Grassland connectivity explains entomophilous plant species assemblages in an agricultural landscape of the Pampa Region, Argentina

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Abstract   The Pampa grassland of Argentina is one of the most highly threatened biomes in the world. A high proportion of the original grassland cover has been transformed into land for agriculture or degraded. In the southern part of the region, fragmented semi-natural grasslands over exposed rock still persist and connectivity between them is assumed to be crucial for maintaining viable populations. We quantified overall connectivity of grassland patches in a sector of the Southern Pampa region, and investigated the degree to which landscape connectivity explains entomophilous plant species assemblages in a subset of patches. We characterized each of the 301 patches in the landscape by their degree of intra-patch and inter-patch connectivity based on graph theory, and considering threshold dispersal distances from 100 to 1000 m. We surveyed entomophilous plant species in 39 grassland patches and classified the species in three categories (annual herbs, perennial herbs and shrubs) considering their different growth form and longevity. The influence of connectivity variables on entomophilous plant species assemblages variation was explored using Canonical Correspondence Analysis. Although grassland patches were poorly connected at all threshold distances, some of them were found to be critical for global connectivity. Connectivity significantly explained total, annual-biennial and shrub assemblages for all threshold dispersal distances (6–13% of total variation). Variation in annual species assemblages was associated with intra-patch and inter-patch connectivity at short distance (100 m), while variation in shrub species assemblages was explained by intra-patch and inter-patch connectivity for distances between 100 m and 1000 m. This study evidenced the low connectivity of the study system, allowed the identification of critical areas for conservation, and provided valuable information to develop management strategies in increasingly human-dominated landscapes.

Key words: assemblage-level connectivity, canonical correspondence analysis, conservation planning, habitat patch networks, Tandilia System.

INTRODUCTION

In current rural landscapes, the expansion of agriculture over natural ecosystems has been one of the main drivers of habitat and species loss (Saunders *et al.* 1991; Hooper *et al.* 2005). Theories and models have been developed to understand how present landscape structure affects distribution patterns of plant and animal species (Macarthur & Wilson 1967; Forman 1995; Farhig 2003; Fahrig *et al.* 2011). In the last two decades, a considerable number of studies have applied those theories and models to different taxa and landscapes (McGarigal & McComb

1995; Metzger 1997; Cagnolo *et al.* 2006; Sabatino *et al.* 2010; Herrera & Laterra 2011; Saura *et al.* 2011). In particular, patch size and connectivity are the most widely used indicators of landscape health (Forman 1995; Farhig 2003). Connectivity is the degree to which the landscape facilitates or impedes the movement of organisms and other ecological fluxes among resource patches (Taylor *et al.* 1993). Connectivity is crucial for biodiversity conservation since it determines animal or plant dispersal at the landscape level, and hence it is tightly related to the chances of population survival in heterogeneous landscapes (Burel & Baudry 2005). Connectivity depends not only on the characteristics of the landscape, but also on the movement abilities of the organism as well as the permeability of the matrix (Tischendorf &

Fahrig 2000; Rey Benayas *et al.* 2008; Manning *et al.* 2009). Graph-theoretic approaches are among the most widely used and advocated methods for analysing landscape connectivity (Pascual-Hortal & Saura 2006; Urban *et al.* 2009; Galpern *et al.* 2011). Their simplicity and flexibility offer much to land practitioners, such as the opportunity to make decisions based on which patches are most critical to uphold landscape connectivity, allowing the scope and effectiveness of resource management to be increased (Urban *et al.* 2009). Studies on connectivity using graph theory approaches have focused mainly on forest ecosystems (Pascual-Hortal & Saura 2008; Shanthala Devi *et al.* 2013; Hernández *et al.* 2014), but to a lesser extent on grasslands, and they have only rarely been carried out for plant species.

Maintaining plant species diversity in intensively managed (and frequently fragmented) systems requires the provision of propagules from external sources (Bakker & Berendse 1999; Blomqvist *et al.* 2003). Seed dispersal is recognized as a key ecological process on community assemblages (Kadoya 2009), and can occur through a variety of vectors such as wind (anemochory), water (hidrochory), plant's own means (autochory) and animals (ectozoochory and endozoochory) (Vittoz & Engler 2007). However, seed dispersal processes are very complex and related not only to plant species traits such as diaspore size, seed dispersal attributes (e.g. awns, wings), seed bank persistence and plant longevity (annuals, perennial with and without clonal ability) (Lindborg 2007; Lindborg *et al.* 2011; Marini *et al.* 2012), but also to landscape composition and configuration (Soons *et al.* 2005). For example, since small seeded species are better dispersers (Kiviniemi & Eriksson 1999) and species with large seeds are better recruiters (Geritz 1995), plants with large seeds would be more sensitive to isolation than small-seeded plants (Soons *et al.* 2005). According to species-specific life-history traits, authors such as Kolb and Diekmann (2004) have demonstrated that short-lived and non-clonal plants are less sensitive to area and/or habitat loss. These species have better dispersal capacities and lower competitive abilities, responding quickly to unsuitable conditions like declining habitat area and changed habitat quality (Lindborg *et al.* 2011). On the other hand, long-lived and clonal plants have lower ability to respond to isolation because of dispersal limitation between patches (Holt 1992).

Entomophilous plant species that inhabit non-cropped habitats in rural landscapes are of particular importance since besides pollinators, insects visiting these groups of species may perform other ecological functions, such as pest population control and crop herbivore regulation (Torretta & Poggio 2013; Bischoff *et al.* 2016). Several studies showed that fruit and/or seed output of most pollinator-dependent crops are favoured by feral honey bees and wild bees that depend on resources provided by these remnant semi-natural habitats (Kremen *et al.* 2002; Chacoff & Aizen 2006). Furthermore, the distribution and abundance of entomophilous plant species in an agricultural landscape can affect the diversity of insects and increase the ecological fitness of pollinator populations through enhanced larval and adult nutrition, and this also provides secondary benefits to the farm and the surrounding landscape (Wratten *et al.* 2012; Sáez *et al.* 2014). However, insects and their function in rural landscapes are also affected by landscape transformation in general and connectivity of semi-natural ecosystems in particular (Winfree *et al.* 2009).

The Southern Pampa region of Argentina constitutes one of the most intensively- and extensively-used agricultural landscapes in South America. Within this region, the Tandilia System forms a mountain range that extends within an area of approximately 1 400 000 ha. It is a discontinuous elevation in the Pampa plain characterized by ancient (lower Paleozoic) eroded hills (sierras) and small rocky outcrops (usually called cerrilladas). Native grasslands and shrublands still persist in sierras and cerrilladas because of steep slopes, shallow soils and exposed bedrock, which do not allow tillage to be used (Herrera & Laterra 2011). Sierras and cerrilladas (hereafter 'grassland patches') are surrounded by a matrix of annual crops and represent important hotspots of biodiversity (Sabatino *et al.* 2010; Herrera & Laterra 2011; Kristensen *et al.* 2014; Gilarranz *et al.* 2015), and a source of ecosystem services (Barral & Maceira 2012). Grassland patches have elements of the temperate grassland biome, which is globally significant for its endangered biodiversity and importance to agricultural production (Sala *et al.* 2000; Herrera *et al.* 2014), and was recently reported to be the biome with the lowest biodiversity integrity in the world (Newbold *et al.* 2016). However, efforts for their conservation and restoration are still incipient because of the general lack of knowledge about their structure and function (but see Aizen *et al.* 2016; Herrera & Laterra 2011; Herrera *et al.* 2013), and the difficulty in including efforts for their conservation into public and private environmental agendas (Logsdon & Chaubey 2013). Research studies in this direction are crucial, especially because these ecosystems are immersed in a region of consolidated agriculture and they are under pressure from different threats (i.e. agriculture, exotic species invasion, overgrazing, among others) (Barral & Maceira 2012). In particular, these semi-natural habitats hold a great diversity of entomophilous plants species (Sabatino *et al.* 2010; Aizen *et al.* 2012; Gilarranz *et al.* 2015), which are important for maintaining crop pollinators and biological controls in these landscapes.

Here, we (i) quantify overall connectivity of grassland patches in a sector of the Tandilia System using

graph-theoretic approaches; (ii) determine the importance of individual grassland patches for maintaining overall connectivity, and (iii) investigate the degree to which landscape connectivity explains entomophilous plant species assemblages in a subset of grasslands patches. We studied the response of entomophilous plant species assemblages to connectivity by separately considering three groups of species according to growth form (herbs and shrubs) and longevity (annuals-biennials, perennials) (Appendix S1). These three groups of species have different traits related to dispersal and persistence in fragmented landscapes (Holt 1992; Soons *et al.* 2005; Lindborg *et al.* 2011). We attempted to provide insights into the role of connectivity in the distribution patterns of entomophilous plant species from the perspective of landscape ecology, and contribute with information about which grassland patches are more important for conservation or restoration according to their role in maintaining connectivity of the global landscape.

MATERIALS AND METHODS

Study area

The study area is located in the Southern Pampa region of Argentina within the Tandilia System (38°01′–36°54′S, 60°14′–57°32′W; Fig. 1). Grasslands are dispersed in an anthropogenic matrix dominated by annual crops such as soybean, sunflower, wheat, corn and potato and perennial pastures. Vegetation consists of grasslands dominated by species of the genera *Nasella*, *Piptochaetium*, *Bromus*, *Aristida*, *Briza*, *Setaria*, among many others; pajonales of *Paspalum quadrifarium*; and shrublands of *Eupatorium* spp., *Colletia spp.*, and *Bacharis* spp., among others (Frangi 1975; Soriano *et al.* 1991; Valicenti *et al.* 2010). The climate of the region is sub-humid–humid mesothermal with no or a small water deficiency, with a noticeable seasonal variation in temperature, and with a short cold period. Mean annual precipitation is 800 mm (Burgos & Vidal 1951). Soils are typical Argiudoll and Hapludoll developed from loessic deposits over cuarcitic rocks (INTA 1991).

Defining grassland patches

Grassland patches in the study area were delineated from Google Earth images with dates corresponding to the vegetation survey period (2002–2009, see below). By grassland patch we mean any area of sierras and cerrilladas that, due to the steep slopes, shallow soils, and exposed bedrock, has not been transformed into land for agriculture, plantation forestry or mining. The resulting grassland patch layer contained 301 patches ranging in area from 1 ha to 7000 ha, and with a total area of 53 400 ha (Fig. 1).

Sampling of grasslands, data collection and characterization

A total of 39 selected grassland patches were surveyed during the flowering season of 2001/2002 (19 patches) and 2007/2009 (20 patches). None of these patches were sown with exotic forage species, or presented symptoms of recent
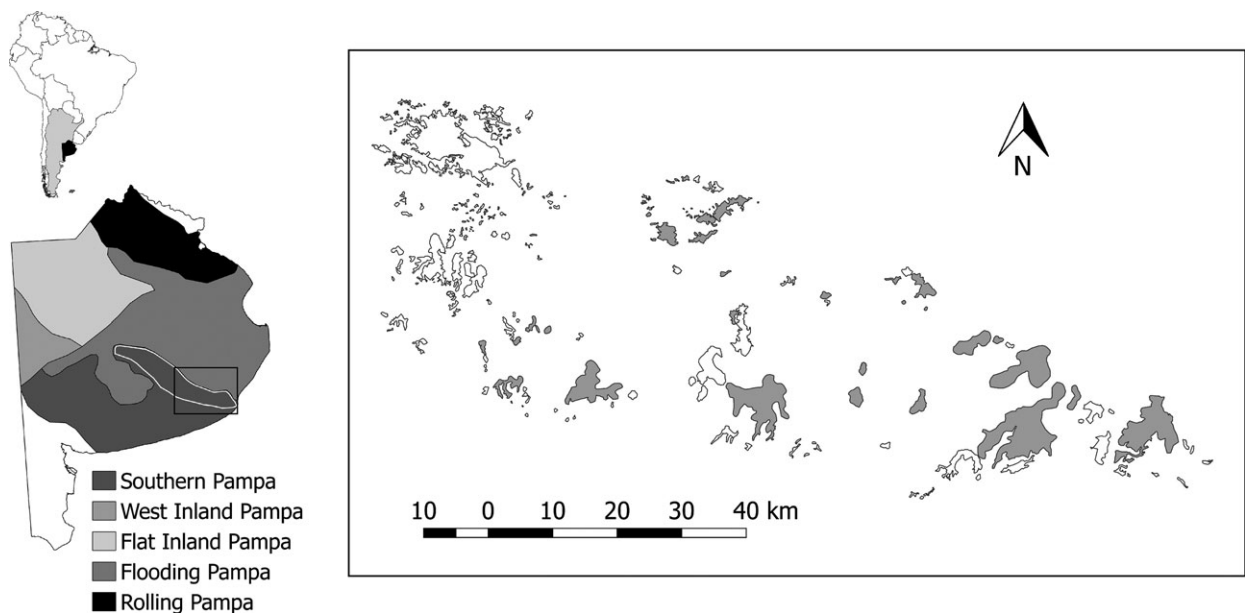

Fig. 1. The figure on the left shows the location of the Pampa sub-regions in Buenos Aires Province, Argentina. Within the Southern Pampa sub-region, the white outline shows the Tandilia System. The figure on the right shows the distribution of grassland patches in the study area. Grey filled patches are those in which entomophiles plant species were sampled.

application of herbicide, and/or subjected to high cattle stocking rates. These patches ranged in size from 3 to 4200 ha (Fig. 1), and their distances from the nearest grassland patch ranged between 0.04 and 7.6 km. Vegetation data (list of species) of the years 2001/2002 were obtained by a modified-Whittaker sampling plot of 20 m × 50 m (0.1 ha) (Stohlgren *et al.* 1999; for more detail see Herrera & Laterra 2011); while vegetation data of the years 2007/2009 were obtained by sampling along two parallel 100 m transects within an area of 0.5 ha. Species were classified according to their growth form (Vázquez-Sánchez *et al.* 2012) and longevity in three combined groups: annual-biennial herbs, perennial herbs and shrubs (Appendix S1).

Quantifying overall connectivity

A graph is a model in which the landscape is represented by a network of spatially explicit *nodes* corresponding to habitat patches (here grassland patches), connected by *links* representing the capability of focal species to disperse between two patches (Urban & Keitt 2001). We used the Integral Index of Connectivity (IIC), which has a set of desirable properties for evaluating landscape-level connectivity and the contribution of individual habitat patches to connectivity (Pascual-Hortal & Saura 2006). The IIC ranges from 0 to 1, increasing with improved connectivity (Pascual-Hortal & Saura 2006), and is calculated as:

$$IIC = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} \frac{a_i \times a_j}{1 + nl_{ij}}}{A_L^2} \qquad (1)$$

where n is the total number of nodes in the landscape, a_i and a_j are the attributes of nodes i and j (they may correspond to any quantitative characteristic of the node that may be relevant; in this study, we used patch area as the attribute), nl_{ij} is the number of links in the shortest path between patches i and j, and A_L is the maximum landscape attribute, in this case the extent of the study area.

The Equivalent Connected Area (ECA) is defined as the size of a single habitat patch (maximally connected) that would provide the same IIC value (same habitat reachability) as the actual habitat pattern in the landscape (Saura *et al.* 2011). ECA is calculated as the square root of the numerator of the IIC index. ECA can never be smaller than the size of the largest patch in the landscape and will never be above the total habitat area in the landscape. Here, we summarized the relative level of grassland connectivity in the study area as the ratio between ECA and the total area covered by grassland patches. We also used the number of components (NC) as another measure of global connectivity. A component is a group of connected nodes. This means that an organism inhabiting any node within the component can potentially move or disperse to any other node in the same component, while by definition, two patches are isolated from each other if they belong to different components. More connected landscapes consist of one big component in which all the habitat patches are connected (Saura & Pascual-Hortal 2007).

As landscape connectivity depends on the dispersal abilities of specific species, the same landscape has different levels of connectivity for different species (Crooks & Sanjayan 2006; Fourie *et al.* 2015). As a consequence, and due to the lack of reliable detailed information available on the seed dispersal distances of plant species in the study area, we selected three threshold dispersal distances (100, 500 and 1000 m) that cover a wide range of responses of seed dispersal of plant species to the landscape pattern (Thomson *et al.* 2011). Species with large dispersal capacities (such as annual herbs) are likely to be more connected at larger dispersal distances (500–1000 m), while species with high dispersal limitation such as shrubs are likely to be more connected at shorter dispersal distances (100 m). The threshold distance specifies under which inter-patch distance two patches would be considered as having a direct connection, that is a link in the graph. These distances were then used to calculate the connectivity metric values (IIC, ECA, NC) for the entire landscape and the patch-level connectivity values that are described in the next section.

Importance of individual grassland patches for overall connectivity

In order to evaluate the contribution of individual grasslands for the maintenance of overall landscape connectivity, each of the 301 patches was systematically removed from the landscape (one at a time), and the impact of their loss was evaluated through the following equation:

$$dIIC(\%) = 100 \times \frac{IIC - IIC_{remove}}{IIC} \qquad (2)$$

where IIC is the index value when all nodes are present in the landscape and IIC_{remove} is the index value after the removal of a given habitat patch (Pascual-Hortal & Saura 2006; Saura & Pascual-Hortal 2007).

The patch-level dIIC values can be partitioned into three distinct fractions (intra, flux, connector) considering the different ways in which a certain landscape element k (here a grassland patch) can contribute to habitat connectivity and availability in the landscape (Saura & Rubio 2010):

$$dIIC_k = dIICintra_k + dIICflux_k + dIICconnector_k \qquad (3)$$

The intra fraction is the contribution of patch k in terms of intra-patch connectivity (amount of grassland habitat resources that exist within the patch). It is independent of how patch k may be connected to other patches. This fraction is equivalent to the variation in a family of fragmentation indices that take the squared patch area as the basis for their computation, such as the area-weighted mean patch size (Li & Archer 1997). The flux fraction estimates the potential amount of dispersal flux expected to depart from or arrive at a particular habitat patch, that is, it measures how well connected a particular habitat patch is to the rest of the habitat areas in the landscape. The connector fraction evaluates how important k is in maintaining the rest of the patches (different from k) connected to each other, that is, how much the patch contributes to connectivity by functioning as a stepping stone in between other patches. For more details on these fractions see Saura and Rubio (2010).

The dIIC index and its fractions were calculated for the three selected dispersal distance (100, 500 and 1000 m), as well as for several larger threshold distances (up to a maximum of 1e+8 m) in order to illustrate the pattern of variation of dIIC fractions in a broader range of hypothetical conditions.

We calculated the area of grassland patches as well as edge-to-edge Euclidian distances between them using QGIS 2.4 and the Conefor Inputs plugin (http://www.conefor.org/gisextensions.html). These data were used as the input for calculating the connectivity indices (IIC, dIIC and its fraction, NC and ECA) using Conefor Sensinode 2.6 (Saura & Torné 2009), available at www.conefor.org.

Influence of connectivity in explaining entomophilous plant species assemblages

In order to evaluate the effect of connectivity (in terms of dIICintra, dIICflux and dIICconnector) of the 39 selected grassland patches on entomophilous plant species assemblages (presence-absence of species) according to their growth form and longevity, a series of Canonical Correspondence Analysis (CCA) were performed for each species group and threshold dispersal distance. A CCA is a canonical analysis that evaluates how much of the variation of the structure of a multivariate data set is explained by independent variables (Legendre & Legendre 1998). The amount of explained variance (inertia) obtained by each set of variables was used as a measure of the strength of the relationships. Since data come from two sources of different information, that is two sampling dates: 2001/2002 and 2007/2009, we included data source as a categorical covariate in all the analyses in order to control for its effect. We calculated significance of relationships by performing a permutation test similar to analysis of variance (99999 permutations) (Legendre & Legendre 1998).

Previously, a DCA (Detrended Correspondence Analysis) was performed on each matrix variable response (total entomophilous plant species, annual-biennial herbs, perennial herbs and shrubs) in order to use an appropriate canonical ordering method (ter Braak 1994). The length of the gradient detected (>2 SD) suggests the suitability of using the unimodal method CCA instead of linear method RDA (Leps & Smilauer 2003). All analyses were carried out with the package Vegan 2.3-2 (Oksanen 2015) of R software (R 2014).

RESULTS

Plant species composition

We registered a total of 247 entomophilous plant species in the 39 studied grassland patches belonging to 55 families: 54 annual-biennial herbs, 136 perennial herbs and 41 shrubs. Sixteen herbs were only identified as genera and they could not be classified according to their longevity; thus, they were not included in the analysis. The most widely represented family was Asteraceae (65 species).

Quantifying overall landscape connectivity

The graph-based analyses showed that overall landscape connectivity in terms of the ratio between ECA and the total area covered by grassland patches was relatively low and quite similar at all threshold dispersal distances (Table 1). The high number of components (NC), many of them with only one patch, also indicated a low connectivity in the study system. NC values decreased from 130 to 59 as the dispersal distance increased from 100 to 1000 m (Table 1). The size of the main component in terms of habitat availability (proportion of patch area and number of patches) increased with threshold dispersal distance; however, values were relatively low (Table 1). Figure 2 shows an example of the main component at the threshold distance of 500 m.

Importance of individual grassland patches for overall connectivity

The highest contributions of individual grassland patches for the maintenance of overall landscape connectivity, as evaluated by dIIC, were 32.3%, 34.1% and 35.5% for 100, 500 and 1000 m, respectively (See Fig. 3 for 500 m threshold distance). These values represent the percentage of connectivity decrease that would result from the loss of a given patch from the landscape. The dIIC decomposition suggests that

Table 1. Overall connectivity indices for the study area and for the three selected threshold distances. ECA/area: Equivalent Connected Area/total area covered by grassland patches

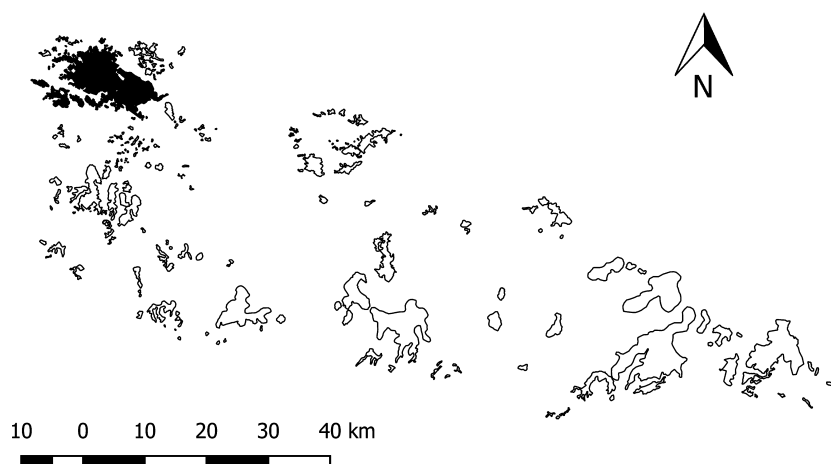| Threshold distance (m)	(ECA/area) 100 (%)	NC	Proportion of patch area in the main component (%)	Number of patches in the main component	NC with only a patch
100	25.3	230	0.04	7	191
500	26.7	103	17.3	47	56
1000	28.0	59	20.0	84	25

NC, Number of components.

Fig. 2. Main component (black grassland patches) and remaining components (white grassland patches) distribution at threshold distance of 500 m.

grassland patches contribute to connectivity mainly depending on dIICintra, followed by dIICflux and dIICconnector (Figs 3, 4).

Influence of connectivity in explaining entomophilous plant species assemblages

Results of CCA showed that connectivity variables significantly explained total, annual-biennial herbs and shrubs assemblages for all threshold dispersal distances. Explained variance ranged between 6 and 13% (Table 2). In all cases, high levels (nearly 70%) of unexplained variance were found. The variance explained by the effect of sampling dates varied among groups of species (from 7 to 20% of total explained variation).

The dIICintra index contributed significantly to explaining total, annual-biennial herbs and shrubs assemblages for all threshold dispersal distances. The dIICflux index contributed significantly to explaining total, annual-biennial herbs and shrubs assemblages at 100 m, while its contribution was marginal for shrubs at 500 m. The dIICconnector index only explained shrubs assemblages at 500 and 1000 m but with marginal levels of significance (Table 2).

DISCUSSION

In this study, we showed evidence of the importance of functional connectivity on entomophilous plant species assemblages in one of the most threatened ecosystems in the world (Newbold *et al.* 2016). At the same time, we identified priority sites for conservation. Connectivity is in general used in a structural way because of its ease of measurement. However,

structural connectivity does not consider the configuration and the spatial distribution of habitats, the capabilities of dispersal of species or specific processes, and/or certain attributes of habitat patches such as size or quality (Chardon *et al.* 2003). The graph-theoretic approach makes it possible to include all these aspects, making more realistic predictions on landscape configuration and its effects on biodiversity.

Grassland patches in the study area are poorly connected for the different threshold distances, as shown by the considered connectivity metrics. Compared with the results of this study, other grasslands of the world have been reported to be better connected, as is the case of Mpumalanga (South Africa), where 93.6% of the total grassland habitat patch area (27.6% of the number of patches) is connected in a single component at a threshold distance of 50 m (Fourie *et al.* 2015). Thus, in our study system, identification of grassland patches that are critical for the persistence of overall connectivity is crucial, and conservation efforts and reserve networks should concentrate on protecting those sites with a higher dIIC. We found that grassland patches with high dIIC are those larger in size. This trend is mainly due to the large distances separating the patches, compared with the focal dispersal distances, which results in a relatively high isolation of individual patches. In this situation, the amount of habitat resources that can be reached by an organism using grasslands is largely determined by the size of the grassland patch in which this organism initially dwells, given the weakness of the connections that would eventually allow it to reach other patches. However, patches have different roles within the landscape network, and as shown in Fig. 3 for 500 m threshold distance, grassland patches with
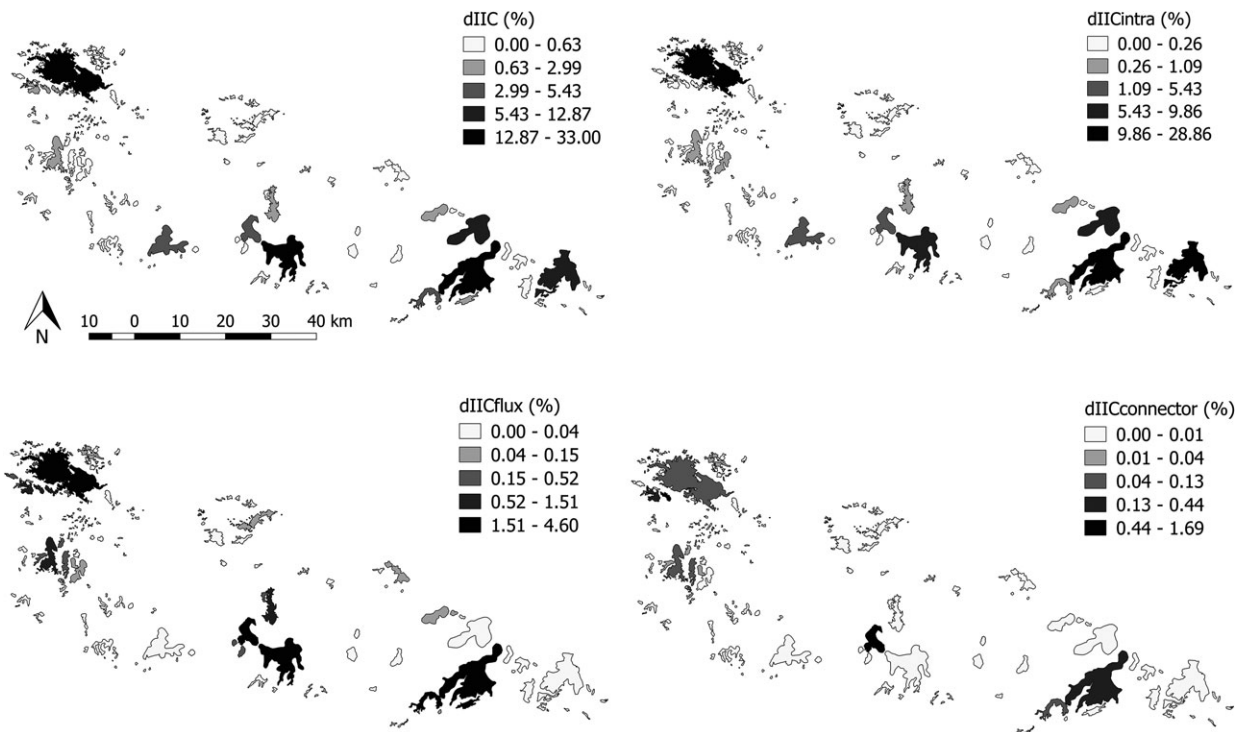
Fig. 3. Contribution of individual grassland patches for the maintenance of overall landscape connectivity at threshold distance of 500 m according to dIIC and the three fractions of this index (intra, flux, connector). Dark colours represent grassland patches more important for overall connectivity.
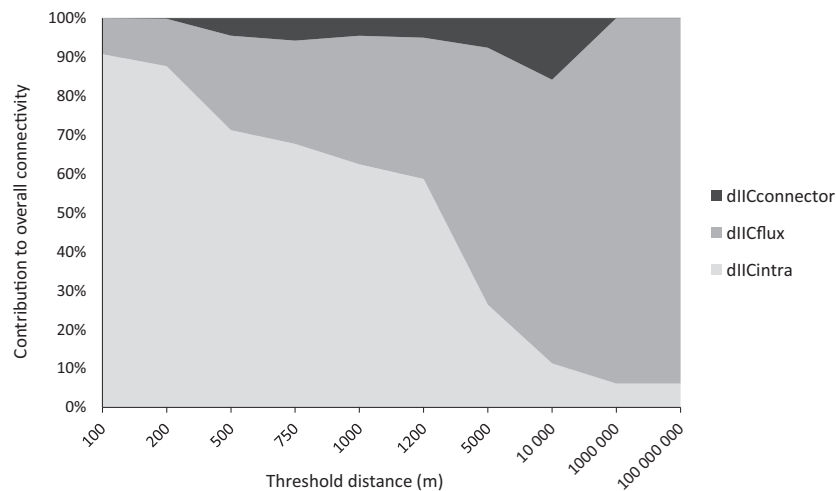

Fig. 4. Relative contribution of each dIIC fraction to the total importance of grassland patches for connectivity in the landscape. To illustrate the broader pattern, we used threshold distances >1000 m.

high dIICintra (large patches) also have higher values of dIICflux (are well connected to other grassland areas in the landscape). According to Saura and Rubio (2010), patches not only serve as sites for shelter, foraging and breeding, but also produce (or receive) dispersal fluxes to (or from) other habitat patches. They also function as stepping stones that facilitate dispersal between other patches, even when they are not the final destination of the dispersal fluxes. Thus, within the same landscape and for the same focal species, different patches may play different roles depending on their topological position and

Table 2. Summary results of Canonical Correspondence Analysis (CCA) between connectivity variables (dIICintra, dIIC-flux, dIICconnector) and entomophilies plant species assemblage's data classified according to growth form and longevity from 39 grassland patches for the three selected threshold distances (100, 500 and 1000 m). Values represent marginal effects (individual variance explained) of explanatory variables

	Threshold distance (m)			dIICintra	dIICflux	dIICconnector
Total species	100	%Var	13.69			
		F		2.03	2.18	
		P-value		0.044**	0.040**	ns
	500	%Var	12.49			
		F		2.52		
		P-value		0.011**	ns	ns
	1000	%Var	12.18			
		F		2.03		
		P-value		0.044**	ns	ns
Annual-biennial herbs	100	%Var	13.69			
		F		2.03	2.18	1.65
		P-value		0.002***	0.004**	ns
	500	%Var	12.49			
		F		2.53	0.98	1.28
		P-value		0.001***	ns	ns
	1000	%Var	12.82			
		F		2.41	1.29	1.08
		P-value		0.001***	ns	ns
Perennial herbs	100	%Var	6.48			
		F		1.14	1.2	0.72
		P-value		ns	ns	ns
	500	%Var	6.62			
		F		1.19	0.81	1.12
		P-value		ns	ns	ns
	1000	%Var	6.26			
		F		0.955	0.801	1.002
		P-value		ns	ns	ns
Shrubs	100	%Var	8.71			
		F		2.29	1.73	1.09
		P-value		0.005***	0.049**	ns
	500	%Var	8.34			
		F		2.97	1.73	1.58
		P-value		0.001***	0.079*	0.087*
	1000	%Var	8.67			
		F		2.61	1.36	1.61
		P-value		0.002***	ns	0.091*

P-value: ns >0.05, *<0.1, **<0.05, ***<0.01.
%Var is explained variation by all connectivity variables.

intrinsic habitat characteristics, as quantified by the three dIIC fractions. On the other hand, the main component in terms of habitat availability and number of patches included the grassland patch with the large dIIC value (See Figs 3, 4 for the 500 m threshold distance). This single patch constitutes a very large area connecting with small patches but with medium and high dIICflux and dIICconnector values, respectively. Therefore, this patch could be an important site for conservation also in terms of inter-patch connectivity, by helping organisms from other patches to access the resources in this large block of habitat and by promoting the likelihood of dispersal between other patches as a stepping stone between them.

Results of this study showed that the variation in assemblages of species with short-life cycles was mainly explained by intra-patch habitat availability (dIICintra) at all threshold distances and by inter-patch connectivity (dIICflux) at short threshold distance (100 m). This group of species is characterized by its dispersal capabilities and ability to respond to unfavourable conditions such as changes in habitat quality or isolation (Lindborg *et al.* 2011). However, if grassland patches are large enough and suffer internal disturbances frequently (grazing, fire, derived herbicide from the matrix, etc.), this group of species could persist within them by colonization processes from other parts of the same patch. The effects of local disturbance on plant species assemblages have

been demonstrated by some authors, especially for ruderal species which are good colonizers under disturbance conditions such as fire or grazing, which change vegetation canopy and litter height, making sites available for recruitment (Grime 1979; Wright *et al.* 2003; Herrera & Laterra 2009, 2011). Thus, the greatest part of the variation in species composition that could not be explained by connectivity variables in this study could be explained by management or disturbance history. However, these aspects were not specifically analysed in this study, and additional research would provide further insights into this topic.

The variation in shrub species assemblages was explained in part by intra-patch habitat availability at all threshold distances. This is consistent with evidence from studies of grasslands that have shown that long-lived and clonal mechanism species tend to develop persistent populations (Eriksson 1996; Lindborg *et al.* 2005) and would be most affected by high isolation between patches. Furthermore, dIIflux explained part of the variation of this group of species at low distances (100 m), and marginally at relatively large distance (500 m). Species of this group with larger seed and fruits can be eaten by birds and other animals transporting them over long distances (Westoby *et al.* 1996). This would explain not only the effect of dIICflux but also the dIICconnector (although its effect is marginal) at higher threshold distances. Thus, the shrub species are also associated with patches that given their topological position work as stepping stones, which could be related to the movement of frugivorous birds dispersing seeds in the landscape.

Variation in perennial herbs assemblages was not explained by intra-patch or inter-patch connectivity. This group of species is probably more diverse in terms of dispersion and persistence traits (Appendix S1) and the connectivity effects could be diluted.

In this study, we did not take into account the role of linear landscape elements (rail and roadside vegetation, verges, etc.) or the characteristics of the matrix (different types of land use and successional states) in explaining plant species assemblages. However, these aspects can also be dealt with through the methods and metrics that we used for connectivity analyses, provided that the relevant landscape and ecological information is available, and this is part of our planned future research. There is interesting evidence of the role of linear elements as connecting elements and of the role of the matrix as potential habitat. For example, Jiménez *et al.* (2015) found that plantings on roadside acted as selective bird attractors, providing food and perches for frugivorous species. In their grassland connectivity study in South Africa, Fourie *et al.* (2015) found that the inclusion of abandoned croplands increased the overall connectivity of the landscape. In a sector of our study area, Sáez *et al.* (2014) investigated the interactive effects of habitat patches at different scales acting as pollinator sources for sunflower and demonstrated that honey bee visitation to crops was strongly affected by proximity to large expanses of natural habitats (sierras). In the same way, previous studies also demonstrated the importance of habitat area and patch centrality to preserve local interaction diversity in pollination webs in the Argentine Pampas (Sabatino *et al.* 2010; Gilarranz *et al.* 2015). However, there is a general lack of knowledge about how spatial structure and dispersal simultaneously affect vegetation structure and dynamics of linear elements in the studied landscape, as well as the geographic distribution of ecosystem services and co-evolutionary processes, which should be addressed in further research.

The identification of key grassland patches for entomophilous plant species generated in this study represents an important input for developing policies of biodiversity conservation and sustainable management of these grasslands. The results of this work provide valuable tools for territorial planning based on patch management aimed at conservation of key grassland patches for connectivity as well as of isolated patches that could provide important refuge for unique or restricted species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Main traits associated with dispersal and persistence in a fragmented landscape of species grouped according to growth form and longevity used in this study.