Dynamics of structural traits in two competing C₃ grass species: influence of neighbours and nitrogen

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Abstract

This work analysed the regulatory structural mechanisms involved in the competitive interactions between the annual grass *Bromus willdenowii* Kunth. (BW = prairie grass) and the perennial C₃ grass *Dactylis glomerata* L. (DG = orchardgrass) during pasture establishment. Four combinations of species (pure BW, pure DG, DG flanked by BW and BW flanked by DG plants), with and without winter nitrogen fertilization, were factorially arranged in a randomized complete block design. Data were recorded at two organization levels: tillers (three tiller age cohorts) and target plants. Annual neighbours caused a decrease in the number of living leaves in tillers of intermediate age of both species. This structural regulatory mechanism led to a decrease in tiller number per plant and, therefore, restricted the development of horizontal space occupation. Annual neighbours did not cause an increase in tiller size, measured as lamina length or pseudostem height, but decreased root biomass. As a consequence, annual neighbours did not lead the hierarchy in light capture, but limited species radical colonization and competitive ability for soil resources. Winter nitrogen fertilization only affected tiller size in older tillers. These findings emphasize the importance of the cultural decisions, as sowing densities and nitrogen fertilization, to optimize pasture floristic composition.

Keywords: *Bromus willdenowii* Kunth., *Dactylis glomerata* L., intra- and interspecific initial competition, tiller size, tiller density, hierarchies among plants, pasture floristic composition

Introduction

Optimizing pasture floristic composition requires an understanding of the growth regulation mechanisms of competition among the coexisting species. Morphogenetic traits – leaf appearance rate, leaf elongation rate, leaf lifespan (Lemaire and Chapman, 1996; Lemaire and Agnusdei, 2000) and tiller appearance rate (Hirata and Pakiding, 2002) – are key components of the structural traits, tiller size (Virkajärvi and Järvenranta, 2001) and tiller density (Sugiyama, 1995), and play a major role in the competitive ability of plants within the community (Lemaire and Millard, 1999). Tiller size is proportional to final leaf size, number of living leaves per tiller and pseudostem size (Hume, 1991; Lemaire and Chapman, 1996; Lemaire and Agnusdei, 2000; Virkajärvi and Järvenranta, 2001; Berone and Dreher, 2007; Berone *et al.*, 2007a,b). Tiller density depends on leaf appearance rate, which determines the maximum tiller appearance rate, but also on the site filling rate (Davies, 1974; Gautier *et al.*, 1999; Bahmani *et al.*, 2000). In addition, all these traits show plastic variations because they are regulated by environmental factors (Lemaire and Chapman, 1996; Gastal and Durand, 2000). The knowledge of morphogenetic traits and of morphological and structural plasticity constitutes the basis for the understanding of the mechanisms affecting species growth and persistence because they are appropriate predictors of species competitive performance (Islam and Hirata, 2005).

The size of tillers is associated with light competitive ability (Hazard and Ghesquière, 1995; Nurjaya and Tow, 2001) and potential productivity (Sugiyama, 1995, 1999), while the density of tillers is associated

with horizontal space occupation and species persistence (Hume, 1991). The life form of coexisting species might condition all these structural traits because they are related to different patterns of carbon partitioning between tiller size and tiller density (Sugiyama, 1995). During vegetative growth, plants of *Lolium perenne* showed a balanced activity at each of the three main centres of meristematic growth: the terminal meristematic region (apical meristem and several expanding leaves or leaf primordia), lateral meristems (tiller buds) and root meristems. Instead, *Lolium temulentum*, an annual grass, showed a meristematic activity concentrated at the terminal meristem; these differences between perennial and annual plants increased with plant age (Ryle, 1970). In addition, Robson *et al.* (1988) showed that perennial grasses often display a greater priority of carbon partitioning towards daughter tillers than annual ones, higher proportion of biomass partitioned to pseudostems and lower to blades and roots (Garnier, 1992). However, it is still not clear whether there is a differential biomass partition between size and density of tillers in seedlings of annual and perennial C_3 grasses.

Also, both structural traits – tiller size and tiller density – would be regulated by direct and indirect signals mediated by neighbour plants (Bahmani *et al.*, 2000) and/or nitrogen supply. These different carbon allocation patterns between coexisting species may be caused by hierarchical differences in light acquisition within the stand, rather than by intrinsic genotypic differences between life forms (Lemaire and Millard, 1999; Lemaire, 2001). These hierarchies, developed under competition for light, lead to soil resource competition and may occur from the initial phases of growth, because they are related to the perception of plants about the existence of neighbours (Lemaire and Millard, 1999). In addition, tiller age, another important factor interfering with structural characteristics, may also influence plant responses by altering sward structure (Paiva *et al.*, 2012). In summary, structural mechanisms are still poorly understood and may be determinants of early hierarchies between coexisting annual and perennial species within a community.

We have previously evaluated morphogenetic traits in miniswards composed of the perennial grass *Dactylis glomerata* (orchardgrass = DG) and the annual/biennial grass *Bromus willdenowii* (prairie grass = BW). This is a common mix of grass species used in humid temperate pastoral systems of Argentina. *Dactylis glomerata* is a slow-establishing species with low seedling competitive ability (Nurjaya and Tow, 2001). *Bromus willdenowii* is native to the South American temperate plains (Maddaloni and Ferrari, 2001), and behaves like an annual plant because it does not produce replacement tillers after the reproductive period (Scheneiter and

Rosso, 2005). As BW shows higher growth rates during the period of low temperatures than the perennial grass, BW can establish rapidly and exclude DG (Harris, 2001; Sanderson *et al.*, 2002), especially when supplemented with nitrogen application (Suding *et al.*, 2005). During the early establishment, a critical stage in multispecies pastures (Skinner, 2005), annual neighbour plants with greater leaf elongation rate may cause a delay in leaf appearance rate of the older tillers of neighbour plants of both species. This delay reduces the tiller appearance rate of plants by light restriction at the base of the canopy (Gatti *et al.*, 2013). However, it is not clear how these morphogenetic mechanisms are associated with the dynamics of structural changes among tiller age cohorts in the presence of different neighbours and with nitrogen fertilization practices. Therefore, are other structural mechanisms involved in the early competitive interactions between annual and perennial species? Are they involved in the early hierarchies among these establishing species and hence, in species persistence and pasture composition?

The objective of this work was to describe the structural mechanisms involved in the competitive interactions between annual and perennial C_3 grass species. This study focused on two organization levels: tillers and plants, in situations of intra- and interspecific competition and under nitrogen fertilization, during the critical establishment phase of the mixture pasture. We associated the structural mechanisms with those morphogenetic ones described in Gatti *et al.* (2013) and with competitive abilities and early hierarchies between coexisting annual and perennial species within a community. We hypothesized that annual neighbours, through reductions of light at soil level, will affect the hierarchies about light and soil resources acquisition; they will improve the competitive ability for light, and they will reduce it for soil resources. The magnitude of these effects will be enhanced by nitrogen application. To test these hypotheses, we analysed the dynamics of structural and growth-related traits of three tiller cohorts in individual plants (target plants) of the coexisting annual and perennial species, flanked in the sowing line by two neighbour annual or perennial plants.

Materials and methods

Environmental conditions

The experiment was carried out in the experimental field of the School of Agronomy, University of Buenos Aires, Buenos Aires, Argentina (FAUBA; 34° 35′ S, 58° 29′ W). The top soil (0–20 cm) had pH (1:2·5) 5·85, electrical conductivity 0·20 dS m^{-1}, carbon content

(Walkley Black) 12·9 mg g^{-1}, nitrogen content (Kjeldhal) 1·5 mg g^{-1} and nitrate content (SNEDD) 1·86 mg kg^{-1}. Current extractable phosphorus content (Bray and Kurtz 1) in the top 20 cm showed a patchy spatial distribution from 5·26 to 10·93 mg kg^{-1}. The experimental period from sowing (27 May 2008) to the last record and harvest (13 September 2008) was 109 days. During this period, precipitation was 142·2 mm, and mean daily temperature was 11·8 ± 2·8°C (mean minimum and maximum daily temperatures of 6·4 and 18·9 °C respectively). The air temperatures were lowest from 25 July to 8 August and then showed a slowly increasing trend towards the end of the experiment (see details in Gatti *et al.*, 2013). Photosynthetic active radiation (PAR) was measured just above and inside the canopy at soil level 60 days after emergence (60 dae) and at harvest time (85 dae), at 12:00 h. (Sims *et al.*, 2005), using a 50-cm-length aluminium data logger bar radiometer (Photosynthetic Photon Flux = PPF = μmol m^{-2} s^{-1}).

Plant material and experimental design

Twenty-four experimental units were sown as miniswards of five alternated rows 0·18 m long, with 0·175 m row spacing (approximately 0·16 m^2). Three of the rows were sown with grasses (*Bromus willldenowii* Kunth 'Fortín Pergamino' (BW = prairie grass; annual/biennial species) and *Dactylis glomerata* L. 'Porto' (DG = orchardgrass; perennial)), whereas the other two were sown with *Trifolium pratense* 'Redgold' (TP = red clover), to simulate a usual pasture composition. In each minisward, every grass line had a combination of BW and DG, which corresponded to four sowing designs of intra- and interspecific competition: BW flanked by BW (BW Bw); DG flanked by DG (DG Dg); BW flanked by DG (BW Dg); and DG flanked by BW (DG Bw) (Figure 1; also in Gatti *et al.*, 2013).

After emergence, seedling density was manually set to obtain approximately 200 grass seedlings m^{-2}, 1·5 cm of distance between plants, the one usually obtained in Argentine pastures 2 months after sowing (Gatti *et al.*, 2011). The four sowing grass combinations (two target species × two neighbour species) and two levels of fertilization (yes/no) (see *Data collection* for definition of target plants) formed a 2 × 2 × 2 factorial design in a randomized complete block design with three replicates. The blocks controlled two variation sources: topographic position and initial soil P content. Due to the high nutrient demand of legumes, we enhanced phosphorus level to 20 ppm, so plants were fertilized by applying between 42 and 68 kg P ha^{-1} in blocks with highest and lowest initial soil P, respectively, in agreement with Rubio *et al.* (2012).

On 19 July, during winter, 53 days after sowing (das) and 29 days after emergence (dae), urea fertilizer was applied at a rate of 170 kg N ha^{-1}, a dose that usually allows a linear response in most C$_3$ grass species of the Humid Pampa (Fernández Grecco *et al.*, 1995). At this time, plants had one to three tillers per plant and five to six living leaves in the main stem (Gatti *et al.*, 2013), a size corresponding to the beginning of the establishment stage (Ries and Svejcar, 1991; Sanderson *et al.*, 2002).

Data collection

Data were collected after fertilization on the three central individual plants – the central target plant and their two neighbours – on the central grass line of each minisward, to avoid border effects from external grass rows. We did not expect a neighbour effect of the rows with legumes due to their very slow initial growth rate and plant biomass during the experimental period, with a low mean daily temperature of
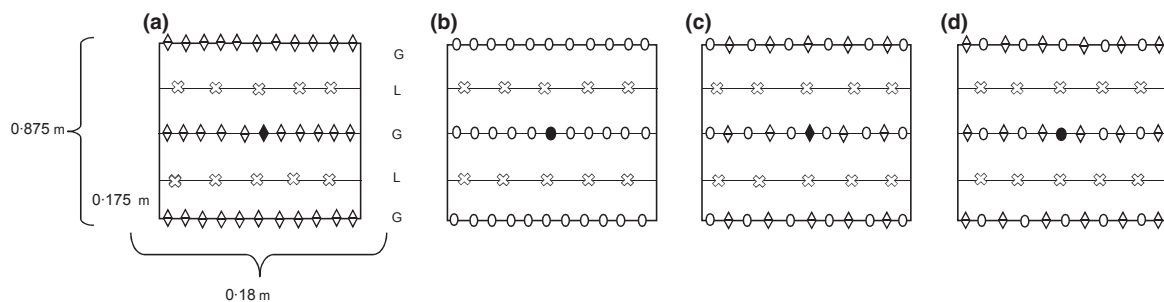
Figure 1 Diagram of the four minisward arrangements: (a) BW flanked by BW (BW Bw), (b) DG flanked by DG (DG Dg), (c) BW flanked by DG (BW Dg) and (d) DG flanked by BW (DG Bw). Black symbols identify the target plant: ♦ BW and • DG; ×TP; G, grass rows; and L, legume rows.

$11\cdot8 \pm 2\cdot8°C$ (Gatti *et al.*, 2013). Tiller cohorts were marked with plastic rings of different colours, and pseudostem height and tiller number per plant were recorded. The number and length of expanded and growing or expanding leaves were monitored weekly on three tiller age cohorts: the main tiller and the largest ones that appeared 20 and 30 days after fertilization (20-daf and 30-daf tillers, respectively), i.e., 50 and 60 days after emergence (50-dae and 60-dae tillers respectively). Data recording ended at the beginning of the reproductive period of BW (13 September; 85 dae and 56 daf) because we considered that competition for resources is directly linked to the vegetative development of plants (Lemaire and Millard, 1999).

Whole target plants were harvested at the end of the experiment, maintaining the identity of the marked tillers. The plant root system was manually excavated; the soil surrounding it was carefully removed, and then the root was rinsed with water (Fang *et al.*, 2012). The harvested material was separated into root and aerial fractions. The aerial fraction was separated into dead and living material, and living mass was separated in laminae and pseudostems. Subsamples of green laminae (i.e. the youngest expanded leaf of each tiller) were selected, and leaf area was determined from digital images obtained with scanner and later software-processed (O' Neal *et al.*, 2002). All root and aerial fractions were oven-dried at 65°C until constant weight and weighed.

Structural variables in tillers and target plants

The number of living leaves per tiller (leaves tiller^{-1}), which represented the total green leaves present in each tiller, was estimated by the quotient between leaf lifespan and leaf appearance rate (Lemaire and Chapman, 1996; Lemaire and Agnusdei, 2000), was reported by Gatti *et al.* (2013). Living leaves were composed of the number of expanded (i.e. those with visible ligule) and that of growing or expanding leaves (i.e. those where the ligule was not visible). The proportion of growing leaves per tiller was calculated as the number of growing living leaves^{-1} ratio. Total green lamina length per tiller (cm tiller^{-1}), a tiller size estimator (Berone and Dreher, 2007; Berone *et al.*, 2007a,b, 2008), was the weekly average of green lamina length per tiller, including both expanded and growing leaves (Virkajärvi and Järvenranta, 2001; Agnusdei and Colabelli, 2008; Berone *et al.*, 2007a,b; Berone and Dreher, 2008). Pseudostem height (cm tiller^{-1}) was the distance between the highest visible ligule and the soil surface (Virkajärvi and Järvenranta, 2001). Tiller density (tillers plant^{-1}) was the number of tillers per target plant at final harvest (109 das; 85

dae; 56 daf). Leaf areas of marked tiller cohorts and target plants (cm^2 tiller^{-1} and cm^2 plant^{-1}, respectively) were estimated in harvested green lamina material.

Growth-related variables in tillers and target plants

At harvest, living tiller mass (green mass; g tiller^{-1}) was composed of lamina and pseudostem mass per tiller; dead tiller mass was dead and senescent material; gross tiller mass was the sum of green and dead material of each of the three tiller cohorts. These variables, recorded in the marked tillers, were similarly estimated at plant scale. Gross and root plant masses (g plant^{-1}) integrated total target plant biomass (g plant^{-1}). Biomass partition towards aerial and root biomass was gross plant mass and root plant mass per unit total target plant biomass respectively (%).

Statistical analysis

Factorial analyses of variance with a significance level of 5% were performed following a randomized complete block design with three factors: target species (two levels) × neighbour species (two levels) × fertilization (two levels) ($n = 3$; 24 miniswards). All traits were assessed for normality using the Shapiro–Wilk test. The differences between tiller cohorts were analysed with a split-plot design (three levels). As there were interactions involving tiller cohorts, the analysis was split for each cohort; within each cohort, partitions by factor were carried out when other interactions subsisted. InfoStat Professional Package, version 1.1 (Universidad Nacional de Córdoba, Argentina), was used to perform statistical analyses.

Results

Microcanopy photosynthetically active radiation (PAR) interception

Sixty-six days after emergence (66 dae and 36 daf; on 25 August), PAR interception was 64% of incident light, and was not affected by the species ($P = 0·68$) or fertilization practices ($P = 0·09$). Nevertheless, PAR interception of target plants with a BW neighbour was higher than with a DG neighbour (70% when the neighbour was BW vs. 58% when the neighbour was DG). Transmitted PAR to the soil was lower when the neighbour was BW than when the neighbour was DG (102 μmol m^{-2} s^{-1} when the neighbour was BW vs. 138 μmol m^{-2} s^{-1} when the neighbour was DG). At harvest date (on 13 September; 85 dae and 56 daf;

towards the end of winter), PAR interception of each species was differentially affected by the identity of the neighbour (species*neighbour interaction, $P = 0.0128$) and nitrogen fertilization ($P = 0.042$). DG PAR interception (92·8%) was not affected by neighbour identity and fertilization. Instead, BW PAR interception was higher when flanked by a neighbour of the same species (92·7% when the neighbour was BW vs. 85·3% when the neighbour was DG) and also higher when it was fertilized (85·7% N$^-$ vs. 92·2% N$^+$).

Structural tiller variables

Leaves per tiller

During the post-fertilization period, the number of growing (Figure 2a) and expanded leaves (Figure 2b), composing the living leaves per tiller (Figure 2c), decreased from the oldest to the youngest cohort in both species. In the main stem, number of living and expanded leaves of BW were lower than those of DG (5·7 vs. 8·6 living leaves tiller^{-1}; $P = 0.0015$, and 2·7 vs. 5·9 expanded leaves tiller^{-1}; $P = 0.0004$, for BW and DG respectively) (Table 1).

While expanded and living leaves of BW were lower than those of DG (Figure 2b and c; Table 1), growing leaves showed the opposite pattern; BW expanded simultaneously more leaves than DG (BW: 3·0 vs. DG: 2·6 growing leaves tiller^{-1} in the main stem) ($P = 0.031$; Figure 2b; Table 1). As a consequence, the ratio between both variables (number of growing. living leaves^{-1} ratio) was higher in the annual grass (BW: 0·54 vs. DG: 0·32) ($P < 0.0001$; Figure 2d; Table 1). The proportion of growing leaves increased from the main stem to the 60-dae/30-daf tiller cohort (0·32–0·71 leaves leaf^{-1}) in DG, but remained without change among tiller cohorts in BW (a mean of 0·58 leaves leaf^{-1}, Figure 2d). No neighbour effects were detected, with the exception of the 50-dae/20-daf cohort, in which both species showed a higher number of expanded (1·1 when the neighbour was BW vs. 2·9 expanded leaves when the neighbour was DG; $P = 0.0012$) and also higher living leaves when they grew with DG neighbours than with BW neighbours (3·0 when the neighbour was BW vs. 4·7 living leaves when the neighbour was DG; $P = 0.0013$; Figure 2c; Table 1). As a consequence, in this cohort, the proportion of growing leaves per tiller was higher with BW neighbours than with DG neighbours (0·7 vs. 0·4 leaves leaf^{-1}, respectively; $P = 0.0017$). In the youngest tiller, no significant effects were observed for any factor ($P > 0.05$; Figure 2d, Table 1). Nitrogen fertilization exerted no significant effects upon the number of leaves per tiller ($P > 0.05$).
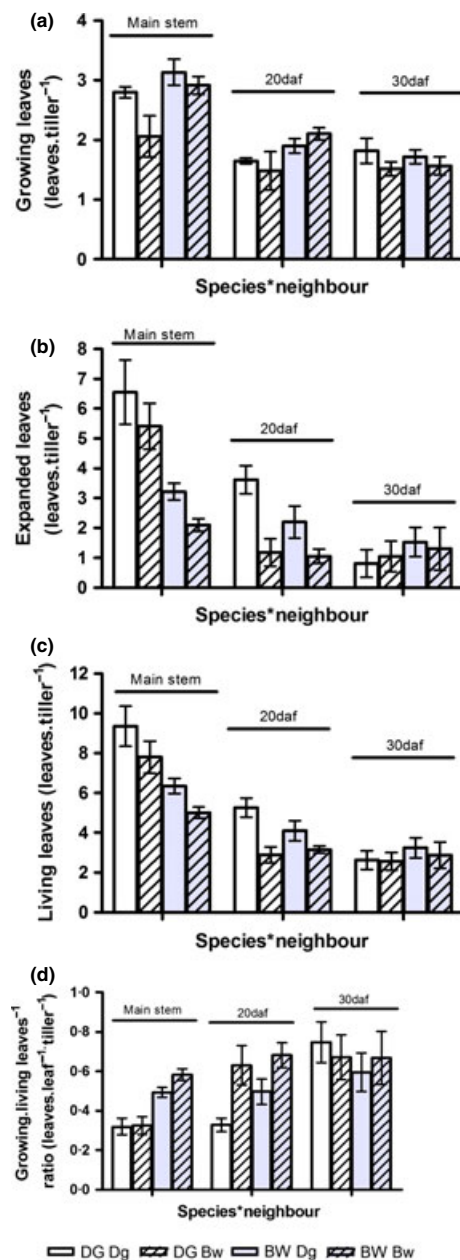
Figure 2 (a) Number of leaves expanding simultaneously (number of growing leaves; leaves tiller^{-1}), (b) number of expanded leaves (leaves tiller^{-1}), (c) number of living leaves (leaves tiller^{-1}) and d) proportion of growing leaves (leaves leaf^{-1} tiller^{-1}), comparing only the effects of the four different situations of intra- and interspecific competition of the perennial *Dactylis glomerata* (DG) and the annual *Bromus willdenowii* (BW). Fertilization effects ≥ 0·05 (see Table 1 for details). Data are means ± standard error. First name abbreviation: target species; Second name abbreviation: neighbour species (e.g. DG Dg).

Table 1 Results of the analysis of variance comparing the effects of species (two levels: *Dactylis glomerata* [DG] and *Bromus willdenowii* [BW]), neighbours (two levels: DG and BW), winter fertilization (two levels: with and without nitrogen) and their interactions, upon key structural variables (n = 3).

Variable	Unit of growth (tiller cohort; plant)	Species effect — Actual values and P		Neighbour effect — Actual values and P		Fertilization effect — Actual values and P		Interaction Type and P	References
Growing leaves (leaves tiller^{-1})	Main stem	DG = 2·6 BW = 3·0	*	DG = 2·9 BW = 2·7	ns	N$^-$ = 2·8 N$^+$ = 2·8	ns	ns	Fig. 2a
	20-daf tiller cohort	DG = 1·7 BW = 2·0	ns	DG = 1·8 BW = 1·9	ns	N$^-$ = 1·8 N$^+$ = 1·9	ns	ns	
	30-daf tiller cohort	DG = 1·7 BW = 1·6	ns	DG = 1·8 BW = 1·5	ns	N$^-$ = 1·7 N$^+$ = 1·6	ns	ns	
Expanded leaves (leaves tiller^{-1})	Main stem	DG = 5·9 BW = 2·7	**	DG = 4·9 BW = 3·8	ns	N$^-$ = 4·3 N$^+$ = 4·3	ns	ns	Fig. 2b
	20-daf tiller cohort	DG = 2·4 BW = 1·6	ns	DG = 2·9 BW = 1·1	**	N$^-$ = 1·8 N$^+$ = 2·2	ns	ns	
	30-daf tiller cohort	DG = 0·9 BW = 1·4	ns	DG = 1·2 BW = 1·2	ns	N$^-$ = 0·8 N$^+$ = 1·6	ns	ns	
Living leaves (leaves tiller^{-1})	Main stem	DG = 8·6 BW = 5·7	**	DG = 7·8 BW = 6·4	ns	N$^-$ = 7·1 N$^+$ = 7·1	ns	ns	Fig. 2c
	20-daf tiller cohort	DG = 4·0 BW = 3·6	ns	DG = 4·7 BW = 3·0	**	N$^-$ = 3·6 N$^+$ = 4·0	ns	ns	
	30-daf tiller cohort	DG = 2·6 BW = 3·0	ns	DG = 2·9 BW = 2·7	ns	N$^-$ = 2·5 N$^+$ = 3·1	ns	ns	
Growing. living leaves^{-1} (leaves leaf^{-1})	Main stem	DG = 0·3 BW = 0·5	**	DG = 0·4 BW = 0·4	ns	N$^-$ = 0·4 N$^+$ = 0·4	ns	ns	Fig. 2d
	20-daf tiller cohort	DG = 0·5 BW = 0·6	ns	DG = 0·4 BW = 0·7	**	N$^-$ = 0·6 N$^+$ = 0·5	ns	ns	
	30-daf tiller cohort	DG = 0·7 BW = 0·6	ns	DG = 0·7 BW = 0·7	ns	N$^-$ = 0·8 N$^+$ = 0·6	ns	ns	
Total green lamina length per tiller (cm tiller^{-1})	Main stem	DG = 40·3 BW = 66·8	**	DG = 53·4 BW = 53·7	ns	N$^-$ = 46·6 N$^+$ = 60·5	**	ns	Fig. 3a
	20-daf tiller cohort	DG = 27·2 BW = 46·4	**	DG = 36·7 BW = 36·9	ns	N$^-$ = 31·6 N$^+$ = 42·0	*	ns	
	30-daf tiller cohort	DG = 19·1 BW = 30·5	**	DG = 25·7 BW = 23·9	ns	N$^-$ = 24·5 N$^+$ = 25·1	ns	ns	
Tiller density (tillers plant^{-1})	Entire plant	DG = 15·3 BW = 13·9	ns	DG = 17·2 BW = 12·0	*	N$^-$ = 14·6 N$^+$ = 14·7	ns	ns	Fig. 3c

Data are means and P values: ** $P < 0·01$; * $P < 0·05$ and ns, non-significant differences.

Total green lamina length per tiller

Bromus willdenowii showed higher total green lamina length per tiller than DG, in the three tiller cohorts (e.g. 66·8 vs. 40·2 cm tiller^{-1} in the main stem of BW and DG, respectively; $P \leq 0·0011$; Figure 3a; Table 1). No neighbour effects were evident in the total green lamina length per tiller of either species. Total green lamina length per tiller increased in response to fertilization in the main stem (46·6 vs. 60·4 in N$^-$ and N$^+$, respectively; $P = 0·004$) and the 50-dae/20-daf tiller cohort (31·6 vs. 42·0 in N$^-$ and N$^+$, respectively; $P = 0·01$), but not in the 30-daf tiller cohort (Figure 3a, Table 1).

Pseudostem height

Pseudostem height was higher in BW than in DG (9·2 vs. 4·7 cm tiller^{-1} in BW and DG, respectively; $P < 0·0001$) and showed a decreasing trend with increasing tiller age (Figure 3b). In the youngest tiller, the pseudostem height of BW plants flanked by BW
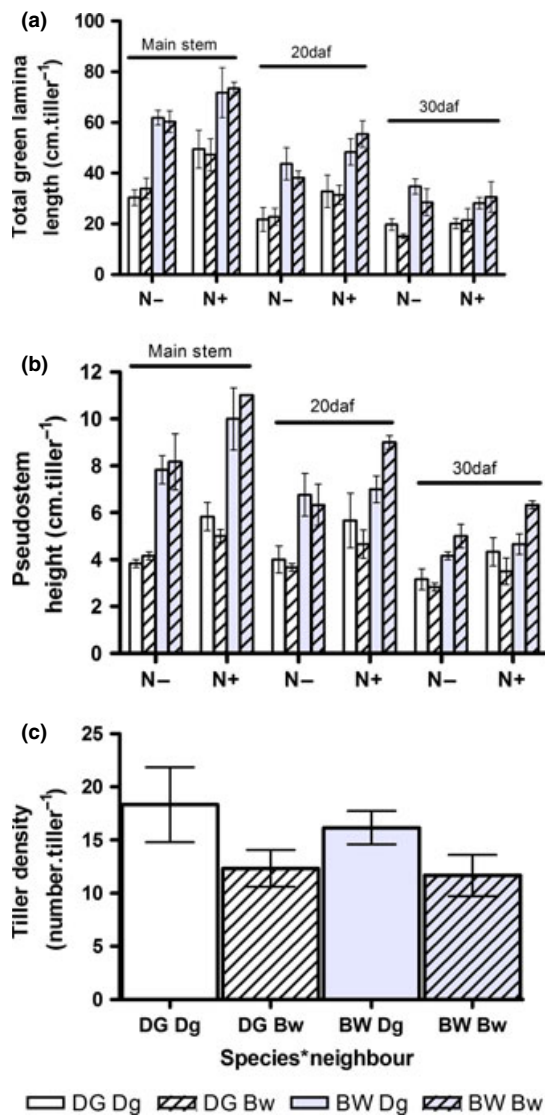
Figure 3 (a) Total green lamina length (cm tiller^{-1}), (b) pseudostem height (cm tiller^{-1}) and c) tiller density (number plant^{-1}) evaluated in four different situations of intra- and interspecific competition of the perennial *Dactylis glomerata* (DG) and the annual *Bromus willdenowii* (BW) in non-fertilized (N$^-$) and fertilized (N$^+$) treatments. In (a) and (b), data are means ± standard error of three tiller age cohorts (main stem and marked tiller cohorts appeared 20 and 30 days after fertilization= daf), whereas in (c), data are means ± standard error of the plant (Fertilization effects ≥ 0·05). First name abbreviation: target species; Second name abbreviation: neighbour species (e.g. DG Dg).

plants (5·7 cm tiller^{-1}) was higher than those of BW plants flanked by DG neighbours (4·4 cm tiller^{-1}), but no neighbour effects were evident in the pseudostem

length of DG plants (3·5 cm tiller^{-1}) (species*neighbour interaction; $P = 0.01$). Nitrogen fertilization increased pseudostem height in all tiller cohorts (e.g. 6·0 in N$^-$ vs. 7·9 cm tiller^{-1} in N$^+$ in the main stem; $P \leq 0.01$).

Individual tiller leaf area

Leaf area of the main tiller was higher for BW than for DG (64·0 vs. 31·7 cm^2 tiller^{-1}, respectively; $P = 0.0001$) and showed an increase in response to fertilization (39·3 in N$^-$ vs. 56·43 cm^2 tiller^{-1} in N$^+$) ($P = 0.0062$). The 50-dae/20-daf tiller cohort showed a similar effect (species effect, BW: 36·9 vs. DG: 17·7 cm^2 tiller^{-1}, respectively; $P < 0.0001$ and fertilization effect, N$^-$: 22·2 vs. N$^+$: 32·4 cm tiller^{-1}; $P = 0.0077$), and there was no effect in the youngest cohort (60-dae/30-daf; $P > 0.05$). No significant neighbour effects were detected in the leaf area of any of the tiller cohorts ($P \geq 0.56$).

Growth-related tiller variables

Tiller biomass

Gross tiller biomass (dead + living masses) of BW was higher than that of DG in the three cohorts (e.g. BW: 0·74 vs. DG: 0·25 g tiller^{-1} in the main stem: $P < 0.0025$) and increased by nitrogen fertilization in the main tiller (N$^-$: 0·42 vs. N$^+$: 0·57 g tiller^{-1}; $P = 0.043$; Figure 4a). Dead masses of BW accumulated in the main and in the 50-dae/20-daf tiller cohort were higher (0·09 and 0·05 g tiller^{-1}, respectively) than those of DG (0·03 g tiller^{-1} in both tiller cohorts; $P < 0.0036$). BW tillers also showed higher living tiller mass than DG, and these differences decreased with tiller age: 0·2 vs. 0·6, respectively, in the main stem ($P < 0.0001$) and 0·1 vs. 0·07, respectively, in the 60-dae/30-daf tiller cohort ($P = 0.0015$). Living tiller mass was composed of laminae and pseudostems (Figure 4b and c), which were higher in BW than in DG tillers (BW laminae mass: 0·4 vs. DG laminae mass: 0·1 g tiller^{-1} and BW pseudostem mass: 0·3 vs. DG pseudostem mass: 0·08 g tiller^{-1} in the main stem; $P < 0.0086$); such differences decreased from the oldest to the youngest tillers. Gross and dead masses of the 50-dae/20-daf tiller cohorts of both species were higher when flanked by DG than when flanked by BW neighbour plants (0·2 vs. 0·3 g of gross mass tiller^{-1} when the neighbour was BW and DG, respectively; $P < 0.04$). The pseudostem mass of the 50-dae/20-daf tiller cohorts of both species increased when the neighbour plants were DG compared with tillers flanked by BW neighbour plants (0·06 vs. 0·09 g tiller^{-1} when the neighbour was BW and DG,
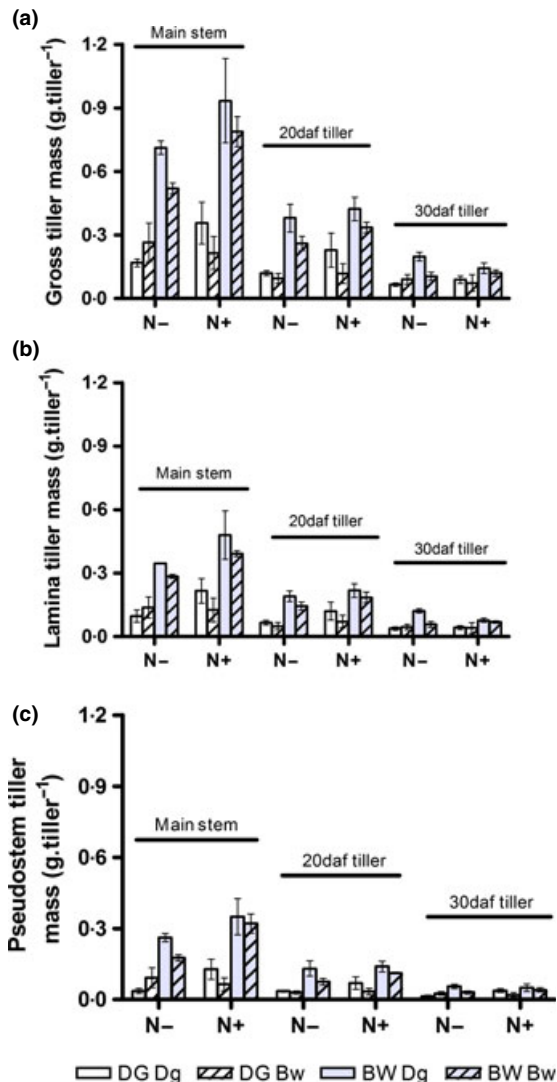
Figure 4 (a) Gross tiller biomass and living tiller biomass components (g tiller^{-1}): (b) lamina mass and (c) pseudostem mass in the final harvest (13th September) of the three tiller cohorts evaluated in four different situations of intra- and interspecific competition of the perennial *Dactylis glomerata* (DG) and the annual *Bromus willdenowii* (BW) in non-fertilized (N$^-$) and fertilized (N$^+$) treatments. (d) Gross plant biomass and living plant biomass components (g plant^{-1}): e) pseudostem mass and (f) lamina mass of target plants. Data are means ± standard error of three tiller age cohorts (main stem and marked tiller cohorts appeared 20 and 30 days after fertilization= daf). First name abbreviation: target species; Second name abbreviation: neighbour species (e.g. DG Dg).

respectively; $P = 0.044$). Laminae mass showed no neighbour effects ($P > 0.05$). Instead, gross and living tiller masses of the main stem were increased by fertil-

ization (0.4 vs. 0.6 g of living tiller mass in N$^-$ and N$^+$, respectively; $P = 0.026$) by means of increasing lamina (N$^-$:0.2 vs. N$^+$:0.3 g tiller^{-1}; $P = 0.038$) and pseudostem (N$^-$:0.1 vs. N$^+$:0.32 g tiller^{-1}; $P = 0.024$) (Figure 4b and c).

Structural plant variables

Final tiller density of the target plant

Tiller density at harvest was similar in both species (14.6 tillers plant^{-1}; $P = 0.53$), but higher with DG than with BW neighbours (17.2 vs. 12 tillers plant^{-1}, respectively; $P = 0.03$). We found no effects of fertilization ($P = 0.97$; Figure 3c; Table 1).

Leaf area of target plants

As observed in individual tillers, the leaf area of target plants was higher in BW than in DG (252 vs. 141 cm^2 plant^{-1}, respectively; $P = 0.01$). No neighbour effects were detected ($P = 0.92$), and although nitrogen had increased leaf area of the older tillers, it exerted no effects at plant organization level (174 in N$^-$ vs. 218 cm^2 plant^{-1} in N$^+$; $P = 0.26$).

Growth-related plant variables

Target plant aerial biomass

According to the pattern observed at tiller level, the gross aerial mass of BW target plants was greater than that of DG target plants (3.30 vs. 1.17 g plant^{-1}; $P = 0.0001$) and DG neighbour plants increased the gross mass of both species compared with BW neighbour plants (2.72 vs. 1.76 g plant^{-1}, $P = 0.0331$; Figure 5a; Dead and living plant masses of BW were greater than those of DG respectively (0.4 vs. 0.1 g of dead mass per plant; $P = 0.0002$ and 2.9 vs. 1.0 g of living mass per plant; $P = 0.0001$). Lamina and pseudostem masses of the annual grass were greater than those of the perennial grass respectively (BW. 1.7 vs. DG. 0.6 g of laminae mass per plant; $P = 0.0002$, and BW. 1.1 vs. DG. 0.4 g of pseudostem mass per plant; $P = 0.0002$).

Living and dead masses of target plants of both species were greater when flanked by DG than when flanked by BW neighbour plants (BW neighbour: 1.5 vs. DG neighbour: 2.4 g plant^{-1}; $P = 0.033$ and BW neighbour: 0.2 vs. DG neighbour: 0.3 g plant^{-1}; $P = 0.016$, respectively; Figure 5b). As a consequence, gross plant mass was greater with DG than with BW neighbours (BW neighbour: 1.8 vs. DG neighbour: 2.7 g plant^{-1}; $P = 0.033$). DG neighbour plants increased lamina mass (BW neighbour: 0.9 vs. DG
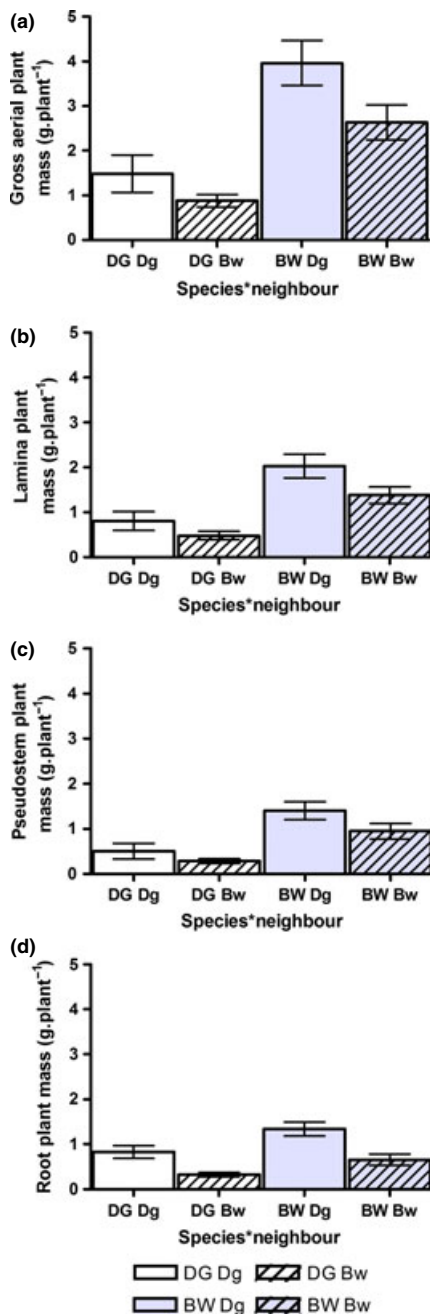
Figure 5 (a) Gross aerial plant biomass and living plant biomass components (g plant^{-1}): (b) lamina mass, (c) pseudostem mass and (d) root biomass (g plant^{-1}) of target plants in the final harvest (13th September) in four different situations of intra- and interspecific competition of the perennial *Dactylis glomerata* (DG) and the annual *Bromus willdenowii* (BW). Data are means ± standard error. Fertilization effects ≥0·05. First name abbreviation: target species; Second name abbreviation: neighbour species (e.g. DG Dg).

neighbour: 1·4 g plant^{-1}; $P = 0.044$) and pseudostem mass of target plants of both species compared with those of plants flanked by BW neighbours (BW neighbour: 0·6 vs. DG neighbour: 1·0 g plant^{-1}; $P = 0.049$; Figure 5b and c). Although both species showed the same final tiller density and fertilization increased pseudostem mass, we detected no fertilization effects on plant gross and living mass ($P > 0.05$).

Target plant root biomass

Root biomass was higher in BW than in DG plants (1·00 vs. 0·58 g plant^{-1}, respectively; $P = 0.0087$; Figure 5d) and lower with a BW than with DG neighbours (0·49 vs. 1·08 g plant^{-1}, respectively; $P = 0.0007$). Fertilization did not affect root biomass ($P = 0.70$).

Total target plant biomass and partitioning

In accordance with gross aerial biomass and root biomass, total target plant biomass (sum of both previous ones) was higher in BW plants (4·30 vs. 1·48 g plant^{-1}; $P < 0.0001$) and lower with BW than with DG neighbours (2·25 vs. 3·52 g plant^{-1}, respectively; $P = 0.0053$). As a consequence, biomass partitioning towards aerial parts of the plant was higher in BW than in DG plants (77 vs. 67%, respectively; $P = 0.018$) and higher with BW than with a DG neighbour (0·76 vs. 0·68, respectively; $P = 0.036$). The opposite pattern was detected in root partitioning, being lower in BW than in DG target plants (0·32 vs. 0·61%, respectively; $P = 0.014$, and 42% lower with BW than with DG neighbours (0·34 vs. 0·59%, respectively; $P = 0.031$). Fertilization did not affect total target plant biomass or partitioning ($P > 0.05$).

Discussion

Species effects

Bromus willdenowii showed ≈60% higher tiller size than DG in all the tiller cohorts due to its individual final lamina length (85% longer, data not shown) and ≈ 70% greater pseudostem height (Figures 2a and 2b; Table 1). Both traits suggest that BW has a superior light competitiveness than DG. Besides longer leaves and pseudostems, tiller size can be composed of higher number of living leaves (Hume, 1991). Nevertheless, BW showed smaller numbers of expanded and living leaves per tiller than DG (BW = 5·7 vs. DG = 8·6 living leaves per tiller; Figures 2b and c; Table 1) in the main stem. These values are similar to those found by Duru and Ducrocq (2000a) (between 4·8 and 7·0 in different growing periods), but greater than those

found by several other researchers (between 4 and 5 living leaves per tiller in different cv. of DG and BW) (Rawnsley *et al.*, 2002; Turner *et al.*, 2006a,b; Berone and Dreher, 2007). BW had ≈90% greater leaf area per tiller, ≈130% greater lamina mass (Figure 4b), ≈170% greater pseudostem mass (Figure 4c) and ≈140% greater living and gross tiller masses (Figure 4a) than DG in the three tiller cohorts.

Despite its smaller tiller size, DG did not show higher plant-tiller density (Figure 3c; Table 1) than BW because it showed a dynamic increase in the proportion of actively growing leaves towards the youngest tiller cohorts. This greater proportion of growing leaves per tiller determined small carbon amounts exportable to sites filling (Berone *et al.*, 2007*a*) and tillering, because the leaf growth zone is a strong sink with priority for the use and accumulation of assimilates (Volenec and Nelson, 1984; Gautier *et al.*, 1999; Schnyder *et al.*, 2000; Nelson, 2000).

Neighbour effects

Differential specific neighbour effects were observed upon tiller density and plant biomass (Figures 5a–c). Annual neighbours restricted 30% tiller density (Figure 3c; Table 1) associated with 62% lower number of expanded and 36% lower living leaves per tiller and 61% higher proportion of growing leaves per tiller in 50-dae/20-daf tillers (Figures 2b–d; Table 1). This may constitute a carbon-partition restriction towards axillary meristems and hence tiller density, as leaves begin to attend axillary bud demands at 80% of their final length (Schnyder *et al.*, 2000). This early adjustment of the number of leaves in tillers of intermediate age acts as a regulatory mechanism, which decreased tillering in plants flanked by the annual neighbours. This decrease in the number of expanded leaves represents a strategy that promotes their early death (associated with a shorter leaf lifespan, Gatti *et al.*, 2013) and not of those in active growth (fewer expanded leaves, but equal growing leaves)**,** which can vegetate in better illuminated canopy strata. As it was shown by other experiments involving C_3 grasses (Bahmani *et al.*, 2000; Nelson, 2000), no neighbour effect upon growing leaves was observed (Figure 2a). This structural regulatory mechanism was associated with an early (66 dae) decrease in transmitted PAR to the soil of 26% caused by annuals (102 μmol m^{-2} s^{-1} when the neighbour was BW vs. 138 μmol m^{-2} s^{-1} when the neighbour was DG).

While annual neighbours did not increase the green lamina length (Figure 3a; Table 1) or pseudostem height (Figure 3b) (similar tiller height; see later in *Hierarchies among cohorts and plants in the canopies*), they decreased gross tiller mass by 31% (tiller size in mass) associated principally with 33% less pseudostem mass (Figure 4a and c) in the 50-dae/20-daf tiller cohort. Probably this was due to a dilution effect of pseudostem dry matter (<g cm^{-1}). We were unable to detect significant results in the 60-dae/30-daf tiller cohort, probably because of its short evaluation period (21 days of evaluation prior to final harvest).

Fertilization effects

As nitrogen fertilization promoted leaf extension rate (Gatti *et al.*, 2013), it was also associated with structural variables, mainly total green lamina length per tiller (Figure 3a; Table 1) and pseudostem height (Figure 3b). This increase in pseudostem height caused by N agrees with that found by Duru and Ducrocq (2000b) and Duru *et al.* (2000). This investment in the length of laminae and pseudostems explains the increase of ≈45% in leaf area per tiller that was positioned in the upper layers of the canopy. In main stems, this positive carbon and biomass balance allowed the increase in gross (Figure 4a) and living tiller masses via increases of 36% in lamina (Figure 4b) and 57% in pseudostem tiller masses (Figure 4c). This increase in pseudostem mass agrees with that reported by Calvière and Duru (1999) and Duru *et al.* (2000). The effect of fertilization commonly resulted in an immediate increase in the cell number of the tillers present at the moment of fertilization (Volenec and Nelson, 1983; Gastal and Durand, 2000), i.e., the oldest and most illuminated tillers, associated with the vertical gradient of leaf nitrogen distribution parallel to vertical light distribution within the canopy (Löscher *et al.*, 2003).

Nitrogen fertilization affected lengths (fresh lengths) more than weights (dry matter) of the tillers evaluated. This effect seems to be diluted from the oldest to the youngest tillers because all of them were statistically significant for the main stem, but only the pseudostem height remained significant until the 30-daf tiller cohort. In the 50-dae/20-daf tillers, the increases in green lamina length (33%) and in pseudostem height (27%) were not accompanied by changes in lamina or pseudostem tiller masses (Figure 4b and c), probably because of the reductions in their weight per unit of length (data not shown). This would represent a mechanism of dry-matter conservation at early sowing stages, linked to water deposition, corresponding to a volumetric expansion of tissues (Schnyder *et al.*, 2000).

Nitrogen fertilization did not affect the number of living leaves per tiller (Figure 2c) as it had no effect on leaf appearance rate and leaf lifespan (Gatti *et al.*, 2013). Nitrogen fertilization did not affect tiller density or masses ($P > 0.05$) at plant scale.

Hierarchies among cohorts and plants in the canopies

Hierarchies of dominant plants inside the canopy are developed during pasture establishment (i.e. the first 85 dae) determined by species genotypic characteristics: BW plants were taller and erect (higher green lamina length and pseudostem height) (Figure 3a and b) than DG plants. This genetic hierarchy in the ability to capture light is imposed early during the pasture establishment stage (from the main stem) in canopies composed of C₃ annual and perennial grasses.

Meanwhile, the lack of increases in tiller size (i.e. shade-tolerance response; Figure 3a) by means of neighbour effects is aligned with the fact that tiller size increases are observed in shaded canopies (Casal *et al.*, 1987; Virkajärvi and Järvenranta, 2001). This is not the case during the canopy establishment phase, because, in this period, it was not possible to fulfil all the stages of the processes of competition for light (Lemaire, 2001) when the light interception was ≤93% of the incident light. The hierarchical environmental position among plants inside the canopy would develop in later stages of the establishment phase (>85 dae and 100 das in these types of mixtures). Nevertheless, a sign of plastic hierarchy led by the annual neighbours was observed: in the youngest tiller (60-dae/30-daf), the pseudostem height of BW plants flanked by BW neighbours was 25% higher than that of BW flanked by DG neighbours (Figure 3b). This was associated with light limitations towards the end of the experimental period. This shade-avoidance response takes place before plants perceive a reduction in the red/far-red ratio and before they became directly shaded, representing an indicator of neighbours' proximity (Ballaré *et al.*, 1987, 1990; Casal *et al.*, 1987), and in this case, before the green lamina length changes (Figure 3a; Table 1).

Neighbours led horizontal aerial occupation as well as underground colonization. The lower number of expanded leaves of intermediate-age tillers of plants flanked by BW neighbours probably induced the decrease in tiller density (Figure 3c and Table 1). This early restriction of horizontal space occupation led by annual neighbours is of great importance during the pasture establishment and/or when the canopies are slightly dense (Virkajärvi and Järvenranta, 2001). In this sense, tillering represents an exploitation or opportunist response (Nelson, 2000) Obviously, it conditions the future community composition (Ross and Harper, 1972; Eagles *et al.*, 1982).

Annual neighbours decreased root biomass (Table 1) and carbon partitioning towards roots. This suggests that during the establishment phase, annual neighbours do not improve plant light competition or horizontal space colonization, but limit the ability to acquire soil nutrients efficiently, which is important for a competitive outcome under high soil nutrient conditions (Sugiyama, 1999). This neighbour effect is especially relevant in slow-establishing species, such as the perennial grass DG, which have low seedling competitive ability because of their slow initial belowground growth and nitrogen capture (Nurjaya and Tow, 2001). This initial growth might determine its later performance because the competitive ability of DG correlates positively with root weight in the second year after establishment (Sugiyama and Nakashima, 1994). Therefore, annual neighbours limit the hierarchy of underground colonization of plants, which is related to water and nitrogen capture in the short term and its future light capture ability and soil nutrient acquisition (Lemaire, 2001).

A hierarchy between tiller age cohorts was observed: those less illuminated had a dominated hierarchy within the plant structure, showing lower green lamina length (Figure 3a; Table 1) and pseudostem height (Figure 3b), while the opposite pattern was shown by the best-illuminated tillers. The relationships between developing branches could be critical for the success of the whole plant because it is essential to allocate resources to the branches with the greatest probability for high lifetime yield (Novoplansky, 2003). In younger grass plants, the largest tiller was at the same time the one with present and future performance, i.e., with dominant hierarchy within the plant structure. In the same sense, nitrogen effects on green lamina length (Figure 3a) were evident only in the older tillers, main stem and 50-dae/20-daf tiller cohort, because added nitrogen may have been incorporated into better illuminated plant tissues, being unavailable to the 60-dae/30-daf tiller cohort. Both changes in allocation patterns with tiller age correspond to a change from a predominantly horizontal to a more vertical strategy of plant growth (Lemaire and Millard, 1999).

Conclusions

In temperate mixtures composed of perennial and annual/biennial C₃ grasses (DG + BW), we recorded changes in structural traits, of genetic and plastic origins, that led the hierarchies among plants within the canopy, during the critical establishment phase and in the absence of defoliation. The much greater final green lamina length and pseudostem height of all the tiller age cohorts evaluated from the annual BW, in comparison with the perennial DG, suggest a greater hierarchy and competitive ability in light capture of BW plants. Despite its smaller tiller size, DG did not show an increase in plant tiller density and,

consequently early horizontal space occupation, probably due to the increase in the proportion of growing leaves per tiller observed in the youngest tiller cohorts. This low space-occupation ability might negatively influence the ability of the perennial grass to persist in the community. Nevertheless, environmental hierarchies in light capture – increases in green lamina length and in pseudostem height – could not be established because the increase in tiller size is only observed in shaded canopies, which was not the case during the establishment phase.

On the other hand, neighbours led aerial horizontal occupation and underground colonization. The early adjustment of the number of expanded leaves and living leaves per tiller in tillers of intermediate age, such as the 20-daf/50-dae tiller cohort, acts as a regulatory mechanism, which decreased tiller density in plants flanked by the annual neighbour. Also, annual neighbours limited the hierarchy of underground colonization (less root biomass of plants) and competition ability for soil resources. This is especially relevant in slow-establishing species like the perennial DG, which has slow initial below-ground growth and nitrogen capture. This can condition the later performance of perennial grass in the pasture.

Winter nitrogen fertilization improved tiller height of all tiller cohorts, and light capture, but not tiller mass or tiller density; thus, its effect did not turn out to be significant at the plant scale.

The hierarchy in light capture would be related directly to the genotypes chosen to mix, while the hierarchies of aerial horizontal occupation and the underground space colonization are clearly neighbour-controlled, and then of environmental or plastic origin. In these circumstances, a higher sowing density of perennial grass will determine a greater frequency of favourable situations of intraspecific competition for DG and interspecific competition for BW that would improve pasture density, reduce weed invasion and optimize pasture floristic composition. The reproductive condition of the annual/biennial species during spring and tiller survival during summer can modify the floristic composition of the community, for which these responses should be evaluated in later stages of the plant life cycle. These findings allowed us to clarify some structural mechanisms involved in early competitive interactions between annual and perennial grasses in the sowing rows and emphasize the importance of the cultural decisions at the moment of planning temperate pastures.

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