Original article

Inhibitory effects of *Eucalyptus globulus* on understory plant growth and species richness are greater in non-native regions

Pablo I. Becerra[1*], Jane A. Catford[2], Inderjit[3], Morgan Luce[4], Krikor Andonian[5], Erik T. Aschehoug[6], Daniel Montesinos[7], and Ragan M. Callaway[8]

[1] Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal and Center of Applied Ecology and Sustainability (CAPES), Pontificia Universidad Católica de Chile. Av. Vicuña Mackenna 4860, Santiago, Chile, [2] Biological Sciences, University of Southampton, Southampton, SO17 1BJ, UK; School of BioSciences, The University of Melbourne, Vic 3010, Australia; Fenner School of Environment and Society, The Australian National University, ACT 0200, Australia; [3] Department of Environmental Studies, Centre for Environmental Management of Degraded Ecosystems (CEMDE), University of Delhi, Delhi 110007, India, [4] MPG Ranch, Missoula, MT, 59801, USA, [5] Department of Ecology & Evolutionary Biology, University of California, Santa Cruz 95064, USA, [6] Department of Environmental Science and Natural Resource Management, Norwegian University of Life Sciences, 1433 Ås, Norway, [7] Centre for Functional Ecology, DCV-FCTUC, Universidade de Coimbra, Calçada Martim de Freitas, 3000 456 Coimbra, Portugal, [8] Division of Biological Sciences and the Institute on Ecosystems, The University of Montana, Missoula, MT, 59812, USA.

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Correspondence: Pablo I. Becerra, Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal and Center of Applied Ecology and Sustainability (CAPES), Pontificia Universidad Católica de Chile. Av. Vicuña Mackenna 4860, Santiago, Chile. E-mail: pablobecerra@uc.cl

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ABSTRACT

Aim: We studied the Novel Weapons Hypothesis in the context of the broadly distributed tree species *Eucalyptus globulus*. We evaluated the hypothesis that this Australian species would produce stronger inhibitory effects on species from its non-native range than on species from its native range.

Location: We worked in four countries where this species is exotic (USA, Chile, India, Portugal) and one country where it is native (Australia).

Time period: 2009-2012

Major taxa studied: *Plants*

Methods: We compared species composition, richness and height of plant communities in 20 paired plots underneath *E. globulus* individuals and open areas in two sites within its native range and each non-native region. We also compared effects of litter leachates of *E. globulus* on root growth of seedlings in species from Australia, Chile, USA and India.

Results: In all sites and countries, the plant community under *E. globulus* canopies had lower species richness than did the plant community in open areas. However, the reduction was much greater in the non-native ranges: species richness declined by an average of 51% in the eight non-native sites versus 8% in the two native Australian sites. The root growth of 15 out of 21 species from the non-native range were highly suppressed by *E. globulus* litter leachates, whereas the effect of litter leachate varied from facilitation to suppression for six species native to Australia. The mean reduction in root growth for Australian plants was significantly lower than for plants from USA, Chile and India.

Main conclusions: Our results show biogeographic differences in the impact of an exotic species on understory plant communities. Consistent with the Novel Weapons Hypothesis, our findings suggest that different adaptations of species from the native and non-native ranges to biochemical compounds produced by an exotic species may play a role in these biogeographical differences.

INTRODUCTION

Many studies have documented that exotic plant species suppress the performance, abundance and diversity of native species (e.g. Richardson & MacDonald, 1989; Pyšek & Pyšek, 1995; Brewer, 2008; Hejda *et al.*, 2009; Flory & Clay, 2010; Abreu & Duringan, 2011; Vila *et al.*, 2011). One of the most striking, but less common, observations about the impact of exotic species has been the stronger negative effect that some of these species produce in their invaded range compared to their native range (Callaway *et al.*, 2011, 2012; Inderjit *et al.*, 2011; Kaur *et al.*, 2012). Such biogeographic differences may be a consequence of the higher abundances that invaders often achieve in their invaded ranges (Richardson & MacDonald, 1989; Pyšek & Pyšek, 1995; Maron & Marler, 2008), and subsequent effects on resource use and competition (Maron & Marler, 2008; Hejda *et al.*, 2009). Invaders may become more abundant in their non-native regions for different reasons. For example, they may experience a greater competitive advantage in invaded regions (Besaw *et al.*, 2011; Callaway *et al.*, 2011), may be released from pathogens and herbivores (e.g. Joshi & Vrieling, 2005; Callaway *et al.*, 2008), better adapted to disturbance than the native species with which they now co-occur (Hierro *et al.*, 2006), and they may be introduced into environmental conditions that are more favorable than those in their home range (Cavieres *et al.*, 2014).

In addition to these explanations, in a few cases invasive species appear to exert stronger effects in their non-native ranges than in their native ranges in ways that are not fully explained by their greater abundance (Thorpe *et al.*, 2011; Shah *et al.*, 2014). For example, Callaway *et al.* (2012) found that the abundance of *Acroptilon repens* in North America, where it is invasive, was twice than that in Uzbekistan, where it is native, but this twofold increase in abundance produced 25-30 times lower biomass of native species in North America than in Uzbekistan. Rather than purely a response to increased abundance of the invader, these effects may reflect that species in the invaded regions are poorly adapted to novel biochemicals produced by the invader (Ni et al. 2010). Novel biochemicals can have strong negative effects on naïve native plant species via direct allelopathic effects to the plant (Callaway & Aschehoug, 2000; Callaway & Ridenour, 2004; Inderjit *et al.*, 2011; Kim & Lee, 2011), alterations to the native soil biota through antibiotic effects (Callaway *et al.*, 2008), and on herbivores (Lankau *et al.*, 2004; Cappuccino & Arnason, 2006; Wikström *et al.*, 2006; Schaffner *et al.*, 2011). Increased invader impact via disproportionate biochemical effects has been formalized under the Novel Weapons Hypothesis and there is growing body of evidence supporting this hypothesis for a number of invasives including *Ageratina adenophora* (Inderjit *et al.*, 2011), *C. diffusa* (Callaway & Aschehoug, 2000), *Foeniculum vulgare* (Colvin & Gliessman, 2011), *Prosopis juliflora* (Kaur *et al.*, 2012), *Chromolaena odorata* (Qin *et al.*, 2013), and the macroalga, *Bonnemaisonia hamifera* (Svensson *et al.*, 2013). Although multiple studies have experimentally evaluated the Novel Weapons Hypothesis in the invaded range of invasive species, few studies have examined biochemical effects of invasive species on plant communities in both invaded and native regions (but see Ni *et al.*, 2010). Here, we compare characteristics of plant communities underneath and adjacent to stands of a globally invasive tree, *Eucalyptus globulus,* in its native range of southeastern Australia with those in its non-native ranges of USA, Chile, India and Portugal.

Forest plantations are a major source of invaders worldwide (Richardson 1998) and many of these planted species have invaded different regions of the world. Species of *Eucalyptus* (Myrtaceae) are among the most widely planted forestry species and of these, *Eucalyptus globulus* Labill. is one of the most common (Boyd, 1996). This species has been introduced around the world for wood products and horticulture (Boyd, 1996; Rejmánek *et al.*, 2005; Becerra, 2006; Joshi, 2012; Dixit *et al.*, 2012). *E. globulus* does not commonly spread into native vegetation and thus is not usually considered a strongly "invasive" species (but see Boyd, 1996; Becerra, 2006). Nevertheless, several *Eucalyptus* species produce leaf litter containing allelopathic compounds that are commonly associated with poor understories due to suppression of co-occurring plant species (Baker, 1966; del Moral & Muller, 1969, 1970; May & Ash, 1990; Sasikumar *et al.*, 2001; Graca *et al.*, 2002). Rabotnov (1982) observed that *E. globulus* trees introduced to California and Western Europe develop species-poor understories in comparison to plant communities just beyond the *E. globulus* stands (also see del Moral and Muller, 1969). However, it is unknown if this also occurs in the understory of *E. globulus* patches in the native range of Australia. Consequently, we evaluated how plant communities vary with the presence of *E. globulus* trees both in its native range in Australia and different regions of the world where it has been introduced as an exotic, and examined if this biogeographic pattern may be related to the Novel Weapons Hypothesis. We measured species richness and plant height of communities under *E. globulus* canopies and in adjacent areas without tree canopies, and compared the effects of litter leachates on the growth of species from the native and non-native ranges in a laboratory experiment.

METHODS

Field study

We conducted vegetation surveys in four countries where *E. globulus* is non-native (India, Chile, USA, Portugal) and Australia, where it is native. *Eucalyptus globulus* is native to New South Wales, Victoria and Tasmania, and different intra-specific taxa have been defined (*E. globulus subsp. bicostata*, *E. globulus subsp. globulus*, *E. globulus subsp. maidenii, E. globulus subsp. pseudoglobulus*). The total native range for this group of taxa is 30°12' – 43°30' S and 115°30' - 152°45' E. In this study we used *E. globulus ssp. globulus* (core native range: 32°25' - 43°30' S, 147°45' - 152°45' E) because it has been widely introduced to other countries. In Portugal, this species is distributed (in plantations or naturalized) between 37° and 42° N, 7° and 9° W. In Chile, *E. globulus* has been planted between 32° and 40° S, mainly near coastal areas between 71°40' and 74°00' W. In North America, *E. globulus* is distributed between 32° and 40° N and between 120° and 124° W, approximately. In India, this species is distributed between 11° and 25° N and between 72°36' and 82°30' E. Within each of these five countries, we sampled two sites (Appendix S1 in Supporting Information). All sites have either Mediterranean, seasonal temperate, or subtropical climates and their original native vegetation ranged from shrublands to forests. All sites had experienced some level of anthropogenic disturbance producing open areas mixed with shrub or forest patches, and thus, vegetation in the study sites corresponded to grasslands, open shrublands or open forests (Appendix S1 in Supporting Information).

Using aerial photographs, we selected two sites in each country that were accessible, extended over 3-5 ha and contained adult *E. globulus* individuals (i.e. 20-50 cm DBH). We avoided recent forestry plantations of *E. globulus*. In the non-native range, study sites were chosen in areas with naturalized individuals of *E. globulus*, although in some cases a few individuals present in the site were probably planted within the last century (Appendix S1 in Supporting Information). In all sites of the non-native range, *E. globulus* trees were growing in areas that were probably treeless or had low woody cover due to historical human disturbances (such as fires, logging, livestock). In the native range of Australia, the sampled *E. globulus* trees were growing in *E. globulus* stands.

The aim of our field study was to examine if plant communities growing under, and potentially influenced by, *E. globulus* trees were different to plant communities growing in areas with no influence of *E. globulus*, the latter of which we used as experimental controls. We preferred to use open areas with no other tree species as controls in order to homogenize controls among countries. Using other trees as controls might have increased (uncontrolled) variability among countries due to different tree species composition among them. Open areas did not have *E. globulus* leaf or bark litter. The open areas may have been shaded for a few hours each day, but light levels would have been higher there than under canopy of *E. globulus*. Although using open areas compromises our study design by confounding light availability and *E. globulus* effects, our study is focused in comparing the magnitude of the difference among countries (i.e. an effect size, not a raw value). As such, this design and analytical approach still enabled us to compare how effects of *E. globulus* might differ between its native and non-native ranges.

In each of the two sites in each of the countries of the native and non-native range, we sampled the plant community in 1 x 1 m plots in areas under canopy of 20 randomly selected adult *E. globulus* trees and 20 randomly chosen open areas (without tree canopy). In each plot, we recorded all vascular plant species present and measured the height of the vegetation at four random locations within the plot (except in India for the height). We selected adult trees similar in size (> 15 m height and at least 20 cm DBH), although variability in size among trees was unavoidable, and may have increased variability in our results. Under the canopy of each tree, we randomly selected a direction (compass bearing) and located 1 plot midway between the canopy edge and the trunk of the tree. In the non-native range, for each under-canopy measurement, another paired 1 x 1 m plot was randomly located in surrounding open areas free of *E. globulus* (or other tree species). To do this, we randomly selected a direction and a distance between 5-20 m from the sampled trees toward an adjacent open area. In non-native sites individual trees of *E. globulus* were isolated and irregularly distributed within each site, which facilitated to find open areas. However, in Australia, the trees of *E. globulus* do not grow as isolated as they do in the non-native ranges, therefore we sampled under individuals of *E. globulus* present in more closed stands. For this reason, in Australia, open plots were located in sites that had been cleared for roads and power lines over 50 years ago, and were adjacent to the sampled *E. globulus* stands. In these open areas, from a starting point, we randomly choose a direction and distance up to 40 m where we located the plot. Then, the next plots were located by the same protocol.

Because *E. globulus* trees in the non-native ranges have been growing for many years, we assumed that other species at these sites have had the chance to disperse and grow either under or outside the canopy of them. Abiotic conditions such as soil type, climate, topography, and elevation were similar for the plots located under *E. globulus* canopies and open areas in each site. Because in each site sampled *E. globulus* trees were near to either grassland, shrubland or forest patches, the local species pool available to grow under *E. globulus* and in open areas was composed of species adapted to grow in closed vegetation as well as species adapted to grow in open areas. Similarly, for Australia, we assumed that after clearing to install roads or power lines, there was enough time for ruderal plants adapted to grow in open areas to colonize these cleared areas and potentially invade adjacent *E. globulus* stands, and time for understory plants to recolonize open areas from the same species pool as *E. globulus* understories. On the other hand, in the native range, plots under *E. globulus* were in some cases located under more than one tree, in contrast to the non-native sites where these plots were located under only one tree. Therefore, potential inhibitory effects from *E. globulus* on the understory might be stronger in native regions than in non-native regions. This made our analyses more conservative since, according to the Novel Weapon Hypothesis, the effect of *E. globulus* in the native range should be weaker than in non-native regions. In any case, and to correct for potential environmental and historical differences, we use proportional values instead of raw counts when comparing among sites and regions.

Glasshouse experiment

The aim of this experiment was to evaluate if leachates produced by *E. globulus* litter produces different effects on species native to the non-native and native range of *E. globulus*. Following the general protocol of del Moral and Muller (1969), we collected naturally occurring litter beneath a stand of mature *E. globulus* trees near Santa Barbara, CA, USA (34.585717° N; -119.501542° W; 24 m elevation). This site was not sampled for community attributes. Acquiring litter from other ranges was avoided because of the possibility of introducing unwanted biota. We used litter recently produced and as it naturally occurred, consisting of about 90% leaves but including some shed bark, small twigs, and seed capsules. The litter was a rough average of what occurred in a 25 x 25 cm area and 5 cm depth in the stand where litter was collected. A 2.5 mm precipitation event would drop a little over 1.5 L in an area of this size. So, this volume of litter was collected and mixed with this volume of water to conduct the irrigation by decantation, simulating as close as possible the conditions experienced by seedlings in the field.

Species used in this experiment were selected because their presence in the same sites were the field study was conducted or because they are distributed in the same geographic area and habitats as *E. globulus* occurs either in its native and non-native range. Seeds from Chile and India were field-collected by authors at the same sites where the field study was conducted. Seeds from California and Australia were field-collected by professional seed companies in the regions where *E. globulus* occurred. We attempted to choose species that were relatively common and widespread in our study sites, but our acquisition and utilization of species for this experiment was determined by availability and germination as well.

We conducted two trials, each with different target species. For the first trial, leachate was produced on Feb 1st, 2011 with a dry weight of 21 g of *E. globulus* litter placed in 1.5 L of distilled water for 72 hours at 5°C. We estimated that this would be roughly equivalent to a 2-cm rainfall event. This leachate was applied to seeds of 13 target species (Appendix S2 in Supporting Information): 10 species that are native to either California, Chile, or India (the non-native ranges of *Eucalyptus*) and three species native to Australia. For the second trial we produced leachate in the same way on May 24th, using the same concentration, and applied this leachate to eight new species: three species native to Australia and five species native to the exotic range (Appendix S2 in Supporting Information). In each trial, 10-15 seeds of each species were placed into each of 6 Petri dishes (60 mm diameter) on top of a sheet of Whatman Grade 1 filter paper (Sigma-Aldrich). One mL of leachate was placed in each of three randomly chosen Petri dishes and 1 mL of distilled water was placed in the other three dishes as a control. Seedlings germinated and grew in the dark for 4-12 days. Root length of all seedlings of each species was measured using the same number of days since germination, but as species germinated at substantially different rates the timing of the measurements differed among them; however, root length measurements were taken at the same time for the two treatments for each species. Although we used a very small fraction of the potential species pool of each site in the experiment, most of the 21 species were observed or collected at the studied field sites in each biogeographical region, thus can be considered at least partially representative of the flora in the non-native and native regions.

Data analyses and statistics

For the field data, we first analyzed the change in species richness between open sites and under *E. globulus* canopy. A global test on the raw number of species present in each of the environments (open vs. under *E. globulus*) could be misleading, since each of the studied countries might have inherent differences in species richness. Consequently, we standardized species richness data by means of Relative Interaction Indexes (RII; Armas et al., 2004). RII's are linear, have defined limits (-1, +1), are symmetrical around zero, and produce approximately normal distributions. We used species richness between under *E. globulus* and in open controls to calculate RII's reflecting the intensity of species suppression caused by *E. globulus* presence. Linear Mixed-Effects models with nested random effects based on Laird and Ware (1982) were built in the statistical platform R 3.10 (R Development Core Team, 2014) with the procedure "lme" from library "nlme" (Pinheiro et al., 2017). We then tested for consistency in the difference (RII) in species richness between each open/under *E. globulus* plot-pair among regions by using "range" (native/non-native) as a fixed factor, "site" and "country" as random factors, with "site" nested within "country". Additionally, we evaluated the percentage of species present under *E. globulus* canopies that were unique to *E. globulus* canopies in relation to the total number of species present in each site. This count was carried out for each site and country separately. Separately, we tested for inter-regional differences in understory height, with "range" and "treatment" (open/under *E. globulus*) as fixed factors, and "country" and "site" as random nested factors, with "site" nested within "country".

For the glasshouse experiment, we assessed changes in root length by means of Linear Mixed-Effects models with the procedure “lme” from library “nlme”, and tested for inter-regional differences in root growth with “range” and “treatment” (leachate/control) as fixed factors, and “country” as a random factor. The factors “species” within “genus” within “family” were also added as nested random factors to the model to account by phylogeny in the model. We also used t-tests ($P < 0.05$) to evaluate the effect of leachate treatment on root growth of each species separately.

RESULTS

Field patterns

The species composition of *E. globulus* understories and open areas at our sites was mainly herbaceous, except in Portugal where shrubs were more diverse than other groups (Appendix S3 in Supporting Information). Understory species at our sites in Chile and USA were mostly exotic, and almost all of these species were Eurasian, whereas in Portugal, India and Australia the species were primarily native to these countries (Appendix S3 in Supporting Information).

There were more species in the open grassland-shrubland surrounding *E. globulus* trees than under canopies at sites in both the native and non-native ranges of *E. globulus* (Fig. 1; Table 1). However, this reduction in species richness under *E. globulus* canopies was stronger in non-native regions than in the native region of Australia (L. ratio=5.605; df=2,5; p=0.0179). Relatively little remaining variation was explained by random effects ($SD_{intercept}=0.097$; $SD_{residuals} = 0.254$) suggesting that differences among and within non-native regions were small. In the native range of *E. globulus*, there was a large number and percentage of species unique to *Eucalyptus* understories among all species present in these plots, varying from 30.4 to 35.6% of the observed species pool (Table 1) with an average of 33.0% ± 2.60 (1 SE). In the non-native ranges, there were far fewer species unique to *E.*

globulus understories, varying from 0 to 21.4% (Table 1), with an average of 7.7% ± 2.66 (1 SE). Across all sites, mean understory height was lower under *E. globulus* canopies than in the open areas (L. ratio=133.160; df=3,7; $p<0.001$) (Fig. 2). However, we found a statistically significant interaction between range and canopy treatment (L. ratio=45.159; df=4,10; $p<0.001$), indicating that the greater understory height in open areas than under *E. globulus* was observed mainly in the non-native ranges (Fig. 2).

Glasshouse experiment

The leachate treatment highly suppressed the root growth of all species originating from the non-native range (USA, Chile, or India, -45% to -100%) but only for some species native to Australia. Consequently, there was significant interaction between the factors "range" and "treatment" (L. ratio=114.737; df=3,8; $p<0.001$). Of the six species native to Australia, the effect of litter leachate varied from two significantly positive responses of the root growth (+18.7 and +48%, t-tests: $P < 0.05$), two non-significant effects, and two significantly negative response (-15.9% and -56.8%; t-tests: $P < 0.05$) (Fig. 3). The mean reduction for Australian natives was -1.0±14.3% versus -71±4.4% for natives from California, Chile and India.

DISCUSSION

The lower richness and height of plants in *E. globulus* understories that we recorded across both the native and invaded range suggests negative effects of *E. globulus* on understory species, and corresponds with a widely but anecdotally reported pattern of species-poor understories beneath *Eucalyptus spp.* canopies. A number of different mechanisms may cause these negative effects, but we found that many plant species exposed to leachates of *E. globulus* were suppressed. These included some species native to the home range of *E. globulus* and all species from regions where *E. globulus* had been introduced, suggesting that the allelopathic effects of *Eucalyptus* litter may contribute to lower species richness and plant growth in *E. globulus* understories.

Other studies of *E. globulus* and other *Eucalyptus* species have also shown strong allelopathic effects on species from different biogeographical regions where *Eucalyptus* species have been introduced (Baker, 1966; del Moral & Muller, 1970; May & Ash, 1990; Sasikumar *et al.*, 2001). We do not know the biochemicals responsible for the effects of litter and leachate from *E. globulus* in our study. However, leaves of this and other *Eucalyptus* species possess a diverse and variable suite of biochemicals, some of which are grouped under the term "eucalyptol" (Elaissi *et al.*, 2012). Oil volatiles, in particular chlorogenic acid, have been identified as possible active allelochemicals in *E. globulus* (May & Ash 1990). In direct and soil-based bioassays, Del Moral and Muller (1969) found that natural fog drip collected from beneath *E. globulus* suppressed the European annual grass, *Bromus diandrus*, probably via chlorogenic, p-coumarylquinic, and gentisic acids. They also found that artificial fog drip inhibited six other species of European grasses now found in California. Konar & Kushari (1989) compared the effects of leaf leachates from three tree species native to India on *Costus speciosus*, another Indian native, to those of *E. globulus*. They found that the Indian natives enhanced the growth of *Costus* whereas *E. globulus* leachate suppressed *Costus*. May and Ash (1990) found that *E. globulus* litter extracts suppressed the growth of several species, both when applied directly to seeds and seedlings, and through soil. Kohli & Singh (1991) found that crude volatile oils from the leaves of *E. globulus* and *E. citriodora* and the pure terpenes from these oils inhibited the growth of a number of crop plants and species native to Asia. Del Moral & Muller (1970), Sasikumar *et al.et al.* (2001), Zhang *et al.et al.* (2010) and Elaissi *et al.et al.* (2012) have reported allelopathic effects of other *Eucalyptus* species.

Any potential allelopathic effects of *E. globulus* are highly likely to interact with other mechanisms, for example, resource competition or acquisition. Suppression of root growth such as we observed in our experiment could impede the ability of understory plants to reach deeper and moister soil layers. We focused on the effects of leachate from litter directly applied to seedlings in artificial conditions, but this mode of biochemical delivery is reasonable as litter can come in direct contact with seeds and seedlings without soil mediation. We did not explore how soil might modify leachate effects (Kaur *et al.*, 2012), but *E. globulus* litter might function indirectly through effects on soil biota (Callaway & Ridenour, 2004; Callaway *et al.*, 2008; Martins *et al.*, 2013; also see Canhoto & Laranjeira, 2007). It is important to note that we used litter only from *E. globulus* trees in California, and we do not know if leachates produced by *E. globulus* in other countries might have different effects.

Reductions in species richness and plant height under *E. globulus* canopies were much greater in the non-native ranges than in the native range. Furthermore, there were more species exclusive to the *Eucalyptus* forest understory in the native range, which also suggests that many Australian understory species might have adapted to any biochemical or other effects of *Eucalyptus*. It is also important to note that some understory species observed in the non-native range were not native to those countries, most notably in Chile and USA. The large majority of these species were native to Europe and thus do not have a long history of interacting with *E. globulus*. Our results are consistent with the Novel Weapons Hypothesis, which poses that some exotic invasive species might take advantage of the production of allelopathic, antimicrobial, or herbivore defense compounds that are not produced by other species in the non-native ranges (e.g. Callaway & Aschehoug, 2000; Callaway & Ridenour, 2004; Inderjit *et al.*, 2011; Colvin & Gliessman, 2011; Lamarque *et al.*, 2011; Kaur *et al.*, 2012; Qin *et al.*, 2013; Svensson *et al.*, 2013).

Although the stronger effect of *E. globulus* trees on plant communities in its invaded ranges compared to its home range might be produced, at least in part, by the weaker tolerance of understory plants to biochemical compounds produced by *E. globulus* litter, other mechanisms are likely to also play a role in these biogeographical differences. First, invasive species are often larger, grow faster, or attain greater population density in their non-native range (Callaway & Ridenour, 2004; Joshi &

Vrieling, 2005), thus they can likely drive greater competitive impact simply through greater abundance or performance. Nevertheless, we evaluated plant communities under individual mature trees of *E. globulus* in all regions, hence the abundance of *E. globulus* was effectively controlled in our survey, although different size among them could produce variability, for example in the impact of each tree. Second, *Eucalyptus* species often use much more water than many other species (Dvorak 2012) and therefore soils under *E. globulus* canopy could be drier than areas without trees of this species in each region. This may also have produced poorer plant communities under *E. globulus* canopies. Third, light reductions under canopies of *E. globulus* may also have influenced the field pattern described in our results since our control plots were not forested. Fourth, species can interact very differently with variation in climate or other abiotic conditions (Cavieres *et al.*, 2014). The climates of the four non-native regions that we studied have strongly seasonal precipitation (Mediterranean climates in USA-California, Portugal, and central Chile and monsoonal seasonal in India). In contrast, the climate in the native range has precipitation spread more evenly through the year, although it is concentrated in winter. If climate in the native region is more favorable for *E. globulus* than climate in non-native regions (Becerra & Bustamante, 2011), according to the stress gradient hypothesis more negative effects from *E. globulus* on species richness would have been more likely in the native range (Cavieres *et al.*, 2014). However, we found the opposite pattern, stronger negative effects in the exotic ranges, thus climate does not seem likely to drive the biogeographic differences we found.

Although *E. globulus* is not highly invasive in the non-native regions included in our study (Becerra, 2006; Cal-IPC, 2006; Kirkpatrick, 1977), *Eucalyptus* species are important forestry species, with thousands of hectares planted around the world (Boyd, 1996; Rejmánek *et al.*, 2005; Joshi, 2012; Dixit *et al.*, 2012). Thus, our results are also relevant to the conservation of biodiversity within plantations. Our regionally explicit results add to the body of knowledge suggesting the possibility of regional evolutionary trajectories involving the biochemistry of plants (e.g. Thompson, 2005), and have interesting implications for community ecology. First, they add to a growing body of literature (e.g. May & Ash, 1990; Callaway & Aschehoug, 2000; Ni *et al.*, 2010) indicating that there is some degree of species-specificity in the effects of plant-released secondary metabolites in plant-plant interactions. Second, our results are consistent with the idea that some invaders may succeed because they possess biologically active biochemicals to which species in the non-native range have not adapted.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Regions and localities where the patterns of naturally growing vegetation were sampled.

Appendix S2 Species included in the glass-house experiment.

Appendix S3 Flora of each country observed in the field study.

DATA ACCESIBILITY

Data will be made available at figshare.org. Data can be also made privately available to reviewers and editors upon request.

BIOSKETCH

The research team is comprised by researchers from different countries, but all of them interested in studying the causes and consequences of biological invasions, as well as ecological mechanisms underlying these processes.

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Table 1 Values of species diversity found in all regions. S-values indicate total number of species observed either under *Eucalyptus globulus* canopies or in open adjacent areas. The total number of shared species between open sites and under canopy is also presented, as well as the percentage of unique species to *Eucalyptus* in relation to the total number of different species in each site ((S-under – shared)/ (S–open + S-under – shared)).

Country	Locality	S – open (N° sp)	S – under (N° sp)	Shared (N° sp)	Percentage of unique species to *Eucalyptus* canopy (%)
India	Finger Post	28	4	1	9.7
	Santinalla	33	20	11	21.4
USA	St. Barbara	30	11	8	9.1
	St. Cruz	20	9	7	9.1
Portugal	Coimbra	21	10	7	12.5
	Lousã	17	4	4	0
Chile	Alhue	14	13	13	0
	Puchuncavi	8	6	6	0
Australia	Lorne	32	30	16	30.4
	Aireys Inlet	47	42	16	35.6

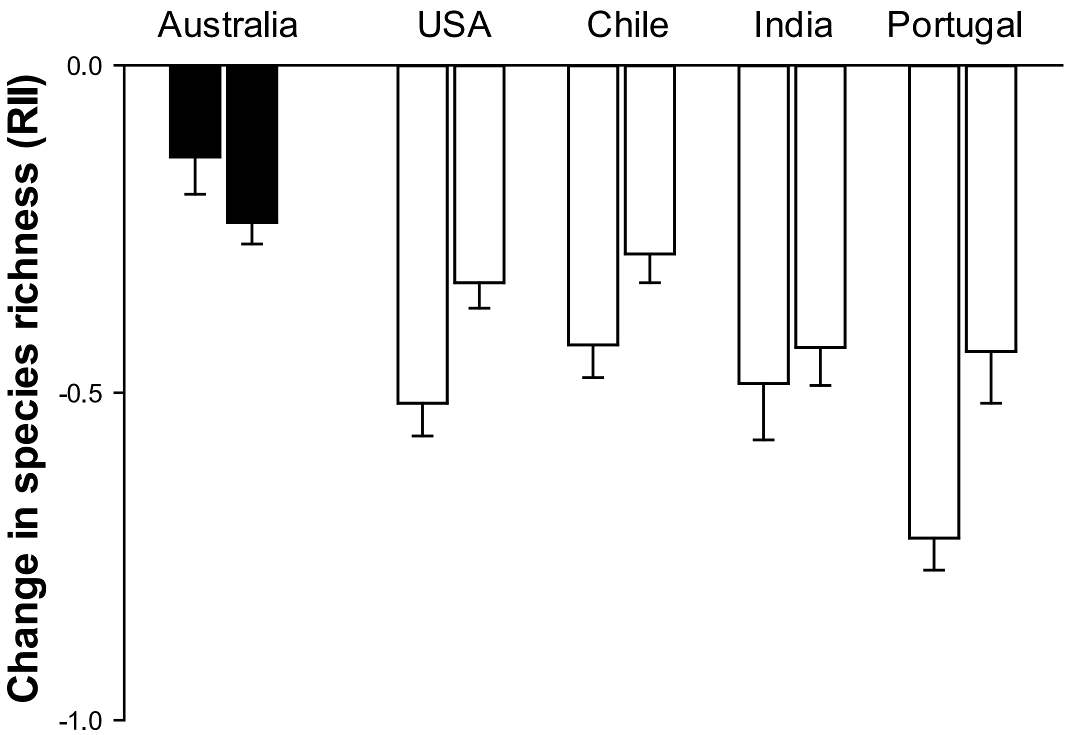

Figure 1 RII values for species richness observed under the canopies of *E. globulus* relative to open areas. Each bar indicates a site sampled in countries corresponding to the native (Australia) and non-native (USA, Chile, Portugal, India) range of *E. globulus*. Error below means represent 1 SE.

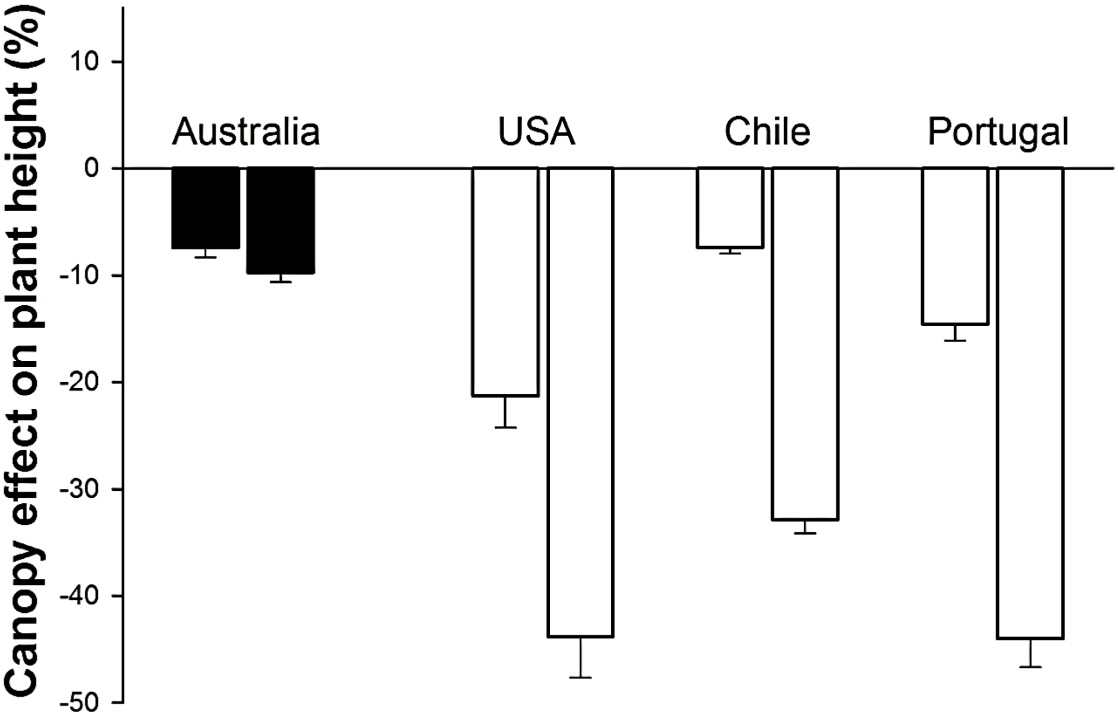

Figure 2 Variation in the effect of the *E. globulus* canopy on understory height between native (Australia) and non-native ranges (USA, Portugal, Chile). Each bar indicates a site sampled in countries corresponding to the native (Australia) and non-native (USA, Chile, Portugal) range of *E. globulus*. Values on the y axis are calculated as the difference between the mean plant height (cm; mean ± 1SE) of vegetation under *E. globulus* patches and in open areas, divided by the height in open areas for all pairs of plots at a given site.

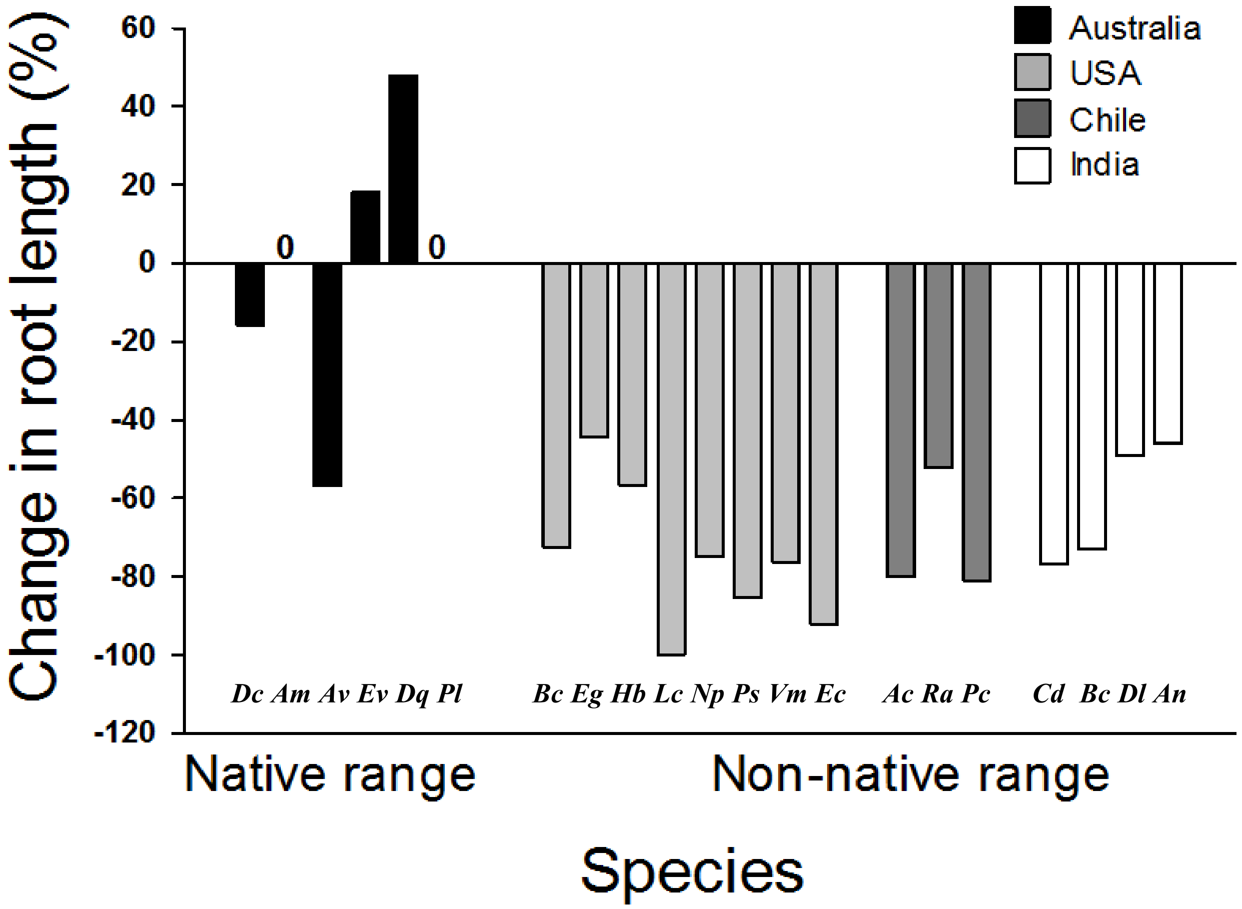

Figure 3 Effect of leachates on root length. Each bar represents a different species either native to the native range (Australia) or non-native range (USA, Chile, India) of *E. globulus*. The *y*-axis shows the mean percentage decrease in root growth in leachates, relative to root growth in the controls for each species. Values correspond to the average among different petri dishes used for each species in controls and treatments with leachates. Species corresponding to each bar are indicated below them (*Dc: Dichelachne crinita, Am: Acacia melanoxylon, Av: Acacia verticallata, Ev: Eucalyptus viminales, Dq:Deyeuxia quadriseta, Pl: Poa labillardieri, Bc: Bromus carinatus, Eg: Elymus glaucus, Hb: Hordeum brachyantherum, Lc: Lasthemia californica, Np: Nasella pulchra, Ps: Poa secunda, Vm: Vulpia microstachys, Ec: Escholzia californica, Ac: Amsincckia calycina, Ra: Rodophiala advena, Pc: Pasithea coerulea, Cd: Chloris dolichostachya, Bc: Brassica campentris, Dl: Dalbergia latifolia, An: Acacia nilotica*).