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Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*

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Abstract Invasive plant species have been suggested to change the composition of the soil community in a way that results in a positive feedback for them and a negative feedback for the native plant community. *Carpobrotus edulis,* a species native to South Africa, is one of the most aggressive exotic species in Mediterranean Europe. Although several aspects of its invasion biology have been studied, the occurrence of plant-soil feedback has been scarcely investigated. We first checked for the existence of biotic resistance in soils from two invaded sites of Mediterranean Europe and one site in the native area. Secondly, we evaluated the effects of soil conditioning on the germination and plant growth of *C. edulis* and two key species of native dunes. Finally, we tested the effects of short- and long-term soil conditioning on the performance and reproductive effort of *C. edulis*. Our results show that at first there is a natural resistance to invasion by the soil biota. Later, biotic resistance in invaded soil is suppressed by the establishment of a soil community that enhances the growth of *C. edulis* and that negatively influences the growth and survival of the native plants. Long-term soil conditioning in the field resulted in shifts in the balance between vegetative growth and sexual reproduction. Long-term invasion was also reflected in high levels of endophyte colonization by chytrids in roots, although the physiological consequences of this colonization remain unknown. The results obtained illustrate a mechanism that explains how *C. edulis* breaks the initial biotic resistance of newly-invaded landscapes. Finally, this study highlights the importance of studying plant-soil interactions on different members of the plant community and temporal stages in order to fully understand invasion.

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Introduction

Plants selectively affect the soil biota associated with their rhizosphere and, as a consequence, feedback interactions are established between plant and soil (Bever et al. 1997; Reynolds et al. 2003; van der Putten 2003). The direction of the feedback, whether negative or positive, depends on the net effect of the

different soil organisms (e.g., soil-borne pathogens, plant mutualists, and root-herbivores) on plant growth and fitness. Plant-soil feedback is crucial to define the spatial and temporal dynamics of natural plant communities (see reviews by Ehrenfeld et al. (2005) and Kulmatiski et al. (2008)).

Currently, there is considerable evidence that the soil biota can play a determinant role in invasion processes by exotic plant species (te Beest et al. 2009; Reinhart and Callaway 2006; Levine et al. 2006; Reinhart et al. 2005). Exotic species may thrive in new areas because of the release of natural soil enemies (Agrawal et al. 2005; Reinhart et al. 2005; Knevel et al. 2004), but they can also modify the soil community of the invaded soil to: (1) establish positive feedbacks that will promote the invasion (Richardson et al. 2000; Rodríguez-Echeverría et al. 2009); or (2) accumulate organisms that are detrimental to the native community, thereby creating a negative feedback for the native plants (Mangla and Inderjit 2008; Eppinga et al. 2006; Nijjer et al. 2007).

Carpobrotus edulis (L.) N. E. Br. is a clonal plant originally native to South Africa. It can also reproduce sexually, producing conspicuous flowers that result in fruits that contain numerous seeds. This species is considered to be highly invasive in coastal areas of Mediterranean Europe. Most studies so far have focused on the effects of *Carpobrotus* spp. on the invaded community (Bartomeus et al. 2008; Jakobsson et al. 2008; Suehs et al. 2001; Weber and D'Antonio 1999; D'Antonio et al. 1993; D'Antonio 1990) as well as clonality and growth (Roiloa et al. 2010. Traveset et al. 2008; Sintes et al. 2007). Based on these studies, it appears that *Carpobrotus edulis* has a significant impact on the diversity, structure and dynamics of the native vegetation. Moreover, the invasion of *C. edulis* has a dramatic impact on the characteristics of the invaded soil, leading to an increase in organic matter content and a decrease of pH (D'Antonio and Mahall 1991; Vilà et al. 2006; Conser and Connor 2009). However, assessing whether plant-soil feedback is important for the establishment and dynamics of *C. edulis* populations has so far received little attention (Van Grunsven et al. 2009).

Interestingly, recent research at multiple sites (i.e. Islands) across the European Mediterranean region has indicated that, in general, these sites showed high levels of resistance to the establishment of *Carpobrotus* spp. (Vilà et al. 2008). A direct release of soil-borne

pathogens has also been proposed as an explanation for the success of *C. edulis* in the Mediterranean (Van Grunsven et al. 2009), which contradicts the study by Vilà et al. (2008), at least for the experimentally-tested soils. Therefore, in spite of the information available about this invasive species, the mechanisms of invasion and the role of plant-soil feedback in this process are still not clearly understood.

Residual effects on soil characteristics due to long-term invasion by *C. edulis* have been shown to hamper the establishment and development of the native plant community in California (Conser and Connor 2009). However, whether or not those residual effects were mediated by changes in the soil community was not studied. The long-term impact of soil residual effects on *C. edulis* performance is also unknown. Environmental conditions (e.g. nutrient and water availability, photoperiod, etc.) can induce clonal plants to allocate resources for vegetative growth or sexual reproduction (Bai et al. 2009; Hesse et al. 2008). The physiological changes that trigger sexual reproduction can also be affected by different soil organisms (Perner et al. 2007) and, therefore, plant-soil feedback may play an important role in determining these reproductive shifts. In the specific case of *C. edulis,* Traveset et al. (2008) found that flower density in patches of different invaded sites was correlated with different levels of soil N and pH, which suggests that soil plays a determinant role in the reproductive plasticity of this species.

Experimental evidence is needed to demonstrate whether: (1) biotic resistance takes place; and (2) plant-soil feedback is responsible for the breakage of the initial biotic resistance. We hypothesized that the introduction of *C. edulis* into a new area may build a soil community that can condition the establishment and success of the invader as well as other native plant species. If that holds true, changes introduced in the soil community by *C. edulis* would contribute to the disruption of the initial biotic resistance, and consequently determine the invasive success of this species. In order to test this general hypothesis, we performed a series of experiments in which the effects of soil biota on this invasive species, as well as on two other key native species, were tested at different stages of invasion. We first checked for the presence of biotic resistance in the soil of two invaded sites in Mediterranean Europe, comparing these samples with soil from an area where *C. edulis*

is native (South Africa). Secondly, we evaluated the effects of soil conditioning on seed germination and plant growth of the invasive *C. edulis* and two native species (i.e. *Ammophila arenaria* and *Cistus salviifolius*). Soil conditioning in plant-soil feedback experiments is performed to mimic and simulate the selection *sensu stricto* that occurs in the soil biota after initial establishment (Bever 2003). These changes may determine the performance of the plant, the interactions with other members of the community, and the success of the establishment of invader offspring. Finally, because perennial plants also cause soil changes that are not easily reproducible by short-term soil conditioning in the laboratory, we also studied the effect of natural field soil associated with different stages of invasion on *C. edulis* performance.

Materials and methods

Site descriptions and collection of soil for experiments

For the establishment of all experiments we collected rhizospheric soil under the dominating vegetation on two localities where *Carpobrotus edulis* is regarded as an invasive species (i.e. in Portugal and Spain) and in one locality where the species occurs within the natural range of distribution (i.e. South Africa).

Portugal: In February 2008, we collected soil at the Mata Nacional das dunas de Quiaios in Central Portugal (40°13′N, 8°53′W). Although Portuguese Atlantic dunes seem quite resilient to the invasion of *C. edulis* (Maltez-Mouro et al. 2010), at this particular site *Carpobrotus edulis* is considered to be a serious threat for the indigenous dune plant community. In terms of phytogeographical typology, the study site consists of the typical coastal dune flora, including *Euphorbia paralias*, *A. arenaria*, *Corema album*, *Medicago marina*, *C. salviifolius*, *Cytisus grandiflorus* and some reintroduced *Pinus pinaster*. The site corresponded with a Mediterranean *Ammophiletea* association (transition from class *Otantho maritimi-Ammophiletum australis* to the class *Querceta ilicis*). For a detailed description of the flora at the place of study, see Roiloa et al. (2010). Ten soil samples of ca 1 kg were collected under patches with natural vegetation mixtures at least 25 m apart from each other.

Spain: In April 2008, we collected soil from the dunes of Es Trenc (39°20′N, 2°59′E) in the island of Mallorca. The dunes behind the beach form a well-preserved set of Mediterranean coastal dunes with typical foredune vegetation, including *A. arenaria* and *Elymus* spp. and inner areas covered with small shrubs, such as *Juniperus phoenicea* and other typical Mediterranean woody species including *Pistacia lentiscus*, *Cistus monspelliensis*, and *C. salviifolius*. In phytosociological terms, the area corresponds with a typical Mediterranean *Ammophiletum* class. Ten soil samples of ca 1 kg were collected under natural vegetation mixtures at least 25 m apart from each other.

South Africa: In May 2008, soil samples were collected in Lamberts Bay. This site (32°04′S, 18°19′E) is situated on the Western coast of South Africa where *C. edulis* grows vigorously in areas adjacent to the shoreline. Ten soil samples of ca 1 kg were collected under mature patches of *C. edulis* at least 50 m apart from each other. Soil was stored in plastic bags and sent to Belgium within 48 h. Upon arrival, soil samples were stored in a refrigerator at 6°C until use.

Preparation of plant material

Carpobrotus edulis seeds were bought from Chiltern Seeds (Bortree Stile, Ulverston, Cumbria, UK). *A. arenaria* and *C. salviifolius* seeds were collected from the dunes of Quiaios, Portugal in summer 2007. Seeds were kept in a cool and dry place until the set-up of the experiments. The seeds used to generate plants for all experiments were surface sterilised by submersion in a 4% household bleach solution for 20 s. They were then washed 10× with distilled water, submerged for 40 s in a 10% ethanol solution, followed by a further 10× washes with distilled water. After surface sterilization, seeds were germinated in plastic incubators (17.5 cm × 13 cm × 6 cm) filled with sterilized river white sand (±2 cm depth) and moistened with demineralized water until soil saturation. Between 75 and 100 seeds per species were placed in each incubator. Scarification was necessary for the germination of the *C. salviifolius* seeds. Seed containers were set under 36-watt lights, with a 16 h light/8 h dark photoperiod regime at 23 ± 1°C and 35% RH. After germination, seedlings were left to grow for 2 weeks and at this stage were transplanted to experimental pots for experiments 1, 2, 3, 5 and 6 (see further).

Commercial white river sand (Hubo®, Belgium) was used in all experiments, unless otherwise stated. Before use, sand was autoclaved (1 atm, 120°C) for 1 h. Soil from Portugal, Spain and South Africa was separated into two portions. One half of the soil sample from each source was autoclaved, and the other half was left unsterilized. Pots (1.2 l) were filled with 1 l of sterilized autoclaved river sand and inoculated with either sterilized or non-sterilized soil from each of the sites (i.e. Portugal, Mallorca and South Africa). Soil was used as the inoculum at a rate of 85 ml/pot. This was done to avoid changes in the nutrient content of the soil due to different abiotic soil characteristics from the different soil origins. All pots were watered every 2 days with 50 ml of demineralized water, and fertilized every 3 weeks with 50 ml of a commercial fertilizer solution (COMPO®, NPK 16-18-25) at the concentration recommended by the manufacturer (i.e. 1 g l^{-1}).

Experiments 1–4 were designed to analyze the effects of soil conditions and plant-soil feedback on *C. edulis* performance. Experiments 5 and 6 tested the effects of soil conditioning by *C. edulis* on germination and growth of the native plant species *A. arenaria* and *C. salviifolius*. Plant biomass was assessed in all experiments as follows: plants were gently uprooted and washed at harvest, put into paper envelopes and oven-dried at 70°C for 48 h.

Experiment 1: biotic resistance of newly-invaded soils

We used an experimental set-up similar to other studies addressing the effect of the soil community on plant performance (Reinhart et al. 2005). Using a full factorial design, we tested the effects of site (Portugal, Mallorca and South Africa) and soil biota (sterilized vs. non-sterilized soil inoculum) on *C. edulis* growth. A 2-week old *C. edulis* seedling was transferred to each pot and grown in the conditions described above for nine weeks. Pots were placed randomly under the lights of an indoor growth chamber at 23 ± 1°C and 35% RH. Seven replicates were used per treatment.

Experiment 2: effects of soil conditioning in the performance of *Carpobrotus edulis*

We used a similar set-up to that in the previous experiment but this time with a previous soil conditioning phase, as used in Bever et al. 1997. The experiment also compared the effects of site (Portugal, Mallorca and South Africa) and soil biota (sterilized vs. non-sterilized soil inoculum). For the soil conditioning phase, two seedlings of *C. edulis* were transplanted to each experimental pot, prepared as described above and grown for 12 weeks. After the 12-week conditioning period, the *C. edulis* seedlings were removed and replaced by one seedling of the same species per pot. Plants were harvested after 10 weeks. We had eight replicas per treatment. Experimental conditions were as in experiment 1.

Experiment 3: effects of soil conditioning on the germination of *Carpobrotus edulis*

Soil conditioning was performed as for experiment 2 but in this case a non-conditioned control was prepared by maintaining pots without plants during the 12-week period. After this conditioning, the soil from each site and type of inoculation was mixed thoroughly and used to fill small pots (7 × 5 × 10 cm) for the germination test. The experiment compared the effects of site (Mallorca vs. Portugal), soil biota (sterilized vs. non-sterilized inoculum), and conditioning (conditioning vs. non conditioning). Each treatment was replicated five times, and each container received 50 seeds. The plastic pots were placed randomly under lights (36 watts, 16 h light/8 h dark regime) in a growth chamber set at 23 ± 1° C and 35% RH. All pots were watered with demineralized water and checked every other day to record the number of germinated seeds. Seed germination was monitored for eight weeks, and screening was stopped when the percentages of germination did not vary after one week.

Experiment 4: effects of long-term natural soil conditioning on *Carpobrotus edulis*

Since it was not feasible to mimic soil conditioned by years of invasion by *C. edulis* in the field, rhizospheric soil was collected from an invaded area (i.e. Dunas de Quiaios, Portugal) and used to set up an experiment to determine the adult plant responses to soil invaded for different lengths of time: (1) bare soil surrounding the natural native plant community, where the presence of *C. edulis* has not been recorded during the past 15 years (hereafter, uninvaded soil); (2) young patches where *C. edulis* grew vigorously and corresponded to

an estimated invasion time of less than 10 years (hereafter, short-term invasion); and (3) patches where decaying mats of *C. edulis* were present, on which the invasion had been recorded for more than 10 years (hereafter, long-term invasion). The first 15 cm of soil was collected at different randomly selected sites but under similar vegetation patch sizes. For each type of soil we took three composite samples of 40 kg each (10 subsamples of ca. 4 l of soil each).

These samples were stored in plastic bags and taken directly to the laboratory. Each sample was thoroughly mixed and sieved to remove large pieces of roots and pebbles. Fragments of *C. edulis* ramets were collected from different equally-sized, young patches where *C. edulis* grew vigorously within the same dune system. The ramets of *C. edulis* collected in the field were chopped into fragments of equal size, length and weight. We discarded fragments that were already showing flower buds, open flowers, or signs of herbivory. All roots were removed from the ramets before transfer to the pots. Two clonal fragments, each with three ramets, were placed in every pot.

The experiment compared *C. edulis* performance in the three types of soils collected (i.e., uninvaded, short term, and long term invaded soil). A total of 36 plants in eighteen 10-l pots with 6 replicas per treatment were included. Soil chemical analysis of a bulk sample of each soil type revealed that the invaded soils had a higher organic matter and nitrogen content, and a lower pH, than uninvaded soil (uninvaded: %OM = 0.03, pH = 7.5, %N = 0.01; short-term invasion: %OM = 0.18, pH = 5.3, %N = 0.02; long-term invasion: %OM = 0.23, pH = 5.3, %N = 0.02). The experiment was conducted in the greenhouse facilities of the University of Coimbra (Portugal) and it started on the 1st March 2008, to run during the natural flowering period of the species in the wild for this region. Pots were kept in a greenhouse for 16 weeks and were watered on a weekly basis with 0.5 l distilled water. The number of flowers and the length of the flowering period were recorded every other day during the 16 week experimental period.

Experiment 5: effects of soil conditioning on the germination of *Ammophila arenaria* and *Cistus salviifolius*

Soil conditioning and preparation of the germination pots and conditions during the germination test were performed as described in experiment 3 using soil from Mallorca and Portugal. Five replicates with 50 seeds each were used per treatment. *A. arenaria* and *C. salviifolius* were selected because they are key species, representative of two succession stages (foredune and inner-dune, respectively) of the invaded areas. The experiment compared the effects of site, soil biota (sterilized vs. non-sterilized inoculum) and conditioning for each species separately.

Experiment 6: effect of soil conditioning on the performance of *Ammophila arenaria* and *Cistus salviifolius*

Preparation of experimental pots and conditions during the experiment were performed as in the previous experiment. After soil conditioning, *C. edulis* plants were removed and replaced by 2-week old *A. arenaria* or *C. salviifolius* seedlings. There were seven replicas per treatment. Plants were grown for 10 weeks and watered as described above. The experiment compared the effects of site, soil biota (sterilized vs. non-sterilized inoculum) and conditioning on plant growth.

Estimation of percentages of root infection by endophytic fungi

In order to determine whether changes in plant growth were being mediated by root colonisation of mycorrhizal or pathogenic fungi, a subsample from each root system (obtained in experiment 4) was stained and subsequently screened under a microscope. Root fragments (1 cm long) were stained with ink (Blue Quink, Parker) following a modification of the protocol of Vierheilig et al. (1998). Roots were cleared in 2.5% (wt/vol) KOH for 1 h at 90°C, rinsed with tap water, and immersed in 1% HCl overnight. They were then stained with 1% (vol/vol) ink in 1% HCl for 30 min at 60°C. Root colonisation was estimated in 50 root fragments per plant under a stereoscopic microscope (Leica MZ 8) using the grid-line intersect method (Giovanetti and Mosse 1980).

Statistical analysis and calculations

All data were checked for normality and homogeneity of variance with the Kolmogorov–Smirnov test

and Levene's test, respectively. If either of these was not fulfilled, data were log $(X + 1)$ transformed. Statistical analyses were performed with the statistical package Statistica 7.0. A factorial ANOVA was used to compare the effects of the factors and interactions mentioned above for each experiment. In all cases, post -hoc Tukey's planned comparisons were performed to address differences within each particular site as a function of soil biota (i.e. sterilized soil inoculums vs. unsterilized) or soil conditioning. Since germination, percentage of seedling survival and percentage of fungal infection showed a binomial distribution, we analysed those variables with a generalised linear models (with a logit-link for binomial data and correction for over-dispersion) using Proc Glimmix (SAS 9.1; SAS Institute 2001).

Feedback effects were calculated using the formula of relative difference: $(X - X_c)/X_b$, where X corresponds to a single value for plants inoculated with soil biota (non-sterilized soil inoculum), X_c is the average biomass for the plants inoculated with sterilized soil inoculum, and X_b is the largest value among X and X_c for every observation.

Results

Effects on *Carpobrotus edulis*

Experiment 1: biotic resistance of newly-invaded soils

The growth of *C. edulis* was clearly affected by soil biota ($F_{1,35} = 4.409$, $P = 0.043$). Overall, non- sterilized soil inoculum had a detrimental effect on the growth of *C. edulis*, but the effect was site dependent ($F_{2,35} = 4.439$, $P = 0.019$). The effect of soil inocula on biomass was not the same across the compared sites ($F_{2,35} = 4.522$, $P = 0.018$; Fig. 1a); in Mallorca and Portugal, plants grew larger in pots inoculated with sterilized soil than in pots inoculated with non-sterilized soil inoculum. No differences were observed between plants treated with sterilized or non-sterilized soil inoculum in South Africa (Fig. 1a). The net effect of soil biota on the growth of experimental plants is easily observed when comparing the relative differences between plants inoculated with soil biota and the control plants (sterilized soil inocula) from the different sites (Fig. 1b).
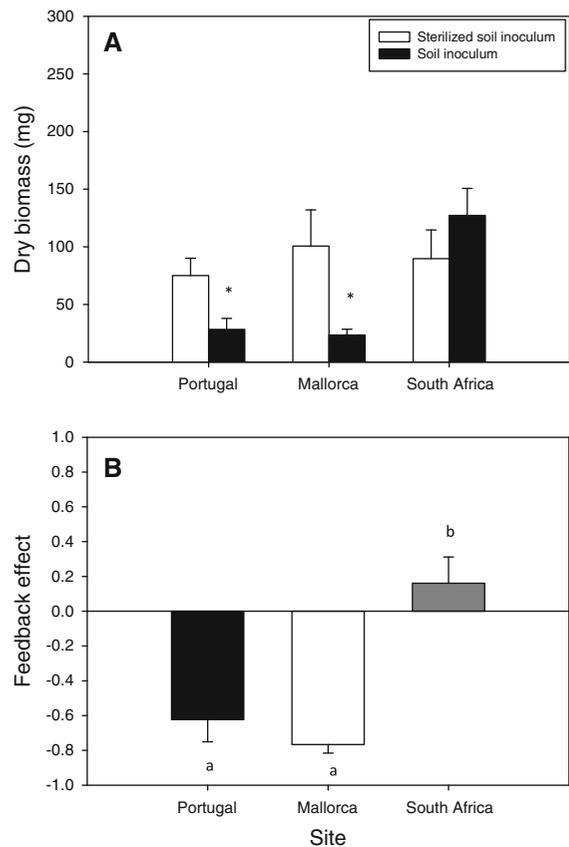
Fig. 1 a Total plant biomass (mean ± SE) of *Carpobrotus edulis* seedlings grown in soil inoculated with either sterilized or non-sterilized soil inocula from 3 different sites (Experiment 1). *Asterisks* indicate significant differences between soil treatments according to a post-hoc Tukey's test ($P = 0.001$, $n = 7$). **b** Feedback effect (mean ± SE) of the soil biota for different sites. *Different letters* indicate significant differences according to post-hoc Tukey test within site ($P = 0.001$, $n = 7$)

Experiment 2: effects of soil conditioning in the performance of Carpobrotus edulis

Soil conditioning with *C. edulis* had a marked impact on its subsequent growth. It is noteworthy that, in experiment 2, plants in general had almost double the biomass, regardless of the soil treatment and site, compared with plants in the previous experiment (see Figs. 1a vs. 2a). Plant growth (dry biomass) was different across the compared sites ($F_{2,42} = 4.241$, $P = 0.021$). The interaction between site and soil biota was also significant ($F_{2,42} = 3.622$, $P = 0.035$), which indicated that the effects of the soil inocula varied across the compared sites: in soil from

Mallorca, there was a strong positive effect of soil inocula (Fig. 2a), whereas in Portugal and South Africa there were no differences (Fig. 2a). The differences per site in the response of plants to soil inoculation are clearly illustrated when feedback effects are calculated ($F_{2,23} = 12.57$, $P = 0.0001$; Fig. 2b).

Experiment 3: effects of soil conditioning on the germination of *Carpobrotus edulis*

Soil conditioning had no effect on the germination of *C. edulis* ($F_{1,32} = 12.57$, $P = 0.32$; Table 1). No significant effect of site (Mallorca vs. Portugal,
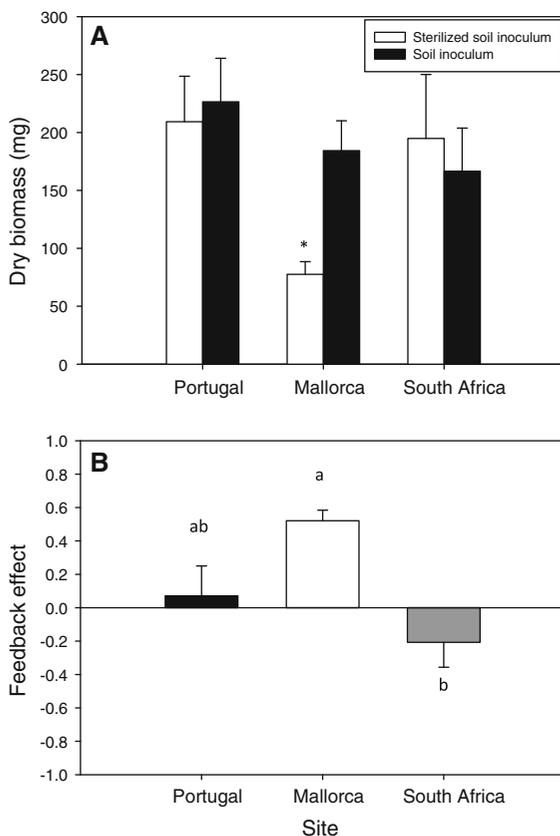
Fig. 2 a Total plant biomass (mean ± SE) of *Carpobrotus edulis* seedlings grown after inoculation with sterilized or non-sterilized soil from 3 different sites and after a previous soil conditioning phase with *C. edulis* (Experiment 2). *Asterisks* indicate significant differences between soil treatments within each site according to post-hoc Tukey's test ($P = 0.001$, $n = 8$). **b** Feedback effects (mean ± SE) of soil biota for the 3 different sites. *Different letters* indicate significant differences after ANOVA and post-hoc Tukey test ($P = 0.001$, $n = 8$)

$F_{1,32} = 11.47$, $P = 0.45$; Table 1) or soil biota (sterilized soil inoculums vs. non-sterilized soil inoculums, $F_{1,32} = 1.57$, $P = 0.7$) was found.

Experiment 4: effects of long-term natural soil conditioning on *Carpobrotus edulis*

There were no significant differences in dry biomass as a function of soil type. However, the difference in biomass for plants growing in long-term invasion soil with respect to uninvaded or short-term invasion soil was nearly significant ($F_{2, 35} = 2.763$; $P = 0.07$; Fig. 3a).

The type of soil in which the plants were grown affected the production of flowers (Fig. 3b). Plants growing in soil collected from uninvaded sites produced up to four times the number of flowers ($P = 0.001$, $F_{2,35} = 4.026$, Fig. 3b) than plants growing in soil previously occupied by *C. edulis* (short-term and long-term invasion soil). The same trend was observed for flower phenology, with a higher number of days with open flowers in VS, although no statistical differences could be detected (see Fig. 3c).

Fungal root colonization was observed in all experimental plants. The structures detected inside the roots did not correspond to mycorrhizal fungi but to colonization by putatively pathogenic fungi of the Chytridiomycota (Order Chytridiales). Significant differences were found between the studied soils ($P = 0.001$, $F_{2,35} = 4.026$; Fig. 3d), with the highest value of root colonization found in the long-term invasion soil.

Effects on native key species

Experiment 5: effects of soil conditioning on the germination of *Ammophila arenaria* and *Cistus salviifolius*

The percentage of germination was significantly different between sites (Mallorca vs. Portugal) for *A. arenaria* ($F_{1,32} = 12.57$, $P = 0.0001$; see Table 1). Inoculation with sterilized or non-sterilized inocula and soil conditioning did not have a significant effect on the germination percentage of *A. arenaria* or *C. salvifoliius* ($F_{1,32} = 2.57$, $P = 0.10$; see Table 1).

Table 1 Percentages of seed germination (mean ± SE) for *Carpobrotus edulis, Ammophila arenaria,* and *Cistus salviifolius* in soils from two invaded areas (Mallorca and Portugal), according to sterilization and soil conditioning status (Experiment 3 and 5). Different capital letters indicate significant differences after post-hoc Tukey's test

Soil inocula	Plant species		
	C. edulis	*A. arenaria*	*C. salviifolius*
Mallorca	23.7 (±6.55)	17.2 (±5.36)A	12.6 (±4.24)
Conditioned	24.5 (±10.1)	16.6 (±5.49)	10.9 (±5.75)
Non-conditioned	22.9 (±9.52)	17.8 (±5.98)	14.3 (±6.71)
Mallorca-sterile	12.6 (±3.54)	11.4 (±2.72)	10.4 (±3.18)
Conditioned	12.1 (±4.91)	11.3 (±3.37)	9.67 (±4.16)
Non-conditioned	13.2 (±5.67)	11.6 (±4.55)	11.2 (±5.19)
Portugal	30.5 (±5.89)	35.5 (±5.11)B	10.7 (±2.78)
Conditioned	30.2 (±8.66)	35.1 (±7.02)	10.5 (±3.88)
Non-conditioned	30.8 (±8.99)	36.0 (±8)	10.9 (±4.35)
Portugal-Sterile	26.5 (±6.81)	32.7 (±5.84)	8.91 (±1.98)
Conditioned	26.4 (±10.08)	33.5 (±8.72)	9.27 (±2.74)
Non-conditioned	26.5 (±10.33)	32.0 (±8.47)	8.54 (±3.1)
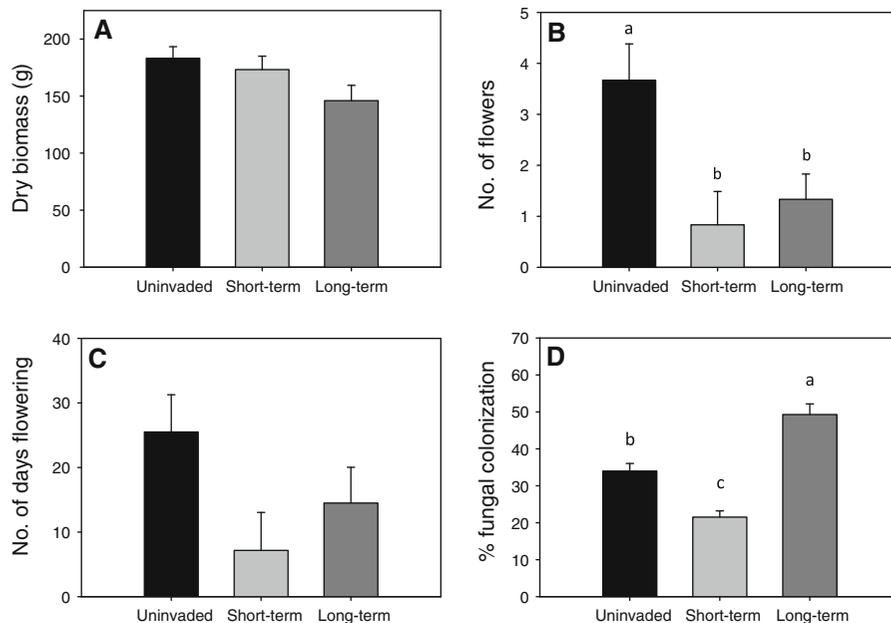
Fig. 3 *Carpobrotus edulis* dry biomass (**a**), number of flowers (**b**), number of days flowering (**c**) and percentage of root colonization by chytrids (**d**) in the three different soils used in experiment 4: uninvaded soil; short-term invasion soil; and long-term invasion soil. All graphs represent mean ± SE. *Different letters* indicate significant differences after ANOVA and post-hoc Tukey's test ($P = 0.001$, $n = 6$)

Experiment 6: effect of soil conditioning in the performance of *Ammophila arenaria* and *Cistus salviifolius*

The soil biota selected after the soil conditioning phase negatively affected the growth (dry biomass) of *A. arenaria*. In conditioned soil, *A. arenaria* experienced a reduction of 23% in dry biomass (Fig. 4a).

This growth response of *A. arenaria* was consistent across the two compared sites (Fig. 4a). For *C. salviifolius*, there was no significant effect of site with or without previous soil conditioning on the growth of plants (see Fig. 4b).

The survival rate of the two plant species was affected after soil conditioning with *C. edulis* in non-sterilized soil ($F_{1,24} = 5.95$, $P \leq 0.0225$ and
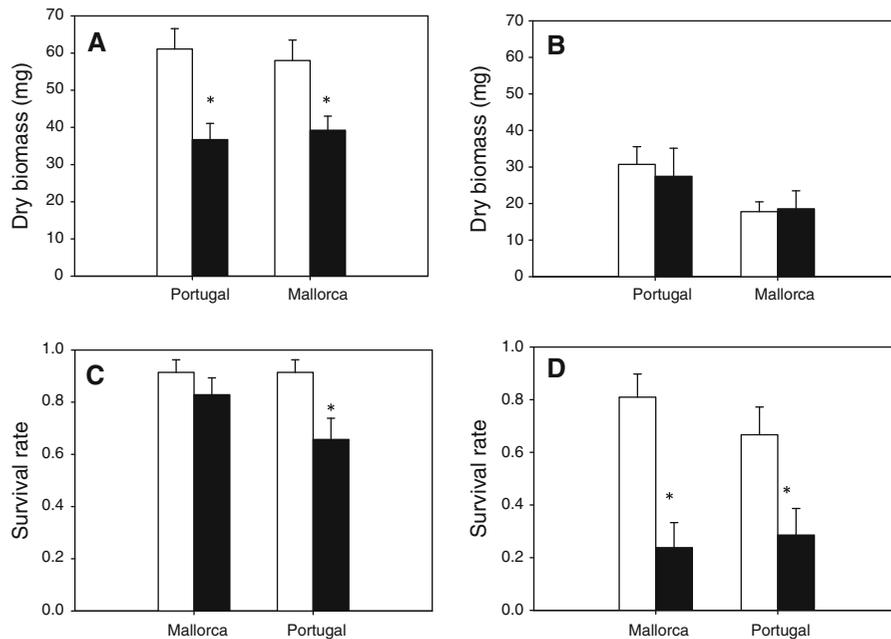
Fig. 4 Biomass and survival percentages (mean ± SE) of *Ammophila arenaria* (**a–c**) and *Cistus salviifolius* (**b–d**) after soil conditioning by *Carpobrotus edulis* (Experiment 5 and 6). *White bars* represent unconditioned soil and *black bars* conditioned. *Asterisks* indicate significant differences after post-hoc Tukey's comparison within each site ($P = 0.001$, $n = 7$)

$F_{1,24} = 17.47$, $P \leq 0.0003$ for *A. arenaria* and *C. salviifolius*, respectively). While seedling survival was very high (approximately 90%) for *A. arenaria* plants growing in unconditioned soil, the percentage of survival dropped to 70% in conditioned soil from Portugal (see Fig. 4c). An even stronger effect was observed regarding *C. salviifolius,* which experienced a survival rate of only 23% in conditioned soils at both sites compared (see Fig. 4d).

Discussion

Overall, the results obtained in this study show that plant-soil interactions (i.e., the plant-soil feedback established and plant reproductive response to soil conditions) may play an important role in the invasion of *C. edulis*.

The results of the first experiment clearly point to a high natural biotic resistance to *C. edulis* of the soil community from the invaded range (Portugal and Mallorca), which is in contrast with the slightly positive effects on *C. edulis* growth of the soil biota

from South Africa. These results suggest that *C. edulis* does not experience a release of soil pathogens on these new soils. On the contrary, our data support the hypothesis of a high initial resistance to *C. edulis* within Mediterranean regions (Vilà et al. 2006), and show that the soil biota is part of this mechanism. Other studies focused on invasive species have shown a direct release of soil pathogens (Reinhart et al. 2005). Even *C. edulis* has been shown to experience a release of soil pathogens in soils from two different invaded sites in France and Southern Spain (Van Grunsven et al. 2009). The contrasting results obtained by Van Grunsven et al. (2009) and in this study indicate that the processes involved in invasion might be site-specific and depend on the initial composition of the soil community (Knevel et al. 2004).

The combined results of experiments 1 and 2 suggest that initial seed pressure must be a key factor in breaking the initial biotic resistance. The initial propagule density at which *C. edulis* is able to "break" the initial resistance is a question that needs to be addressed experimentally. However, previous

studies have shown that the production of seeds in *Carpobtrotus* species is quite abundant (Suehs et al. 2001; Vilà and D'Antonio 1998), so propagule pressure can be considerably high once a plant is established in a new area. Since the germination experiment showed that soil conditioning does not affect the germination capacity of *C. edulis* seeds, it is reasonable to suggest that, given the large amount of seeds produced per fruit (>1,000), a significant percentage of seedlings are able to establish in each growth season.

The assessment of plant performance after long-term conditioning with field soil yielded interesting results; pointing out that the differences in soil attributes resulting from different durations of *C. edulis* invasion can produce shifts in the vegetative/reproduction balance of the plant. *Carpobrotus edulis* plants experienced an enhanced flower production in the uninvaded soil as compared with short- and long-term invaded soils. Plant response to soil conditions is especially important for perennial species that can shift between clonal and sexual reproduction, because those strategies result in different cost-benefit balances that determine their success in establishment (Honnay 2008). The higher investment in flowers can have consequences on the invasion dynamics, an effect that needs to be further considered. *Carpobrotus edulis* is a self-fertile, self-compatible species and experiences no inbreeding depression (Suehs et al. 2001). In such a case, an increase in the number of flowers and in flowering phenology may result in increased seed production and dispersal and, ultimately, in the successful establishment of plant populations (Suehs et al. 2004).

Changes in floral traits and phenology as a function of soil characteristics have been observed with other invasive species (Chun et al. 2007; Mal and Lovett-Doust 2005). In our case, the physiological mechanism behind the observed changes was not investigated, but we may attribute these flexible responses in plant growth to phenotypic plasticity triggered by the contrasting conditions of the soils used (Traveset et al. 2008). Clonal plants, as seen in other study systems, might switch between vegetative growth and sexual reproduction, depending on habitat quality (Hesse et al. 2008; Roiloa and Retuerto 2006). The soils used in this experiment (with invaded soils having a lower pH and a higher percentage of organic

matter and available nitrogen) show a pattern similar to other studies with *Carpobrotus* spp. (D'Antonio and Mahall 1991; Vilà et al. 2006). However, because biotic and abiotic factors were confounded in our experimental design, we cannot exclude the possibility that the changes observed were a response to different belowground biotic interactions. Interestingly, we observed higher levels of chytrid infection in the long-term invaded soils. Chytrids can be commensalist or pathogenic, depending on the host plant (Hibbett et al. 2007). The differential accumulation of soil fungi in the invaded soils compared could also be associated with the plant responses observed.

The residual effects on soil caused by *C. edulis* in invaded areas of California and Europe can affect the germination and establishment of other plant species (Conser and Connor 2009). The soil conditioning time used for this work (12 weeks) did not affect the percentage of germination of the two native species tested, but it did have negative effects on their survival and growth rates. These results show that seed germination was independent of the soil community, and probably subject to changes in abiotic conditions, while plant growth was more sensitive to changes in the soil biotic composition. Nevertheless, differences were observed between plant species and also between sites. The soil biota conditioned by *C. edulis* had a more negative effect on the survival of *C. salviifolius* than on *A. arenaria*. In fact, the conditioned soil from Mallorca did not have any effect on the survival of *A. arenaria* seedlings. Surprisingly, soil conditioning did not have an effect on *C. salviifolius* growth, which suggests that the effects of soil conditioning occur during the very early stages of plant development. The mechanisms involved in this process were not studied and, therefore, we cannot rule out any possibilities, which include allelopathy, a negative effect on soil mutualists, and accumulation of pathogens. In the case of *A. arenaria*, which to a certain extent is a mycorrhizal-dependent species (de la Peña et al. 2006), the effect might be due to suppression of beneficial mycorrhizal interactions, but this potential mechanism needs to be tested experimentally. For *C. salviifolius*, the strong effects observed in seedling survival point to pathogens or allelopathic compounds as being responsible for the mortality of native seedlings. As pointed out before, we observed

an accumulation of chytrids in the long-term invaded soils and the role of these symbiotic fungi in the plant community needs to be elucidated. The accumulation of soil fungi in the invaded soils could be detrimental for other plants and not for *C. edulis*, a mechanism that has been observed in other invasive plants (Mangla and Inderjit 2008; Eppinga et al. 2006; Westover and Bever 2001).

Carpobrotus edulis is one of the most aggressive and studied invasive plant species in Mediterranean areas. The results obtained here illustrate a complex array of plant-soil interactions and point to a mechanism that may explain how this species becomes very successful in newly-introduced areas. The modification of the soil community produced by *C. edulis* establishment not only breaks the initial biotic resistance of the newly-invaded landscapes, but it also is beneficial for the invader and detrimental for the native plant community. The performed experiments show that plant-soil interactions may affect different events (i.e. establishment, growth, phenotypic plasticity) at different temporal moments during the invasion process, which provides new insights to understand the invasiveness of this species. The observed changes in sexual and vegetative growth in soils with different invasion history are particularly interesting. Nevertheless, there are some aspects that need further consideration. We only used soil from one site where *C. edulis* is a native species and also the replication within the invaded range is limited to two dune systems. Therefore, extrapolating the present results to other geographical regions or invaded systems should only be done with caution. Moreover, in order to have a clearer picture on the functioning of plant-soil feedbacks further studies need to elucidate the effect of soil conditioning by native species on *C. edulis* and also address the particular changes in the soil biota that take place once *C. edulis* is established.

References

Agrawal AA, Kotanen PM, Mitchell CE, Power AG, Godsoe W, Klironomos J (2005) Enemy release? an experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86:2979–2989

Bai WM, Sun XQ, Wang ZW, Li LH (2009) Nitrogen addition and rhizome severing modify clonal growth and reproductive modes of *Leymus chinensis* population. Plant Ecol 205:13–21

Bartomeus I, Vilà M, Santamaria L (2008) Contrasting effects of invasive plants in plant-pollinator networks. Oecologia 155:761–770

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465–473

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. J Ecol 85:561–573

Chun YJ, Collyer ML, Moloney KA, Nason JD (2007) Phenotypic plasticity of native vs. invasive purple loosestrife: a two-state multivariate approach. Ecology 88:1499–1512

Conser C, Connor EF (2009) Assessing the residual effect of *Carpobrotus edulis* invasion, implication for restoration. Biol Invasions 11:349–358

D'Antonio C (1990) Seed production and dispersal in the non-native, invasive succulent *Carpobrotus edulis* (Aizoaceae) in coastal strand communities of Central California. J Appl Ecol 27:693–702

D'Antonio C, Mahall BE (1991) Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis,* and two native shrub species in California coastal scrub. Am J Bot 78:885–894

D'Antonio CM, Odion DC, Tyler CM (1993) Invasion of maritime chaparral by the introduced succulent *Carpobrotus edulis*. Oecologia 95:14–21

de la Peña E, Rodriguez-Echeverria S, van der Putten WH, Freitas H, Moens M (2006) Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. New Phytol 169:829–840

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant-soil system. Annu Rev Environ Resour 30:75–115

Eppinga MB, Rietkerk M, Dekker SC, De Ruiter PC, Van der Putten WH (2006) Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. Oikos 114:168–176

Giovanetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Hesse E, Rees M, Muller-Scharer H (2008) Life-History variation in contrasting habitats: flowering decisions in a clonal perennial herb (*Veratrum album*). American Naturalist 172:E196–E213

Hibbett DS et al (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Honnay O (2008) A meta-analysis of the relation between mating system, growth form and genotypic diversity in clonal plant species. Evol Ecol Res 22:299–312

Jakobsson A, Padron B, Traveset A (2008) Pollen transfer from invasive *Carpobrotus* spp. to natives—a study of pollinator behaviour and reproduction success. Biol Conserv 141:136–145

Knevel IC, Lans T, Menting FBJ, Hertling UM, van der Putten WH (2004) Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien *Ammophila arenaria* in South Africa. Oecologia 141:502–510

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: a meta-analytical review. Ecol Lett 11:980–992

Levine JM, Pachepsky E, Kendall BE, Yelenik SG, HilleRis-Lambers J (2006) Plant-soil feedbacks and invasive spread. Ecol Lett 9:1005–1014

Mal TK, Lovett-Doust J (2005) Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, *Lythrum salicaria* (Lythraceae) in response to soil moisture. Am J Bot 92:819–825

Maltez-Mouro S, Maestre F, Freitas H (2010) Weak effects of the exotic invasive *Carpobrotus edulis* on the structure and composition of Portuguese sand-dune communities. Biol Invasions

Mangla S, Inderjit, Callaway RM (2008) Exotic invasive plant accumulates native soil pathogens which inhibit native plants. J Ecol 96:58–67

Nijjer S, Rogers WE, Siemann E (2007) Negative plant-soil feedbacks may limit persistence of an invasive tree due to rapid accumulation of soil pathogens. Proc R Soc B-Biol Sci 274:2621–2627

Perner H, Schwarz D, Bruns C, Mader P, George E (2007) Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants. Mycorrhiza 17:469–474

Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. New Phytol 170:445–457

Reinhart KO, Royo AA, Van der Putten WH, Clay K (2005) Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. J Ecol 93:890–898

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281–2291

Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmanek M (2000) Plant invasions—the role of mutualisms. Biol Rev 75:65–93

Rodríguez-Echeverría S, Crisóstomo JA, Nabais C, Freitas H (2009) Belowground mutualists and the invasive ability of *Acacia longifolia* in coastal dunes of Portugal. Biol Invasions 11:651–661

Roiloa SR, Retuerto R (2006) Development, photosynthetic activity and habitat selection of the clonal plant *Fragaria vesca*, growing in copper polluted soil. Funct Plant Biol 33:961–971

Roiloa SR, Rodríguez-Echeverría S, de la Peña E, Freitas H (2010) Understanding the role of clonality in plant invasions: a field experiment with *Capobrotus edulis*. Biological Invasions

Sintes T, Moragues E, Traveset A, Rita J (2007) Clonal growth dynamics of the invasive *Carpobrotus* aff. acinaciformis in Mediterranean coastal systems: a non-linear model. Ecol Modell 206:110–118

Suehs CM, Médail F, Affre L (2001) Ecological and genetic features of the invasion by the alien *Carpobrotus* (Aizoaceae) plants in Mediterranean island habitats. In: Brundu G, Brock J, Camarda I, Child L, Wade M (eds) Plants invasions: species ecology and ecosystem management. Backhuys Publisher, Leiden, pp 145–158

Suehs CM, Affre L, Medail F (2004) Invasion dynamics of two alien *Carpobrotus* (Aizoaceae) taxa on a Mediterranean island: II. reproductive strategies. Heredity 92:550–556

te Beest M, Stevens N, Olff H, van der Putten WH (2009) Plant-soil feedback induces shifts in biomass allocation in the invasive plant *Chromolaena odorata*. J Ecol 6:1281–1290

Traveset A, Moragues E, Valladares F (2008) Spreading of the invasive *Carpobrotus* aff. acinaciformis in Mediterranean ecosystems: the advantage of performing in different light environments. Appl Veg Sci 11:45–54

van der Putten WH (2003) Plant defense belowground and spatiotemporal processes in natural vegetation. Ecology 84:2269–2280

Van Grunsven RHA, Bos F, Ripley BS, Suehs CM, Veenendaal EM (2009) Release from soil pathogens plays an important role in the success of invasive *Carpobrotus* in the Mediterranean. S Afr J Bot 75:172–175

Vierheilig H, Coughlan AP, Wyss U, Piche Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007

Vilà M, D'Antonio CM (1998) Fitness of invasive *Carpobrotus* (Aizoaceae) hybrids in coastal California. Ecoscience 5:191–199

Vilà M, Tessier M, Suehs CM, Brundu G, Carta L, Galanidis A, Lambdon P, Manca M, Medail F, Moragues E, Traveset A, Troumbis AY, Hulme PE (2006) Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. J Biogeogr 33:853–861

Vilà M, Siamantziouras ASD, Brundu G, Camarda I, Lambdon P, Medail F, Moragues E, Suehs CM, Traveset A, Troumbis AY, Hulme PE (2008) Widespread resistance of Mediterranean island ecosystems to the establishment of three alien species. Divers Distrib 14:839–851

Weber E, D'Antonio CM (1999) Phenotypic plasticity in hybridizing *Carpobrotus* spp. (Aizoaceae) from coastal California and its role in plant invasion. Can J Bot-Revue Canadienne De Botanique 77:1411–1418

Westover KM, Bever JD (2001) Mechanisms of plant species coexistence: roles of rhizosphere bacteria and root fungal pathogens. Ecology 82:3285–3294