



# Uncropped field margins to mitigate soil carbon losses in agricultural landscapes



Luciana D'Acunto\*, María Semmartin, Claudio M. Ghersa

IFEVA, Departamento de Recursos Naturales, Facultad de Agronomía, Universidad de Buenos Aires CONICET, Argentina

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## ABSTRACT

Agricultural intensification is a major cause of habitat transformation. Continuous cropping alters ecosystem services, such as biodiversity and carbon sequestration. Empirical evidence from agricultural lands in Argentina has shown that permanently vegetated areas imbedded in the agricultural matrix (uncropped margins) play a critical role in plant and animal communities compared to the usual situation of crops surrounded by other crops (cultivated margins). However, the potential impact of uncropped margins on their own carbon stocks and fluxes and on those of their neighbouring cropped fields remains unknown. We investigated the impact of uncropped (herbaceous and woody) and cropped margins (cultivated fields) on their own topsoil carbon stocks and fluxes and on those of their neighbouring croplands (soybean fields). We identified soybean fields adjacent to one of three possible margin types: herbaceous or woody permanent vegetation, and field crop, which acted as control because it is the most frequent situation in the region. In each of these margin–soybean pairs, we sampled transects from the margin towards the centre of the soybean field (50 m). Woody margins showed the greatest soil carbon content, the least decomposable plant litter and the greatest influence on the neighbouring crop. Conversely, herbaceous margins had the lowest litter accumulation and the most decomposable litter. Only woody margins influenced soil properties in the first metres of the cropped neighbourhood. Centres of soybean fields were similar, irrespective of margin type. The decomposition of common substrates was not affected by margin type. These findings suggest that woody margins are the unique element of the current landscape with a potential to mitigate soil carbon loss from agroecosystems, albeit spatially limited. In contrast, the low biomass and highly decomposable litter of herbaceous margins reveal the urgent need to re-think their current management strategies.

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## 1. Introduction

Agricultural intensification is a major cause of landscape fragmentation and losses of biodiversity and soil carbon. A large number of studies, involving different scales and approaches, confirm the negative effects of agriculture expansion and intensification (Matson et al., 1997; Burel et al., 1998; Benton et al., 2003; Tscharrntke et al., 2005; Norris, 2008). Overall, increasing cultivated area, reducing crop diversity, homogenizing crop management and replacing perennial permanent and semi-permanent habitats for annual crops resulted in a reduction of spatial and temporal landscape heterogeneity (Tscharrntke et al., 2005; Poggio et al., 2010). In temperate grasslands of South America (Soriano, 1991), and particularly in the Rolling Pampa region (Baldi et al., 2006), there mixed farming systems that combined extensive husbandry with annual crops have been largely replaced by continuous cropping.

This replacement reduced landscape heterogeneity and altered the provision of ecosystem services such as biodiversity (de la Fuente et al., 2006; Bilenca et al., 2007) and carbon sequestration (Viglizzo et al., 2011a,b; Caride et al., 2011).

Landscape ecology has provided valuable approaches to understand the impact of agriculture expansion and intensification on ecosystem properties. Permanent or semi-permanent landscape elements imbedded in the agricultural matrix are critical for preserving ecosystem services (Klein et al., 2003; Follain et al., 2007). Corridors of uncropped permanent vegetation (margins) constitute both habitat and refuge for many species (Burel et al., 1998; Marshall and Moonen, 2002; Tscharrntke et al., 2005). They also serve as connectors for metapopulations (Gonzalez et al., 1998) and as barriers that reduce wind speed and soil loss by erosion (Burel et al., 1998; Walter et al., 2003; Brandle et al., 2004; Follain et al., 2007). Moreover, in European landscapes, where corridors represent a large proportion of the landscape (Baudry et al., 2000), hedgerows tend to increase local soil carbon content (Walter et al., 2003; Follain et al., 2007). Nevertheless, the effect of margins on ecosystem functioning will depend on

\* Corresponding author. Tel.: +54 11 4524 8070/71; fax: +54 11 4514 8730.  
E-mail address: [dacunto@agro.uba.ar](mailto:dacunto@agro.uba.ar) (L. D'Acunto).

margin type, target species traits and landscape context (Aviron et al., 2005). The agro-ecosystems of the Rolling Pampa largely differ from the well studied European systems. They form an extensive and homogeneous cropland mosaic made of large arable fields and sparse, less disturbed wire-fencerow networks composed of spontaneous woody patches scattered and herbaceous fencerows that frequently receive intentional or unintentional spraying of total herbicides from the neighbouring soybean crops (Ghersa et al., 2002; de la Fuente et al., 2010). Empirical evidence suggests that, in spite of the large structural and functional differences between the Rolling Pampa and the European agricultural landscapes, Pampean uncropped margins also impact on plant and animal communities. Studies performed at detailed spatial scales revealed more diverse weed communities (Poggio et al., 2010, 2013) and a greater abundance of small mammals (Bilenca et al., 2007) in uncropped margins than in their neighbouring cropped fields. Furthermore, weed richness gradually decreased with distance from the uncropped margins (Poggio et al., 2010), which suggests that margins, despite their limited proportion in the landscape, act as a source of material and information (e.g. propagules), while the cropped matrix acts as a sink.

Carbon cycling in agroecosystems is largely affected by crop management and landscape context. Crop sequence, tillage techniques and fertilization levels regulate the amount of carbon fixed by plants and exported by harvest, and the quantity and quality of the residue incorporated into the soil (Follett, 2001). In turn, agricultural practices indirectly control other carbon cycling sub-processes by modifying soil temperature, moisture and fertility (Knorr et al., 2005; Luo et al., 2010). At the landscape scale, margins may also play a critical role against soil erosion, particularly in semiarid and rolling regions (Okin et al., 2006; Alvarez et al., 2012). Yet in humid and flat landscapes margins may affect carbon cycling. For instance, different vegetation types impact on decomposition and carbon storage through differences in their litter quality among other factors (Liao et al., 2008; Castro et al., 2010). In the case of soil carbon stocks and fluxes, we have no evidence at the landscape scale of the potential impact of low-disturbed margins either on their own carbon stocks and fluxes or on those from their neighbouring cropped areas.

Here we investigated this impact of low disturbed margins. We also investigated the role of decomposition as a mechanism partially responsible for the eventual effects of margins on soil carbon stocks. We identified adjacent pairs of “uncropped herbaceous margin–soybean field”, “uncropped woody margin–soybean field” and “cropped margin–soybean field”, as a control treatment (i.e. crop–crop interface). By setting sampling points along 50 m-long transects, we first sampled carbon and mass stocks in standing vegetation, litter, and topsoil inside the margin system in order to describe the three aforementioned margin types. Second, by sampling along transects from the margin towards the adjacent soybean fields we investigated the effect of each margin type on the neighbouring soybean field. Finally, by means of complementary field and greenhouse decomposition experiments, we investigated the role of decomposition as a critical carbon sub-process that may partially account for variation of carbon stocks among margin types. Our experimental design discriminated among margin effects related to *in situ* microenvironment, and biological and physicochemical features inherent to soil and litter quality. We expect (1) woody margins to have the largest carbon stocks (plant, litter and topsoil) and herbaceous margins to have intermediate values, between woody and cropped margins. The greater carbon accumulation of woody margins would partially result from a slower *in situ* decomposition rate, due to differences in litter quality rather than in the soil environment; (2) woody margins to display the greatest effect on carbon stocks of neighbouring soybean fields, with a decreasing effect as distance from the margin

increases, and herbaceous margins to display intermediate values. Along uncropped margin–soybean field interface, differences in litter quality and soil microenvironmental conditions are expected to vanish as distance from margin increases.

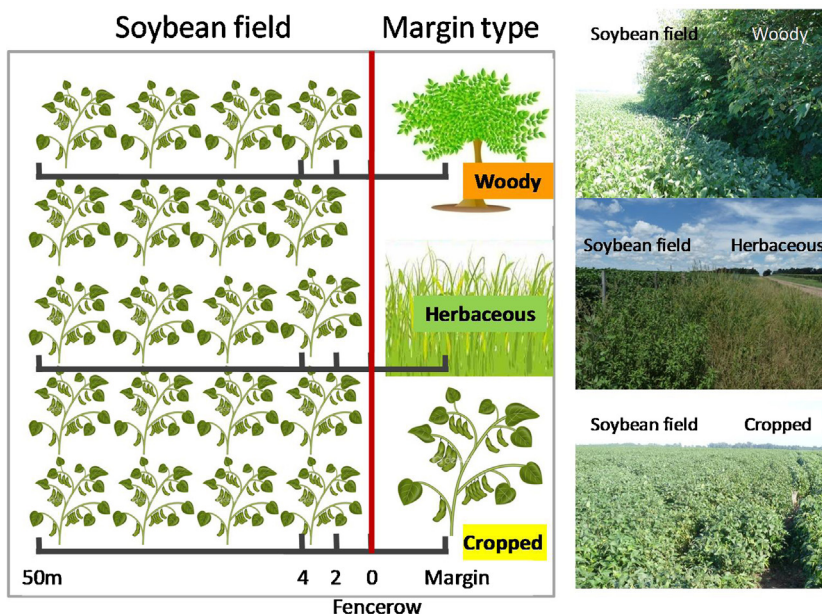
## 2. Materials and methods

### 2.1. Study area

The study was carried out in 2010 in the central Rolling Pampa, which extends from 32° to 34° S and 60° to 61° W in the North of Buenos Aires province, eastern Argentina. Climate is temperate sub-humid, with warm summers and no marked dry season. Mean annual rainfall is 1000 mm and mean annual temperature is 17 °C. The frost period extends from mid-April to late-September. Soils are mainly Argiudolls, characterized by a clay accumulation subsurface horizon (Soriano, 1991). During the expansion of agriculture in 1880–1914, the original grassland vegetation was extensively ploughed and converted into an area of cattle and crop production, which resulted in extensive farmland mosaics fragmented by intricate networks of wire-fencerows, railroads, roads, streams and rivers (Ghersa and León, 1999). Since the 1990s, technology (no-tillage and genetically modified crops), as well as the increased international prices for soybean, led to an intensification of agriculture with the replacement of the mixed cattle and crop systems by continuous cropping. Nowadays, cropping is the dominant land use and has been accompanied by the removal of fencerows to enlarge and simplify the cropped area. Therefore, in the current landscape, native species occur only as small, scattered populations in fragments of semi-natural vegetation in grazing paddocks, wire-fencerows and roadside verges (Rapoport, 1996; Ghersa and León, 1999).

### 2.2. Description of field margins

We identified two representative uncropped margin types: (i) dominated by spontaneous herbaceous vegetation (hereafter herbaceous margin) and (ii) dominated by spontaneous woody vegetation (hereafter woody margin). We also considered fields cultivated with soybean and maize (hereafter cropped margin), representing the most frequent situation, and considered as control situation (Fig. 1). Herbaceous margins were dominated by annual and a few perennial species and they are vegetated most of the year, with lower cover during winter. The most abundant species are grasses (*Cynodon dactylon*, *Digitaria sanguinalis*, *Lolium multiflorum*, *Poa annua* and *Paspalum dilatatum*) and forbs (*Apium leptophyllum*, *Artemisia annua*, *Anthemis cotula*, *Bidens subalternans*, *Capsella bursa-pastoris*, *Chenopodium album*, *Hypochoeris radicata*, *Matricaria chamomilla*, *Portulaca oleracea*, *Silene gallica*, *Tagetes minuta* and *Trifolium repens*). These margins are linear environments, 5–10 m wide. Woody margins cover an average area of 1 ha, are permanently covered and the most abundant tree species of the overstory are *Broussonetia papyrifera*, *Fraxinus* spp., *Gleditsia triacanthos*, *Ligustrum* sp., *Melia azedarach* and *Morus alba*. These margins also have an herbaceous understorey (*Ammi majus*, *Bromus catharticus*, *Chenopodium album* and *Tagetes minuta*), however, unlike the herbaceous margins, woody margins are not directly sprayed with herbicides. They receive drift from the neighbouring crops fields' application at a very low frequency. Cropped margins (represented by crop fields averaging 50 ha in size), cultivated with soybean and maize, are sprayed with systemic and contact insecticides during the spring and harvested in the first half of autumn; then soil remains covered with crop residue until the following crop. They are also sprayed with non-selective herbicides (e.g. glyphosate) to reduce weeding (Ferraro et al., 2003). Our



**Fig. 1.** Diagram of the experimental design and photographs of soybean fields adjoining to one of three possible margin types: herbaceous or woody permanent vegetation and cropped margins (cultivated fields). In each of these margin–soybean pairs we sampled transects from the margin towards the centre of the soybean field (50 m).

sampling took place in regular commercial fields belonging to three farms in the area.

### 2.3. Sampling design and analysis

Within an area of approximately 15,000 ha, we selected 15 soybean fields averaging 50 ha in size. All fields had had maize as previous crop. The fifteen selected fields were surrounded by one of the three field margin types introduced above ( $n=5$ ). In order to describe margins and to investigate their effects along the margin–soybean interface, in each experimental unit (margin–soybean field), we established sampling points along two perpendicular transects from the margin towards the soybean fields. Each transect was randomly located along the field margin (set 20 m apart from each other), avoiding corners, gates, troughs, ditches and any other margin discontinuity (Poggio et al., 2010). We randomly sampled each margin type, whereas points inside soybean fields were sampled at increasing distances from the margin to the soybean field centre: 0 or fencerow, 2 m, 4 m and 50 m. Distances were established based on the assumption that differences in soil carbon properties would be more likely near the margin.

We estimated plant, litter and topsoil (0–15 cm) carbon stocks. Plant biomass from herbaceous margins and from the understory of woody margins was estimated by clipping standing biomass at ground level in ten randomly located frames (0.4 m × 0.4 m). Harvested biomass was oven-dried to constant weight (60 °C for 48 h) and weighed. In woody margins, we also estimated tree biomass in 6 circular plots (14 m diameter) (Bechtold and Zarnoch, 1999). In each plot we measured the diameter at breast height of all the individuals and recorded their species identity. To estimate woody biomass per unit area we used allometric and empirical equations based on the diameter at breast height and individual density (Toky and Bisht, 1993; Dascanio and Barrera, 1994; Jiang et al., 2008; Blujdea et al., 2012). Litter mass was determined by collecting all plant litter lying on soil surface, from six randomly located frames (0.4 m × 0.4 m) in each margin type and in the sampling points along the transects. Finally, following the same spatial design, we sampled soil cores to a depth of 15 cm and measured soil organic carbon, labile carbon and total nitrogen in the topsoil. Total organic carbon and nitrogen were determined by Walkley Black and

Kjeldahl methods respectively. Labile carbon was determined by the density fractionation method (Richter et al., 1975).

### 2.4. Decomposition experiments

We performed two complementary litterbag experiments at field and glasshouse conditions in order to estimate litter decomposition constants, and to isolate the *in situ* conditions (e.g. soil moisture and temperature) from conditions inherent to soil (e.g. carbon and nitrogen contents). For the field experiment, we collected freshly senesced litter from each margin type, and soybean and maize litter that were used as common substrates. In herbaceous and woody margins we collected freshly senesced leaves and stems of each vegetation community, whereas in cropped margins we collected only freshly senesced leaves when the margin was maize and senesced leaves and stems when the margin was soybean. The collected litter mass was cut into pieces of approximately 5 cm long in order to simulate the action of the macrofauna (Seastedt, 1984). We assembled litterbags (15 cm × 15 cm for woody margin litter and 15 cm × 20 cm for all other litters, 2 mm fibreglass mesh) containing 3 g of air-dried litter from each margin and common substrates. Only for woody margins, whose litter was denser, we used 5 g of air-dried litter. Litter of maize and soybean used as common substrates were collected in cropped fields within the study area different from those used as cropped margins.

After soybean harvest (April), four litterbags corresponding to each litter type (soybean, maize and margin) were placed at each sampling point per transect. Prior to placing litterbags, we manually removed all superficial crop residues in order to maximize litter contact with soil. Litterbags were fixed to soil with metal clamps and covered with the removed crop residues. Two litterbags of each litter type were collected from each transect after 30 and 90 days of incubation and analysed for changes in mass over time. Ash-free dry mass was determined to eliminate contamination with soil (Harmon et al., 1999). Mass loss over time was estimated using a single exponential decay model,  $\ln(M_t/M_0) = -kt$ , where  $M_0$  is the initial ash-free dry mass,  $M_t$  is the ash-free dry mass at time  $t$  and  $k$  is the decomposition constant, calculated as the slope of the natural logarithm of remaining mass against time, at 0, 30 and 90 days (Swift et al., 1979). We quantified the loss of material from

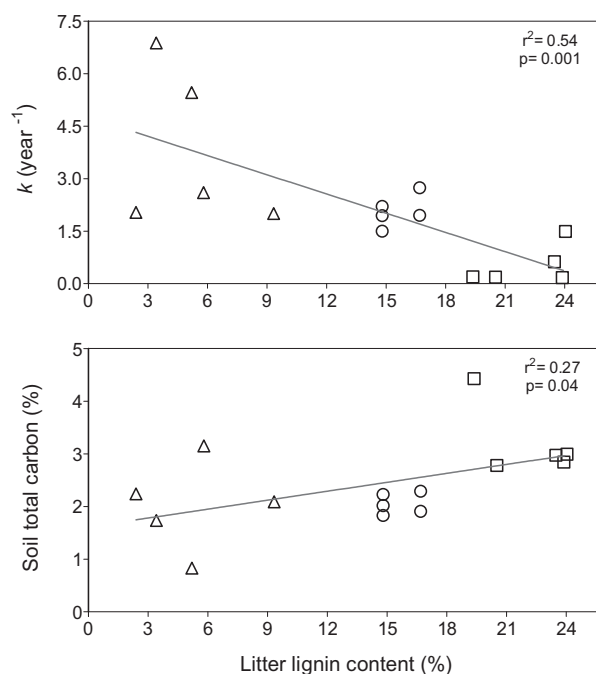
the litterbags due to handling by using 5 additional litterbags of each substrate that received the same procedure as the incubated litterbags but were immediately removed and processed in the laboratory. Then we used this information to correct the decomposition constant estimates.

Factors known to affect the litter decomposition constant, such as litter quality and micro-environmental conditions were also measured. Initial litter carbon and nitrogen content was determined by dry combustion (LECO Corporation) and soluble compounds, hemicelluloses and lignin concentration were determined by successive extractions with acid detergent reactions (Van Soest, 1963; Van Soest et al., 1991). Soil temperature and volumetric moisture was logged during 48 h, after soybean harvest, in each margin type and in the centres of the soybean fields. The logged measurements between 8:00 p.m. and 6:00 a.m. were considered as night. Gravimetric moisture content was also sampled at the beginning of the experiment, at 30 and 90 days.

Finally, and in order to isolate the *in situ* conditions on litter decomposition, mediated by eventual microclimatic differences in the field, from those accumulated on soil properties, we performed a complementary litter decomposition experiment under greenhouse controlled common conditions, where a common substrate was decomposed in microcosms containing soil from the three margin types. Since decomposition constants of soybean and maize, used as common substrate in the field, did not interact with margin type, in this experiment we only included soybean litter. We collected soil from the field experimental units coming from each margin type and placed it in individual trays of 20 cm × 15 cm and 5 cm in height. The design was factorial with three margin types (herbaceous, woody and cropped) and two harvest dates (30 and 90 days after incubation). In each tray we placed a litterbag with 1 g of soybean litter ( $n=5$ ), previously collected from the study site. Litterbags were covered with 1 cm of soil and were softly pressed to enhance soil–litter contact. The experimental units (tray+litterbag) were randomly assigned to a site in the greenhouse and incubated at 25 °C for either 30 or 90 days. Gravimetric water content of soil was maintained constant by adding distilled water throughout the experiment after daily evaluation. Five litterbags were harvested from each treatment after 30 and 90 days of incubation and analysed for mass loss over time as described above for the field experiment. Ash content determination, corrections for losses due to litterbag handling, and decomposition constant estimation were determined as described above.

### 2.5. Statistical analysis

We compared margin types by one-way ANOVAs for plant biomass, litter mass and quality, decomposition constant, soil organic matter, total and labile carbon. When statistical effects were detected ( $p \leq 0.05$ ) means were compared by LSD tests. We also compared the litter decomposition constant ( $k$ ) of maize and soybean (in the field) and of soybean (under greenhouse conditions) and quality of both common standard litters using analyses of variance. Means were compared by LSD tests. We also related litter quality and its decomposition constant with soil variables by linear and non linear regression analysis. We evaluated the effects of margins on the margin–soybean fields interface by linear and non linear regressions for each plant and soil variable evaluated against the distance from the margin. Finally, we analyzed the soil temperature and water content in margin and soybean fields centres by analysis of variance where margin type, time of the day (day or night) and their interactions were the sources of variation.



**Fig. 2.** Relationship between decomposition constant ( $k$ ) (upper panel) and soil total carbon (lower panel) of each margin type (*i.e.* cropped, herbaceous and woody) and initial lignin concentration of litter. Circles, triangles and squares correspond to cropped, herbaceous and woody margins respectively. Data are means and the line is the regression equation.

## 3. Results

Margin type influenced plant, litter and soil carbon stocks. Overall, stocks were largest in woody margins and smallest in herbaceous margins (Table 1). Plant biomass of woody margins was nearly 130-fold and 22-fold larger than herbaceous and cropped margins, respectively. Plant litter of herbaceous margins was half of that in cropped and woody margins. Soil organic matter and carbon were 50% greater in woody margins than in the other margins, whereas the labile fraction showed larger differences.

Margin types modulated the litter decomposition constant by changes in litter quality (Tables 2 and 3). Litter of woody margins decomposed significantly more slowly than the rest and showed the highest carbon and lignin contents. Conversely, litter from herbaceous margins decomposed the fastest and had the lowest lignin concentration. In turn, cropped litter showed intermediate decomposition values. Initial lignin concentration of litter was inversely correlated with litter decomposition constants and accounted for 54% of variation of litter decomposition throughout a 90-day decomposition period ( $p=0.001$ ,  $n=15$ ) (Fig. 2, upper panel). Initial lignin litter concentration was positively correlated with soil total carbon (Fig. 2, lower panel).

Margin type, however, did not influence the decomposition constant of the common standard substrates (maize and soybean) ( $p > 0.1$ , Table 2). In the field, these substrates differed in quality (Table 3) and decomposition constant ( $F_{1,53} = 7.48$ ;  $p = 0.008$ ). However, their decomposition constants did not vary due to margin type (Table 2). In the greenhouse, soybean decomposition constant did not differ among margin types ( $F_{2,12} = 0.83$ ;  $p = 0.4$ ).

Woody margins affected litter and soil carbon stocks of their neighbouring soybean fields (Fig. 3). Soil total carbon and litter mass of woody margin–soybean pairs decreased as the distance from the margin towards the soybean field centre increased. Labile carbon was also lower in soybean fields but its reduction was not

**Table 1**  
Plant, litter and soil carbon properties of the three studied margin types.

|   | Margin type                  |                             |  |
|---|------------------------------|-----------------------------|--|
|   | Cropped                      | Herbaceous                  | Woody  |
| <i>Plant</i>  |                              |                             |  |
| Growth form   | Annual (crops + weeds)       | Annual/perennial grasses    | Deciduous/evergreen woody and herbaceous understorey |
| Standing biomass (g/m <sup>2</sup> ) <sup>***</sup> | 588.32 <sup>b</sup> (207.23) | 100.4 <sup>a</sup> (14.58)  | 13,147 <sup>b</sup> (2751)                           |
| <i>Litter</i>                                       |                              |                             |  |
| Litter mass (g/m <sup>2</sup> ) <sup>**</sup>       | 711.33 <sup>b</sup> (52.28)  | 104.94 <sup>a</sup> (30.64) | 662.76 <sup>b</sup> (70.61)                          |
| <i>Soil</i>   |                              |                             |  |
| Organic matter (%) <sup>*</sup>                     | 3.56 <sup>a</sup> (0.20)     | 3.58 <sup>a</sup> (0.82)    | 5.53 <sup>b</sup> (0.53)                             |
| Total carbon (%) <sup>*</sup>                       | 2.07 <sup>a</sup> (0.11)     | 2.08 <sup>a</sup> (0.48)    | 3.21 <sup>b</sup> (0.31)                             |
| Labile carbon (%) <sup>*</sup>                      | 0.28 <sup>a</sup> (0.04)     | 0.37 <sup>ab</sup> (0.11)   | 0.94 <sup>b</sup> (0.28)                             |

Margins types corresponded to uncropped areas dominated by either herbaceous or woody permanent vegetation, and cropped areas cultivated with soybean or maize. The three margin types were adjoining soybean fields. Data show means with standard error in parentheses ( $n = 5$ ). Different letters indicate significant differences among margin types from an ANOVA test.

<sup>\*</sup>  $p < 0.05$ .

<sup>\*\*</sup>  $p < 0.01$ .

<sup>\*\*\*</sup>  $p < 0.001$ .

**Table 2**  
Litter decomposition constant ( $k$ ).

| $k$ (year <sup>-1</sup> ) | Margin type               |                          |                          |
|---------------------------|---------------------------|--------------------------|--------------------------|
|                           | Cropped                   | Herbaceous               | Woody                    |
| Margin litter             | 2.03 <sup>ab</sup> (0.26) | 3.02 <sup>b</sup> (0.82) | 0.53 <sup>a</sup> (0.25) |
| Common litter             |                           |                          |                          |
| Soybean                   | 1.48 <sup>a</sup> (0.13)  | 1.32 <sup>a</sup> (0.3)  | 1.94 <sup>a</sup> (0.16) |
| Maize                     | 2.01 <sup>a</sup> (0.3)   | 2.02 <sup>a</sup> (0.37) | 2.01 <sup>a</sup> (0.21) |

$k$  of litters from different origins (margins) decomposing in each of the three margin types. 'Margin litter' refers to litter originated in cropped and uncropped herbaceous and woody margins. 'Common litter' refers to maize and soybean substrates used as standards. Data show means with standard error in parentheses ( $n = 5$ ). Different letters indicate significant differences of  $k$  among margin types,  $p < 0.05$ .

as gradual as total carbon and litter (Fig. 3). Conversely, herbaceous margin and cropped margin pairs were similar and did not exhibit a spatial pattern ( $p > 0.05$ , Fig. 3). Although woody margins influenced several soil properties of their cropped neighbourhood, centres of soybean fields were not affected by margin type (Fig. 3).

The decomposition constants of litter from margins consistently denoted the differences of litter quality (Fig. 4) already detected in the margins (Table 2). However, neither litter from margins (Fig. 4) nor common litters used as standard (Fig. 5) were affected by the position along the margin–crop interface.

Soil temperature showed an expected daily pattern, with nights colder than diurnal temperatures (Fig. 6). Nevertheless, we also found an interactive effect between margin type and daily dynamics as woody margins tended to have a lowest thermal amplitude between days and nights ( $p_{\text{margin} \times \text{time}} = 0.09$ , Fig. 6). In soybean field centres, temperature also followed the expected daily pattern and those adjoining woody margins tended to be slightly colder ( $p = 0.07$ ). Soil volumetric moisture was similar among margins

types ( $p = 0.75$ ) and in soybean field centres ( $p = 0.65$ , Fig. 6). The daily pattern of soil moisture did not show significant differences between day and night in margins ( $p = 0.11$ ) nor in the field centres ( $p = 0.53$ , Fig. 6).

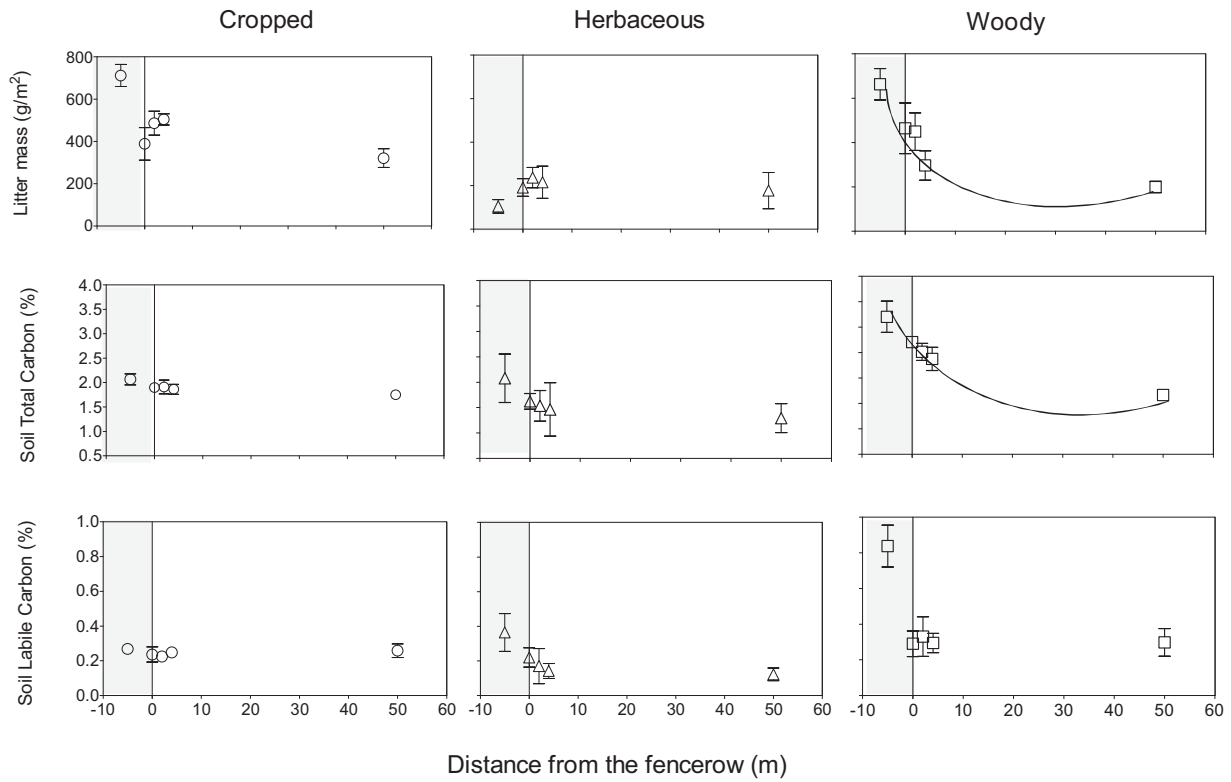
#### 4. Discussion

We showed that uncropped margins imbedded in the agricultural landscape vary in their own plant, litter and soil carbon stocks and modify those of their neighbouring croplands. Overall, margins dominated by woody vegetation combined the greatest plant and soil carbon stocks with the slowest litter decomposition. In contrast, herbaceous margins had reduced carbon stocks with respect to the cropped control situation with a lower standing plant biomass and litter accumulation, and a faster litter decomposition. Woody margins were the only margin type that significantly influenced their neighbouring soybean fields, with effects that exponentially vanished from the margin to the centre of the

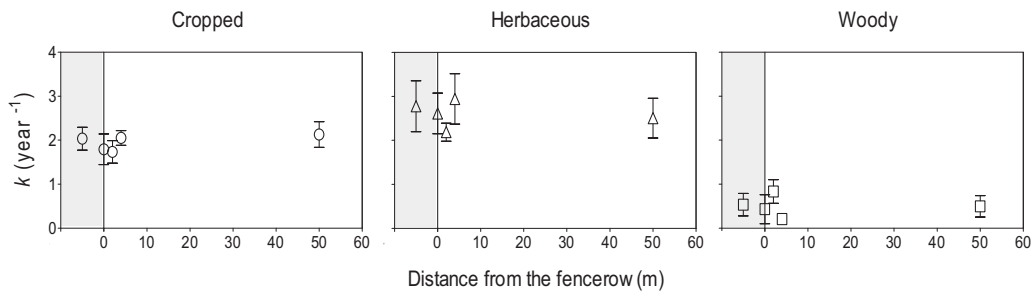
**Table 3**  
Litter quality properties.

|                          | Margin litter              |                            |                            | Common litter             |                            |
|--------------------------|----------------------------|----------------------------|----------------------------|---------------------------|----------------------------|
|                          | Cropped                    | Herbaceous                 | Woody                      | Soybean                   | Maize                      |
| <i>Litter properties</i> |                            |                            |                            |                           |                            |
| Carbon (%)               | 40.83 <sup>ab</sup> (1.08) | 41.28 <sup>b</sup> (0.27)  | 49.03 <sup>d</sup> (0.63)  | 44.08 <sup>c</sup> (0.44) | 39.08 <sup>a</sup> (0.36)  |
| Nitrogen (%)             | 2.11 <sup>c</sup> (0.17)   | 1.93 <sup>bc</sup> (0.08)  | 1.87 <sup>bc</sup> (0.08)  | 1.06 <sup>a</sup> (0.10)  | 1.76 <sup>b</sup> (0.04)   |
| C/N                      | 19.66 <sup>a</sup> (1.13)  | 21.69 <sup>ab</sup> (0.78) | 26.44 <sup>b</sup> (1.32)  | 42.66 <sup>c</sup> (4.59) | 22.29 <sup>ab</sup> (0.59) |
| Lignin (%)               | 15.56 <sup>c</sup> (0.46)  | 5.23 <sup>a</sup> (1.19)   | 22.26 <sup>d</sup> (0.97)  | 10.61 <sup>b</sup> (1.37) | 3.31 <sup>a</sup> (0.36)   |
| Lignin:N                 | 7.64 <sup>b</sup> (0.68)   | 3.03 <sup>a</sup> (0.51)   | 11.94 <sup>c</sup> (0.68)  | 11.10 <sup>c</sup> (2.23) | 1.85 <sup>a</sup> (0.22)   |
| Soluble (%)              | 24.32 <sup>a</sup> (1.28)  | 65.71 <sup>b</sup> (3.10)  | 59.50 <sup>b</sup> (4.76)  | 55.08 <sup>b</sup> (4.55) | 55.74 <sup>b</sup> (1.36)  |
| Hemicellulose (%)        | 16.15 <sup>a</sup> (1.13)  | 30.43 <sup>c</sup> (2.56)  | 17.17 <sup>ab</sup> (2.28) | 13.70 <sup>a</sup> (0.53) | 23.23 <sup>b</sup> (1.36)  |

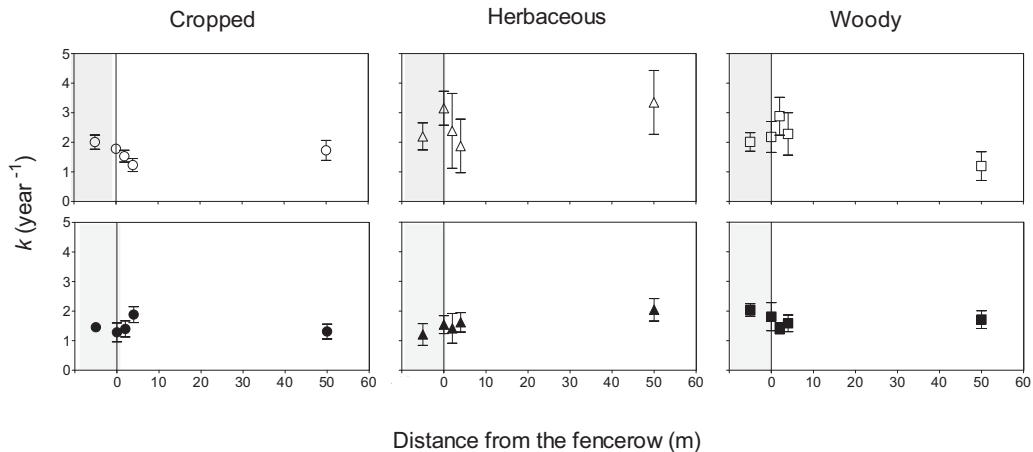
Initial litter properties reflecting litter quality. 'Margin litter' refers to litter originated in cropped and uncropped herbaceous and woody margins. 'Common litter' refers to maize and soybean substrates used as standards. Data show means with standard error in parenthesis ( $n = 3$ ). Different letters indicate significant differences among margin types from an ANOVA test ( $p < 0.001$ ).



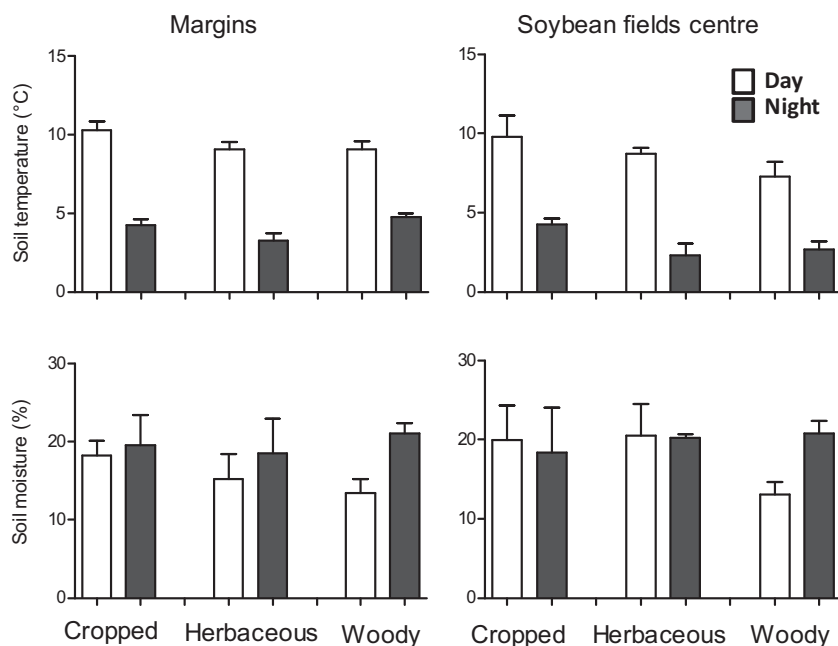
**Fig. 3.** Litter mass, soil total and labile carbon contents across the margin–soybean field interface for three different margin types (cropped, herbaceous and woody). Zero on the x-axis corresponds to the intersection zone (fencerow) between different vegetation fragments, and left (shaded) and right areas correspond to outside and inside soybean fields respectively. Data are means ( $n=5$ ), and error bars represent  $\pm 1$  standard error. An exponential function of the form  $y = \alpha * e(\beta * x)$ , where  $y$ =litter mass, soil total carbon or soil labile carbon,  $\alpha$  and  $\beta$  are fitted constant and  $x$ =distance, was applied to the data.



**Fig. 4.** Decomposition constant ( $k$ ) of litter of each margin type (cropped, herbaceous and woody) in their corresponding margins (left shaded area) and in the soybean fields (right area) at increasing distances from fencerow. Zero on the x-axis corresponds to the intersection zone between different vegetation fragments. Data are means ( $n=5$ ), and error bars indicate  $\pm 1$  standard error.



**Fig. 5.** Decomposition constant ( $k$ ) of two standard substrates: maize (top panels) and soybean (bottom) across the margin–soybean field interface for three different margin types (cropped, herbaceous and woody). Zero on the x-axis corresponds to the intersection zone between different vegetation fragments, and left shaded and right areas correspond to outside and inside soybean fields respectively. Data are means ( $n=5$ ), and error bars indicate  $\pm 1$  standard error.



**Fig. 6.** Soil temperature and volumetric moisture of topsoil in each margin type (cropped, herbaceous and woody) (left panels), and in centre of each soybean field adjoining to a different margin type (right panels). Vertical bars indicate  $\pm 1$  standard error ( $n = 3$ ).

neighbouring cropped field. Differences of litter decomposition among margin types were exclusively related to litter quality, as decomposition of common standard litters was not affected by margin type or by the position along the margin–soybean field interface. In conclusion, our findings suggest that woody margins represent the single current landscape element with an effective, although spatially limited, potential to mitigate soil carbon losses in this intensively managed agricultural landscape. Furthermore, this role is not only given by differences of carbon gains and stocking in woody structures, but also by the lower losses during litter decomposition. Conversely, the low production of highly decomposable litter of herbaceous margins reveals the urgent need to re-think current management strategies of these landscape elements to improve their role in carbon sequestration.

#### 4.1. Uncropped woody and herbaceous margins

Margins dominated by woody vegetation played a critical role for local soil carbon sequestration in our Rolling Pampa croplands. These results are important because of two major reasons. On the one hand, they provide a more precise estimate of the upper limit to soil carbon accumulation of these soils, revealing that this value is about 55% greater than croplands. This limit seems to be larger than those reported by recent studies that have indicated 20–35% soil carbon reductions due to cropping. We believe that previous evidence underestimated soil potential to store carbon as they contrasted croplands with long term pastures or relict grassland patches instead of woodlands (Álvarez et al., 2009; Caride et al., 2011). Therefore, our results demonstrate that this new, human induced element of the Rolling Pampa landscape, which is spontaneously spreading (Ghersa and León, 1999; Ghersa et al., 2002), enhances soil carbon stocks even with respect to the undisturbed climatic grassland vegetation. On the other hand, these results are of particular interest because the invasion of European tree species (e.g. *Gleditsia triacanthos*, *Melica alba*), seems to have reached a point of no spontaneous return to a grassland state (Ghersa et al., 2002). The woody encroachment of these ecosystems, as a natural process or as commercial plantations, dramatically alters water dynamics, because of a greater

evapotranspiration and increases the risk of soil salinization (Jobbágy et al., 2008). Instead, its impact on carbon balance seems to be more difficult to predict. A recent analysis showed a gradient from negative to positive effects depending on factors such as the age of the woody patch among others (Eclesia et al., 2012). Ultimately, the assessment of the inclusion of woody margins as part of a landscape design oriented to developing sustainable agro-ecosystems should consider all the relevant dimensions regarding ecosystem services other than goods provision. In this context, our results suggest that, in terms of soil carbon storage, woody margins might constitute local spots of carbon sequestration embedded in a cropped matrix with a negative soil carbon balance.

Plant and soil carbon pools of margins dominated by spontaneous herbaceous vegetation were lower than expected. While we expected lower pools than in woody margins, standing plant biomass and litter of the herbaceous margins were even lower than the control cropped situation. The combination of  $C_3$  and  $C_4$  plant species in the herbaceous margins, which assures a year round production (Semmartin et al., 2007), suggests that potential primary production of these communities should be greater than that of croplands, which have shorter periods of high plant cover. Nevertheless, the management of these margins likely counteracts this potentially greater carbon fixation capacity. First, the herbaceous margins frequently receive intentional total herbicide application (de la Fuente et al., 2010) with the purpose of controlling the high weed abundance and diversity of these habitats (Poggio et al., 2010, 2013). Therefore, herbicide reduces the potential plant cover of soil. Second, crops receive external nutrient addition by fertilization, which increases their primary productivity. In other words, current agricultural practices impair herbaceous margins by reducing their potential to fix carbon in such a way that reductions in soil carbon are also expected (Caride et al., 2011). A reduction on herbicide use will allow perennial vegetation dominate and subsequently increase litter and soil carbon accumulation. Moreover, maintaining the fencerow networks (with herbaceous vegetation) in the landscape is critical for sustaining agroecosystem biodiversity (Poggio et al., 2010).

#### 4.2. Woody margins influence on neighbouring soybean fields

Woody margins also displayed a spatially limited, but significant, influence on litter and soil total carbon stocks of their neighbouring soybean fields. Our results showed that the influence of woody margins along their interface with soybean fields decreased as the distance from the fencerow increased. These results were expected since the interface area is subjected to the same disturbance regime of agricultural activities as the field centres, but they are also physically and biologically influenced by their uncropped margins (Poggio et al., 2010). Studies in Europe have also documented the influence of the surrounding landscape on the diversity and abundance of different organisms inhabiting the crop fields (Aviron et al., 2005). In relation with soil carbon stocks, a few studies revealed areas of greater soil carbon accumulation as the imprint of ancient hedgerows (built c.a. 800 AC), currently absent in the landscape (Walter et al., 2003; Follain et al., 2007). Moreover, studies in agroforestry have shown the positive correlation between tree presence and soil organic carbon stocks up to distances of 8 m from the trees (Simón et al., 2013). Nevertheless, most of the empirical evidence comes from ecosystems with different relevant landscape features, as ratios between cropped and uncropped areas, which in Western Europe are, by far, lower than those of the Rolling Pampas (Baudry et al., 2000; Aviron et al., 2005; Poggio et al., 2010). Our results highlight how spontaneous and uncultivated margins have also an effect on matter transfer (carbon) from neighbouring margins to their adjacent cropped areas in Rolling Pampa. This indicates that the conservation of woody vegetation patches can have an influence beyond their limits, even in landscapes where these elements constitute a minority group. Therefore, the management effort should be oriented to strengthen the interaction between croplands and uncropped margins (Tscharntke et al., 2005). Herbaceous margins did not influence their neighbouring cropped fields, which suggests that the reductions in plant richness from uncropped herbaceous margins to their adjacent cropped fields documented in these ecosystems (Poggio et al., 2010) are probably not strong enough to result into greater soil carbon stocks.

#### 4.3. Uncropped margin effects on litter decomposition

Margin type effects on soil carbon were mediated by differences in the litter quality and the consequent variation in litter mass dynamics during decomposition. Margin type did not significantly affect litter decomposition of common litters by changes in the micro-site environment. As hypothesized, it appears that the control of the margin type on decomposition, mediated by changes in litter quality, is stronger than the effects mediated by changes in the soil environment. The two uncropped margin types here studied displayed the greatest variation of litter quality and decomposability, as woody margins showed the most recalcitrant and less decomposable litter and the herbaceous ones showed the opposite pattern. The decomposition of common substrates, used to isolate effects of litter quality from the environmental ones, was insensitive to margin type. Our results showed that only one litter trait, lignin initial content, accounted for 54% of the variation in litter decomposition constants. The negative significant correlations between lignin litter content and litter decomposition constants and soil carbon that we found suggest that woody margins significantly affected soil carbon accumulation through the recalcitrancy of their litter. On the other hand, although margins included large differences of soil and plant properties, as well as soil microbial composition and diversity (D'Acunto, unpublished data), differences in soil temperature and moisture were not significant. The lack of effects of the environment over litter decomposition was expected, since climate (temperature and moisture) is one of

the controls on litter decomposition through microbial activity at a large scale (Dyer et al., 1990). However, Zhang et al. (2008) showed that the combination of climatic conditions and litter quality controls the litter decomposition process, accounting for 87.5% of the variation in litter decomposition constants. It seems that only in regions where water is the limiting factor (deserts or semi-arid regions) water availability could become the dominant influence of litter decomposition (Coureaux et al., 1995). At the patch scale, our results agree with the notion that litter quality (i.e. total nutrient and C:N ratio) and decomposer community are the strongest control of litter decomposition (Wardle et al., 2003).

### 5. Conclusion

Here we showed that uncropped, permanently vegetated margins imbedded in the agricultural landscape vary in their own plant, litter and soil carbon stocks and modify those of their neighbouring croplands. Our results showed that even in areas where this low disturbed habitats represent a minor fraction of the landscape (Ghersa et al., 2002), understanding the factors that control carbon cycling at the landscape level is particularly important. Both crop and non-crop elements with positive and negative carbon balances ought to be combined in a way that will mitigate climatic change by sequestering carbon in plant and soil pools.

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