7 | 244.9±23.6 |
| Total 0–15 cm | 544.3±3.3 | 590.4±37.5 | 558.0±134.6 | 555.7±14.1 | 496.3±92.9 | 494.2±34.7 | 582.6±46.9 | 472.0±32.3 |
| Renewal time (y) | | | | | | | | |
| 0–5 cm | 5.8 | 6.0 | 6.4 | 5.9 | 7.2 | 6.8 | 6.7 | 8.5 |
| 5–15 cm | 2.1 | 1.7 | 2.3 | 2.1 | 2.6 | 3.3 | 3.3 | 4.1 |
| Total 0–15 cm | 3.5 | 3.5 | 4.3 | 4.1 | 4.6 | 5.0 | 4.8 | 6.3 |

[a] BGB values are the average of all samplings within each period

In summer 2004, the productivity rates of grazed plots in Alinyà decreased by 29% and 37% in the 0–5 cm and 5–15 cm layers, respectively (Table 3, Fig. 4). In Prat Llong, there was also an overall reduction of belowground relative productivity rates in the grazed plots at the 5–15 cm layer (Fig. 4). However, season-by-season analyses pooling both sites together (Table 3) only indicated a significant reduction of relative productivity rates at both layers in the summer season. This summer decline in productivity rates in the grazed plots reached as high as 25.3 $gm^{-2}$ in Alinyà and 87.4 $gm^{-2}$ in Prat Llong, i.e. a decrease of 20% and 39% in grazed plots compared with ungrazed ones in Alinyà and Prat Llong respectively.

## Discussion

### Belowground production

Annual belowground production in the studied grasslands (472–590 $gm^{-2}$ $y^{-1}$) was higher than the

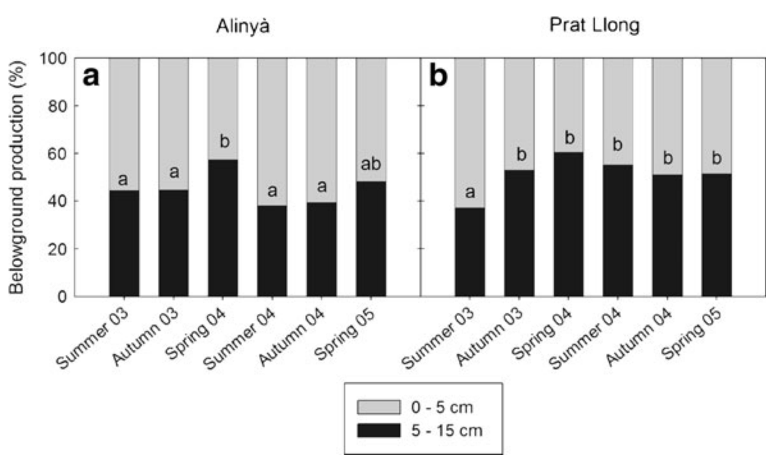



**Fig. 3** Contribution of each layer (0–5 cm and 5–15 cm) to total (0–15 cm) production in Alinyà (**a**) and Prat Llong (**b**). Bars show the mean values of the four plots of each site ($n=4$) at each period. Different letters indicate significant ($p<0.05$, Duncan test) differences between seasons

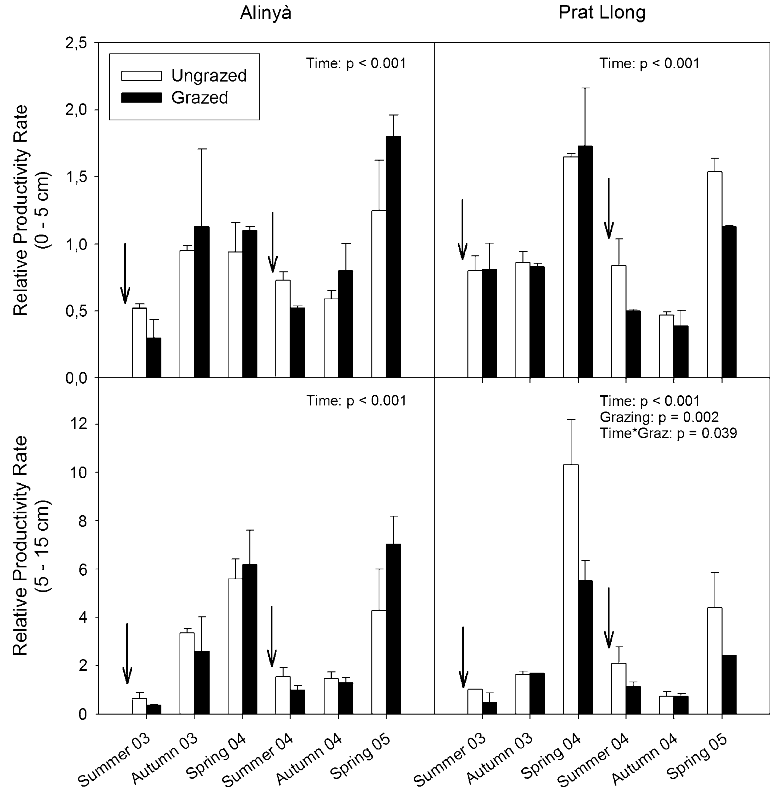



**Fig. 4** Relative belowground productivity rates (g $kg_{BGB}^{-1}$ $d^{-1}$) of grazed and ungrazed plots by site and depth (0–5 and 5–15 cm). Arrows indicate grazing events. Vertical bars show the standard error of the mean ($n$=2). ANOVA results only refer to productivity rates in 2004. There were no significant effects in 2003

estimates reported by Montané et al. (2010) in Pyrenean grasslands (130 $gm^{-2}$ $y^{-1}$) using ingrowth cores or by Hitz et al. (2001) (214–385 $gm^{-2}$ $y^{-1}$) using sequential cores in Alpine grasslands, and similar to the estimates reported by Fisk et al. (1998) on ingrowth cores to 15 cm depth in moist and wet meadows of the North-American alpine tundra (410 and 600 $gm^{-2}$, respectively). Annual production represented between 14% and 22% of the peak annual BGB (mean: 21% and 20% in Alinyà and 18% and 17% in Prat Llong for the first and second year, respectively), which is similar to the values (~17%, estimated from Fisk et al. 1998) found in moist alpine tundra.

Assuming steady-state conditions (i.e. the yearly amount of roots produced is similar to the amount of

**Table 3** Differences between sites and grazing treatments in belowground relative productivity rate (g $kg_{BGB}^{-1}$ $d^{-1}$) at 0–15 cm depth and by depth layers (0–5 cm and 5–15 cm). Significant p-values are in bold ($p$<0.05)

| | Summer 03 | Autumn 03 | Spring 04 | Summer 04 | Autumn 04 | Spring 05 |
|---|---|---|---|---|---|---|
| 0–15 cm | | | | | | |
| Site | **0.035** | 0.459 | **0.006** | 0.189 | **0.031** | **0.019** |
| Grazing | 0.108 | 0.572 | 0.712 | **0.008** | 0.754 | 0.380 |
| S × G | 0.940 | 0.761 | 0.071 | 0.430 | 0.749 | 0.065 |
| 0–5 cm | | | | | | |
| Site | **0.012** | 0.495 | **0.018** | 0.874 | **0.021** | 0.253 |
| Grazing | 0.321 | 0.852 | 0.479 | **0.047** | 0.711 | 0.557 |
| S × G | 0.383 | 0.969 | 0.579 | 0.725 | 0.252 | 0.074 |
| 5–15 cm | | | | | | |
| Site | 0.827 | 0.329 | 0.308 | 0.186 | **0.012** | **0.001** |
| Grazing | 0.072 | 0.647 | 0.304 | **0.004** | 0.677 | 0.269 |
| S × G | 0.279 | 0.682 | 0.215 | 0.562 | 0.661 | 0.229 |

roots that die) and a root C content of 366±2 $mg g^{-1}$ ($n$=30, from ingrowth roots, data not shown), our results would suggest that gross C inputs into the soil from BGB (excluding root exudates) could be between 173 and 216 gC $m^{-2}$ $y^{-1}$ at the top 15 cm soil layer. This represents about one third of the total ecosystem gross primary production estimated by tower $CO_2$ flux measurements at a nearby grassland in Alinyà during 2003–2004 (605 gC $m^{-2}$ $y^{-1}$; Gilmanov et al. 2007). These potential belowground C inputs into the soil would represent a yearly input of about 2–3% of total soil C stocks of the site in the top 15 cm (8.0 $kg m^{-2}$ and 8.5 $kg m^{-2}$ in Alinyà and Prat Llong, respectively).

Biomass density and production was much higher in the first 5 cm than in the 5–15 cm subsurface layer. Nevertheless, belowground relative productivity rates give some indication on root dynamics. Although we did not measure root birth and mortality, our results suggested that root activity increased with depth, causing higher root turnover and shorter renewal times at the subsurface layer. Other studies carried out in grasslands and forests generally found no layer effects (Gill et al. 2002) or a decrease (Arnone et al. 2000) in root turnover with depth. These divergences could be related to differences in root activity associated with branch order and diameter, which also varies with depth (Guo et al. 2008).

## Seasonal patterns in belowground productivity

Changes in BGB over time were about twice as high as root production at both sites. This may be partially due to increasing uncertainty when subtracting two estimates subjected to a significant variability, as is the case when estimating changes in BGB.

Dynamics in seasonal belowground production is determined by several factors related to climate, plant communities, soil characteristics and land management. In mountain areas, the length of the growing season is determined by snow cover or duration of soil freezing (Körner 2003). However, at our study sites, in spite of lower temperatures and shorter growing seasons in Prat Llong (169 d) than in Alinyà (228 d), there were no significant between-site differences in total annual belowground production. The shorter growing season at Prat Llong was counteracted by its higher productivity, particularly in the first 5 cm layer in summer 2003 and spring 2004. The higher rainfall values in Prat Llong than in Alinyà could explain it. This pattern had already been reported for aboveground biomass in alpine meadows where, at the late-snowmelt sites, the larger LAI and higher N content of canopies compensated their shorter growing season by enhancing aboveground productivity (Baptist and Choler 2008).

There was a clear seasonality in the belowground productivity in our grasslands and, although seasonal microclimate variations are typically stronger in the surface layer, the seasonal peaks of belowground production were more pronounced in the subsurface layer. It had also been reported by Hendrick and Pregitzer (1996) in temperate North American forests. Between-layer differences in seasonal variability of relative belowground productivity rates could respond to differences in plant species phenology with different rooting depths or more dynamic root systems at the subsurface layer, with higher growth rates under suitable conditions in spring together with higher mortality in the harshest seasons.

Root production has been reported to be related to climate parameters such as temperature (Gavito et al. 2001; Steinaker and Wilson 2008), but in our study the high July and August temperatures did not result in high summer productivity. This finding indicates that temperature alone is not a reliable predictor of seasonal root growth in mountain grasslands. Thus spring was the most productive season, which accounted for more than 50% of annual production, before a sharp decrease in summer. This early peak may be related to the acquisition of nutrients, as reported by Jaeger et al. (1999), who found that plants in alpine tundra acquire N early in the growing season, when microbial N pools are low and N is more readily available, allowing higher productivity. Then, as soil temperature increases, N may be more immobilised by microbial biomass. Steinaker and Wilson (2008) also found that the major peak of root production in Canadian grasslands and forests occurred at the beginning of summer, and reported a second smaller peak at the end of the season (late summer and autumn). In our case, relative productivity rates in autumn differed between years, and there was no other peak as clear as the spring peak.

In spring, plant root systems were not only more productive but also grew deeper. These seasonal changes could be related to differences in the timing of species development with different rooting depths, or to microclimatic subsoil conditions. Fitter (1986) observed seasonal differentiation in the depth of root activity, explained by roots seeking more suitable microclimate conditions. Although our data showed that temperature was not the only factor determining root activity, it does determine the freeze-thaw cycles of the soil surface layer at the beginning and the end of the growing season. Most soils (and particularly mountain soils) are characterized by a thermal gradient along the profile (Körner 2003), which may explain these differences in the seasonality of belowground productivity at each layer. Such conditions at the beginning of the growing season, when snow has melted and there are more frequent freeze-thaw events in the topsoil, could restrict the plant's ability to grow and limit water and nutrient uptake by species with shallower roots. The more stable temperatures in deep layers may (if they are not frozen) allow BGB development of deep-rooting species earlier in the growing season. This effect was not detected in autumn, when freeze-thaw cycles also occur, perhaps because plants are much less active in this season.

### Short-term effects of grazing exclusion on belowground biomass and production

At short term, excluding grazing increased relative belowground productivity rates in summer at both sites. Although aboveground biomass consumption was lower in 2004, the reduction in productivity rates in grazed plots was especially relevant after the second grazing event. This may suggest that increased root productivity in ungrazed plots may be potentiated by the removal of grazing events occurring as years pass. Reduced belowground productivity in response to defoliation or grazing had previously been found in pot (Guitian and Bardgett 2000; Zhao et al. 2008) and field (Ruess et al. 1998; Johnson and Matchett 2001; Smit and Kooijman 2001) experiments. However, although in our experiment, relative productivity rates in summer were lower in grazed grasslands, there were no significant differences in total annual production between grazed and ungrazed plots. This is probably due to the low root production observed in summer and the high variability in both seasonal and whole-year production.

Although grazing reduced relative belowground productivity rates, its effect on BGB was site-dependent. While in Alinyà, the reduction of belowground productivity in summer coincided with a decrease in BGB, this effect was not observed in Prat Llong, where BGB at 0–5 cm even remained higher in the grazed plots throughout the experimental period. These discrepancies may occur because such changes in BGB are the result of both belowground production and root lifespan. Additional data on the effect of grazing on root lifespan and on changes in soil biological activity and decomposition rates could help explain these between-site differences. It has been shown that decomposition rates may also be influenced by grazing (Klumpp et al. 2009). Nevertheless, consistent increases in belowground productivity in abandoned grasslands for longer periods of time could be expected to have consequences on BGB and on the incorporation of organic C into the soil organic matter.

## Conclusions

Seasonal belowground production dynamics seem to be determined, first of all, by microclimate whereas grazing exclusion has a discrete short-term effect. Thus, as expected, relative productivity rates were higher at the uppermost soil layer than in the subsurface layers of the subalpine grasslands. However, roots at the subsurface layer showed higher relative activity and stronger seasonality. The most productive season (spring) did not coincide with the warmest season, indicating that factors other than temperature, such as nutrient availability, influence the seasonal variation of root production. The hypothesis that root production in spring and autumn would be biased to the subsurface layer is only partially supported by our data, which show higher contribution of subsurface production only in spring, not at the end of the growing season.

Compared with excluding grazing, grazing causes a short-term decrease of root production just after the event, without having apparent effects on yearly belowground C input into the soil.

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