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Pinus contorta invasion into treeless steppe reduces species richness and alters species traits of the local community

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Abstract *Pinus contorta*, one of the most invasive tree species in the world, has been proposed as a model species for improving our understanding of invasion ecology. In this study, we assessed the impact of *P. contorta* invasions on the species richness, diversity and species traits of a resident treeless steppe community. In a *Pinus contorta* invasion gradient (Patagonia, Chile), we surveyed vegetation from high canopy closure pine invasion to treeless steppe, and computed species richness, diversity and Sørensen similarity indexes.

For all species, we determined functional trait values from the literature, data bases, and personal observations. Species richness and diversity were related to canopy cover (a proxy for invasion intensity) using generalized linear mixed-effects models. Changes in species traits due to canopy cover were analyzed using RLQ ordination analysis and the fourth-corner analysis. We found that *Pinus contorta* canopy cover significantly reduced the number of native species by 70 %, implying a strong effect on species exclusion. A few native species, however, prevail in the novel conditions (e.g. *Baccharis magellanica*, *Acaena integerrima*). Species traits changed significantly with increasing pine canopy cover, where *P. contorta* promoted the existence of traits related to shade-tolerance and conservative reproductive strategies. We conclude that the negative impacts of *Pinus contorta* into the treeless steppe, including a reduction in the number of species and the shifting to traits adapted to tolerate shade and associated with conservative reproductive strategies, can have severe implications for the conservation of biodiversity and ecosystem functioning where it invades.

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Introduction

Plant invasions are an important component of global change, which have been traditionally associated with

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a loss of local biodiversity, productivity and cultural heritage (Alpert et al. 2000; Lockwood et al. 2005). Although the significant impacts of plant invasions on local species diversity and composition in the invaded communities have been largely examined in several systems (e.g. Powell et al. 2011; Vilà et al. 2011), few studies have determined the impact of invasions on the species composition and functional diversity of invaded systems (Chabrerie et al. 2010; Rundel et al. 2014) and the functional response of the invaded community (Levine et al. 2003). Given that in many cases naturalized populations of woody invasive species have occurred in areas without native woody vegetation, the novel presence of a tree canopy cover will necessarily imply strong changes in functional trait diversity, i.e. because of a change in environmental filtering conditions. Thus, woody invasive species have the potential to have a major detrimental effect on ecosystem functioning not only through a reduction in biodiversity but also through a change in functional diversity (Thuiller et al. 2012).

Afforestations with conifer species, particularly *Pinus* species, constitute an important form of land-use change globally (Simberloff et al. 2010). In the southern hemisphere, species of the genus *Pinus* have been preferred for plantation because of their relatively high growth rate, which is a trait highly desired for soil conservation and biomass production purposes (Gundale et al. 2014; Richardson 1998; Simberloff et al. 2010). Some of the *Pinus* species, however, have proved to be highly invasive, especially in treeless areas (Pauchard et al. 2016). Among these, *Pinus contorta*, native from western North America, is considered by many one of the most invasive tree species in the world (Richardson and Rejmanek 2004). Because of its widespread invasive range, which includes Scandinavia, southern South America and New Zealand, *Pinus contorta* has even been proposed as a model species for improving our understanding of invasion ecology in general (Gundale et al. 2014). Aspects associated with the patterns of invasion of *P. contorta* and the mechanisms invoked to explain successful invasion have been studied in the invaded areas (e.g. Langdon et al. 2010; Ledgard 2001; Peña et al. 2008; Richardson and Rejmanek 2004; Richardson et al. 1994). In southern Chile, for example, *Pinus contorta*—along with *P. ponderosa*, *P. sylvestris* and *Pseudotsuga menziesii*—was intensively planted to afforest treeless areas experiencing a high occurrence

and risk of landslide activity after large–scale, anthropogenic fires occurred in the mid–twentyth century (Fajardo and Gundale 2015; Fajardo and McIntire 2010). Later, *P. contorta* was planted for timber production and, more recently, to obtain carbon sequestration credits. However, few attempts have been conducted to study how invasions of *P. contorta* alter the vegetation composition and structure of the resident community. With time, successful pine invasions will tend to encroach (continuous recruitment) and increase height and canopy cover, thus reducing the light availability for the resident species. This simple effect should change community parameters such as species diversity and richness but also community functional diversity. A continuous reduction in light availability will necessarily modify the environmental conditions of the site. These new conditions should favor some traits while filtering others out in the *new* community. Therefore it is expected that some species will disappear and others will enter the community, i.e. some species will not tolerate the new abiotic conditions.

According to the trait-based approach for plant ecology, functional traits reflect how plants adapt to variation in both their physical environment and their biotic interactions. It is henceforth expected that functional traits are involved in community structuring (Adler et al. 2013). Environmental filtering (i.e. species sorting) and limiting similarity (i.e. niche partitioning) have been proposed as two contrasting forces responsible of community assembly processes (Weiher and Keddy 1995). The concept of environmental filtering suggests that species with particular traits that are within a certain range of values (the filter or sieve) will establish and persist in a community and all other species whose trait values are out of the range will be absent of the community. Limiting similarity, in contrast, states that species being too similar in ecological terms (traits' similarity) will inevitably compete stronger for resources leading to the extinction of one of the species (MacArthur and Levins 1967). Thus, the appearance of invasive pines in treeless areas that will eventually close the canopy is challenging for the resident community in two ways: (1) it changes the prevailing abiotic conditions (it modifies the *rules of the game* of species assemblage) mainly by decreasing light availability, i.e. the environmental filter is somehow different, and (2) it adds a new dominant species that impinges novel biotic

interactions in the community. Which species are going to quit, adapt or improve to the novel conditions is an open question and will necessarily depend on the collection of traits and the capability of adaptation of each species.

The main objective of this study was to assess the impact of the invasion of *Pinus contorta* on a historically treeless steppe community in Patagonia, Chile. To achieve this objective we studied the effects *P. contorta* has on species composition and abundance of the resident community and their functional traits across a gradient of invasion, from non-invaded, treeless steppe to high-density invaded areas dominated by the novel tree life form. Although treeless steppe constitutes a harsh environment for tree establishment, including low nutrient availability, cold temperature, and strong winds, as soon as *P. contorta* plantations became mature, a very aggressive invasion begun that now constitutes one of the highest invasion expansions in natural areas in southern South America (Langdon et al. 2010) (Fig. 1). In particular, we were interested in responding the following question: what is the impact of *P. contorta* invasions on the species richness, abundance and species traits of the resident community? Because of the most conspicuous effect of *P. contorta* invasion is a reduction in light availability of a plant community that, in principle, has not evolved under such a condition, we expected a drastic reduction in species richness and abundance with the intensity of invasion. We hypothesized that *P. contorta* creates a new environmental filter to understory species of the local community and thus it filters for specific traits that will likely differ from the original ones imposed solely by the environment. In particular, we expected that traits associated to low light conditions (e.g. bigger leaves and seeds) should be promoted under high invasion intensity, which can bring potentially new species to the community and also displace other more shade-intolerant species out of the community.

Methods

Species and site description

Pinus contorta Douglas is a pioneer, shade intolerant and fast growing tree species, which naturally occurs from northwest Canada to southwest USA, thus covering an ample variation of biotic conditions (Ledgard 2001). *Pinus contorta* can reach maturity as early as 5–10 years, with an annual cone production including abundant seeds (500,000–800,000 seeds per hectare per year), where seeds are dispersed mostly by wind and their viability is between 65 and 90 % (Ledgard 2001). Seed germination and posterior seedling establishment are enhanced at full sunlight and on mineral soil conditions (Ledgard 2001).

Our study was conducted in the area of Coyhaique Alto (45°33′S, 72°04′W, 740 m a.s.l.), 35 km east of the city of Coyhaique, Región de Aysén, Chile. This area has been described as an eco-region of cold steppe formation (Luebert and Pliscoff 2006; Scheu et al. 2008). Total annual precipitation here is around 450 mm and it occurs mostly in winter time. The mean annual temperature is 7 °C, with a mean between 0 and 3 °C in winter, while in summer mean temperature varies between 10 and 15 °C (Dirección General de Aguas, Servicio Meteorológico Nacional). Vegetation is dominated by tussock grasses, which

Fig. 1 *Pinus contorta* invasion into the treeless steppe in Coyhaique Alto sector, Patagonia, Chile. The extension from the plantation (seed source) to the area without invasion is of about 1000 m. Picture was taken facing east. *Photo credits* Pablo Bravo–Monasterio

corresponds to a temperate steppe dominated by *Festuca pallescens* and *Mulinum spinosum* (Luebert and Pliscoff 2006). The steppe is a short-stature vegetation formation where tussocks are spatially distributed in a regular arrangement and a great portion of space is represented by bare soil. Here, *Nothofagus antarctica*, a native treelet, occasionally dominates the steppe in more humid locations. Afforestations of *P. contorta* in the Chilean Patagonia were initiated in the early 70's (Löewe and Murillo 2001). The *P. contorta* plantation (seed source) in the study area was established in 1981 and it is separated by a 25 m road from the highest-density invasion area (Fig. 1). Langdon et al. (2010) found that at the very edge of the *P. contorta* invasion (next to the road) tree density can reach up to 16,650 individuals per hectare in >30 years, with the most distant seedling found 3 km away from the seed source. Pine density is highly associated with distance from the seed source (plantation) (Pauchard et al. 2016), allowing for the isolation of the effect of the invasion in plant community. Given that the steppe constitutes a short-stature, homogeneous-structure vegetation system, we confidently assumed that no other factor (biotic or abiotic) affected the invasion progress, but the wind dispersion of seeds.

Sampling and species identification

Sampling was conducted in February and March 2013. In order to cover most of the invasion gradient, we set seven ∼1000 m sample transects perpendicular to the road. Each transect was spaced 70–100 m from its neighbor. At each transect, we set 27 1-m^2 plots that covered from high to moderate density pine invasion, up to areas of no pine presence. These plots were established using a random walk design (Underwood 1997), where distances (constrained from 20 to 50 m) were randomly generated. In these plots we identified all species and estimated their cover. Species were identified following nomenclatures by Correa (1998), Rodríguez et al. (2008), Zuloaga et al. (2008), Silva (2010), and Fuentes et al. (2013). Specimens of unknown species were collected and compared with specimens belonging to the collection of the Botany Department Herbarium, Universidad de Concepción, Concepción, Chile (CONC) for posterior identification. Species cover was estimated by two observers following a visual classification based on the structure

of the vegetation. The classification was as follows: species cover class 1, <1 % of the plot area; class 2, 1–5 %; class 3, >5–15 %; class 4, >15–25 %; class 5, >25–50 %; class 6, >50–75 %; and class 6, >75 % of the plot area. By using the same visual classification for species cover, we also recorded plot area covered by bare soil. Finally, we also considered the potential effect of herbivory as a biotic variable affecting the composition of species; potential herbivory in the study area is represented by the presence of two alien mammals, *Lepus europaeus* (hare) and *Ovis aries* (sheep). By accounting for feces amount in each plot, we recorded a potential herbivory index cover following the same classification than above.

To characterize *P. contorta* canopy cover, we took digital hemispherical photographs of the canopy from a height of ∼50 cm above the center of each plot. We used a 7-mm Nikon f 7.4 fisheye lens (the lens has an orthographic projection of 180° angle of view), mounted on a Nikon Coolpix 5000 digital camera (Nikon Corporation, Tokyo, Japan). Photographs were taken under cloudy sky conditions or late in the day to avoid the direct exposure to sun. Photographs were also under-exposed by two stops, to increase contrast between the sky and foliage (Zhang et al. 2005). The canopy cover was computed for each plot using the Gap Light Analyzer (GLA ver. 2) software (Frazer et al. 2000).

Species traits

From the 57 species found in the study site we selected eight functional traits that we classified into reproductive-, vegetative-, and environment-associated traits. Within the reproductive-associated traits, we had: (1) dispersion mode (anemochory, endo and epizoochory, and others); (2) fruit type (succulent, dehiscence and indehiscent); (3) seed mass (small <0.10 mg, medium 0.12–2.0 mg, medium–high 2.01–9.99 mg, and heavy >10 mg). Within the vegetative-associated traits, we had: (4) life form (annual and perennial plants); (5) growth form (erect, prostrate, cushion); (6) plant height (small <15 cm, intermediate 15–70 cm and tall >70 cm). Finally, within the environment-associated traits, we had: (7) leaf shape (lanceolate, linear, obovate, and others); and (8) leaves arrangement or phyllotaxis (alternate, opposite, decussate, basal and others). All these traits are contained in trait lists compiled by

Weiher et al. (1999), and Westoby et al. (2002). Trait values for the 57 species found in the study area were obtained from literature, data bases, and personal observations (Correa 1998; Fuentes et al. 2013; Silva 2010; Teillier et al. 2013; Török et al. 2013).

Statistical analysis

We computed native, exotic and total species richness, Shannon's diversity (H) (natural logarithm) (McCune and Grace 2002) and Pielou's evenness (J) (Mulder et al. 2004) indexes at the plot level. First, to test whether the resident community species richness and diversity differed according to *P. contorta* canopy cover—a proxy for invasion intensity—, we fitted generalized linear mixed-effect model regressions (GLMM) with a Poisson distribution and a log link function using lme4 (Bates and Maechler 2009) in R2.15.3 (R-Development-Core-Team 2013). For the model under study, we considered *P. contorta* canopy cover (%) as fixed effect with transects as random factors to account for among-transects variation.

We also computed the Sørensen index (Chao et al. 2005) of composition similarity between invaded and non-invaded plots. The Sørensen index has been traditionally used to estimate compositional similarity of plant assemblages. Non-invaded plots were all those plots that were located far away from the seed source, did not have any pine in the neighborhood and had a canopy cover <10 %. Commonly, non-invaded plots were in one extreme of each transect. The Sørensen index was computed using the software EstimateS 9.0 (Colwell 2013), which compares each invaded plot's species composition with an average non-invaded plots' species composition. Sørensen index is expressed as a species similarity index that goes from 0 for complete dissimilarity and 1 for complete species overlap between both communities (Chao et al. 2005). From the same analysis we also computed species shared between both communities, the number of species lost and gained under the invasive condition. Finally, we fitted linear regression models between species similarity values (and species shared, lost and gained) and *P. contorta* canopy cover; a negative and significant slope would mean that the higher the *P. contorta* invasion success (higher canopy cover values) the lower the species similarity between invaded and non-invaded communities.

In order to assess the species trait responses of the native community to the *P. contorta* invasion gradient we conducted first an RLQ co-inertia analysis and then the fourth-corner statistic. In the first analysis we aimed to identify which species traits of the resident community were associated to *Pinus contorta* invasion. Thus, we estimated the trait–environment relationship conducting an RQL co-inertia analysis by considering the abundance of species in the plots. We considered as environmental variables the *P. contorta* canopy cover, the bare soil cover and the potential herbivory cover, even though the latter is a biotic component. This type of analysis is a multivariate technique that provides ordination scores to summarize the joint structure among three tables of information: L, species distribution across samples; R, environmental characteristics of samples (e.g. *P. contorta* canopy cover); and Q, species traits (Dolédec et al. 1996; Dray et al. 2003). This allowed us to estimate the trait–environment relationship (Dray et al. 2014). In more detail, the RLQ analysis combines table R (plots × environmental parameters) with table Q (species × traits) with a link expressed by a contingency table L (plots × species). These three matrices are simultaneously combined by means of a Correspondence Analysis (CA) on table L (optimal correlations between plots and species scores), a Principal Component Analysis (PCA) on tables R and L, and a Multiple Correspondence Analysis (MCA), which all maximize the covariation between the plot scores constrained by the environmental variables of table R and the species scores constrained by the traits of table Q (Dolédec et al. 1996; Dray et al. 2003). The significance of the relationship between table R (environmental variables) and table Q (traits) was tested with a Monte Carlo permutation test ($n = 999$) (for more details, see Dolédec et al. 1996). All these analyses were done using the ade4 (Chessel et al. 2004), vegan (Oksanen et al. 2013) and maptools (Bivand et al. 2015) packages in R.

Second, in order to quantify trait–environment relationship and test for its significance, we conducted the fourth-corner statistic (Dray and Legendre 2008). This analysis measures the link between species traits and environmental variables using a Pearson correlation coefficient and a pseudo-F. A first model tests whether species assemblages are dependent on environmental variables of the plots; permutation of plot vectors in the table L, model 2 in Legendre et al.

(1997). In the second model, a permutation procedure tests whether the distribution of species depends on the traits they possess; permutation of species vectors of the table L, model 4 in Legendre et al. (1997). The two models were combined in order to obtain a correct level of Type I error (Dray and Legendre 2008). We considered a trait to be significantly correlated if both p values associated to models were lower than $\alpha = 0.05$.

Results

Species composition, richness and diversity

Across the invasion gradient, we found a total of 57 species, most of which were herbs and forbs, with only three species of small shrubs: *Baccharis magellanica* (Asteraceae), *Berberis empetrifolia* (Berberidaceae) and *Mulinum spinosum* (Apiaceae) (For a list of all 57 species found, see Table S1). As expected, the percentage of canopy cover of *Pinus contorta* significantly reduced species richness (Fig. 2a, b), diversity (Fig. 2c, d, Fig. 3) and altered community functionality (Table 1; Fig. 4). In particular, the percentage of canopy cover had a significant and negative impact on the number of native species ($z = -10.34$, $P < 0.001$, Fig. 2b). Pine invasion reduced the number of native species by 70 %; species like *Mulinum spinosum* (Apiaceae), *Leucheria candidissima* (Asteraceae) and *Chloraea alpina* (Orchidaceae) were absent in the presence of *P. contorta*. Other species like *Loasa bergii* (Loasaceae), *Phacelia secunda* (Boraginaceae), and *Valeriana carnosa* (Valerianaceae) could tolerate the presence of *P. contorta* in areas where the tree canopy cover was not >30 %. However, species like *Baccharis magellanica* proved to be capable to tolerate and eventually prevail under the new conditions imposed by a closed tree canopy cover. Although some species, mostly exotics, like *Holcus lanatus* (Poaceae), *Rumex acetocella* (Polygonaceae), and *Hordeum murinum* (Poaceae) were present under *P. contorta* canopy cover, this did not have a significant effect on the number of alien species ($z = -1.51$, $P = 0.130$, Fig. 2b). Thus, as most of the species in the community were native, the total decrease of species with tree canopy cover was significant (Fig. 2a). Similarly, when the whole community (native and exotics) was considered, both Shannon's

diversity index (Fig. 2c) and Pielou's evenness J index (Fig. 2d) were significantly reduced with *P. contorta* canopy cover. The number of shared species (Fig. 3a) and the Sørensen index of composition similarity (Fig. 3b) among plots across the invasion gradient were also significantly reduced with *P. contorta* canopy cover.

Species trait relationships with canopy cover

The RQL analysis showed a significant association between environmental variables and species traits. The first two axes of the RQL ordination contributed to 98 % of the total inertia or variance (91 and 7 %, respectively). The Monte Carlo test indicated that the co-structure between R and Q matrices was significant ($P < 0.001$, based on 999 permutations). The first RQL axis correlated best with an increase of *P. contorta* canopy cover ($r = 0.91$), and with a decrease of bare soil cover ($r = -0.66$) and, with less strength, of presence of potential herbivores ($r = -0.11$, Table 1). The second RQL axis was positively correlated mainly with bare soil ($r = 0.74$) and weakly with tree canopy cover ($r = 0.18$), and negatively correlated with the presence of potential herbivores ($r = -0.67$). Complementarily, the RQL analysis revealed a clear concentration of invaded plots in the upper right corner of the ordination graph (Fig. 4a), whereas most of the species are concentrated in *half-invaded* and *non-invaded* plots, in the left side of the graph (Fig. 4a). It is noteworthy that in the invaded plots not only exotic species like *Holcus lanatus* (Poaceae) prevailed, but even some native species thrived, like *Baccharis magellanica* and *Acaena intergerrima* (Asteraceae).

When we analyzed the trait–environment relationship, we found that the first RQL axis was (1) positively and significantly correlated with plant height, prostrate growth form, obovate leaf shape, opposite phyllotaxis, epizoochory dispersion mode, and (2) negatively and significantly correlated with basal phyllotaxis, dehiscence fruit type, climber and cushion growth forms, and lanceolate leaf shape (Fig. 4b). The second RQL axis was (1) positively and significantly correlated with prostrate growth form, linear leaf shape, endozoochory dispersion mode, and (2) negatively and significantly correlated with basal and decussate phyllotaxis, climber and cushion growth forms, dehiscence fruit type, and

Fig. 2 *Pinus contorta* invasion—as the percentage of canopy cover—correlation with total species richness (**a**), native and alien species richness (**b**), Shannon's index of diversity (**c**), and evenness (**d**) of a resident (native and alien species), previously treeless, steppe community in Coyhaique Alto, Patagonia, Chile. For statistical inference see the "Results" section
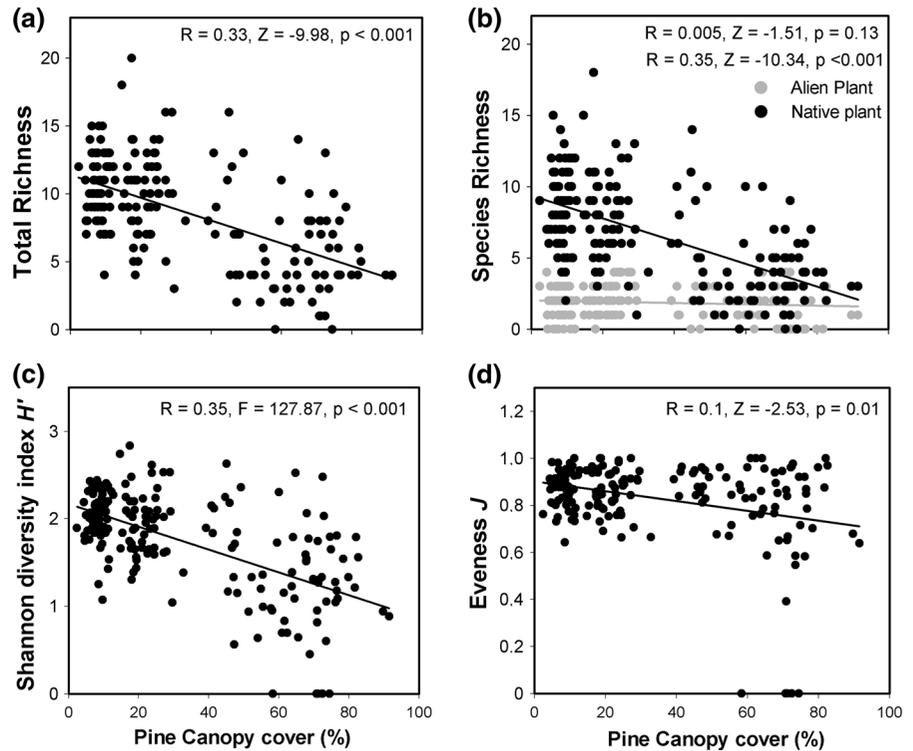
epizoochory dispersion mode (Fig. 4c). Other traits showed only a low correlation. Finally, the results of the permutation tests conducted by the fourth-corner analysis showed significant correlations between the environmental variables and the traits (Table 1). We found a strong correlation ($P < 0.05$) between leaf shape (lanceolate, linear, and others), phyllotaxis (basal), dispersion mode (endozoochory), seed mass (small), and growth form (cushion) with the environmental variables, especially ($P < 0.01$) fruit type (dehiscent) and plant height (small) (Table 1).

Discussion

The *Pinus contorta* invasion in the Patagonian steppe has a significant and negative effect on the local community; it significantly reduces species richness, diversity and alters the distribution of traits in the community. In general, it is expected that the impact on local communities due to tree invasions be very important at the ecosystem level (Richardson 1998; Richardson and Rejmánek 2011), especially in treeless areas (Rundel et al. 2014; Franzese, unpublished).

Although some authors have claimed that the inherent susceptibility of a specific community to be invaded—invasibility—depends on the local diversity of native species (e.g. Alpert et al. 2000), it is clear that here *P. contorta* invasion has an overarching negative effect that goes beyond the fact of how diverse the local community is, especially in treeless vegetative systems such as the steppe (MacDougall et al. 2009; Taylor et al. 2016b). In the Patagonian steppe, where the environment is harsh (cold temperature, dry and windy), spatial pattern analyses at the stand scale have even shown that *P. contorta* establishes in all vegetation microsites depending mostly on seed dispersal and not on the resident vegetation diversity (Pauchard et al. 2016) and therefore pines should be affecting the whole plant community.

According to our results, it is difficult to claim then that *P. contorta* is just exploiting an empty niche and therefore is having no effect of competition on the invaded community. Successful invasion of pine species in other treeless areas was due to pines having different functional traits that allow them to fill an empty niche (Grotkopp et al. 2002). One prediction of this situation is that invaders
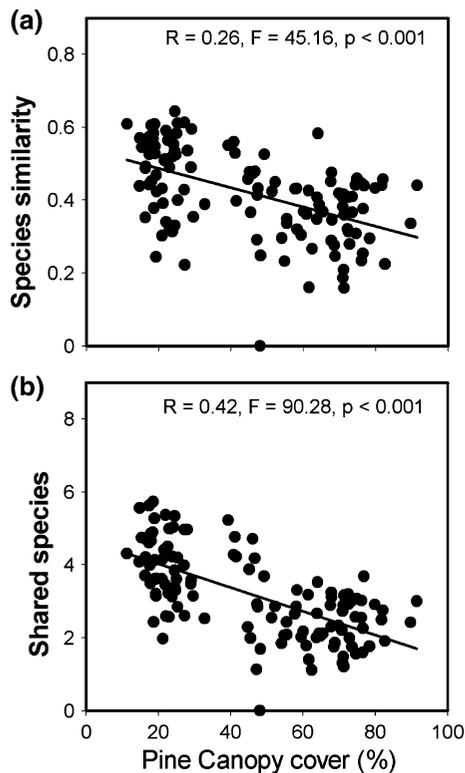
Fig. 3 Species similarity (a) and shared species (b) between treeless steppe community composition plots and the species composition of plots under *Pinus contorta* invasion at varied canopy cover percentages in Coyhaique Alto, Patagonia, Chile

should then coexist with little interaction with the native flora (MacDougall et al. 2009). However, this may be true for the early stages of invasion (i.e. establishment); later on, pines with their higher stature overshadow the steppe community. In fact, in our study the presence of *P. contorta* at high density (high canopy cover) implies the displacement of many local species, including *Mulinum spinosum* (Apiaceae), *Chloraea alpina* (Orchidaceae) and *Leucheria candidissima* (Asteraceae): *P. contorta* generates a community reassembly in composition and functionality. According to MacDougall et al. (2009), the *breakdown* of a local community by the invasion of an alien species (i.e. *invasion that excludes residents* in their Fig. 1) signifies that the average fitness differences surpass the stabilizing niche differences, the latter responsible for the maintenance of stable species coexistence, the former responsible of species exclusion. Although some native species like *Baccharis magellanicum* and *Acaena intergerrima* are able to endure under *P. contorta* canopy, suggesting niche differences (e.g. possessing traits related to shade-tolerance), some other native species are excluded from the original community. We suggest that an access to a deeper water source by *P. contorta* in this dry steppe may allow it to grow faster and

Table 1 Species traits and environmental variable correlation coefficients (Pearson's r) of a steppe community under a *Pinus contorta* invasion in Patagonia, Chile

Species traits	Pine canopy cover	Bare ground	Potential herbivory
Vegetative–associated			
Growth habits			
Cushion	−0.572	−1.504*	−0.996
Plant height (cm)	6.199**	−1.363	−1.667
Reproductive–associated			
Fruit type			
Dehiscent	−9.604**	−1.437	−1.894
Dispersal mode			
Endozoochory	−2.313*	0.672	0.894
Seed mass (mg)	1.063	−1.985*	0.104
Environment–associated			
Leaf shape			
Lanceolate	−1.932*	−0.195	0.003
Linear	−2.132*	0.892	1.038
Others	2.083	−1.635	−1.601*
Leaf arrangements			
Basal	−1.31	−1.046	−2.033*

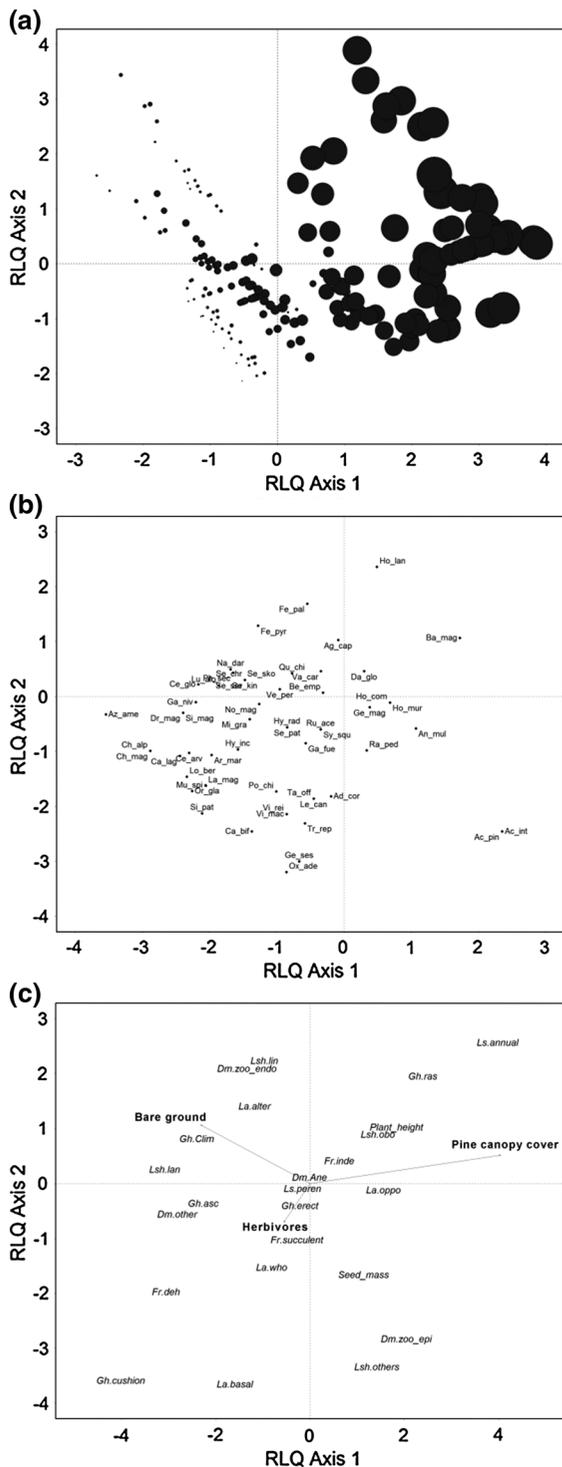Inferential significance is given by * $P < 0.05$, and ** $P < 0.01$

Fig. 4 RLQ diagram as defined by the two axes with projections of plots—where dot size is proportional to *Pinus contorta* canopy cover (a)—, species (b), and the relationship between environmental variables and species traits (c)

larger (fitness differences) and better intercept light than the short-stature resident species that are not used to tolerating shade. Therefore, the actual impact of *P. contorta* on resident species is most likely mediated via above-ground competition, but other processes such as litter accumulation and changes in soil biota cannot be discarded; the well-established mycorrhizal community could be responsible for the competitive advantage of pines in the steppe, especially in heavily invaded areas (e.g. Hayward et al. 2015; Gundale unpublished).

In invasion biology much effort has been placed so far on the search for traits that allow us to predict invasiveness. In contrast, less interest has been addressed on what the functional composition of the invaded community is and how it will respond to invasion. Species of the resident community with similar functional traits to those of the invader should have the greatest competitive effect on invaders (biotic resistance) (Hooper and Dukes 2010). However, in cases where the invasive species constitutes a different life-form from the resident, invaded community, i.e. invasive pines in a treeless steppe, the successful invader will inevitably change the local distribution of traits. Then, how does *P. contorta* invasion alter functionality in the treeless steppe? According to trait-based predictions, in dry environments species having e.g. linear leaf shape, small seeds, and being short in stature should prevail. In our study, seven traits belonging to the resident community showed significant changes across the invasion gradient: dispersion mode (from endo—to epizoochory), seed mass (from light to heavier), plant height (from short to relatively high), leaf shape (from linear and lanceolate to obovate), growth form (cushion plants decrease), fruit type (from dehiscent to indehiscent or succulent), and phyllotaxis (from opposite to basal). All these changes on the frequency of certain trait values can be interpreted as the prevalence of traits more associated with or adapted to the new environmental and biotic conditions: a shady and less windy environment under the pine canopy. Species with higher foliar area (less linear and lanceolate leaf species), taller stature and heavier seeds become more prevalent under *P. contorta* canopy cover, being all these traits associated with shade-tolerance. This may involve a shift from resource-acquisitive to resource-conservative (i.e. shade-tolerance) traits, but it is rather difficult to

establish the shift in these terms because the steppe is indeed an environment where there is a prevalence of resource-conservative traits. In areas of high-density invasion, community-weighted mean traits—which represent the average trait value for a unit of biomass within a community—will be largely driven by pine traits (including a high litter production), leaving little leeway for resident shade-tolerant species to have an important effect on ecosystem processes that underlie ecosystem services. Alternatively, the high amount of litter produced by pines could initially create favorable microsites that increase species richness (Rejmánek and Rosén 1992). With tree age, however, we anticipate that a thicker litter layer (relatively acid when compared to the one produced by the steppe community) will alter the N dynamics of the soil (Fajardo and Gundale 2015; Hess and Austin 2014), and together with increased shading, will lead to a decline of plant species richness and diversity. In this respect, Fajardo and Gundale (2015) found that nitrate and ammonium concentrations were the lowest under *P. contorta* when compared to other pine species and *Nothofagus pumilio* (a native deciduous species) stands in the same region, which impoverishes ecosystem soil nutrient levels.

The case of *B. magellanica* seems to represent an exception to the filtering effect of *P. contorta*. This species—along with *Acaena intergerrima*—may be able to adapt to the new environmental filter positing by a reduction in light availability and also to the negative interactions effects, i.e. competition for light. *Baccharis magellanica* is a prostrate, low-stature cushion-shrub that has been suggested to act as a nurse plant that can facilitate other species, including *P. contorta*, to establish in the steppe. We do not know, however, whether these native species will continue to prevail under higher *P. contorta* canopy cover in the long-term. Although *P. contorta* invasion can reach a tree density up to 16,650 individuals per hectare (Langdon et al. 2010), it still constitutes a young population, where trees are short and canopy have not completely closed. In few decades, *P. contorta* invasion will reduce even more the light availability reaching the ground where most of native species cannot tolerate deep shade. We need to be cautious though in forecasting future scenarios as *P. contorta* invasion may show important spatial heterogeneity as has been shown in other countries such as New Zealand and Argentina (Taylor et al. 2016a).

Conclusions

Clearly, communities that are naturally treeless will be more affected by pine invasions since their species have little or no adaptation to withstand shade or above ground competition, which can have severe implications for the conservation of biodiversity and ecosystem functioning in the Patagonian steppe (Franzese, *unpublished*). We anticipate that the negative impacts of *Pinus contorta* into the treeless steppe—reducing the number of species and changing the functionality by promoting the existence of traits adapted to tolerate shade and associated with conservative reproductive strategies—will have profound and irreversible alterations at many other levels and scales, including primary production and biogeochemical cycles. This is particular worrisome because the invasion process still continues by encroaching already invaded areas but also by advancing and creating new patches that shortly become new seed sources. *Pinus contorta* is reassembling the community, filtering some traits (and species) out of it and bringing other traits (and species) in the community. Finally, we think that there is a great opportunity to study community reassembly processes by monitoring current *P. contorta* invasions and by conducting removal experiments in the invasion gradient to assess the capacity of the system to resist and recover (i.e. ecosystem resilience) from the great event of invasion and to probe the existence of tipping points in pine invasions.

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