Plant functional composition affects soil processes in novel successional grasslands

Laura Yahdjian[a*], Pedro M. Tognetti[a, b], Enrique J. Chaneton[a]

[a] Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA),

Facultad de Agronomía, Universidad de Buenos Aires and CONICET, Av. San Martín 4453,

C1417DSE, Buenos Aires, Argentina.

[b] Departamento de Métodos Cuantitativos y Sistemas de Información, Facultad de

Agronomía, Universidad de Buenos Aires.

*Corresponding author: yahdjian@agro.uba.ar

Running headline: Ecosystem functioning in novel grasslands

Summary

1. Secondary succession may lead to novel, exotic-dominated community states differing in

structure and function from the original native counterparts. We hypothesized that grassland

soil processes associated with C and N cycling decelerate with community turnover from

short-lived forbs and grasses to long-lived native grasses, whereas invasion by exotic

perennial grasses maintains fast cycling rates.

2. We measured litter C and N turnover during decomposition, soil respiration, and soil N dynamics in synthetic plant communities resembling four successional stages, established on abandoned farmland in the Inland Pampa, Argentina. We also compared litter chemistry and decay rates of dominant species from each community stage in a common garden, and assessed mass loss for a standard litter type incubated in all communities.

3. Litter decomposition and soil respiration decreased, while litter N retention increased from early, through mid to late community stages dominated by forbs, short-lived grasses and native perennial grasses, respectively. Soil process rates in exotic perennial grass communities were faster than in native grass communities, but similar to annual grass communities. Further, the standard litter decomposed more slowly in the native perennial than in the exotic perennial grass community. In the common garden, short-lived forbs and grasses decomposed faster than native or exotic perennial grasses, with species' decay rates being negatively related to initial litter C:N ratio.

4. Our results show that changes in soil processes across old-field communities arise chiefly through differences in the quality of litter produced by dominant functional groups. A dominance shift from native to exotic perennial grasses prevented the deceleration of C and N cycling expected with plant successional turnover. Thus invasion by fast-growing exotic grasses may fundamentally alter ecosystem functioning in novel grasslands.

Introduction

Human activities have deeply altered land-cover patterns through conversion to croplands and pastures (Tilman *et al.* 2001; Foley *et al.* 2005), particularly affecting temperate grasslands worldwide (Ramankutty *et al.* 2008). Land-use changes also involve the abandonment and spontaneous re-vegetation of formerly cultivated fields (Lepers *et al.* 2005), a process that is increasingly perceived as an opportunity for conservation (Young, Petersen & Clary 2005; Fischer *et al.* 2015). However, native plant species recovery in old fields is often impeded by invasion from fast-growing exotic species (Cramer, Hobbs & Standish 2008), which may lead to alternative, 'novel' community states (Suding, Gross & Houseman 2004; Hobbs, Higgs & Harris 2009). It has been suggested that the persistence of exotic-dominated vegetation states may be related to the extent to which exotic species traits differ from those of native resident species (Wilsey *et al.* 2009), and how such traits influence soil processes and nutrient cycling (D'Antonio & Hobbie 2005; Baer & Blair 2008). Yet, how functional attributes of novel ecosystems vary across stages of succession remains little understood (Hobbs *et al.* 2014; Martin *et al.* 2014).

Plant succession comprises directional, often predictable, changes in species composition and ecosystem function over time (Odum 1969). Early successional, old-field communities are typically dominated by fast growing species such as annual/biennial forbs and grasses, which are replaced by slow-growing perennial grasses, tall herbs or woody species (Tilman 1988; Bazzaz 1996). These compositional shifts are in turn expected to influence carbon and nutrient cycling rates (Odum 1969; Knops & Tilman 2000), with potential feedback effects on community dynamics (Suding *et al.* 2004; Kardol & Wardle 2010). For instance, litter turnover and nutrient cycling are generally predicted to decelerate through succession, as early plant colonizers with nutrient-rich, more decomposable tissues are replaced by late seral species with greater investment in carbon-based, more recalcitrant

structures (Tilman 1988; Bazzaz 1996). Underground processes may not only reflect the quality of litter shed by plant species at different seral stages, but also changes in soil biotic and abiotic conditions as influenced by dominant plant species traits (Quested *et al.* 2007; Kardol & Wardle 2010). However, it is as yet unclear the extent to which litter quality and soil environment contribute to drive changes in soil processes across successional stages.

Exotic plant species can exert profound ecosystem-level impacts (Ehrenfeld 2003) when they differ from native residents in traits associated with resource acquisition and nutrient cycling such as tissue chemistry (D'Antonio & Hobbie 2005) and growth rate (Daehler 2003; Ashton *et al.* 2005; Van Kleunen *et al.* 2010). Invasive exotic species often exhibit high relative growth rates and resource uptake in nutrient-rich environments (Daehler 2003; Ehrenfeld 2003), and may thus produce more decomposable litter than the average species in the resident community (D'Antonio & Hobbie 2005). A meta-analysis of 94 invasion studies revealed that exotic plant species significantly alter C and N fluxes and pools, generally leading to increased productivity and decomposition rates (Liao *et al.* 2008). Noticeably, however, most field studies have relied on comparisons between invaded and non-invaded sites (Molinari & D'Antonio 2014). This approach has limitations since exotic species may not be the necessary cause of observed ecosystem changes in invaded sites. Many invasive species take advantage of ecosystem alterations created by habitat disturbance rather than being the drivers of changes themselves (Didham *et al*. 2005). Further, a litter incubation experiment comparing co-familial native and invasive exotic species found that exotics decomposed more slowly than natives (Godoy *et al.* 2010). These conflicting results suggest that effects of exotic plants on soil processes may be difficult to predict and that fresh experimental approaches are needed to reveal the mechanisms whereby exotic species alter ecosystem function (Levine *et al.* 2003). Ideally, one would aim to compare the functional

effects of native vs. exotic species established on a common soil substrate and under the same environmental conditions (Walker *et al.* 2010).

Here we examined soil process rates across stages of an old-field grassland succession leading to alternative end-points dominated by either native or exotic perennial grass species. Our objectives were twofold. First, to assess how successional shifts in plant functional composition influence litter C and N turnover, decomposition, soil respiration and soil N cycling. Second, to compare the effects of late-successional, exotic vs. native perennial grasses on soil processes. To these aims, we created synthetic communities resembling early, mid and late stages of grassland succession, and established them side-by-side under initially common environmental conditions, on newly abandoned farmland in the Inland Pampa, Argentina. The Pampa grasslands of eastern Argentina have been extensively transformed by agricultural use over the last century (Baldi, Guerschman & Paruelo 2006; Vega *et al.* 2009). Old-field studies in this system have shown consistent shifts in community composition from fast-growing forb species, through annual/biennial grasses to perennial grasses (Tognetti *et al.* 2010). These functional groupings reflect contrasting species *responses* to environmental factors (e.g., disturbance, fertilization; (Tognetti & Chaneton 2012), while their *effects* on key ecosystem functions (e.g. litter decomposition) are yet unknown. Early- and mid-successional stages comprise mixed native/exotic species assemblages, whereas later seral stages are often dominated by exotic perennial grasses (Tognetti *et al.* 2010). Native perennial grasses are less frequently found in old fields, but dominate remnant grassland fragments (Burkart *et al.* 2011; Tognetti & Chaneton 2015). Recovery of native grasses on abandoned fields is limited by seed dispersal and competition from exotic grasses (Tognetti & Chaneton 2012).

Specifically, we tested the following hypotheses: (i) litter decomposition and soil respiration rates decrease as community dominance shifts from short-lived forbs and grasses to perennial grasses; (ii) establishment of exotic perennial grasses during old-field succession

accelerates soil processes, relative to native-grass dominated systems, thus maintaining fast C and N cycling in late community stages; and (iii) functional groups dominating different stages of succession control decomposition via the quality of litter they produce and by altering the soil environment (i.e. microclimate, microbial activity). To distinguish functional group effects on soil processes mediated by litter quality and local soil environment, we compared the litter chemistry and decay rate of dominant plant species in a common garden, and we also incubated a standard litter in all four synthetic communities representing different stages of succession.

Materials and Methods

Study site

The study was conducted at "Estancia San Claudio", a farm owned by the University of Buenos Aires, in Carlos Casares county, Buenos Aires province, Argentina (35º 55' S, 61º 09' W). The site is located on the eastern Inland Pampa, a subunit of the Río de La Plata Grasslands (Soriano 1992). The climate is temperate sub-humid, with 1022 mm annual precipitation for the last 25 years. Mean monthly temperatures vary from 7.2ºC in July to 23.8ºC in January. Soils are Typic Hapludolls developed from loess materials, with a deep and well-drained A horizon, containing 3% organic matter (Soriano 1992). The landscape is a mosaic of crop fields, sown pastures, and semi-natural grasslands under livestock grazing. Native grassland remnants occur along roadside corridors and abandoned railway tracks, and are dominated by the tall tussock grass *Paspalum quadrifarium* and other native perennial grasses (Burkart *et al.* 2011; Tognetti & Chaneton 2015).

Land abandonment leads to the establishment of novel plant assemblages comprising varied mixtures of native and naturalized exotic species (Omacini *et al.* 1995; Tognetti *et al.* 2010). During the initial 2 years of succession, species richness of native and exotic forbs is

similarly high, but exotic forbs account for nearly 50% of the total cover, including *Carduus acanthoides, Conium maculatum, Hirschfeldia incana*, and *Rumex crispus* (Tognetti *et al.* 2010). After 3-4 years, forbs are replaced by short-lived grasses, mainly *Lolium multiflorum* (exotic) and *Bromus catharticus* (native), which together account for over 60% of total plant cover (Tognetti *et al.* 2010). After 8-10 years of succession, old fields become dominated by exotic perennial grasses from disparate origins, including *Schedonorus arundinaceum* (ex *Festuca arundinacea*), *Dactylis glomerata, Sorghum halepense*, and *Cynodon dactylon*. Exotic grasses may establish persistent community states for over 25 years of succession (Tognetti *et al.* 2010). Native perennial grasses may become established in old fields depending on the site history of land use, seed availability and climate conditions (Omacini *et al.* 1995; Tognetti & Chaneton 2012).

Experimental design

We established four plant communities representing early, mid and late seral stages in a fallow field with a long history of row-crop cultivation. The last crop (soybean) was harvested in April 2005, just before the start of the experiment. In May 2005, we delimited 24 plots (2.2 m x 1.1 m each) distributed in six blocks, each comprising four plots separated by 2 m-wide walkways. Blocks were placed *c.*30-50 m apart in a grid pattern. Individual plots were randomly assigned to a given community type and were sowed with a species mix that represented the dominant functional group in each of four, well-defined successional stages. Early seral communities received a mix of short-lived native and exotic forbs (10 species); mid seral communities comprised short-lived native and exotic grasses (3 spp); and late seral communities were sown with either native (5 spp) or exotic (5 spp) perennial grasses. Species composition was determined from long-term cover data of post-agricultural succession in 10 fields (Omacini *et al.* 1995; Tognetti *et al.* 2010). Seeds were collected during 2004 from old

fields and grasslands in the study area. Plots were sown at a density of 2000 viable seeds/m^2; seeds were equally distributed among species. This sowing rate was intended to swamp the local seed bank with a particular functional group, while also overcoming natural variation in background emergence. Live plant cover at the time of sowing was below 25%. After three years (March 2008), the percent cover of all species was measured in two 1-m^2 quadrats placed on each plot. These synthetic communities adequately matched the functional composition and species richness expected for each stage of succession (Fig. 1).

Litter decomposition experiments

In May 2008, we established a litterbag experiment and measured soil attributes for the subsequent 18 months. To separate the relative contributions of litter quality and decomposition environment created by the plant community, we used a three-tiered approach (e.g. Austin & Vitousek 2000; Quested *et al.* 2007). First, litter of the dominant species in each community was incubated '*in situ*' (*home plot experiment*). *C. acanthoides* was incubated in the early-seral forb community; *L. multiflorum* in the mid-seral grass community; *S. arundinaceum* in the late-seral exotic grass community; and *P. quadrifarium* in the late-seral native grass community. Second, a common litter type was incubated in all four communities (*standard litter experiment*). We used wheat straw (*Triticum aestivum*) as the standard litter to assess the influence of the decomposition environment created by each community after three years, independent of litter origin (Quested *et al.* 2007). For these two experiments the vegetation in each experimental plot was left intact. Third, litter of the 2-4 main species from each community stage (Table 1) was incubated in a common environment to compare species' decay rates under standard conditions (*common garden experiment*). This experiment was established in 10 adjacent, 2 m × 2 m plots located within the same field as the other two. All aboveground vegetation was initially removed from the common garden

plots, which were kept clear by frequent weeding. Species used in the common garden experiment were incubated during the same time window and using the same protocol as in the other two experiments. For the *home plot* and *standard litter* experiments we placed replicate litterbags per treatment per block for each of five collection dates ($6 \times 4 \times 5 = 120$ litterbags per experiment). In the *common garden* experiment, we incubated litter of 12 species (Table 1) in 10 replicate blocks, allowing for five sampling dates ($12 \times 10 \times 5 = 600$ litterbags).

To evaluate litter decomposition we used the litterbag technique (Harmon *et al*. 1999). Litterbags were made of 2 mm-mesh fiberglass screen (10 cm x 20 cm) and contained 2 g of air-dried, field-collected litter material. Bags were anchored to the ground with metal pins. We collected litterbags at 1, 3, 6, 12, and 18 months of incubation by randomly removing one bag per treatment-block in each date. In the laboratory, the remnant litter was carefully cleaned to remove any foreign material (lichens, plants, soil), and was then oven-dried at 60 °C and weighed to the nearest 0.001 g. Litter dry mass values were corrected for ash content (Harmon *et al*. 1999). Data for the proportion of litter mass remaining in each sampling date were fitted to a negative exponential model (Olson 1963): $x_t/x_o = e^{-kt}$, where x_o is the initial litter mass, x_t is the residual litter mass at time *t* and *k* is the decay constant (year^{-1}). We performed separate linear regressions of ln (x_t/x_o) through time (intercept set to one) for each litter type per plot to provide independent estimates of *k* in the various decomposition environments. All regression models were significant (P<0.01, r^2 values > 0.80). For the home plot experiment, litter subsamples from each collection date and for the initial litter materials were analyzed for total C and N concentrations using a TrueSpec elemental analyzer (LECO®, St. Joseph, Mich., USA). The amounts C and N remaining in the litter for each collection date were calculated as percentage of the initial C and N contents to examine patterns of N retention vs. release as well as changes in litter stoichiometry (C:N ratio) during

decomposition. For the initial litter, we also determined C fractions present as soluble carbohydrates, hemicellulose and lignin, by successive extractions with acid detergent reactions ($n = 5$) (Van Soest 1963). These data were used to calculate standard indices of litter quality (C:N and lignin:N ratios).

Soil respiration, N mineralization, and microclimate

In October and November 2008, and in March and April 2009, we measured soil respiration fluxes across all four community types in the home plot experiment using a portable EGM-4 CO_2 Analyzer, a non-dispersive infrared gas analyzer connected to a soil respiration chamber SRC-1 (PP Systems, Hitchin, United Kingdom). On each sampling date, we took three measures within each plot by randomly placing the chamber on natural bare-soil gaps near the litterbags. These measures were averaged to obtain a single value for each plot and date. As plant cover in all plots was close to 100%, bare-soil gaps were typically smaller than 350 cm^2, being densely surrounded by standing plants. Thus, soil respiration provided an integrative measure of whole microbial community function and plant belowground activity, including root respiration.

In May and October 2008 and April 2009, we measured mineral N concentrations in the top soil (0-10 cm) of each plot. Samples were extracted using 5 cm diameter PVC tubes; inorganic N fractions (NH_4^+ and NO_3^-) were assessed for 2M KCl extracts using an elemental analyzer (Alpkem$^®$ autoanalyzer O-I Corporation, College Station, Texas, USA). For each sampling date, a soil subsample was dried at 105ºC for 48 hs to determine the soil gravimetric water content (%); soil N levels were expressed on a soil dry-mass basis. We estimated *in situ* net N mineralization rates during a one-month incubation period (October – November), by taking the difference between initial and final total mineral N (nitrate + ammonium) contents in tubes that prevented plant uptake (Raison, Connell & Khanna 1987). To characterize the

soil microclimate in each community, we measured topsoil (0-5 cm depth) daily temperatures (ºC) with sensors connected to data loggers. Soil temperature was recorded every hour during 2-3 consecutive days in May 2008, October 2008 and April 2009. For each sampling period, we obtained soil mean daily temperatures and mean thermal amplitude (daily maximum minus minimum temperature).

Data analysis

Litter species and community effects on litter decomposition were analyzed using mixed-model ANOVAs, with blocks as the random factor. For the common garden experiment, differences in the initial chemical traits and decay rates (*k* constant) among litter species were analyzed using a nested ANOVA model (Zar 2010), with species (n = 12) nested within functional groups (n = 4) in 10 blocks. We then used Spearman rank correlations to relate the litter species' *k* values in the common garden to their initial chemistry (Zar 2010). For the standard litter and home plot experiments, we tested for differences across seral communities in litter decay rates (*k*) and amount of organic matter (ash-free litter mass) remaining in the litterbags at the end of the study (n = 6 blocks). Because the number of established plant species varied considerably across communities (Fig. 1), we tested the relationship between litter decay rate and species richness per plot in the standard litter experiment through least-squares regression (n = 24).

Patterns in remnant litter %N (release vs. retention) and C:N ratio were analyzed separately for each sampling date using mixed, two-way ANOVA models with block as random effect, followed by pairwise treatment comparisons (Tukey tests, *P*<0.05). Differences in soil respiration, soil mineral N (total, nitrates and ammonium) and microclimatic variables (water content, daily mean temperature and thermal amplitude) were examined using repeated measures, blocked ANOVAs, with sampling dates as the repeated

measure (4 levels). Whenever the community by time interaction was significant, we performed simple ANOVAs within dates followed by *post-hoc* pairwise comparisons (Tukey tests, *P*<0.05). Data transformations were applied as needed to meet assumptions of normal errors and variance homogeneity. All analysis were performed using the software INFOSTAT (Di Rienzo *et al.* 2011).

Results

Initial litter chemistry

Litter chemistry varied among species and functional groups that dominated the different stages of succession (Table 1). Functional groups differed significantly with regard to litter N content ($F_{3,10}$=32.51, P<0.0001) and C:N ratio ($F_{3,10}$=32.3, P<0.0001). Forbs contained higher N than annual grasses (1.30 ± 0.06 % vs. 0.98 ± 0.07 %, respectively, P<0.05), which in turn had higher N than native perennial grasses (0.65 ± 0.02 %, P<0.05). In contrast, on average, the exotic perennial grass species (0.91 ± 0.04 %) did not differ from the annual grasses in litter N content, but had significantly higher N content than native perennial grasses (P<0.05). Total carbon, soluble carbohydrates, hemicellulose, and lignin contents in the litter varied among species, but did not consistently differ between functional groups (Table 1).

Common garden experiment

When incubated in a common environment, litter decay rates differed marginally among the four functional groups (nested ANOVA: $F_{3,10}$=3.54, P=0.07). On average, forbs decomposed faster than annual grasses (1.97± 0.13 year^{-1} vs 1.18± 0.17 year^{-1}, P<0.05) and native perennial grasses (0.81± 0.06 year^{-1}, P<0.05). Average litter decay rates for both perennial grass groups did not differ from that of annual grasses (P>0.10), but exotic perennial grasses decomposed significantly faster (1.19 ± 0.03 year^{-1}, P<0.05) than their native perennial

counterparts. These patterns emerged even though broad differences in decomposition rate were apparent among litter species within functional groups ($F_{11,114}$=50.51, P<0.001; see Table 1). Species-specific decay rates (k) ranged between 2.68 ± 0.15 year $^{-1}$ for the forb *C. maculatum* and 0.61 ± 0.01 year $^{-1}$ for the native perennial grass *P. quadrifarium*. Overall, the species' mean decay constant was negatively correlated to the initial C:N ratio of the litter (Fig. 2). Including lignin and other carbon fractions as predictors of species' k did not significantly improve the model fit (P>0.10, least-squares multiple regression).

Standard litter experiment

The rate of decomposition for the common litter type (*T. aestivum*) did not differ significantly across the four plant communities resembling different stages of succession ($F_{3,21}$=1.86, P<0.17, Fig. 3a), and was not related to the realized species richness per plot (r^2=0.063, P=0.39, n=24). On average, the standard litter decomposed marginally faster when incubated in the exotic than in the native perennial grass community (k difference = 0.43 yr^{-1}, P<0.07; Fig. 3a, inset). Nevertheless, the amount of organic matter remaining in the standard litter bags at the end of the experiment did not differ across communities ($F_{3,21}$=0.43, P=0.74, Fig. 3a).

Home-plot experiment

Litter decay rates decreased significantly from the forb dominated community (test species *C. acanthoides*), through the annual grass community (*L. multiflorum*) to the native perennial grass community (*P. quadrifarium*), which resembled early, mid and late stages of succession, respectively ($F_{3,23}$=77.11, P<0.0001, Fig. 3b inset). In contrast, litter decomposition in the late-successional, exotic perennial grass community (test species *S. arundinaceum*) was faster than in the native grass community, and did not differ from that

measured in the mid-successional, annual grass community (Fig. 3b inset). As a result, after 18 months of incubation, the amount of organic matter remaining in litterbags differed significantly across treatments (Fig. 3b). The native perennial grass community had the largest fraction of remnant matter, whereas the forb community retained the least organic matter in litterbags ($F_{3, 23}$=63.5, P<0.0001). Furthermore, litterbags incubated under exotic perennial grasses had significantly less matter remaining than those incubated under native perennial grasses, but retained a similar fraction as litterbags placed in the annual grass community (Fig. 3b).

On average, 'in situ' soil respiration rates were higher in the exotic perennial grass community than in the other three treatments, especially in spring ($F_{3, 68}$=8.95, P<0.0001; Fig. 4a). This pattern held despite large seasonal variation in soil respiration (time: $F_{3, 68}$=37.8, P<0.0001; community x time: $F_{9, 68}$=1.90, P<0.07). Moreover, throughout the study, soil respiration rates were higher in the exotic perennial than in the native perennial grass community ($F_{1, 11}$=18.9, P<0.001), whereas differences between the short-lived forb and grass communities were mostly not significant (P>0.10; Fig. 4b). Soil respiration rates beneath the four community types were positively correlated to the litter decay rates measured in the respective home plots (r=0.51, P=0.016; Fig. 4b).

Litter N turnover during decomposition exhibited broadly different dynamics depending on the community type ($F_{3, 20}$=299, P<0.0001; time: $F_{4, 80}$=3.1, P<0.02; community x time: $F_{12, 80}$= 30.80, P<0.0001; Fig. 5a). In the forb community, litter of *C. acanthoides* released N throughout the study period, whereas litter N content remained nearly constant for *S. arundinaceum* in the exotic perennial grass community. Conversely, in their respective home plots, the litters of both the annual grass *L. multiflorum* and the native perennial grass *P. quadrifarium* tended to immobilize N throughout the experiment, but %N

retained in the litter was significantly greater (P<0.05) for the native perennial grass community in two out of four sampling dates (Fig. 5a).

As a consequence, the C:N stoichiometry of decaying litter varied markedly across these communities ($F_{3, 20}$=210, P<0.0001; Fig. 5b). While litter C:N ratios generally decreased through time ($F_{4, 80}$=188, P<0.0001), the rate of decline differed among the four community types (community x time: $F_{12, 80}$=20.5, P<0.0001; Fig. 5b). Litter C:N ratios changed only slightly in the forb community, indicating that C and N were released at similar rates during decomposition (Fig. 5b). In contrast, the litter C:N ratio in the annual grass community declined steadily through time, as N was retained in the remnant organic matter while C was being released (see Fig. 3b). The two perennial grass litters had similar C:N dynamics, but with a clear trend for the exotic grass litter to release C and N faster than the native grass litter in their respective home plots (Fig. 5b).

Microenvironmental conditions

Levels of inorganic N (ammonia + nitrate) in the topsoil did not differ among communities in the home plot experiment ($F_{3, 11}$=1.82, P<0.15), but varied widely across sampling seasons ($F_{2, 11}$=34.1, P<0.0001; Table 2). Soil mineral N was mostly found as NH_4^+–N and occurred at higher concentrations in October (mid spring) than in April or May (autumn) (time: $F_{2, 11}$=29.6, P<0.001), varying only marginally among communities ($F_{2, 11}$=2.56, P<0.08; data not shown). On average, NO_3^-–N accounted for 0.4–2.0 % of the soil mineral N. Soil NO_3^-–N levels were highly variable and showed no significant pattern across communities (P>0.10; data not shown). Net N mineralization rates were similar in the four community types (P>0.10; Table 2); neither net ammonification nor nitrification rates differed across communities (P>0.05; data not shown).

Soil microclimatic variables in the home plot experiment depicted clear seasonal changes, but did not always vary among communities (Table 2). Soil moisture content ($F_{3, 11}=1.90$, $P<0.20$) and mean daily temperatures ($F_{3, 11}=2.50$, $P<0.11$; Table 2) were similar across treatments. However, while structurally very different, the short-lived forb and exotic grass communities created the most buffered soil microclimate, as shown by their narrower daily thermal amplitude, compared to the annual grass and native perennial grass communities ($F_{3, 11}=9.3$, $P<0.0025$; Table 2).

Discussion

Our results show that ecosystem functioning varied significantly among experimental communities resembling different stages of old-field succession, after they were grown for three years on a common soil substrate. Litter mass loss and soil respiration decreased, whereas litter N retention increased, from early-successional communities dominated by short-lived forb or grass species to later-successional communities made up of native perennial grasses. However, when later seral communities were dominated by exotic perennial grasses, which is the most common condition for post-agricultural grasslands in the Argentine Pampas, soil process rates did not differ between mid and late seral communities, and were faster in exotic grass communities than in their native grass counterparts. Thus, contrary to expectations from classical succession theory (Odum 1969), we found that the establishment of exotic grasses maintained relatively fast C and N cycling rates, which are typical of younger, less developed and more 'open' ecosystem states. These findings add to growing evidence that invasive plant species may alter key ecosystem processes (Ehrenfeld 2003; D'Antonio & Hobbie 2005; Liao *et al*. 2008). Moreover, our experiments demonstrate that successional changes in soil functioning can be driven chiefly by the litter quality of dominant functional groups, rather than by changes in the soil environment.

Litter decay and soil respiration rates decreased when comparing communities dominated by short-lived forbs or grasses with those of native perennial grasses, as proposed in our first hypothesis. As succession proceeds, plant communities tend to produce litter with reduced nutrient concentrations and, consequently, litter decomposition often declines from early to late seral stages (Wardle *et al.* 2009). This pattern was observed in post-agricultural fields in southern Sweden, where litter decomposition rate decreased with time since land abandonment (Quested *et al.* 2007). However, such a trend was described by comparing management units that differed not only in plant composition, but also in various other biotic and environmental attributes, which blurred the causal link between ecosystem function and species composition (see Walker *et al*. 2010). Here, we incubated litter of dominant species from different seral stages in their corresponding 'home' communities, which were established from seed under initially identical environmental conditions. Therefore, we can be confident that the functional changes observed across community stages reflected differences in plant composition. Specifically, the observed decrease in litter C and N turnover was driven by a predictable shift from short-lived to longer-lived functional groups (Tilman 1988; Bazzaz 1996), yet only when late seral communities were dominated by native grass species producing high C:N litter (see Table 1, Figs 1, 3).

Old-field communities often become dominated by invasive exotic species that replace former native plant residents (Cramer, Hobbs & Standish 2008; Hobbs, Higgs & Harris 2009). Ecosystem function in such novel communities may be quite different from that in their native counterparts (Molinari & D'Antonio 2014; Domínguez *et al.* 2014). However, the mechanisms underlying these effects have remained poorly understood (Wilsey *et al*. 2009; Martin *et al*. 2014). We found that, within just 3 years from establishment on a common substrate, soil functioning diverged between native and exotic perennial grass communities, and that soil process rates were generally faster beneath the exotic

communities. This finding agrees with a meta-analysis of plant invasion impacts showing that litter decomposition increased by 120% in invaded ecosystems, which on average had 40% higher plant and litter N concentrations, compared to native ecosystems (Liao *et al*. 2008). Our experimental results were also consistent with a comparative study in the Inland Pampa where old-field grasslands exhibited faster decomposition rates than nearby native grassland remnants (Spirito *et al.* 2014). Further, in support of our second hypothesis, the exotic perennial community had similar litter turnover rates as the annual grass community typical of mid seral stages. Thus, establishment of exotic grasses may prevent the deceleration of element cycling that is expected as succession proceeds towards dominance by longer-lived plant species (Odum 1969; Tilman 1988, Scherer-Lorenzen 2008). Dominance by exotic perennial species would maintain a fast cycling system, one that is typical of early stages of succession and is less prone to retain C and nutrients in plant and soil compartments.

The deceleration of litter decomposition from early to late seral communities was associated with a dominance shift in functional composition towards species producing litter of a lower quality (i.e. higher C:N ratio). Likewise, differences in soil process rates between late successional, native vs. exotic perennial communities were associated with differences in litter C:N ratio of dominant grasses. Taken together, the results from the home plot and common garden experiments indicated that variation in litter decay rates among resident test species arose chiefly from their contrasting litter chemistry, not changes in local soil environment. We found that dominant species with low C:N ratios decomposed faster in both experiments. In contrast, decomposition rate of the standard litter was similar in all four communities, except for a marginally slower decay under native than exotic perennial grasses (Fig. 3a), suggesting little effect of plant composition mediated by the soil environment. Indeed, we did not detect significant differences in soil moisture content, mean daily temperatures, and N availability among communities. Although we did find differences in

soil thermal amplitude (Table 2), they seemed to have little influence on the standard litter turnover. Topsoil microclimate is primarily controlled by ground cover, and our experimental communities did not substantially differ in terms of total plant cover (Fig. 1). Therefore, species-specific shifts in the quality of litter entering the soil, and associated differences in soil decomposer activity (Fig. 4), appeared to exert an overriding effect on litter decomposition in the context of this study.

Patterns of litter decay in our experimental plots reflected differences in litter chemistry of dominant species associated with their initial C:N ratios. Other litter quality indexes such as lignin content, lignin:N ratio, and carbohydrates fractions did not contribute to explain differences among litter species in the common environment. In contrast, lignin content seems to be the best quality index to predict patterns of litter decomposition when woody species are included in the experiment (Bontti *et al.* 2009). Indeed, in the large-scale Long-Term Intersite Decomposition Experiment (LIDET), litter lignin content was the best predictor of leaf and root decomposition rates (Bontti *et al.* 2009). Our study encompassed a much narrower litter quality range, including only non-woody species, but still highlights the importance of litter chemistry in driving decomposition among community stages of a novel grassland succession. Worldwide, litter decomposition is known to depend on both climate conditions and the legacy of plant functional traits on litter quality. However, Cornwell *et al.* (2008) found that the effect of species-driven differences on litter mass loss was much larger than previously thought and greater than climate-driven variation.

Changes in litter decomposition among community types correlated with soil respiration rates, but were not mirrored by differences in net N mineralization or soil N content. This may be due to soil N mineralization in the topsoil responding more slowly than decomposition in litterbags to variation in plant functional composition and litter quality across treatments (see Knops, Bradley & Wedin 2002). Alternatively, it is possible that the

lack of change in net N mineralization between early and late seral communities reflected a balance between gross N mineralization and N uptake by soil microbes, which are both expected to decrease with time of succession (Odum 1969). Similarly, previous studies had reported a decoupling between C and N cycling processes. In a Patagonian arid steppe, the experimental decrease of rainfall water inputs reduced litter mass loss rates, but did not alter soil net N mineralization (Yahdjian, Sala & Austin 2006). In a Hawaiian rainforest, exotic species invasion accelerated litter decomposition, whereas soil N availability and mineralization rates remained similar between invaded and non-invaded plots (Funk 2010). The fact that, in our study, differences in litter C and N turnover among communities were not related to soil environmental conditions, but were mostly accounted for by the litter quality of dominant species, may have contributed to decouple the response of surface litter turnover and topsoil N cycling to our manipulation of community composition (Knops, Bradley & Wedin 2002).

To study ecosystem changes with secondary succession, our approach was to determine how plant communities resembling contrasting seral stages influenced soil function after just a few years of being established under the same initial conditions. We then assessed several key processes at a given point in time across these surrogate community stages. Comparable designs swapping time for space have been used frequently to study community trajectories during succession as well as to infer long-term changes in ecosystem properties (Odum 1969; Knops & Tilman 2000; Quested et al. 2007; Walker *et al.* 2010). There are, however, inherent limitations to space-for-time substitution, because historical legacies generated by stochasticity in the sequence of species replacement may result in different dynamic patterns through space and time (Walker *et al*. 2010). Our synthetic communities mirrored coarse differences in functional group composition across stages of a well-studied, old-field succession (Omacini *et al*. 1995; Tognetti *et al*. 2010). Interestingly, successional

trajectories in functional group abundances are generally more deterministic than changes observed at the species level (Fukami *et al*. 2005). Therefore, despite its limitations, our approach may be promising to study ecosystem changes through succession within realistic time frames. In addition, our experimental design emphasized the importance of plant functional composition in driving ecosystem function, as different functional groups can shed litter of varying quality to decomposers, and may also potentially create distinct micro-environmental conditions. While we recognize that seral stages are more complex ecological entities than just a group of co-occurring plant species, we showed that measuring simple plant chemical traits may help to link shifts in community composition with ecosystem function during succession (see also Kardol & Wardle 2010).

Our findings are relevant from a conservation standpoint. Only 0.3% of the Pampa grasslands are under some protection status (Baldi & Paruelo 2008), so abandoned farmland areas are of special interest as they offer opportunities to restore secondary grasslands. The spontaneous occurrence of exotic species in old fields can strongly alter soil processes as shown here. We suggest that such ecosystem-level changes may be instrumental in promoting the persistence of fast-growing exotic grasses through a positive feedback mechanism, which may hamper the recovery of native species assemblages, as demonstrated for invaded grasslands elsewhere (Suding *et al.* 2004, 2013). Further research is needed to demonstrate this potential feedback mechanism, which may have strong implications for the re-establishment of native species during post-agricultural succession. Restoring native grasslands will help maintain biodiversity at both local and landscape scales, and thus the chances of retaining ecosystem services such as carbon sequestration in soil organic matter.

Authors' Contributions statement

LY and EJC conceived the questions and designed the study; PMT set up the experiment; LY, PMT and EJC collected the data; LY and PMT analyzed the data; LY and EJC led the writing of the manuscript. All authors contributed to successive drafts and gave final approval for publication.

Data Accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.gn818 (Yahdjian et al, 2017)

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Figure Legends

Fig. 1. Percent cover of plant functional groups (forbs, annual grasses, and native and exotic perennial grasses) and realized species richness (S) in the four synthetic community types resembling different stages of old-field succession in the Inland Pampa. Data shown are means ± SE (n = 6). Species cover and richness were estimated at the beginning of the litterbag experiments. Communities were established by supplementing the seed bank of replicated plots in a recently abandoned field with seeds of dominant functional groups from early, mid and late seral stages.

Fig. 2. Litter decomposition rate in the common garden experiment as a function of initial litter C:N ratio for species in the four plant functional groups. Each point shows the mean decay constant (k, year^{-1}) and C:N values (n = 10) for forbs (3 species), annual grasses (2 species), native perennial grasses (2 species), and exotic perennial grasses (4 species); k values estimated from five litterbag collections over 18 months. The regression line shows the best least-squares fit (k = -0.018 C:N + 2.47; r = -0.62, P = 0.04).

Fig. 3. Litter mass loss through time (main panel) and litter decay rates (k, year^{-1}, insets) in the standard litter (a) and home plot (b) decomposition experiments. Litter of (a) *T. aestivum* and (b) dominant species from each community type (forbs: *C. acanthoides*, short-lived grasses: *L. multiflorum*, native perennial grasses: *P. quadrifarium*, and exotic perennial grasses: *S. arundinaceum*) were incubated during 18 months (May 2008–October 2009). Data points are mean values of ash-free organic matter remaining (± SE, n = 6) at each sampling date. Different letters denote significant differences among treatments for the last sampling date (main panels) and for experiment-wide decay rates (insets) at P<0.07 (a) and P<0.05 (b).

Fig. 4. (a) Soil respiration rates measured in the four plant communities resembling different stages of old-field succession. Data points show means ± 1 SE, n = 6, for spring (October-November) and summer-autumn (March-April). Different letters indicate significant differences among communities within dates (P<0.05, Tukey tests). (b) Correlation between soil respiration (g CO_2 m^{-2} $hour^{-1}$) and litter decomposition rates (k, $year^{-1}$) measured in the four plant community types (r = 0.51, P = 0.016, n = 24). Data points represent replicate plots for each community (n = 6).

Fig. 5. (a) Litter nitrogen (N) turnover during decomposition expressed as percent of the initial N content, and (b) change in the C:N ratio of decaying litter for the dominant species in the four community types during the home plot experiment. Data points show means ± 1 SE (n = 6) for each litterbag collection date. Different letters denote significant differences among communities within dates (P<0.05, Tukey tests).
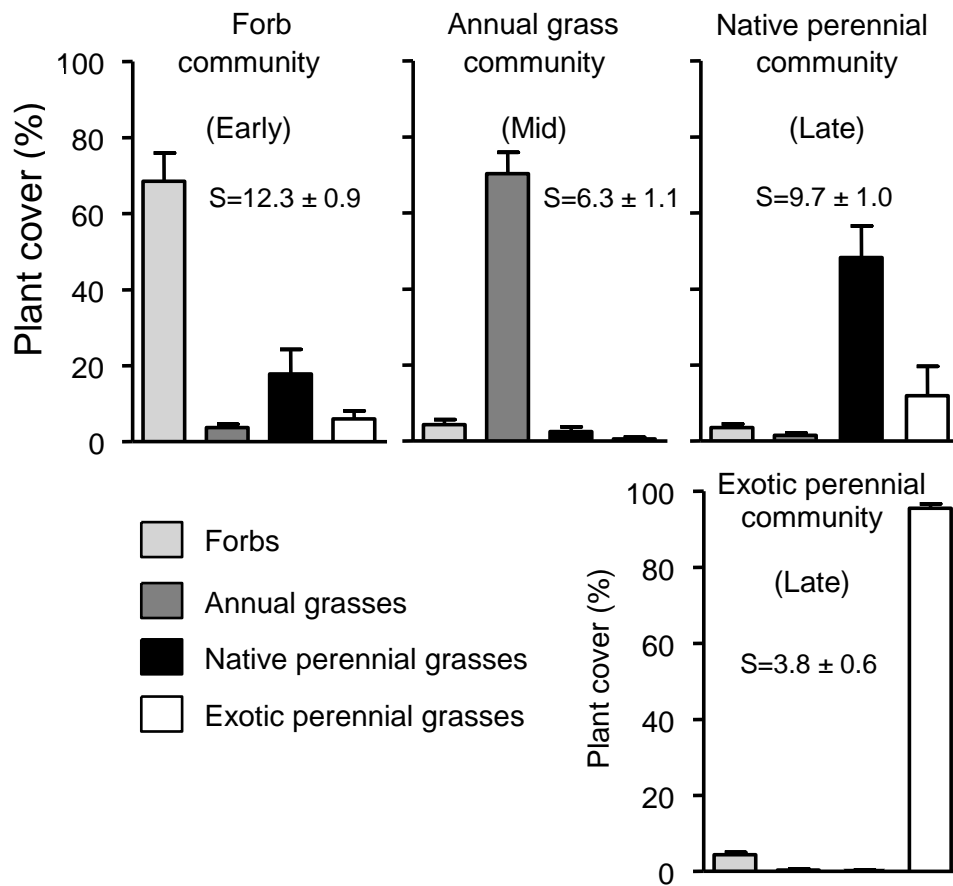
Figure 1

Figure 2

Figure 3

a.

b.

Organic matter remaining (%)

Time (months)

Forb community
Annual grasses community
Native perennial community
Exotic perennial community

Litter decomposition (k, year^{-1})

Forbs   Annual   Native   Exotic
grasses   perennials perennials

Figure 4

a.

b.

Figure 5

a.

Legend:
- Forb community (*C. acanthoides*)
- Annual grasses community (*L. multiflorum*)
- Native perennial community (*P. quadrifarium*)
- Exotic perennial community (*S. arundinacea*)

b.
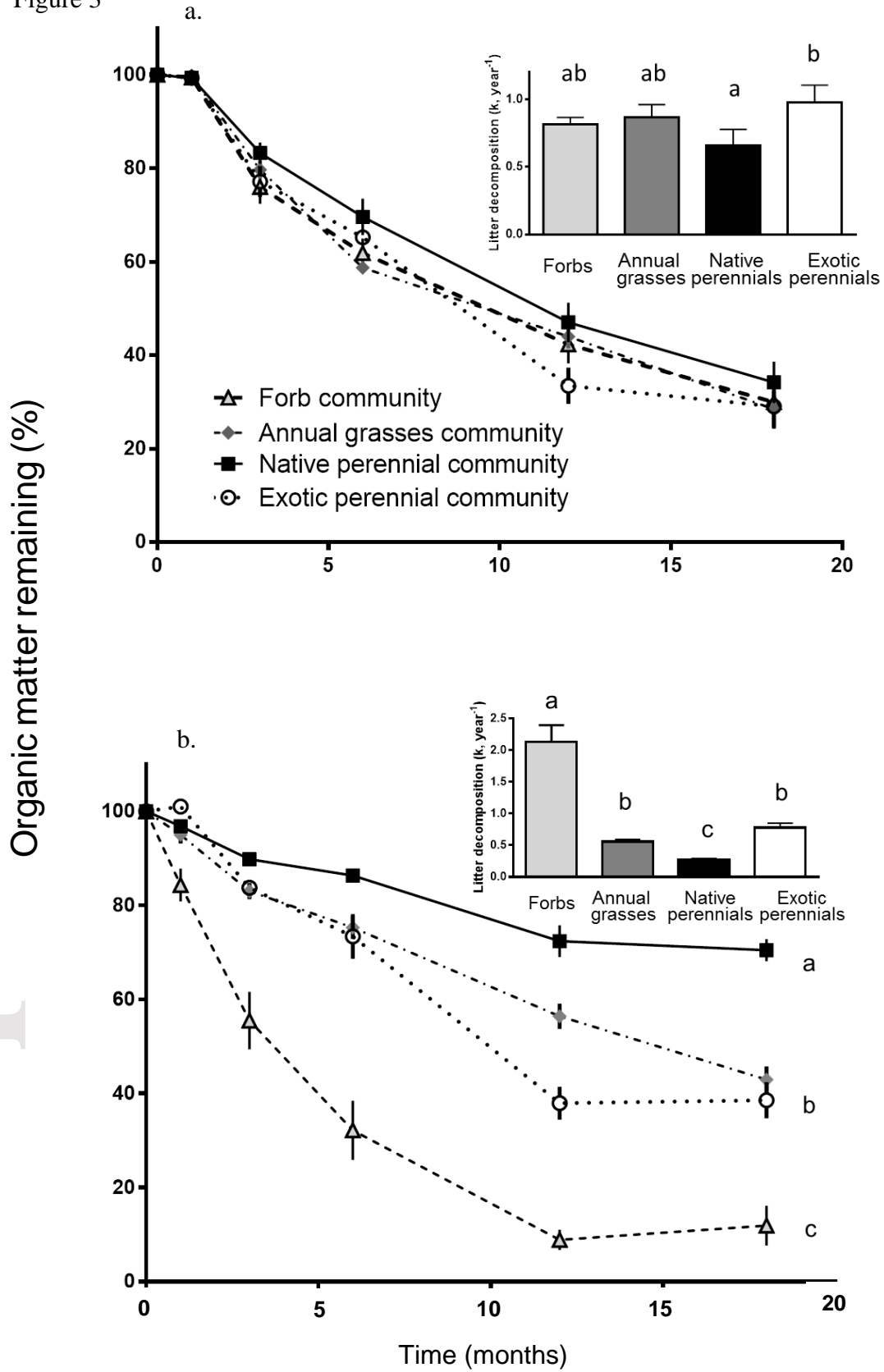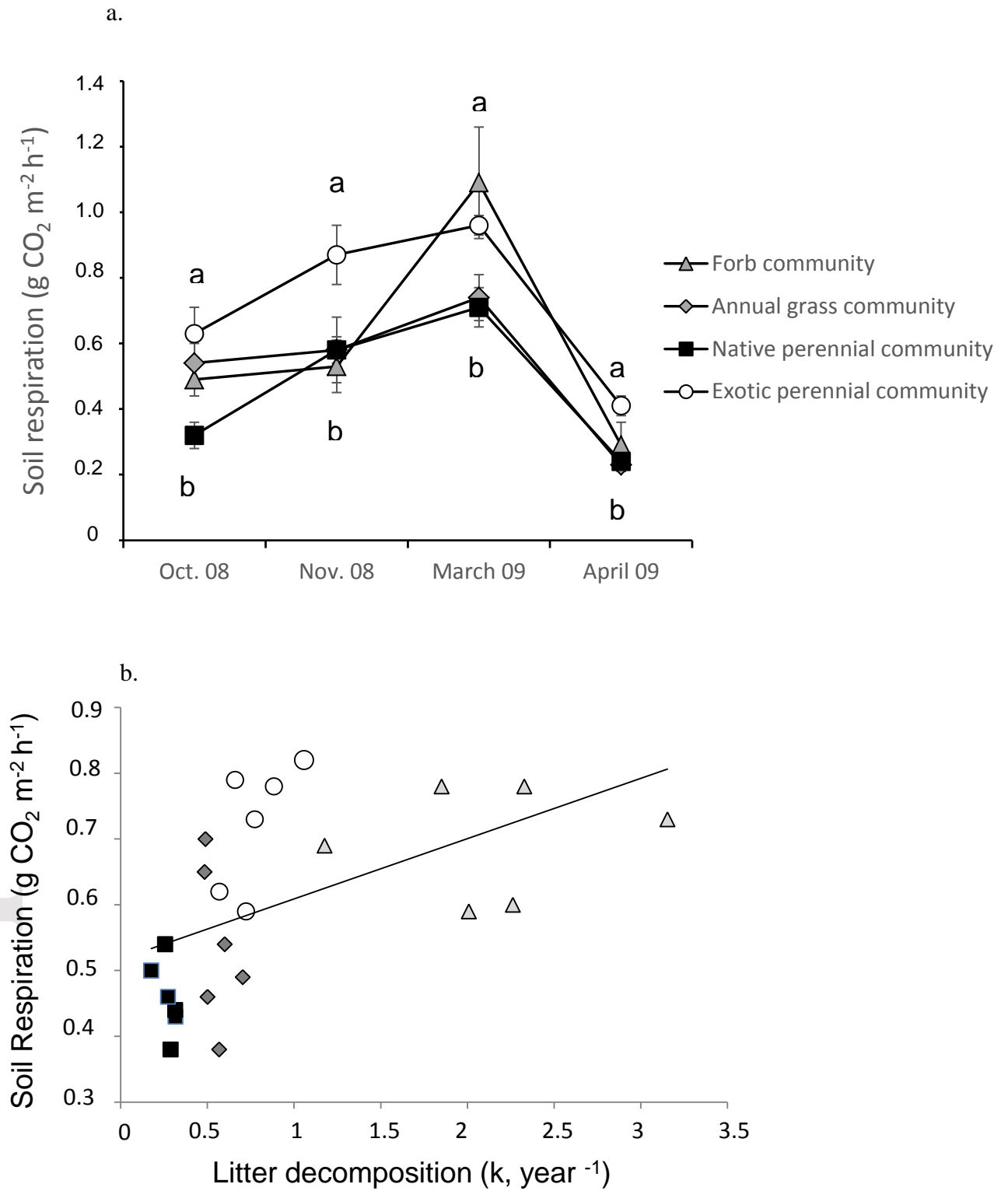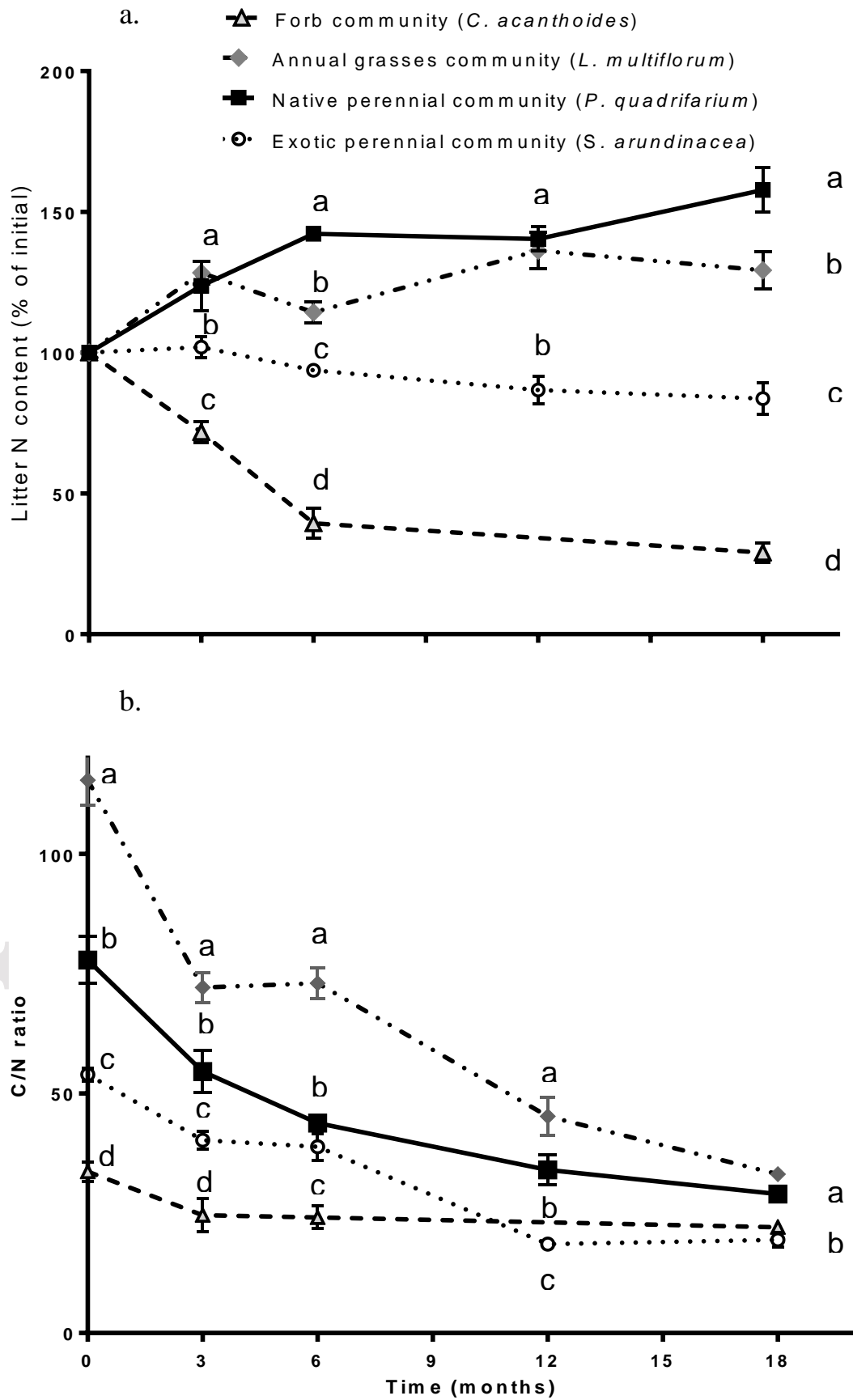
Table1. Initial leaf litter chemistry and decomposition rates (k) for the species used in the home plot and common garden experiments. Species ordered by functional group (FG). Values are means with standard errors in brackets (n = 5 and 10, for litter traits and k values, respectively). Different superscript letters indicate significant differences ($P < 0.05$) between species within columns.

Species	FG	Soluble carbohydrates	Hemi cellulose	Lignin	% C	% N	Lignin:N	C:N	k
Conium maculatum*	F	59.2 [a] (0.51)	31.27 [a] (1.81)	8.01 [a] (0.64)	45.57 [a] (0.14)	1.06[a] (0.02)	7.46 [a] (0.45)	42.88[a] (0.87)	2.68[a] (0.18)
Carduus acanthoides*	F	49.2 [b] (2.96)	41.05 [b] (3.09)	9.74 [a] (0.22)	43.31 [b] (0.27)	1.30[a] (0.08)	7.79 [a] (1.01)	33.66 [b] (2.05)	1.73[b] (0.17)
Rumex crispus*	F	39.14 [c] (1.96)	31.78 [a] (1.02)	29.08 [b] (1.66)	48.25 [a] (0.37)	1.52[a] (0.08)	19.33 [b] (1.76)	31.98 [b] (1.74)	1.48[bc] (0.06)
Bromus catharticus	AG	58.9 [a] (2.37)	35.63 [ab] (2.31)	5.47 [c] (0.47)	40.94 [c] (0.28)	0.99[ab] (0.07)	5.92 [a] (0.77)	42.07[ab] (2.52)	1.63[b] (0.06)
Lolium multiflorum*	AG	49.3 [b] (3.09)	47.58 [c] (2.88)	2.79[d] (0.73)	46.46 [a] (0.18)	0.41[c] (0.02)	6.64 [a] (1.54)	115.34 [d] (5.17)	0.68[f] (0.03)
Paspalum quadrifarium	NPG	36.39 [c] (9.26)	25.06 [d] (7.37)	4.82[c] (0.19)	47.17 [a] (0.22)	0.62[c] (0.04)	8.40 [a] (0.80)	77.83 [d] (4.91)	0.61[f] (0.04)
Schizachirium spicatum	NPG	52.92 [ab] (7.04)	37.62 [b] (4.61)	8.44 [a] (2.13)	42.80 [b] (0.05)	0.69[c] (0.02)	12.08 [c] (3.49)	62.26 [c] (1.85)	1.02[e] (0.05)
Cynodon dactylon*	EPG	57.4 [a] (0.91)	34.42 [ab] (0.69)	8.16 [a] (0.25)	43.00 [b] (0.28)	0.77[c] (0.02)	10.45 [c] (0.57)	55.70[c] (1.52)	1.30[cd] (0.04)
Dactylis glomerata*	EPG	62.05 [a] (0.64)	33.05 [ab] (0.98)	4.56 [c] (0.36)	43.84 [b] (0.39)	1.19[ab] (0.03)	3.77 [d] (0.44)	36.83[ab] (1.05)	1.22[d] (0.06)
Schedonorus arundinaceum*	EPG	54.48 [ab] (2.76)	39.92 [b] (1.82)	5.60 [c] (0.99)	43.05 [b] (0.39)	0.80[b] (0.02)	6.97 [a] (1.37)	53.96 [c] (1.33)	1.10[d] (0.06)
Sorghum halepense*	EPG	51.8 [b] (1.96)	40.48 [b] (2.48)	7.64 [a] (0.56)	45.80 [a] (0.11)	0.91[ab] (0.03)	8.88 [a] (0.89)	50.70[ab] (2.03)	1.12[d] (0.07)
Triticum aestivum*	ST	52.3 [b] (1.41)	42.43 [b] (1.04)	4.43 [c] (0.56)	39.47 [c] (0.36)	0.42[c] (0.01)	13.28 [c] (0.70)	94.31[d] (2.79)	1.53[c] (0.08)

Functional groups are forbs (F), annual grasses (AG), native perennial grasses (NPG), and exotic perennial grasses (EPG). *Triticum aestivum* was used as the standard litter (ST). * Exotic species.

Table 2 Soil micro-environmental properties in the four synthetic communities resembling different stages of old-field succession in the Inland Pampa. Soil mineral nitrogen, water content and daily temperatures were measured in May 2008, October 2008, and April 2009. Net N mineralization was assessed during October–November 2008. Values are means, with standard errors in brackets (n = 6). Different superscript letters indicate significant differences ($P < 0.05$) across sampling dates within communities, except for daily thermal amplitude where letters denote significant differences across communities.

Soil property	Forb community	Annual grass community	Native perennial grass community	Exotic perennial grass community
Net N mineralization (μg N g^{-1} mo^{-1})	0.198 (0.049)	0.044 (0.059)	0.190 (0.141)	0.109 (0.100)
N-NO$_3^-$+NH$_4^+$ (ug/g dry soil)				
May 2008	6.71 [a] (1.35)	5.91[a] (0.29)	5.56[a] (0.44)	7.53[a] (1.06)
Oct 2008	8.93[ab] (0.68)	13.11[b] (1.28)	10.90[b] (1.10)	11.52[b] (0.99)
April 2009	10.75[b] (0.61)	11.17[b] (1.27)	9.26[b] (1.21)	10.97[b] (1.65)
Soil moisture (%)				
May 2008	16,25 [a] (2.86)	17,96 [a] (2.00)	15,39 [a] (1.81)	15,48 [a] (1.54)
Oct 2008	30,70 [b] (2.68)	31,73 [b] (2.42)	29.30 [b] (2.08)	30,63 [b] (2.41)
April 2009	15,32 [a] (1.20)	13,98 [a] (1.39)	15,78 [a] (1.35)	16,45 [a] (1.99)
Soil daily mean temperature (ºC)				
May 2008	7.18 [a] (0.44)	7.60 [a] (0.67)	7.29 [a] (0.39)	7.71[a] (0.61)
Oct 2008	10.67 [b] (0.42)	12.07 [b] (0.47)	10.68 [b] (0.33)	10.79 [b] (0.24)
April 2009	11.94 [b] (0.64)	13.04 [b] (0.37)	12.14 [b] (0.29)	12.49 [b] (0.63)
Soil daily thermal amplitude (ºC)				
May 2008	3.21[a] (0.29)	4.83[b] (0.92)	4.11[ab] (0.29)	3.02[a] (0.47)
Oct 2008	2.92[a] (0.61)	5.21[b] (1.01)	4.44[ab] (1.25)	2.83[a] (0.45)
April 2009	3.71[a] (0.63)	6.96[b] (1.27)	6.33[ab] (1.01)	4.38[a] (0.57)