

## ORIGINAL RESEARCH ARTICLE

## Environment

# Weed effects on the establishment and nutritive value of pastures with different annual/perennial ratio

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## Abstract

Livestock intensification is increasing weed invasion in pastures. We performed an experiment near Buenos Aires, Argentina, to evaluate the impact of weeds on temperate cultivated pastures. We sowed 1,700 viable seeds m<sup>-2</sup> in 24, 1.5-m<sup>2</sup> plots. Each plot had seven sowed lines, three of legumes (red clover, *Trifolium pratense* L.; 50% of seeds) alternating with four of grasses arranged in two sowing designs differing in the annual/perennial ratio: 70% prairie grass (*Bromus willdenowii* Kunth, Bw, annual species) + 30% of orchardgrass (*Dactylis glomerata* L., Dg, perennial species) (>Bw), or 30% of Bw + 70% of Dg (>Dg). In the inter-lines of each plot, we sowed the weed brown mustard (*Brassica juncea* L.) at four growing densities. At 123 d after sowing, we harvested aerial biomass of weeds and forage species (legume biomass was almost negligible at this date). We quantified aboveground net primary production (ANPP) of weeds and grasses (separated into blades, sheaths, and blades + sheaths), tiller density (indicating potential persistence), blade crude protein (CP), and neutral detergent fiber (NDF) concentrations of grasses. We made regressions to analyze the direct effect of weed biomass on each grass species, their indirect effects on the proportion of each species in the pasture, and their net effects on both species taken together. Weeds had negative direct and net effects. However, they did not have any indirect effect on species proportion. Pastures with lower annual/perennial ratio were more productive and potentially more persistent. Weeds also directly increased blade CP of the annual species.

## 1 | INTRODUCTION

An intensive model of agriculture expanded across developed countries since the 1960s (Ewers, Scharlemann, & Balmford, 2009). Prior to the beginning of the 20th century, almost all increases in crop production occurred as a result

of increases in the cultivated area, whereas toward the end of the century, they came from increases in land productivity per area unit (Ruttan, 2002). In the Argentine Pampas prevailed a low-input, rotational cattle–crop production system (Solbrig, 1997), but an intensive crop production expansion process started in Argentina just before the 1980s. This process included peripheral areas that had previously been considered marginal for crop production and were predominantly dedicated to extensive livestock production (Satorre, 2005). As crop occupied all places suitable for its production, a

**Abbreviations:** ANPP, aboveground net primary production; Bw, *Bromus willdenowii* Kunth (prairie grass); CP, crude protein; das, days after sowing; Dg, *Dactylis glomerata* L. (orchardgrass); NDF, neutral detergent fiber.

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process of livestock intensification and an increase of animal stocking rate occurred in places not suitable for agriculture, that consequently remain available for livestock production (Pruel et al., 2006). Pastures constitute a valid alternative to satisfy the animal requirements of these more intensified systems because of their high potential productivity, if they are sown in high productive environments, with improved genotypes and fertilization (Gatti, Ayala Torales, Cipriotti, & Goluscio, 2012).

The most important consequences of the livestock intensification process are deterioration of sown canopies, lower production of desirable species, and the subsequent weed invasion (Rodríguez, Jacobo, & Deregibus, 1998). However, the impact of weed competition on production and persistence of cultivated pastures and grasslands is not yet well known. Consequently, control decisions of the undesired species are usually based on visual tools and intuition (Kemp & Dowling, 2000).

Within this conceptual framework, the use of models to make decisions related to weed control has received growing attention in grain crops (Doyle, Cousens, & Moss, 1986). Thus, models of weed invasion, population growth, and control have served as a framework for organizing biological information on weeds and for developing weed control strategies (Mortimer & Putwain, 1984). In particular, they have helped to identify information gaps, set research priorities, and suggest control strategies (Maxwell, Wilson, & Radosevich, 1988). Therefore, it was firstly suggested that we use a sigmoidal relation between crop yield and weed density (Zimdahl, 1980), but this model has been refuted in theoretical terms (Cousens, Peters, & Marshall, 1984), and a hyperbolic response has been proposed instead (Cousens et al., 1985). However, there is very little information related to this issue for forage production.

Weed infestation directly affect the production of desirable species (Tozer, Bourdot, & Edwards, 2011) through competition for nutrients, water, light, and space, which eventually cause the reduction of their productive ability (Abaye, Scaglia, Teutsch, & Raines, 2009). Weeds can also affect the potential persistence of pastures (Tozer et al., 2011), perceived by the farmers as the most limiting factor in the pasture behavior (Reeve, Kaine, Lees, & Barclay, 2000). The loss of productivity of the pastures under weed competition (Tozer et al., 2011) can be explained by the decrease in the size of the tillers, a variable associated with the competitive ability by light (Nurjaya & Tow, 2001), and the potential productivity of the species (Gatti et al., 2012, 2013). On the other hand, the reduction of the persistence of the pastures is attributed to a lower tiller density, which determines the horizontal occupation of space and potential pasture persistence (Hume, 1991; Tozer et al., 2011). These two structural characters—size and density of tillers—define the carbon partitioning pattern and play an important role

### Core Ideas

- Livestock intensification is causing serious weed invasion processes in pastures.
- Pastures with lower annual/perennial ratios were more productive and potentially persistent than those with higher annual/perennial ratios.
- Weeds decreased production and persistence of annual and perennial species and increased blade crude protein of the annual species.

in the competitive ability of plants (Lemaire & Maillard, 1999).

Besides their negative effect on the productivity and potential persistence of pastures, weeds can also affect the nutritional value of the forage. The nutritional value of forage grasses has been extensively studied in terms of two main traits: (a) neutral detergent fiber content (NDF), both at canopy (e.g., Insúa, Agnusdei, & Di Marco, 2017) and species (e.g., Turner, Donagy, Lane, & Rawnsley, 2006) levels, or (b) crude protein content (CP), either as a function of time (Belanger & Gastal, 1999) or plant age (Lemaire & Gastal, 1997; Marino et al., 2004) or in among-species comparisons (Lardner, Ward, Darambazar, & Damiran, 2013). Many authors suggest that annual species have higher forage quality than perennials (e.g., Van Arendonk & Poorter, 1994) but others found opposite results (Turner et al., 2006) or no differences between those plant functional groups (Niemman, Pureveen, Eijkel, Poorter, & Boon, 1992). Forage quality is severely affected by environmental conditions (Deinum, 1966); the effect of shade on the nutritive value of the forage has been extensively evaluated (e.g., Lin, McGraw, George, & Garrett, 2001). There are two conflicting effects of shading on the quality of forage: on the one hand, part of the negative effects of shading is related to an increase in the sheath/blade ratio due to the elongation of the internodes (e.g., Kephart, Buxton, & Taylor, 1992). On the other hand, shading could positively affect forage quality by changes on specific leaf area of blades (SLA; i.e., the leaf area/leaf weight ratio) (Meziane & Shipley, 1999).

In the temperate–humid environments of Argentina, floristic composition of pastures usually includes annual and perennial C<sub>3</sub> grasses, together with legumes. Prairie grass (*Bromus willdenowii* Kunth, Bw) has heavy tillers, conferring it high competitive ability for light (Nurjaya & Tow, 2001) and productive potential (Gatti et al., 2012, 2013). On the contrary, orchardgrass (*Dactylis glomerata* L., Dg), has low competitive ability due to its initial slow root growth, which is related to a lower nitrogen capture (Nurjaya & Tow, 2001). However, it has higher tiller density than the annual component, being

this trait an indicator of a greater potential persistence (Hume, 1991). Both prairie grass and orchardgrass are usually sown in a design of alternating lines with erect legumes, such as lucerne (*Medicago sativa* L.) or red clover (*Trifolium pratense* L.) (Scheneiter, Mattew, & Rimieri, 2008).

Based on the conceptual model proposed by Golluscio et al. (2009), we differentiated direct, indirect, and net effects of weeds on grass aboveground net primary production (ANPP), persistence, and forage quality. Weeds and/or annual/perennial sowing density ratios would directly affect the ANPP, tiller density, and forage quality of each species (direct effect). They also would change the proportions of both species in the overall pasture: the most affected species would lose importance in the overall pasture if weed biomass increases. If both species have different ANPP, tiller density, or forage quality, then the overall values of the pasture (net effect) will change because the floristic composition changes (indirect effect), even if weeds or sowing design did not directly affect any species. In the same way, even if no indirect effects exist, the overall values of the pasture (net effect) could change as an average of the direct changes caused by those factors on each species. The net effect results from the conjunction of direct and indirect species.

Our hypotheses for our study were:

1. Weeds will have a negative direct effect on both ANPP and potential persistence (estimated from tiller density) of each species.
2. Given its lower competitive ability due to its slow growth, the perennial Dg will be more affected by weeds in terms of forage production and persistence. Therefore, the annual/perennial ratio of the pasture will increase as weed biomass increases.
3. As a result, weeds will have a higher negative net effect on ANPP, and persistence of both grass species taken together when the annual/perennial ratio is low than when it is high.
4. Weed competition will directly increase forage quality of blades of both grass species.
5. As Dg is more affected by weed competition, its quality will be more affected by weed competition than that of Bw.
6. Given the higher effect of weeds on perennial Dg, forage quality of both grass species together will be more affected by weeds when the annual/perennial ratio is low than when it is high.

## 2 | MATERIALS AND METHODS

The experiment was carried out in the experimental station Area Metropolitana de Buenos Aires of INTA, located at Castelar, province of Buenos Aires, Argentina (34.6 S, 58.6 W). Soil is a Typic Argiudoll, with 3.6% of organic matter, 0.443 dS m<sup>-1</sup> of electrical conductivity, and 4 g Mg<sup>-1</sup>

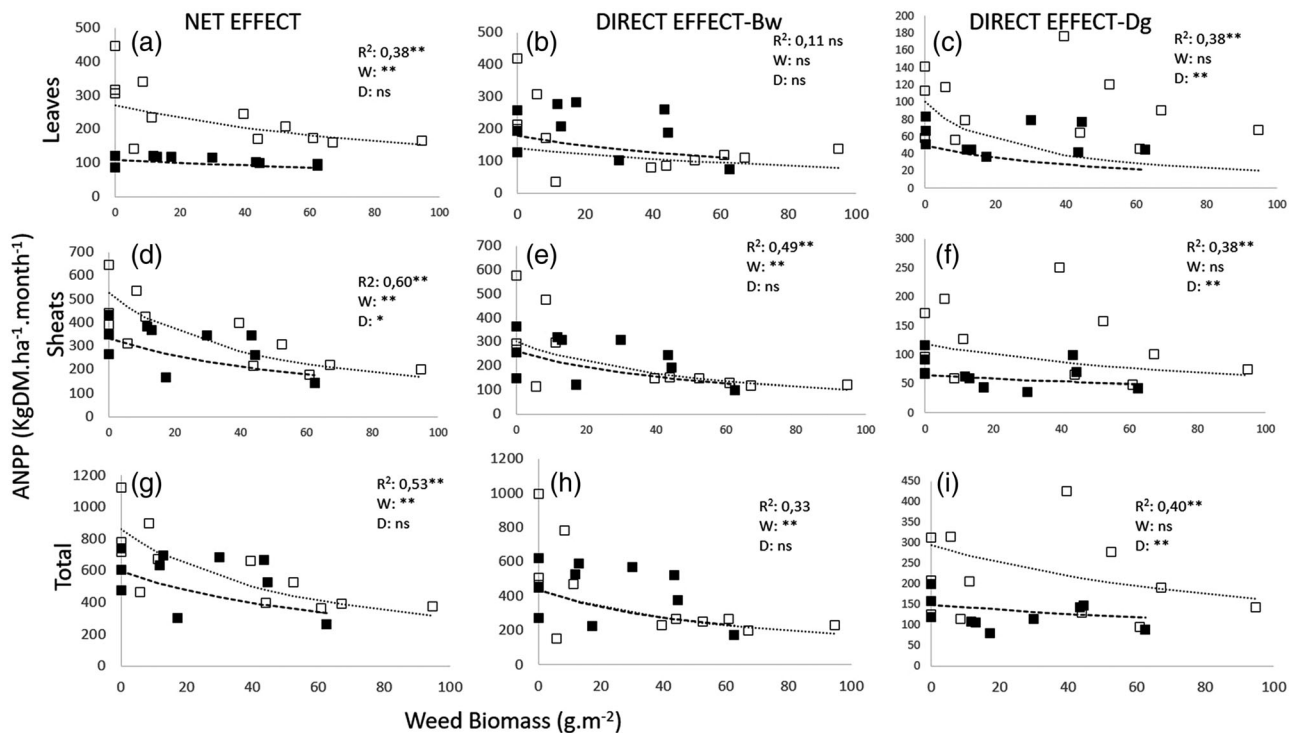
(ppm) of extractable P. Given such P deficiency, we fertilized at sowing with 55 kg P ha<sup>-1</sup>. The experiment was from 15 May to 15 Sept. 2015 (123 days after sowing [das]). Rainfall during the experiment was 371 mm, somewhat higher than the 1995–2015 mean of 298 mm. Average temperature was 13.5 ± 5.9 °C, similar to the 1995–2015 mean of 13.22 ± 1.92 °C (data provided by the Institute of Climate and Water of INTA).

### 2.1 | Experimental design

We established a randomized complete block design (RCBD) of eight treatments (4 weed densities × 2 annual/perennial ratios) with three replications per treatment. We sowed 1,700 viable seeds of forage species per m<sup>2</sup> in 24, 1.5-m<sup>2</sup> plots. Each plot had seven sowed lines separated from each other by 0.21 m, four sowed lines of grasses alternating with three sowed lines of legumes. The grass lines had 850 viable seeds m<sup>-2</sup>, arranged in two possible combinations of grass densities: 70% Bw (annual species) + 30% Dg (perennial species) (>Bw; 12 plots) or 30% Bw + 70% Dg (>Dg; 12 plots). The remaining 850 viable seeds m<sup>-2</sup> corresponded to red clover (see details in Gatti et al., 2012, 2013). In the inter-lines of each micro-plot, we manually sowed one of four different densities of an annual fall–winter–spring cycle weed (brown mustard, *Brassica juncea* L., seeds provided by the FAUBA Seed Laboratory): 0, 40, 80, and 120 viable seeds m<sup>-2</sup>, respectively. Forage and weed species were sown simultaneously to simulate the common joint growth occurring at real field conditions.

### 2.2 | Data gathering

At 123 das, we harvested the aerial aboveground biomass of the central 50 by 50 cm portion of the plot to eliminate border effects, including the two central rows of grasses, the central row of the legume, and all brown mustard individuals growing inside. The biomass harvested was separated into the sown species (differentiating sheaths and blades in the two grasses). As we harvested at ground level, we assumed that biomass represented the ANPP reached by each species from sowing until the end of the study period. The response variables were density and ANPP of weeds, tiller density, ANPP (blades, sheaths, and blades + sheaths), and tiller weight (calculated as the quotient between ANPP and tiller density) of each grass species. Considering its non-destructive measurement method, we quantified tiller density not only at harvest date (123 das) but also at 91 das. We sent the leaf blades of grasses to the Animal Nutrition Laboratory (Facultad de Agronomía, University of Buenos Aires) to analyze NDF (Van Soest & Wine, 1967) and CP concentrations (Kjeldahl, 1983). We



**FIGURE 1** Net effect of weed aboveground net primary production (ANPP) and sowing design on: blade (ANPP, a; sheath ANPP, d; and total, blades + sheaths, ANPP, g). Direct effect of weeds on blades, sheaths, and blades + sheaths ANPP of prairie grass (Bw) (b, e, and h, respectively), and blades, sheaths, and blades + sheaths ANPP of orchardgrass (Dg) (c, f, and i, respectively). White squares represent >Dg design. Black squares represent >Bw design.  $R^2$  (determination coefficient of the regression model), and the significance of  $W$  (weed slope) and  $D$  (sowing design slope) are inserted at the upper right corner of each panel (\*\* $p < .001$ ; \* $p < .05$ ; ns  $p > .05$ )

analyzed the impact of weeds on forage quality of blades instead of the entire canopy because blades are the most consumed fraction (Insúa et al., 2017) and the effect of weed on forage quality of the entire canopy can mix the positive effect on SLA (Meziane y Shipley, 1999) with the negative effect on the sheaths/blades ratio. The ANPP and the concentrations of NDF and CP of the legume were not analyzed because it represented only 7% of the ANPP of the whole pasture (data not shown).

### 2.3 | Statistical analysis

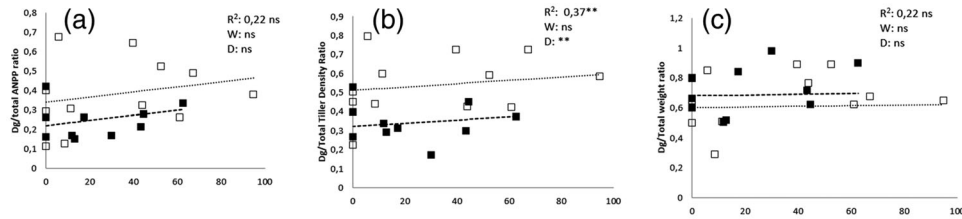
To analyze the effect of weed biomass and the annual/perennial ratio on the different response variables, we fitted a modification of the hyperbolic model proposed by Cousens (1985):  $1/y = a + bW + cD$ . In this multiple regression,  $y$  is the variable measured on grasses (ANPP, tiller density, tiller weight, CP, or NDF),  $a$  is the  $y$  intercept of the model,  $b$  is the slope of the response to weed density ( $W$ ), and  $c$  is the slope of the response to sowing design ( $D$ , dummy variable: Bw dominant = 1 and Dg dominant = 2). This model shows that the negative effect of weeds on grasses is higher as  $b$  increases and the positive effect of annual/perennial ratio is higher as  $c$  increases. The

model has been developed for each grass species separately (direct effects) and for both grass species taken together (net effects). A preliminary analysis showed that weed ANPP had more explicative power than weed density (data not shown). In all cases, the regressions had 24 points (2 sowing designs  $\times$  4 weed sowing densities  $\times$  3 replications) and were carried out with the InfoStat 1.1 package (Di Rienzo et al., 2008) with a significance level of  $p = .05$ .

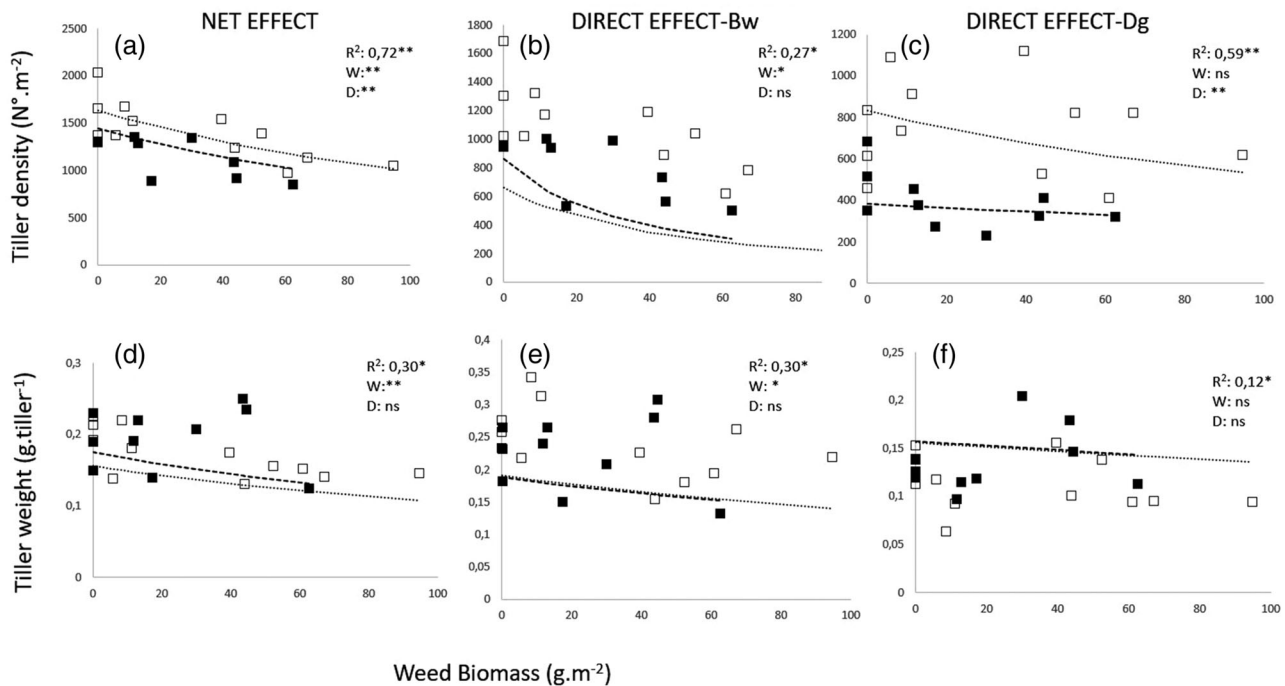
## 3 | RESULTS

### 3.1 | Weed impact on grass ANPP

Weed ANPP had a negative net effect on ANPP for all variables (blades, sheaths, and blades + sheaths) of both grass species together (Figures 1a, 1d, and 1g, respectively). Total ANPP tended to be greater when Dg was dominant than when Bw was, although this net effect of design only was significant for sheath ANPP (Figure 1d). Weed ANPP also had a direct negative effect on Bw ANPP (except in the case of blades; Figure 1b), and was not affected by the sowing design (Figures 1b, 1e, and 1h). Dg ANPP showed the opposite pattern: weed ANPP has not directly affected Dg ANPP, whereas sowing design did: Dg blades, sheaths, and their sum were higher



**FIGURE 2** Indirect effect of weed aboveground net primary production (ANPP) and sowing design on: (a) orchardgrass (Dg) ANPP over total ANPP ratio; (b) tiller density of (Dg) with respect to total tiller density; (c) Dg tiller weight with respect to mean tiller weight. White squares represent >Dg design. Black squares represent >prairie grass (Bw) design.  $R^2$  (determination coefficient of the regression model), and the significance of  $W$  (weed slope) and  $D$  (sowing design slope) are inserted at the upper right corner of each panel (\*\* $p < .001$ ; \* $p < .05$ ; ns  $p > .05$ )



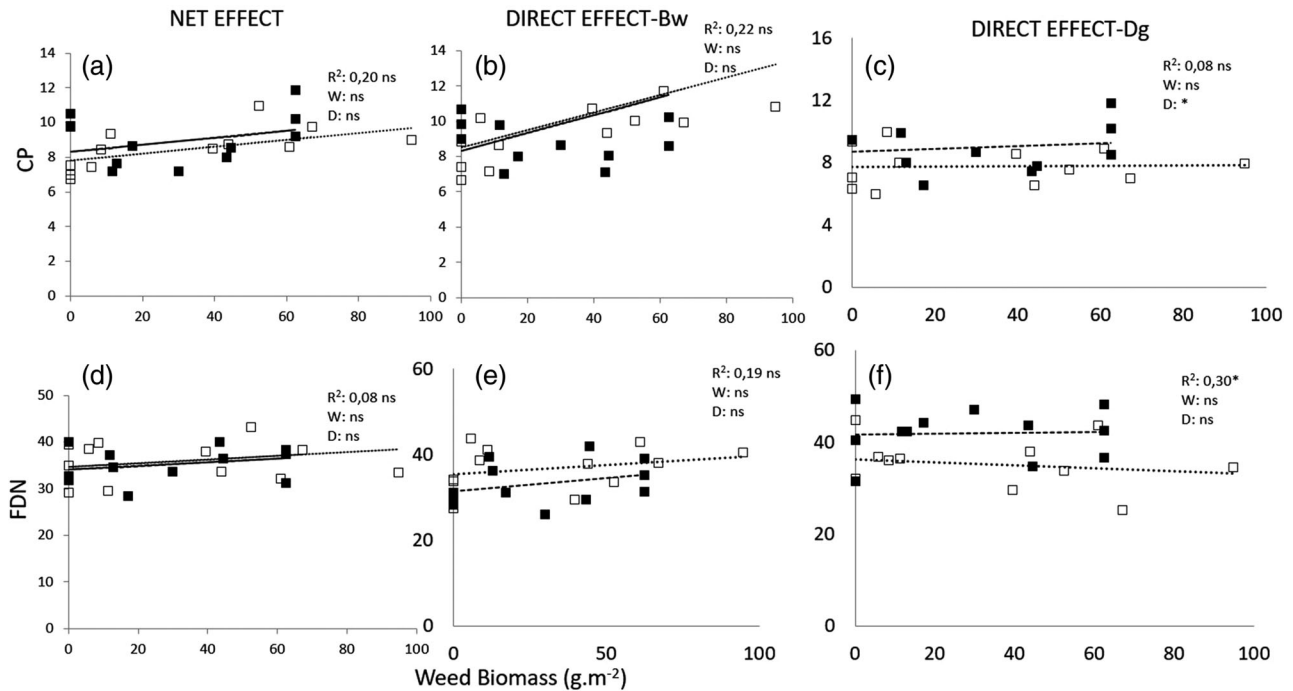
**FIGURE 3** Net effects of weed aboveground net primary production (ANPP) and sowing design on total tiller density (number  $m^{-2}$ ) (a) and mean tiller weight ( $g\ tiller^{-1}$ ) (b). Direct effect of weed ANPP and sowing design on tiller density of prairie grass (Bw) (b) and orchardgrass (Dg) (c); and on tiller weight of Bw (e) and Dg (f). White squares represent >Dg design. Black squares represent >Bw design.  $R^2$  (determination coefficient of the regression model), and the significance of  $W$  (weed slope) and  $D$  (sowing design slope) are inserted at the upper right corner of each panel (\*\* $p < .001$ ; \* $p < .05$ ; ns  $p > .05$ )

when Dg was dominant than when Bw was (Figures 1c, 1f, and 1i).

Neither weed ANPP nor sowing design had indirect effects on the final proportion of the overall ANPP explained by Dg (Figure 2a). This lack of indirect effects could explain the similar pattern observed in direct and net effects of weed ANPP and sowing design on ANPP. Weed ANPP did not affect the proportion of Dg over total tiller density, but pastures with >Dg always had a higher proportion of Dg tillers (Figure 2b). Finally, neither weed ANPP nor sowing design affected the ratio between Dg tiller weight and total tiller weight (Figure 2c).

### 3.2 | Weed impact on potential persistence of grasses

Weed ANPP had a negative net effect on tiller density and weight of both grass species taken together. The dominance of Dg had a positive net effect on tiller density, but it did not affect tiller weight (Figures 3a and 3d). Bw tiller density (Figure 3b) and Bw weight (Figures 3e) showed a negative direct effect as weed ANPP increased but were not affected by sowing design. Instead, weed ANPP did not affect Dg tiller density (Figure 3c) or Dg weight (Figure 3f) but Dg had significantly more



**FIGURE 4** Net effects of weed aboveground net primary production (ANPP) and sowing design on crude protein (%CP) (a) and neutral detergent fiber (%NDF) (b). Direct effect of weed ANPP and sowing design on %CP of prairie grass (Bw) (c) and orchardgrass (Dg) (d) and on %NDF of Bw (e) and Dg (f). White squares represent >Dg design. Black squares represent >Bw design.  $R^2$  (determination coefficient of the regression model), and the significance of  $W$  (weed ANPP effect) and  $D$  (sowing design effect) are inserted at the upper right corner of each panel (\*\* $p < .001$ ; \* $p < .05$ ; ns;  $p > .05$ )

tillers when it was in higher proportion in the sowing design.

Tiller density of the two grass species combined increased from 91 to 123 das ( $F = 43.56$ ;  $p < .0001$ ; data not shown). However, this increase was significant on Dg ( $F = 21.47$ ,  $p < .0001$ ) but did not on Bw ( $F = 2.11$ ;  $p = .1535$ , ns), suggesting that tiller production of Dg may have continued later on Dg than on Bw.

### 3.3 | Weed effect on pasture quality

Weed ANPP directly increased the %CP of Bw blades, regardless of which species was in higher proportion (Figure 4b). However, this direct effect was not reflected in the blade %CP of both grass species together, that is, of both species combined (Figure 4a). In contrast, weed ANPP did not directly affect blade %NDF, neither on Bw (Figure 4e) nor on Dg (Figure 4f), and then it did not have any net effect on blade %NDF of the overall pasture (Figure 4d). Both Dg %CP (Figure 4c) and Dg %NDF were lower when Dg was dominant than when Bw was dominant (Figure 4f) but these direct effects of Dg dominance were not reflected in the overall pasture (Figure 4d).

## 4 | DISCUSSION

As stated in our first hypothesis, weeds directly reduced both ANPP and potential persistence (estimated from final tiller density) of both grass species. These results show once again the negative effect of weeds on the seedling growth of forage species, which has been documented in the literature for several decades (e.g., Tozer et al., 2011). Weeds also produced a severe negative net impact on tiller production, with the expected decrease in the potential persistence of pastures that this would trigger (Hume, 1991). The reduction in potential persistence due to weeds is highly relevant since this property has been defined by farmers as the most limiting factor in the behavior of pastures, followed by drought (Kelly & Smith, 2010).

Contrary to our second hypothesis, direct effect of weeds on ANPP was much higher on the annual component than on the perennial one. Although weed effect was higher on Bw than on Dg, it did not significantly change species proportion, and did not produce an indirect effect on ANPP production, tiller density, or tiller weight. This trend is surprising given the high rate of growth of Bw during the winter season (Sanderson, Skinner, & Elwinger, 2002), the great size of their seedlings, its great productivity, and competitive ability (e.g.,

Aarsen et al., 2002), which would confer capacity to establish quickly and exclude other species with slower growth than Dg (Sanderson et al., 2002). The more pronounced effect of weeds on Bw than on Dg could have two complementary explanations. First, it is well known that the growth reduction under a context of limiting resources (shade made by weeds in this case) is more pronounced on species with higher production potential than in species with lower growing rates (Garnier, Farrar, Poorter, & Dale, 1999). Second, the slow establishment of Dg causes an asynchrony between its moment of maximum demand for resources and that of the weed studied here (Andrews et al., 1997). In addition, both grass species sown in this experiment have different thermal requirements. Dg presents its greatest capacity for growth toward spring–summer (Velasco et al., 2001), whereas both Bw and the weed present a growth cycle faster, shorter, and earlier (Pitelka, 1997). Therefore, it could be considered that the greatest overlap between the annual component and brown mustard would result in a greater competition between those two components than between Dg and brown mustard. It should be noted, however, that the weed used in this essay has an annual life cycle. The pattern described can change in case of a perennial weed or under a community of weeds of different life cycles.

Our results also contradicted our third hypothesis because as weed biomass did not directly affect the proportion of Dg in the overall pasture and Dg was less affected by weeds than Bw, pastures with >Dg tended to be more productive because they presented heavier sheaths and denser tillers. Such a pattern could be explained by the differences in life cycle between weeds and Dg mentioned above. These differences in terms of ANPP and tiller density between pastures of different annual/perennial ratios suggest that pastures with a higher proportion of perennial grasses tended to be more productive and persistent.

Partially supporting our fourth hypothesis, weeds produced a positive direct effect on blade %CP of Bw but did not affect blade %NDF. Also it did not affect %CP and %NDF of Dg. These results could be explained by the above proposed increase of SLA (which unfortunately we did not measure), but also by nitrogen dilution where nitrogen concentration decreases as biomass accumulation increases (e.g., Lemaire and Gastal, 1997; Marino et al., 2004). Indeed, the ANPP reduction due to weed competition could produce an increase of CP concentration. Therefore, since weeds reduced Bw ANPP more than Dg, ANPP nitrogen may tend to concentrate more on Bw than on Dg (Meziane & Shipley, 1999; Van Arendonk & Poorter, 1994).

Our results also contradicted our fifth hypothesis. Dg quality was affected by the annual/perennial ratio: CP and NDF were higher when Bw was dominant than when Dg was. However, this effect was not caused by our initially proposed higher competitive effect of weeds on Dg than on Bw, but by the nitrogen dilution (e.g., Lemaire and Gastal, 1997; Marino

et al., 2004). The dominance of Bw directly reduced sheath, blade, and total ANPP of Dg (Figures 1c, 1f, and 1i), thereby causing a higher concentration of CP and NDF in Dg, in comparison to the dominance of Dg.

Finally, also contradicting our sixth hypothesis, the positive direct effect of weed biomass on %CP of Bw, and of >Bw on %CP and %NDF of Dg were partially diluted and did not appear in the net effects. This can be explained because (a) weed biomass did not produce any indirect effect on the annual/perennial ratio of the pasture, and (b) both effects showed little magnitude and affected only one of the species.

## 5 | CONCLUSIONS

1. Weeds decreased productivity and potential persistence of pastures.
2. Despite its greater competitive ability broadly assumed, the annual component (Bw) was proportionately more affected by weed biomass than the perennial one (Dg).
3. Although weed ANPP affected Bw more than Dg, it was not enough to change the Bw/Dg ratio.
4. Pastures with a higher proportion of the perennial component were more productive and presented a greater density of tillers, and therefore a greater potential persistence, than those with a higher proportion of the annual component.
5. Weed ANPP only had a positive direct effect on the CP concentration of Bw and did not affect the %NDF of either of the two species studied. As indirect effects were not found, the positive direct effect of weeds on %CP of Bw was diluted in terms of net effects.

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## CONFLICT OF INTEREST

There is no conflict of interest.

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