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# Perennial warm-season grass monocultures and mixtures: Biomass production and soil improvement in semiarid and shallow soil conditions

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ABSTRACT

Perennial warm-season grasses (WSG) represent a viable alternative for cultivation in marginal lands. Moreover, mixtures of WSG can be expected to exceed monocultures in terms of biomass production since biodiversity and ecosystem functions are predominantly positively related. Our objective was to evaluate (1) stockpiled forage accumulation and quality and (2) root biomass in monocultures and mixtures of *Panicum virgatum*, *P. coloratum* and *Eragrostis superba*, and to compare them with a standard monoculture of *E. curvula*. Additionally, the soil properties under WSG pastures and annual forage crops were compared at the end of the study. The research was conducted in the Dry Pampas of central Argentina, under shallow soil conditions. Stockpiled forage accumulation and root biomass were higher in the most diverse pasture and the mixture of *P. virgatum* – *E. superba* than in the monoculture of *E. curvula*. Stockpiled forage represented an important source of feed for livestock in fall and winter, although of low quality. Soil aggregate stability and initial water infiltration rate were higher in WSG pastures than in adjacent annual forage cropland. Our results suggest that mixtures of WSG can be highly productive in semiarid environments even in shallow soils. Moreover, they can positively influence soil properties.

## 1. Introduction

Perennial warm-season grasses (WSG) are characterized by high use efficiency of radiation, water and nutrient (Pearcy and Ehleringer, 1984; Sage and Kubien, 2003). These attributes make them suitable for cultivation in semiarid regions that are limited by soil fertility. Native and introduced WSG can be used for grazing during the growing season or as stockpiled forage to extend the grazing period into fall and winter (e.g., Scarbrough et al., 2001; Tracy et al., 2010). Perennial warm season grasses have also been considered as feedstock for biofuel production (e.g., Gelfand et al., 2013; Porensky et al., 2014) and they are used in reserve conservation programs for soil protection and soil improvement (e.g., Mulkey et al., 2006). When cultivated, WSG can be grown in monocultures or as mixtures.

Mixtures of WSG can be expected to exceed monocultures of WSG in terms of biomass production, since species richness and ecosystem functions are predominantly positively related (Hooper et al., 2005). The underlying mechanisms proposed are greater complementarity in resource acquisition and use, higher probability of facilitative interactions, and/or higher probability of including highly productive species (Tilman et al., 1997). However, the intensity of these relationships can be modulated by both functional strategy (e.g. intra-vs. interspecific competition intensity) and functional plasticity (e.g. the degree of response to disturbance and/or stress) of the component species (Pontes da Silveira et al., 2012).

Potential enhanced root production of WSG mixtures represents an impending significant contribution for soil improvement in marginal lands (reviewed by Blanco-Canqui, 2010). It can be anticipated that increases in below-ground production will improve soil physicochemical properties, such as organic carbon, bulk density, aggregate stability and the water infiltration rate. Moreover, the presence of species with roots that are able to penetrate into compacted soil layers and to access water stored in the subsoil can alleviate the effects of droughts in reducing WSG productivity (Williams and Weil, 2004).

In the Dry Pampas of central Argentina, as in other semiarid regions of the world, plant growth is normally limited by scarce and variable precipitation and low soil fertility. Moreover, shallow, compacted soils frequently have a reduced water-holding capacity (Quiroga et al., 1996,

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1998). Soils are also susceptible to erosion, particularly when used for annual cropping employing conventional tillage systems (Mendez and Buschiazzo, 2010). In this context, WSG may represent a valuable alternative to enhance plant production and improve soil physicochemical properties, when used for grazing either during the growing season or as stockpiled forage in the fall and winter. However, the area cultivated with WSG is scarce and mainly of *Eragrostis curvula* (Stritzler, 2008) which is quite productive under water shortage and low soil fertility (Stritzler et al., 1996; Montani et al., 1996). However, its main limitation is in forage quality, particularly when stockpiled and used to feed animals in the late fall and winter months, requiring protein supplementation (Rabotnikof et al., 1986; Gargano et al., 2001a,b; Stritzler, 2008). Better stockpiled forage quality has been observed for the WSG *Eragrostis superba* and *Panicum coloratum,* among others (Stritzler, 2008). The quantity and quality of stockpiled forage are critical in fall and winter since alternative pastures, such as annual winter grasses, are risky (in terms of production and soil protection) and too costly to feed breeding livestock. Annual grass productivity is in general strongly limited by water shortage (Bazazz, 1979), and the need of seeding them every year represents an important cost to farmers. On the other hand, restoration of native grasslands is limited by the lack of commercially available seed. These antecedents raise the need to evaluate WSG growing as a monoculture or in mixtures as an alternative to *E. curvula*. In the present study we used a *E. curvula* monoculture as a standard of comparison because we were interested in evaluating production and quality of novel WSG for the study region, but we did not include it in mixtures. However, in view of its local adaptation it may be worth considering *E. curvula* for WSG mixtures in future studies.

The objective of this study was to evaluate (1) stockpiled forage accumulation and quality and (2) root biomass and root presence in the soil profile in monocultures and mixtures of *Panicum virgatum*, *P. coloratum* and *E. superba* (target WSG), and to compare them with a standard monoculture of *E. curvula*. Additionally, the soil physicochemical properties of WSG pastures and adjacent annual forage cropland subjected to conventional tillage were compared at the end of the study. The reason for this comparison is that annual forage cropland represents a common land use form in the study region.

## 2. Materials and methods

### 2.1. Study area

The research was conducted near to Guatraché, La Pampa province, Argentina (37° 41′S and 63° 31′ O), between 2008 and 2012. The general characteristics of the region concerning climate, soil, and vegetation are described in INTA et al. (1980). The climate is temperate and semi-arid. Mean monthly air temperatures range from 6.9 °C in July to 23.2 °C in January, with an annual average of 14.8 °C. The frost-free period averages 165 days. Long-term mean annual rainfall is 640 mm (CV = 32%). Precipitation peaks in the spring and summer months, although the highest water deficit occurs in summer. Annual precipitation during the study period was 448 mm in 2008, 407 mm in 2009, 607 mm in 2010, 713 mm in 2011 and 825 mm in 2012. Dominant soils are classified as shallow Petrocalcic Calciustolls of a sandy-loam texture.

### 2.2. Experimental design

The experiment was conducted at a site (18 m by 72 m) with soil characteristics representative of those at regional level, located in a paddock with a long history of conventional tillage for annual forage cropping. The soil profile showed an A (0–20 cm), AC (20–30 cm), C (30–50 cm), and petrocalcic (50 cm) horizon development. The top soil (0–20 cm) was characterized by sandy-loam texture (50% sand, 46% silt, 4% clay), 2.3% organic matter, 7.5 mg kg$^{-1}$ phosphorus, and a pH of 6.7. In August 2008, the central area (10 m by 20 m) of the site was tilled with a chisel and afterwards with double disc plow, and fenced to exclude grazing. The rest of the site continued under conventional tillage for annual forage cropping (*Avena sativa*). Then, eight 3m by 2 m plots surrounded by 1-m-wide path were established in each of three blocks within the fenced area, and individual species or a combination of species was planted randomly in every plot in September 2008. Single species were *P. virgatum* cv. Alamo, *P. coloratum* cv. Verde, *E. superba* cv. Palar and *E. curvula* cv. Tanganyka, the latter as the standard species for comparison. Mixtures of species were represented by *P. virgatum – P. coloratum*, *P. virgatum – E. superba*, *P. coloratum – E. superba* and *P. virgatum – P. coloratum – E. superba*. All the species studied start their growing cycle in spring and end it in fall. Plants were obtained from pastures cultivated under similar ecological conditions to those of the study area, and transplanted in each plot 0.3 m apart in four lines separated by 0.5 m. Transplants were used instead of seeds to speed up plant establishment. The species in mixtures were mixed within and between lines. In this way, the individuals of any one species in the two-species mixtures were surrounded by four individuals of the other species, whereas in the three-species mixture individuals of one species were surrounded by two individuals of each of the other two species. Total plant density in monocultures and mixtures was 6.67 plants per m$^{-2}$. Afterwards, and during the first growing season, plots received small amounts of supplementary water and were weeded out to warrant plant survival. The experimental pastures were not fertilized during the study period since fertilization was not a variable considered in the objectives of this study. In July 2009, the plots were hand clipped to 5-cm in height to remove any end-of-season standing biomass. Clipping WSG to a 5-cm stubble height in the dormant season would not be expected to reduce the yield in the following growing season (Vogel and Bjugstad, 1968).

### 2.3. Stockpiled forage accumulation and quality

In July 2010 and July 2012, the aboveground standing crop of six plants located at the centre of every plot (0.9 m$^2$) was clipped to 5-cm stubble height, sorted by species in the case of mixtures of WSG. Afterwards, the entire plot was clipped in a similar way and the biomass removed from the study site. We only made one cut per year since our objective was to quantify the stockpiled forage accumulation throughout the growing cycle of WSG. In July 2011, we proceeded as already explained, except that we were impeded in evaluating the aboveground standing crop since unwanted cattle grazing took place on April 15 in that year. A subsample from the harvested material weighing approximately 200 g was dried to constant weight under forced air (50 °C) to determine the water content and it was ground to pass through a 1-mm sieve for subsequent laboratory analysis. In plots with mixtures of WSG, the subsample was representative of the proportional contribution of each species.

The functional mean contribution in terms of production of each species in the mixtures was determined by calculating the relative overyielding index (Loreau, 1998) as: $O_i = (Y_{oi} - YR_{oi} * M_i)/(YR_{oi} * M_i)$, where $Y_{oi}$ is the observed yield of species i in the mixture, $YR_{oi}$ is the observed relative abundance of species i in the mixture, and $M_i$ is the yield of species i in monoculture. A positive $O_i$ indicates that species produce more biomass when in a mixture than in a monoculture, whereas a negative $O_i$ indicates that species produce less biomass in a mixture than in a monoculture. Furthermore, the two-way partitioning of Loreau and Hector (2001) was used to partition the net diversity effect (NE) into complementarity (CE) and selection effects (SE): NE = CE + SE = N $\overline{\Delta RY}\,\overline{M}$ + $N\mathrm{cov}[M,\Delta RY]$, where N is the number of species in the mixture, $\overline{\Delta RY}$ is the average (across all component species) deviation between observed yield and expected relative yield (which is its proportion seeded or planted) in the mixture, $\overline{M}$ is the average (across all component species) monoculture yield, and cov $[M,\Delta RY]$ is the covariance between monoculture yields and species' deviations from expected relative yield. Positive CE indicate patterns of niche partitioning or facilitation, whereas positive or negative SE indicate that overyielding is controlled by one or a few species with fast or slow monoculture growth, respectively.

Stockpiled forage was analysed for neutral detergent fibre (hemicelluloses, cellulose, lignin and cutin) and acid detergent fibre (cellulose, lignin and cutin) by the detergent method (Van Soest and Robertson, 1985), total nitrogen (N) by semi-micro Kjeldahl, and *in vitro* dry matter disappearance (an estimation of dry matter digestibility) through the two-stage technique of Tilley and Terry (1963). Crude protein (CP) was calculated by multiplying N x 6.25. All these parameters are used routinely to characterize forage quality for animal nutrition.

### 2.4. Root biomass, abundance, and distribution

At the end of the study period (July 2012) we evaluated root mass down to 25 cm depth by extracting four soil cores (494 $cm^3$ each) per plot; two along the rows and two in between the rows. The soil cores collected in each plot were then pooled, washed in a 30-mesh sieve to separate roots from mineral soil, and oven dried at 60 °C until constant weight. Additionally, we evaluated root abundance and distribution in all treatments by a semi-quantitative method (Massé, 1982). A soil pit (100 cm by 100 cm by 50 cm depth) was excavated in each plot in one block, and at one side parallel to the rows the soil was loosen up carefully with the help of a spatula and washed with pressurized water to expose the roots. Afterwards, a wire mesh grid (70 cm with by 45 cm depth, with cells of 5 cm by 5 cm) was placed over the exposed roots, and the root abundance was estimated at cell level according to the following scale: 0 = absence; 1 = low presence; 2 = middle presence; 3 = high presence; 4 = very high presence. Data for each 5-cm of soil depth intervals were averaged over 14 cells. Since the scale is only based on root presence or absence, there is not necessarily a relationship between root abundance and root biomass.

### 2.5. Soil physicochemical properties

At the beginning of the study, we collected three composite samples (10 subsamples each) of the superficial soil (first 20 cm) from the study site to determine the bulk density and organic carbon (Walkley and Black, 1934). The same procedure was followed at the end of the study to determine the bulk density and organic carbon of soil from the WSG treatments and the adjacent cropland. At the end of the study we also measured soil aggregate stability (De Leenheer and De Boodt, 1967) in three composite samples (0–20 cm) collected from WSG, adjacent cropland, and from a reference nearby of historically uncultivated land; and we determined (n = 4) the initial and final stable soil infiltration rates under either WSG or adjacent cropland by using a single ring cylinder (Álvarez et al., 2012). Composite soil samples taken at the end of the study included all the WSG treatments, except in the case of soil organic carbon which was determined at the monoculture or species mixture level.

### 2.6. Statistical analyses

Untransformed or transformed data (proportions were transformed to arcsine square root) were checked for normality and homogeneity of the variance and analysed by ANOVA according to a randomized complete block design (stockpiled forage accumulation and quality, root biomass) or a completely randomized design (soil physicochemical properties). When data were collected in a second year, a two-way repeated measures ANOVA was used. Since the year by treatment interaction was not significant ($p > 0.05$) for these data (forage yield and quality), the average results are presented. Multiple mean comparisons were made using LSD tests with Bonferroni corrections, at $p \leq 0.05$. All analyses were performed using the Infostat software (Di Rienzo et al., 2009). Since there were no true replications for root abundance and distribution in the soil profile, only the average and dispersion measurements are presented.

**Table 1**
ANOVA table for stockpiled forage accumulation at varying grass treatment, year and their interaction.

| Sources | DF | F | p |
|---|---|---|---|
| Block | 2 | 0.89 | 0.419 |
| Grass treatment | 7 | 12.24 | < 0.001 |
| Year | 1 | 20.23 | < 0.001 |
| Grass treatment x year | 7 | 2.28 | 0.082 |

## 3. Results

### 3.1. Stockpiled forage accumulation and quality

There was a significant effect of the WSG treatment ($p < 0.001$) and the year ($p < 0.001$) on stockpiled forage accumulation, although the treatment by year interaction was not significant ($p = 0.082$) (Table 1). The average forage yield (kg $ha^{-1}$) was higher in 2010 (6876) than in 2012 (5355). Stockpiled forage was highest in the most diverse WSG mixture and in the mixture of *E. superba*-*P. virgatum*, lowest in the monoculture of *E. superba*, and intermediate for the rest of monocultures and mixtures (Fig. 1). In the mixtures, *P. coloratum* contributed the most to the total stockpiled forage accumulation when growing with *E. superba* and/or *P. virgatum*, whereas the latter species contributed the most when growing with *E. superba* (Fig. 2). The $O_i$ was positive for *P. coloratum* in all mixtures, for *P. virgatum* when combined with *E. superba*, and for the latter species in the most species-rich mixture (Fig. 3). On the other hand, the two-way partitioning of the net diversity effect revealed that the complementarity effect was positive overall ($p < 0.001$), whereas the selection effect was zero on average and varied from positive to negative depending on whether species with a higher- or lower-than-average biomass dominated the mixtures (Fig. 4).

There was a significant effect of both treatment and year on *in vitro* dry matter disappearance ($p < 0.001$ and $p = 0.024$, respectively), crude protein ($p = 0.023$ and $p < 0.001$, respectively) and acid detergent fibre ($p = 0.023$ and $p = 0.003$, respectively), whereas the neutral detergent fibre was not affected (p = 0,405) by treatment although it was affected ($p = 0.003$) by the year. The interaction treatment by year was not significant ($p > 0.219$) for any of the forage

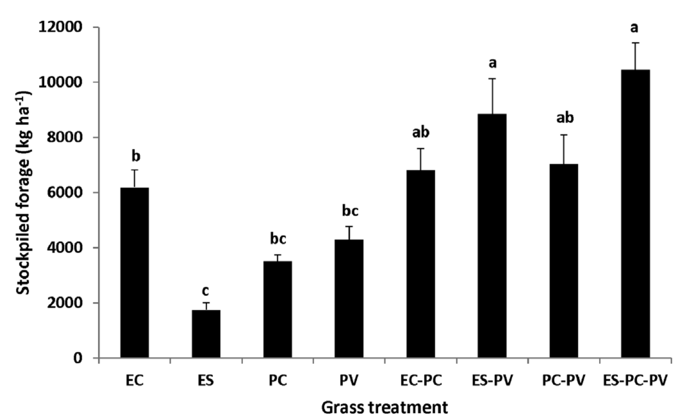


**Fig. 1.** Stockpiled forage accumulation ( ± s.e.) in monocultures and mixtures of warm-season grasses. Grass treatment codes stand for monocultures of *Eragrostis curvula* (EC), *Eragrostis superba* (ES), *Panicum coloratum* (PC), and *Panicum virgatum* (PV), and for two- or three-species mixtures of the last three species. Data are the means of three blocks and two growing cycles (n = 6). Bars with a lowercase letter in common are not significantly different at the 0.05 probability level.

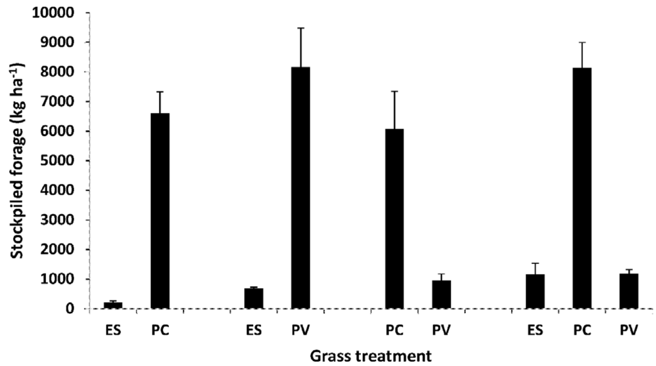


**Fig. 2.** Stockpiled forage contribution to the mixtures by component warm-season grass species. Data are the means ( ± s.e.) of three blocks and two growing cycles (n = 6). Grass treatment codes stand for two- or three-species mixtures of *Eragrostis superba* (ES), *Panicum coloratum* (PC), and *Panicum virgatum* (PV).

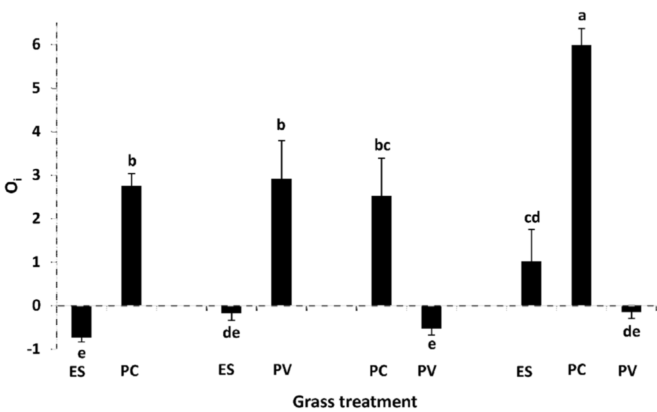


**Fig. 3.** Relative overyielding ( ± s.e.) for each species in mixtures of warm-season grasses, calculated as in Loreau (1998) by: $O_i = (Y_{oi} - YR_{oi} * M_i)/(YR_{oi} * M_i)$, where $Y_{oi}$ is the observed yield of species i in mixture, $YR_{oi}$ is the observed relative abundance of species i in mixture, and $M_i$ is the yield of species i in monoculture. Each value represents the mean of three blocks and two growing cycles (n = 6). An $O_i > 0$ indicates that the species performs better in mixtures than in monocultures. Values with a lowercase letter in common are not significantly different at the 0.05 probability level. Grass treatment codes stand for two- or three-species mixtures of *Eragrostis superba* (ES), *Panicum coloratum* (PC), and *Panicum virgatum* (PV).

quality variables, which on average were better in 2010 than in 2012. *Eragrostis superba* consistently showed the highest values of *in vitro* dry matter disappearance and crude protein, whereas *P. virgatum* showed the lowest ones (Table 2). The content of acid detergent fibre was highest in the mixtures including *P. virgatum* and in the monoculture of *E. superba*.

### 3.2. Root biomass, abundance, and distribution

Root biomass in the superficial soil (0–25 cm) differed ($p < 0.001$) between treatments (Table 3). It was highest in the mixture of the three target WSG, in the monoculture of *P. virgatum* and when the latter species was growing with *E. superba*; and it was lowest in the rest of the WSG treatments. In the monocultures of WSG, root abundance over the soil profile was highest in *P. virgatum* and lowest in *E. superba*; however, there were only small differences between the monocultures in the first 20 cm of soil, except for the relatively low root abundance of *E. superba* (Table 3). In the mixtures of WSG, the root abundance in the first 20 cm of soil was high in all treatments, whereas it was below the root abundance of the *P. virgatum* monoculture at deeper soil layers.

### 3.3. Soil physicochemical properties

There was no difference (p = 0.405) in the bulk density (Mg $m^{-3}$ ± s.e.) of the soil (0–20 cm) between the initial conditions (1.27 ± 0.01) and conditions at the end of the study under either WSG (1.30 ± 0.03) or adjacent cropland (1.32 ± 0.04). Similarly, there was no difference (p = 0.559) in the organic carbon content (Mg $ha^{-1}$ ± s.e.) of the soil (0–20 cm) between the initial condition (34.4 ± 0.6) and conditions at the end of the study under either WSG treatments (34.7 ± 1.0, average across treatments) or adjacent cropland (30.1 ± 0.6). There was no difference (p = 0.938) in organic carbon content between WSG treatments. Aggregate soil stability (0–20 cm) differed (p = 0.002) between the non-cultivated soil and cultivated soil either under WSG or adjacent cropland; reduction in the mean weight diameter of soil aggregates (mm ± s.e.) was different in the three conditions (0.45 ± 0.02, 1.04 ± 0.04, and 1.53 ± 0.24, respectively). Initial soil infiltration rate (mm $h^{-1}$ ± s.e.) was higher ($p < 0.001$) in pastures of WSG (1200 ± 75) than in adjacent cropland (600 ± 35), whereas the stable soil infiltration rate was similar (p = 0.879) in both conditions (39 ± 9 and 37 ± 7, respectively).

## 4. Discussion

As expected, the most diverse pasture of WSG showed the highest value of stockpiled forage accumulation (Fig. 1). The diversity effect in the most species-rich mixture was mainly explained by the complementarity effect, indicating patterns of niche partitioning or facilitation, although there was also competitive dominance of *P. coloratum* over *E. superba* and *P. virgatum* (Fig. 4). *Panicum coloratum* produced more biomass in the mixtures than in the monoculture (Fig. 2) and had a positive $O_i$ (Fig. 3), showing that intraspecific negative interactions were more intense than interspecific competition in this species. Something similar happened with *P. virgatum* when it was growing in combination with *E. superba* (Fig. 3). The strong complementarity effect observed in the present study deserves highlighting. It has been proposed that diverse communities can capture a greater fraction of the available resources and produce more biomass than even the most productive species ("transgressive overyielding", Schmid et al., 2008). The large plant size and higher per capita production of dominant species in mixture than in monoculture may have contributed to enhanced pre-emption of light, water and nutrients (Caldwell et al., 1996; Schwinning and Weiner, 1998; Craine and Dybzinski, 2013). On the other hand, the great performance in terms of production of the standard monoculture of *E. curvula* (it was exceeded by the mixture of the three target WSG and the mixture of *P. virgatum* - *E. superba* only) was most probably related to its outstanding adaptation to the site conditions (Covas and Carnie, 1985), which in the conceptual framework for biodiversity is referred to as the habitat effect (Fridley, 2001). Nevertheless, it is worth noting that functional plasticity of the studied WSG in response to disturbance and stress may affect the intensity of the above relationships (Pontes da Silveira et al., 2012), and that the diversity effects and their underlying mechanisms can change over time as species interactions change the structure of the experimental communities (Cardinale et al., 2007).

Overall, the higher yield in 2010 than in 2012 could be explained in part by greater precipitation in the growing cycle September 2009–April 2010 (619 mm) than in the growing cycle September 2011–April 2012 (511 mm). In addition, nutrient limitations, particularly nitrogen, may also explain in part the observed decay in pasture production over time (Gargano et al., 2001a). The tissue chemistry of WSG is high in C: N ratio and lignin concentration, which slows organic matter decomposition and causes nitrogen immobilization in the microbial biomass (Wedin and Tilman, 1990).

Potentially higher soil nitrogen limitation in 2012 than in 2010 may also partially explain the drop in forage quality in 2012. However, beyond the observed differences in chemical composition and

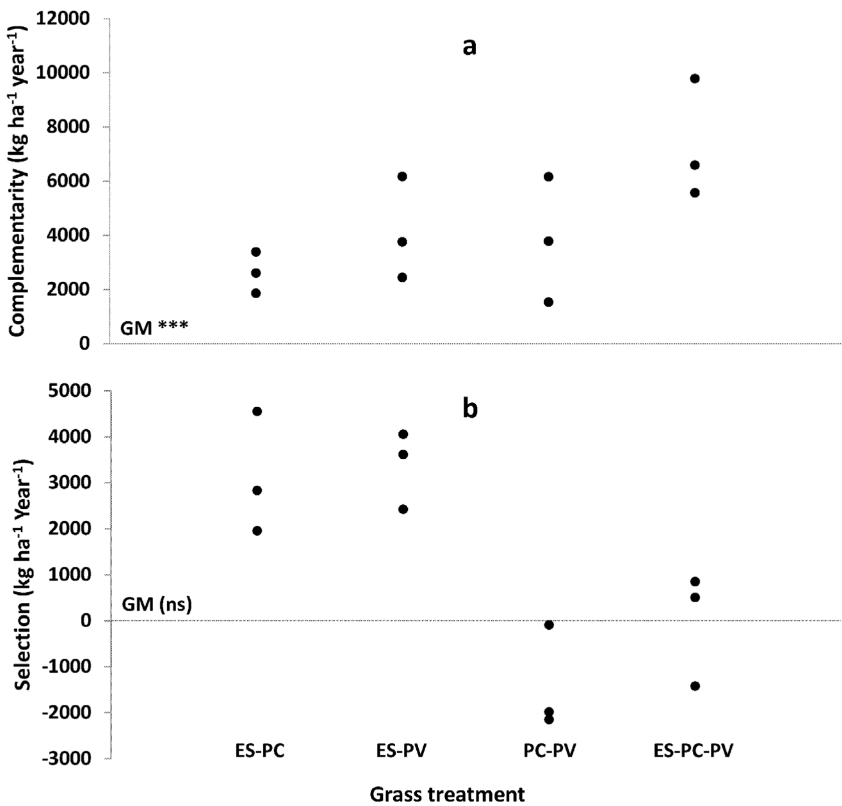


**Fig. 4.** Complementarity (a) and selection (b) effects as a function of species mixtures, according the two-way partitioning methodology of the net diversity effect (Loreau and Hector, 2001). Grass treatment codes stand for two- or three-species mixtures of *Eragrostis superba* (ES), *Panicum coloratum* (PC), and *Panicum virgatum* (PV). The significance of the grand mean (GM) against zero is indicated with a *** ($p < 0.001$) or ns for non-significance ($p > 0.05$). Positive complementarity effects indicate niche partitioning or facilitation, whereas positive or negative selection effects indicate a high biomass or low biomass species overyield, respectively.

**Table 2**
Stockpiled forage *in vitro* dry matter disappearance (IVDMD), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF). Each value represents the mean (± s.e.) of three blocks and two growing cycles (n = 6). Values with a lowercase letter in common within each quality parameter are not significantly different at the 0.05 probability level. Grass treatment codes stand for monocultures of *Eragrostis curvula* (EC), *Eragrostis superba* (ES), *Panicum coloratum* (PC), and *Panicum virgatum* (PV), and for two- or three-species mixtures of the last three species.

| Grass treatment | IVDMD | CP | NDF | ADF |
|---|---|---|---|---|
| | (%) | | | |
| EC | 32.9 (1.5) c | 3.0 (0.2) ab | 85.4 (1.2) | 44.2 (1.1) bc |
| ES | 49.5 (1.5) a | 3.2 (0.2) a | 81.5 (0.8) | 47.1 (1.2) abc |
| PC | 41.7 (1.6) abc | 3.0 (0.3) ab | 81.9 (0.7) | 44.4 (1.3) bc |
| PV | 33.4 (3.3) bc | 2.3 (0.3) cd | 82.6(1.7) | 43.7 (1.5) c |
| ES-PC | 43.9 (2.6) ab | 2.8 (0.2) abc | 81.2 (1.4) | 45.2 (1.2) bc |
| ES-PV | 35.9 (4.4) bc | 2.2 (0.3) d | 83.5 (0.9) | 49.0 (1.9) a |
| PC-PV | 41.0 (1.6) abc | 2.6 (0.2) bcd | 82.3 (1.1) | 47.6 (2.4) ab |
| ES-PC-PV | 40.5 (2.3) abc | 2.6 (0.1) bcd | 83.7 (1.9) | 47.3 (1.1) abc |
| p | < 0.001 | 0.023 | 0.405 | 0.023 |

digestibility between years and treatments, the quality of the stockpiled forage in the present study (Table 2) was inadequate to meet the nutritional requirements of ruminants, even for animal maintenance (NRC, 2016). It should be noted that, in the present study, forage was stockpiled throughout the annual growth cycle of WSG and up to the following winter, without any management actions to improve the quality. Nitrogen fertilization, stockpiling initiation date and harvested date represent some of the management variables that have been used to improve the nutritive value of stockpiled forage of WSG towards meeting the nutrient requirements of beef cattle in late fall and winter (Johnson et al., 2001; Scarbrough et al., 2006; Mbatha and Ward, 2010; Kering et al., 2011). Alternatively, supplementation with protein can help to overcome the low nutrient density of stockpiled forage (e.g., Wheeler et al., 2002).

The extensive root system of the studied WSG, particularly in the mixture of the three WSG and the monoculture of *P. virgatum* (Table 3), was in agreement with the general characterization of the root systems of perennial $C_4$ grasses (Jackson et al., 1996) and it is particularly relevant under edaphic limitations and a semiarid climate. Under conditions of superficial soils and water and nutrient scarcity, a large root system is critical to capture a greater fraction of the available soil resources (Casper and Jackson, 1997). It is also important to improve soil physicochemical properties (Gebhart et al., 1994; Angers and Caron, 1998; Bonin et al., 2013). Root biomass contributes in building soil organic matter, which leads to enhanced soil aggregate stability and water infiltration (Blanco-Canqui, 2010), as was observed in the current study. It also normally contributes to organic carbon sequestration; however, we did not find a significant increase in the organic carbon in the soil under WSG, which may have been due to the relative short time since WSG establishment (four years) and lack of nitrogen fertilization (Lemus and Lal, 2005). Nonetheless, we did observe a numerically higher organic carbon in the soil under the mixture of the three WSG and under *P. virgatum*, which was consistent with their highest root biomass in the upper soil layers. It is worth noting that WSG are commonly characterized by a deep rooting system, thereby promoting carbon sequestration in the deep soil layers (Omonode and Vyn, 2006).

## 5. Conclusions

In semiarid environments, soil water and fertility frequently limit plant growth, which is aggravated in shallow soils. Nevertheless, even under these constraints, our results suggest that mixtures of WSG can be highly productive. Moreover, they can have favourable impacts on soil protection and improvement. In the current study, the most diverse mixture of WSG exceeds monocultures of WSG in above- and belowground biomass production. However, the quality of stockpiled forage was below the nutrient requirement for livestock maintenance, pointing out the need for implementing management actions to overcome this limitation. On the other hand, compared to cropland soil under a conventional tillage system, the soil under WSG showed a higher initial

**Table 3**
Root abundance and root biomass under monocultures and mixtures of warm-season grasses. Each value of root abundance represents the mean ( ± s.d.) of 14 observations from one block, and was estimated according the following scale: 0 = absence; 1 = low presence; 2 = middle presence; 3 = high presence; 4 = very high presence. Each value of root biomass represents the mean ( ± s.e.) of three blocks; values with a lowercase letter in common are not significantly different at the 0.05 probability level. Grass treatment codes stand for monocultures of *Eragrostis curvula* (EC), *Eragrostis superba* (ES), *Panicum coloratum* (PC), and *Panicum virgatum* (PV), and for two- or three-species mixtures of the last three species.

| | Soil depth (cm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0–5 | 5–10 | 10–15 | 15–20 | 20–25 | 25–30 | 30–35 | 35–40 | 40–45 | 0–25 |
| Grass treatment | Root abundance | | | | | | | | | Root biomass (Kg ha$^{-1}$) |
| EC | 4.0 (0) | 3.9 (0.3) | 3.9 (0.4) | 3.9 (0.4) | 3.2 (0.4) | 3.1 (0.3) | 3.1 (0.3) | 3.5 (0.5) | 2.9 (1.1) | 4765 (514)d |
| ES | 3.0 (0.7) | 2.7 (0.9) | 2.5 (0.9) | 2.6 (1.0) | 2.3 (1.0) | 2.0 (0.6) | 1.8 (0.9) | 1.0 (0) | 1.0 (0) | 6368 (768)d |
| PC | 4.0 (0) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 3.2 (0.4) | 3.2 (0.4) | 3.2 (0.4) | 3.4 (0.5) | 2.6 (0.5) | 6452 (763)d |
| PV | 4.0 (0) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 9700 (1288)ab |
| ES-PC | 3.5 (0.5) | 3.9 (0.4) | 3.9 (0.4) | 3.1 (0.5) | 2.6 (0.5) | 2.2 (0.4) | 2.3 (0.6) | 2.2 (0.4) | 1.6 (0.5) | 6832 (1461)cd |
| ES-PV | 4.0 (0) | 4.0 (0) | 4.0 (0) | 3.8 (0.4) | 3.2 (0.4) | 3.0 (0) | 2.4 (0.5) | 2.3 (0.6) | 1.9 (0.4) | 9320 (375)bc |
| PC-PV | 3.7 (0.6) | 4.0 (0) | 4.0 (0) | 4.0 (0) | 2.7 (0.8) | 3.0 (0.8) | 2.9 (0.9) | 3.2 (0.6) | 2.1 (0.5) | 6579 (887)d |
| ES-PC-PV | 3.9 (0.4) | 3.8 (0.6) | 3.7 (0.5) | 3.8 (0.4) | 3.6 (0.5) | 3.2 (0.6) | 3.2 (0.6) | 3.3 (0.6) | 2.9 (0.3) | 11,850 (609)a |

infiltration rate and aggregate stability after four years of implantation. Important questions that still need answers are to what extent do biodiversity effects and their underlying mechanisms change over time as species interactions structure WSG mixtures, and to what extent do the functional strategy and functional plasticity of the component WSG species influence the biodiversity effects.

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