Annals of Botany 1–9, 2007
doi:10.1093/aob/mcl289, available online at www.aob.oxfordjournals.org

OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

Sensitivity of the Invasive Geophyte *Oxalis pes-caprae* to Nutrient Availability and Competition

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Received: 29 June 2006 Returned for revision: 30 August 2006 Accepted: 4 December 2006

- *Background and Aims* Invasion by alien plants may be partially related to disturbance-related increases in nutrient availability and decreases of competition with native species, and to superior competitive ability of the invader. *Oxalis pes-caprae* is an invasive winter geophyte in the Mediterranean Islands that reproduces vegetatively via bulbs. An investigation was made into the relative responses of *O. pes-caprae* and the native annual grass *Lolium rigidum* to nutrient availability and to competition with each other in order to understand patterns of invasion in the field. Because *Oxalis* accumulates oxalic acid in its leaves, which could ameliorate soil phosphorous availability, field observations were made to determine whether the presence of *Oxalis* alters soil P availability.
- *Methods* A full-factorial glasshouse experiment was conducted with nutrient availability (high and low) and competition (*Lolium* alone, *Oxalis* alone, and *Lolium* and *Oxalis* together). Plant performance was assessed by determining (1) above- and below-ground biomass at the time of *Oxalis* maximum biomass and (2) reproductive output of *Oxalis* and *Lolium* at the end of their respective growth cycles. Measurements were also taken for leaf N and P content. Soil samples were taken in the field from paired *Oxalis*-invaded and non-invaded plots located in Menorca (Balearic Islands) and available P was determined.
- *Key Results* High nutrient availability increased *Oxalis* and *Lolium* vegetative biomass and reproductive output to a similar degree. Competition with *Lolium* had a much stronger negative effect on *Oxalis* bulb production than reduced nutrients. *Lolium* was a superior competitor than *Oxalis*; the latter did not affect *Lolium* maximum biomass and spike production. Significantly greater soil-P availability in *Oxalis*-invaded field soils relative to paired non-invaded soils suggest that *Oxalis* influences soil P cycling.
- *Conclusions Oxalis* is a poor competitor. This is consistent with the preferential distribution of *Oxalis* in disturbed areas such as ruderal habitats, and might explain its low influence on the cover of native species in invaded sites. The results also suggest that certain disturbances (e.g. autumn ploughing) may greatly enhance *Oxalis* invasion.

Key words: *Oxalis pes-caprae*, invasive species, competition, nutrients, *Lolium rigidum*, asexual reproduction, Mediterranean Islands.

INTRODUCTION

Invasions by alien species are a global phenomenum that can cause irreversible changes in community structure and ecosystem function (Levine *et al.*, 2003). The success of introduced species depends on their biological traits, propagule pressure and ecosystem invasibility (Lonsdale, 1999; Lloret *et al.*, 2005). The invasibility of plant communities (i.e. the intrinsic vulnerability to invasion) is strongly influenced by the interplay between available resources (e.g. water, nutrients and light) and plant–plant interactions (Keane and Crawley, 2002). For example, disturbance is known to increase invasibility (D'Antonio *et al.*, 1999) mainly due to reduced competitive pressure from other plants and increased resource levels (Hobbs and Huenneke, 1992). While many studies have examined the relative competitive strength of exotic vs. native species (see Vilà and Weiner, 2004; Bruno *et al.*, 2005) and the influence of resource availability on invasive success (e.g. Davis *et al.*, 2000; Blumenthal *et al.*, 2003), relatively fewer studies have examined experimentally the combined effects of competition and resource availability (Hansjörg *et al.*, 2002; Suding *et al.*, 2004). These type of studies are necessary in order to understand the abilityof exotic plants to spread in new areas.

In Mediterranean regions, invasions by non-native plant species are common (di Castri *et al.*, 1990) and are regarded as one of the most important drivers of global change (Sala *et al.*, 2000). One of the most widespread invasive species of many temperate and Mediterranean areas of the world is *Oxalis pes-caprae* (Oxalidaceae, hereafter *Oxalis*), a winter annual geophyte from South Africa (Peirce, 1997). *Oxalis* invasion is particularly evident in many islands of the Mediterranean basin and is probably enhanced by its distinct winter phenology and its short life cycle relative to many native species (with spring–summer phenology and longer life cycles), and by its prolific vegetative reproduction by daughter bulbs

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(Pütz, 1994). *Oxalis* occurs in a variety of habitats, although it is more abundant in ruderal, disturbed and well-drained fertile soils, such as abandoned agricultural fields and orchards, compared with less-disturbed sites such as shrublands and forests (Gimeno *et al.*, 2006). In addition to propagule pressure, *Oxalis* invasion along disturbance and nutrient availability gradients may partially depend on its sensitivity to nutrient availability and to competitive interactions with dominant native species. Such interactions may occur if warm temperatures advance the phenology of native species (Peñuelas *et al.*, 2002; Gordo and Sanz, 2005), therefore increasing the temporal overlap between *Oxalis* and native species. Therefore, knowledge of *Oxalis* sensitivity to nutrient availability and to competition with native species may prove useful for predicting *Oxalis* invasion along disturbance gradients and with climate change. Experimental manipulations of single factors are needed to disentangle the relative effect of these two factors on *Oxalis* invasion in different environments.

Oxalis invasion in Mediterranean islands has been shown to decrease overall native plant diversity by about 10 %, especially with regard to annual grasses (Vilà *et al.*, 2006*b*). However, this effect was lower than that measured for other invasive plant species. The lower negative impact of *Oxalis* on native species relative to other plant invaders may relate to its winter phenology, but also to species-specific traits. For instance, *Oxalis* is known to accumulate oxalic acid in its leaves, which is toxic to herbivore vertebrates if eaten in large quantities (Hulme, 2004). Oxalate is known to be a chelating agent for phosphorous, thus increasing its availability to other plants (Cannon *et al.*, 1995). This raises the possibility that heavy *Oxalis* invasion increases soil phosphorous availability due to the release of oxalic acid-rich litter. If so, spring pulses of phosphorous in *Oxalis*-invaded fields could enhance subsequent germination and growth of native species, thereby contributing to their maintenance.

In this study, a full-factorial greenhouse pot experiment was conducted with nutrient availability (high and low) and competition (*Oxalis* alone, the annual grass *Lolium rigidum* alone, and the two species together) in order to address the following questions. (1) Does nutrient availability affect the growth and vegetative reproductive output (i.e. bulb production) of *Oxalis*? (2) Does competition with *Lolium* reduce the reproductive output of *Oxalis*? (3) Is *Oxalis* as affected by nutrient availability and competition as *Lolium*? *Lolium rigidum* was chosen as a target native species because it commonly co-occurs with *Oxalis* (Vilà *et al.*, 2006*b*). Soil sampling in the field was also conducted in Menorca (Balearic Islands, Spain) in order to address an additional question: does the presence of *Oxalis* alter soil P availability? Due to the distribution of *Oxalis* in disturbed habitats, it was hypothesized that both nutrients and competition would have significant effects on *Oxalis*, although we did not have specific predictions on the relative magnitude of these effects. It was anticipated that competition by *Oxalis* on *Lolium* would be moderate due to differences in size between the two species (*Oxalis* is small relative to *Lolium*).

MATERIALS AND METHODS

Study species

Oxalis is a small annual, bulbous herb native to South Africa (Peirce, 1997) that has invaded the Mediterranean Basin, Australia, India, New Zealand, Portugal, the south-west of England and warm regions of the USA (Peirce, 1997). *Oxalis* was introduced into the Mediterranean Basin in 1796 and it is now found in Italy, Greece, the Iberian Peninsula and North Africa (Damanakis and Markaki, 1990; Brandes, 1991).

Oxalis spreads vegetatively by rooting at nodes and via numerous daughter bulbs (or bulblets) produced by each mother plant during late spring and early summer (Pütz, 1994). On average, each plant produces 20 daughter bulbs (Vilà *et al.*, 2006*a*). Daughter bulbs remain dormant in the summer and sprout in the autumn (Vilà *et al.*, 2006*a*). The peak of vegetative growth occurs during autumn and winter (from November to March), after which flowering starts. During vegetative growth an underground tuber develops. Because viable seed production is rare or absent (see Marshall, 1987), the invading potential of *Oxalis* relies largely on bulb production. *Oxalis* leaves senesce in spring (March–May), at which time bulblet buds start to fill from reserves stored in the tuber (Chawdhry and Sagar, 1973).

Glasshouse experiment

Commencing on 6 October, 2003, a factorial greenhouse experiment was conducted with species, nutrient availability (high and low) and interspecific competition (*Oxalis pes-caprae* and *Lolium rigidum* alone or in competition with each other). The experiment was conducted at the glasshouse facilities of the University of Barcelona (north-east Spain). Bulbs of *Oxalis* were harvested from plants grown in a common garden at the University of Barcelona, which originated from bulbs collected in the field (Menorca, Spain; see Vilà *et al.* 2006*a*). *Lolium rigidum* (hereafter *Lolium*) seeds were collected from natural field populations in Lleida (Catalonia, Spain), which had similar distribution, growth patterns and characteristics as populations in Menorca. *Oxalis* bulbs from 50 different plants were mixed in a bag and sorted into ten size classes (from an average weight of 0·04 g in the smallest size class to 1·06 g in the largest size class). Eight sets of ten bulbs each were prepared, with each bulb in the set selected from a different bulb-size class. Bulb-size distribution was similar among sets, resulting in similar average bulb weight per set [0·39 ± 0·03 g (±s.d.); $P > 0{\cdot}05$]. Two randomly selected sets were assigned to each of the following four treatments: (1) high nutrients with *Lolium*; (2) high nutrients alone; (3) low nutrients with *Lolium*; and (4) low nutrients alone. Two sets of bulbs per treatment allowed for one set to be harvetsed at the time of peak *Oxalis* above-ground biomass, and the other for a final harvest at the end of the experiment.

Each *Oxalis* bulb was sown 3 cm below the soil surface in 2-L plastic pots (11·5 × 21 cm, diameter × depth) filled with silica sand. Several *Lolium* seeds were planted on the soil surface in all pots assigned to the competition treatments, and in 40 additional pots assigned to the *Lolium*-alone treatments under high and low nutrients. *Lolium* seedlings were subsequently thinned to one seedling per pot. Initial *Oxalis* bulb emergence occurred 10 d after sowing. The experimental design was set up to test the effects of nutrients and interspecific competition, and had a total of 160 pots (2 species × 2 nutrient levels × 2 competition levels × 2 sets × 10 pots each). Effects of intraspecific competition are addressed in Vilà *et al.* (2006*a*).

All pots were completely randomized and initially watered to field capacity (200 mL). During the first 2 weeks, pots were subjected to a 60-s surface misting three times a day in order to maintain surface soil moisture and ensure emergence of *Oxalis* and germination of *Lolium*. Fertilization treatments started approximately 3 weeks after sowing (20 October). Pots assigned to the high and low nutrient treatments were watered every 6 d with 200 mL of 1/2 and 1/16 strength modified Hoagland solution ($NO_3^- : NH_4^+ : PO_4^- : K^+ : Ca^+ : Mg^+ : SO_4^-$ at concentrations of 7 : 1 : 1 : 3·25 : 2 : 0·5 : 0·5 mM), respectively. High nutrient levels were estimated to provide approximately 0·015 g mineral N Kg^{-1} soil, to mimic nitrogen availability in productive Mediterranean soils (Serrasolsas *et al.*, 1999). Low nutrient levels were chosen to represent unfertile field soils, such as those in areas where *Oxalis* is native (Read and Mitchell, 1983). After 8 weeks, pots were watered every 3 d until the end of the experiment, with nutrients added every 6 d. Pots were rotated every 2–3 weeks to prevent glasshouse location effects. Glasshouse temperature was set at 20/10 °C day/night (with daylight from 0900 to 1800; there was an approx. 3-h transition period), providing an average temperature of 15·5 °C during the experiment, with average maximum and minimum temperatures of 19·6 and 13·0 °C, respectively. No supplemental light was supplied in the glasshouse. The average temperature in Menorca (1977–2000) from September to April (when *Oxalis* is present in the field) is 14·3 °C, with average maximum and minimum temperatures of 17·8 and 11·0 °C, respectively. Thus, temperature fluctuations in the glasshouse were representative of the mild climate in the field. It is worth noting that the slightly warmer conditions in the glasshouse (approx. 2 °C) fall within the variation recorded in the field.

On 14–15 January, 2004, at the time of maximum *Oxalis* biomass and flowering (but before *Lolium* peak biomass), one of the two sets per treatment was assigned to the first harvest. Above-ground biomass of *Oxalis* and *Lolium* was removed and roots were carefully washed from the sand. At the time of this harvest, *Oxalis* had a well-developed tuber, which was separated from the rest of the fine roots. For pots assigned to the competition treatment an initial attempt was made to separate *Oxalis* and *Lolium* roots. However, roots of the two species were very fine, tightly interwoven and of similar appearance, and the separation process was not possible. Therefore, only above-ground biomass per species was measured for the competition treatment. All biomass fractions harvested for the two species were oven-dried at 65 °C to constant weight, and this value was measured.

On 10 May, 2004, at the onset of *Lolium* leaf senescence and maximum flowering, the final *Lolium* harvest was conducted using the second set of pots. At this point, *Oxalis* above-ground biomass had completely decayed, although bulblet development was still in progress. Therefore, only above-ground *Lolium* biomass was harvested. Tillers and spikes were harvested separately, oven-dried as before, and weighed. Pots that had *Oxalis* (alone or in competition with *Lolium*) were left untouched until 10 July, 2004, when they were emptied and daughter bulbs were harvested.

Soil field survey

In December 2002, twenty-three 2 × 2 m paired invaded and non-invaded plots were selected across Menorca (Balearic Islands), one of the most invaded islands of the eastern Mediterranean Basin (Gimeno *et al.*, 2006). One plot of each pair was centred on an *Oxalis* patch (invaded plot). The other plot of each pair was then selected at approx. 2 m distance from the edge of the invaded plot (control plot). Paired-plots were selected based on a subjective decision following dedicated land surveys to make sure that the two paired-plots shared similar substrate and habitat characteristics. To reduce invaded plot variability, all plots were selected on the basis of having a high cover of the invader (present in ≥14 out of a total of 16 subplots within each plot). The minimum distance between different paired-plots was 1 km.

During the spring of 2002, at the peak of native vegetative cover and species' richness, soil was collected at random from three different subplots and subsequently pooled for each plot. For each sample, surface litter (if present) was removed, and the top 12 cm of soil was sampled to a total plot volume ranging from 0·0005–0·001 m^3 of bulk soil. Samples were air-dried to constant weight, sieved with a 2-mm pore-size sieve and ground in a standard ball mill grinder.

Chemical analyses

Oxalis and *Lolium* leaves from the first glasshouse harvest were dried and ground to a powder (passing through a 2-mm mesh screen) for measurement of total nitrogen (N) and phosphorous (P) content, conducted at the Chemical Analyses Services of the Autonomous University of Barcelona, Spain. Leaf N was analysed with a CSN analyser, and leaf P by atomic emission spectroscopy with Inductively Coupled Plasma analysis after bi-acid digestion (nitric : percloric, 2 : 1) in a microwave oven (Mateo and Sabaté, 1993).

Soils from invaded and non-invaded field sites were air-dried, sieved and analysed for available P (Olsen method) at the Escudero Laboratories (Reus, Spain) following standard protocols (Klute, 1986). No attempt was made to examine differences in soil P availability from the glasshouse experiment because we predicted minimal differences due to the short duration of the experiment and the minimal P retention in the irrigated silica sand.

Statistical analysis

Statistical analyses were first performed separately per species. For *Oxalis*, the effects of competition and nutrient treatments on above-ground biomass (first harvest), and on number, total and average weight of daughter bulbs (second harvest) were tested using an ANCOVA with initial bulb mass as covariate. In addition, linear and non-linear regression analyses were used to test the relationship between initial *Oxalis* bulb weight and the total biomass of bulbs produced.

Nutrient effects on root biomass and root mass ratio (RMR: biomass of roots/total biomass) at the first harvest were tested using a one-way ANCOVA with initial bulb mass as covariate for *Oxalis*, and using a *t*-test for *Lolium*. The competition effect on roots was not tested as the roots of the two species could not be separated when grown together. Two-way ANOVAs were used to test the effects of nutrient and competition treatments on *Oxalis* and *Lolium* leaf N, P and N/P ratio, and on *Lolium* biomas at the first (tillers) and final (tillers and spikes) harvests.

A three-way ANOVA with species, nutrients and competition was used to test whether responses to nutrients and to competition differed between species (i.e. whether the interactions nutrient × species and competition × species were significant). In this case, bulb mass was not used as a covariate since there was no such covariate for *Lolium*.

A paired *t*-test was used to test differences between field soil P content. Variables were Ln-transformed (*Lolium* and *Oxalis* above-ground biomass, leaf nitrogen and phosphorous concentrations, and *Oxalis* mother bulb weight) or square-root transformed (total number and total biomass of *Oxalis* daughter bulbs) to meet model assumptions. All tests were considered significant at $P < 0{\cdot}05$. All statistical analyses were conducted using SPSS 11·5 (SPSS, Inc., Chicago).

RESULTS

Ten days after planting (16 October, 2003), 12·5 % of the bulbs had emerged. Emergence rapidly increased to 72 % after a further week, reaching a maximum of 95 % one month after planting. Emergence tended to be delayed in the smallest bulbs, with 5 % of bulbs (all less than 0·09 g) failing to emerge. While failure to emerge tended to occur in smaller bulbs, the three smallest bulbs (less than 0·033 g) did emerge. *Oxalis* attained maximum biomass and flowering 3·5 months earlier (mid-January) than *Lolium* (mid- to late-April), with complete die-back of *Oxalis* above-ground biomass at the time of *Lolium* maximum biomass.

Oxalis responded to high nutrients with a significant increase in above-ground biomass ($F_{1,36} = 106{\cdot}2$, $P < 0{\cdot}0001$; Fig. 1), and a significant decrease in root biomass ($F_{1,18} = 12{\cdot}6$, $P = 0{\cdot}003$; Fig. 2A) and root mass ratio (RMR; $F_{1,18} = 47{\cdot}8$, $P < 0{\cdot}0001$; Fig. 2B). High nutrients also caused a significant increase in leaf N ($F_{1,36} = 28{\cdot}0$, $P < 0{\cdot}0001$; Fig. 3A) and, to a lesser degree, P concentration ($F_{1,36} = 11{\cdot}5$, $P = 0{\cdot}002$; Fig. 3B), but did not significantly change N/P ratios ($F_{1,36} = 3{\cdot}7$, $P = 0{\cdot}064$; Fig. 3C). High nutrients increased the total

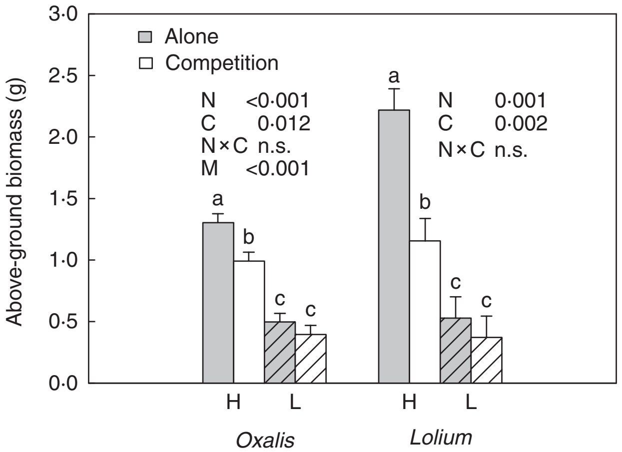

FIG. 1. Above-ground biomass of *Oxalis* and *Lolium* grown under high (H, no hatching) and low nutrients (L, hatched) and either alone or in competition with the reciprocal species. Data are for the first harvest at the time of *Oxalis* maximum growth (see text). Error bars are s.e. of the mean. Different letters indicate statistical differences between treatments. For each species, *P*-values for the effects of competition (C), nutrients (N), their interaction, and initial bulb mass (M, for *Oxalis* only) are also indicated (n.s., not significant).

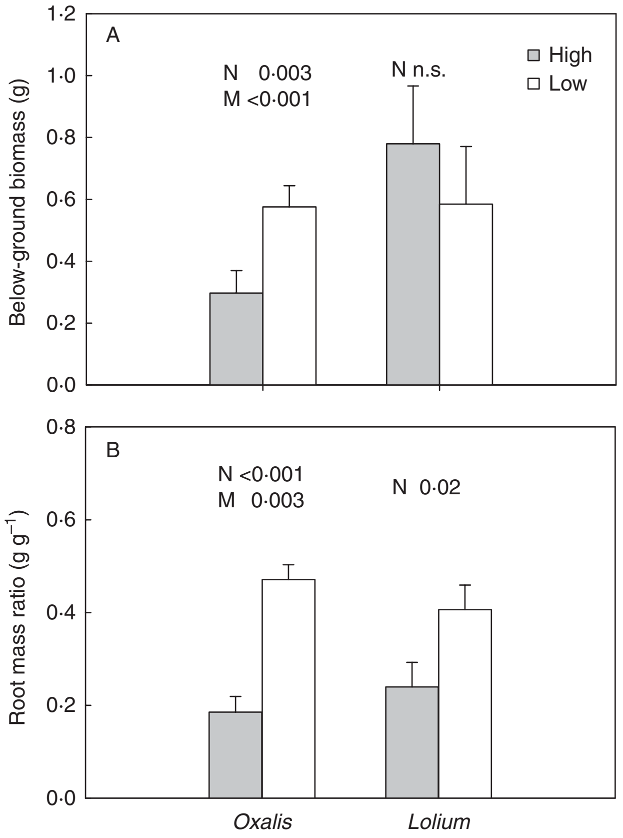

FIG. 2. (A) Below-ground biomass and (B) root mass ratio (g roots g^{-1} total biomass) of *Oxalis* and *Lolium* grown under high and low nutrients. Data are for the first harvest at the time of *Oxalis* maximum growth (see text). Error bars are s.e. of the mean. For each species, *P*-values for the effects of nutrients (N) and initial bulb mass (M, for *Oxalis* only) are also indicated (n.s., not significant).

number of *Oxalis* daughter bulbs ($F_{1,38} = 73{\cdot}2$, $P < 0{\cdot}0001$; Fig. 4A), but did not have an overall effect on total daughter bulb biomass (Fig. 4B). However, the increase in the number of daughter bulbs was due to much higher values under no competition compared with all other treatments, while under competition the total number and biomass of bulbs was as low as under low nutrients ($F_{\mathrm{N\times C;\ 1,38}} = 46{\cdot}2$, $P < 0{\cdot}0001$ and $F_{\mathrm{N\times C;1,38}} = 13{\cdot}4$,

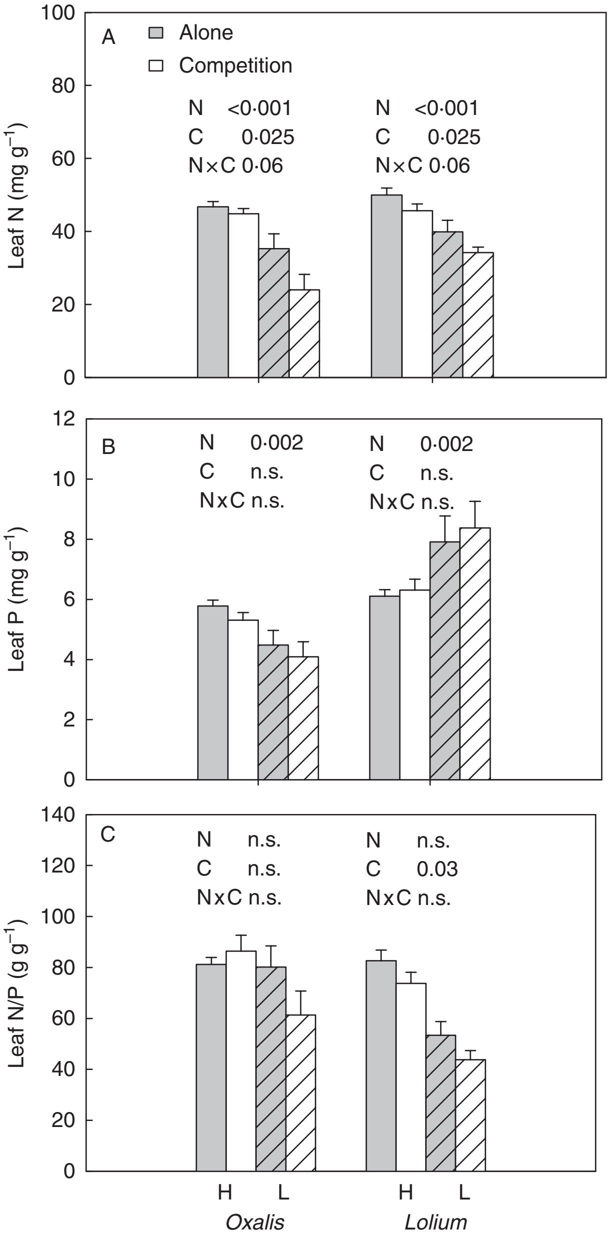

FIG. 3. (A) Leaf nitrogen concentration, (B) phosphorous concentration and (C) N/P ratios of *Oxalis* and *Lolium* grown under high (H, no hatching) and low nutrients (L, hatched) and either alone or in competition with the reciprocal species. Data are for the first harvest at the time of *Oxalis* maximum growth (see text). Error bars are s.e. of the mean. For each species, *P*-values for the effects of competition (C), nutrients (N), their interaction, and initial bulb mass (M, for *Oxalis* only) are also indicated (n.s., not significant).

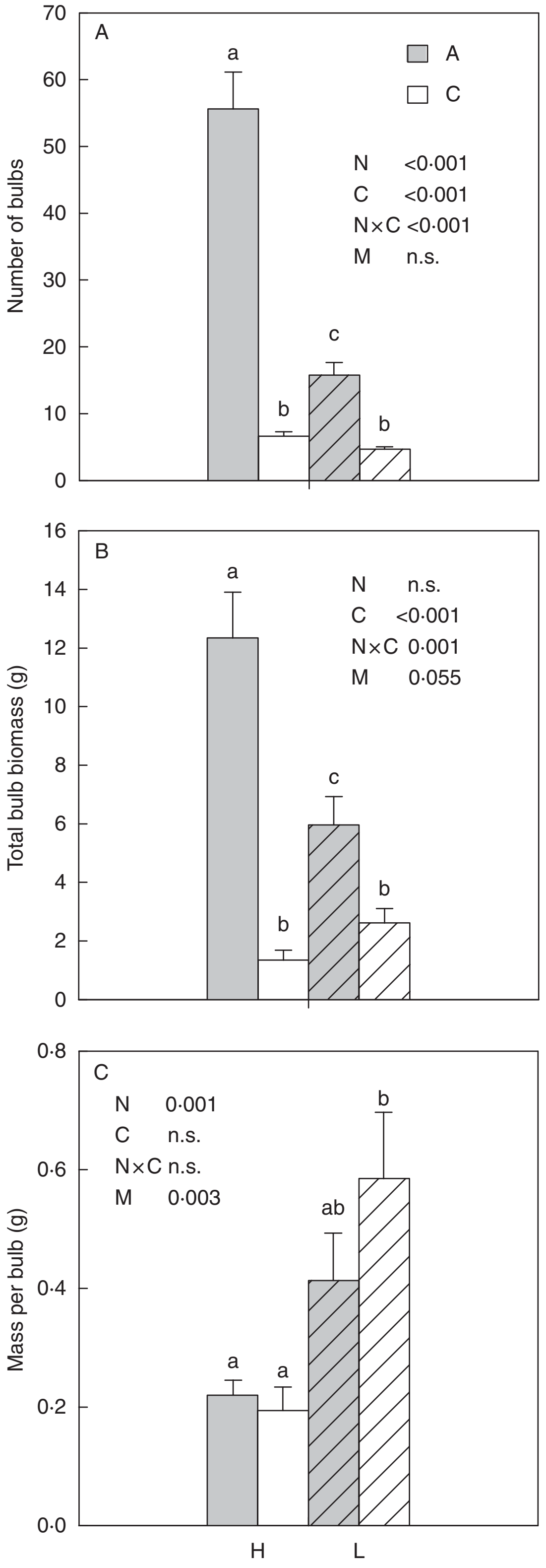

FIG. 4. (A) Total number of daughter bulbs, (B) total daughter bulb biomass and (C) mean mass of individual daughter bulbs of *Oxalis* grown under high (H, no hatching) and low (L, hatched) nutrients and either alone or in competition with *Lolium*. Error bars are s.e. of the mean. Different letters indicate statistical differences between treatments. P values for the effects of competition (C), nutrients (N), their interaction and initial bulb mass (M) are also indicated (n.s. indicate not significant).

$P < 0\cdot001$, respectively; Fig. 4A, B). Daughter bulbs were smaller (lower weight) at high than at low nutrient levels ($F_{1,38} = 21\cdot3$, $P < 0\cdot0001$; Fig. 4C).

Competition by *Lolium* caused a significant decrease in above-ground biomass ($F_{1,36} = 7\cdot0$, $P = 0\cdot012$; Fig.1) and of leaf N concentration in *Oxalis* ($F_{1,36} = 5\cdot5$, $P = 0\cdot025$; Fig. 3A) but it did not affect leaf P concentration and N/P ratios ($F_{1,36} = 0\cdot39$, $P = 0\cdot54$, and $F_{1,36} = 0\cdot99$, $P = 0\cdot33$, respectively; Fig. 3B, C). The effect on leaf N concentration tended to be stronger under low nutrients ($F_{\mathrm{N\times C};1,36} = 3\cdot8$, $P = 0\cdot06$; Fig. 3A). Competition by *Lolium* strongly reduced the total number and total biomass of *Oxalis* daughter bulbs ($F_{1,38} = 215\cdot4$ and $60\cdot2$, respectively; $P < 0\cdot0001$ for both), with a much stronger effect at high nutrients (see above; Fig. 4A, B); however, it did not affect the mass of individual daughter bulbs (Fig. 4C).

Larger initial bulbs in *Oxalis* resulted in higher above- and below-ground biomass ($F_{1,36} = 40\cdot0$, $P < 0\cdot0001$, and $F_{1,18} = 21\cdot2$, $P < 0\cdot0001$, respectively; Fig. 5A, B). However, this effect was mostly restricted to smaller bulbs up to approx. 0·2 g, after which there was no change in the response variables (Fig. 5). An exception was when *Oxalis* was grown alone under low nutrients, where below-ground biomass continued to increase (although not linearly) with initial bulb mass (Fig. 5B). Larger mother bulbs also produced larger daughter bulbs (i.e. higher mass per bulb; $F_{1,38} = 10\cdot2$, $P < 0\cdot003$; Fig. 4C) and tended to result in an increase in the total biomass of daughter bulbs (i.e. a nearly significant effect of the initial bulb mass covariate in the ANOVA; $F_{1,38} = 3\cdot9$, $P = 0\cdot055$). When treatments were considered separately, a positive linear correlation between initial bulb mass and total daughter bulb biomass was only significant when *Oxalis* was grown alone under low nutrients ($r^2 = 0\cdot4$, $P = 0\cdot04$, $F_{1,8} = 6\cdot4$; data not shown).

In *Lolium*, high nutrients increased tiller biomass in the first ($F_{1,38} = 94\cdot8$, $P < 0\cdot0001$) and final ($F_{1,40} = 89\cdot9$, $P < 0\cdot0001$) harvests, and the total spike biomass in the final harvest ($F_{1,40} = 41\cdot8$, $P < 0\cdot0001$; Figs 1 and 6). While *Lolium* root biomass in the first harvest was not affected by the nutrient treatment (Fig. 2A), RMR decreased significantly under high nutrients ($F_{1,20} = 6\cdot3$, $P = 0\cdot02$; Fig. 2B). Nutrients resulted in a significant increase of *Lolium* leaf N concentration in the first harvest ($F_{1,38} = 28\cdot8$, $P < 0\cdot0001$; Fig. 3A), but a significant decrease in leaf P ($F_{1,38} = 11\cdot4$, $P = 0\cdot002$; Fig. 3B). Consequently, *Lolium* leaf N/P ratios for the first harvest decreased under low nutrients ($F_{1,38} = 50\cdot5$, $P < 0\cdot0001$; Fig. 3C).

Competition by *Oxalis* significantly reduced the first-harvest above-ground biomass of *Lolium* regardless of nutrient treatment ($F_{1,38} = 12\cdot0$, $P = 0\cdot001$; Fig. 1). However, *Oxalis* competition did not affect final *Lolium* tiller and spike biomass ($F_{1,40} = 0\cdot5$, $P = 0\cdot13$ and $F_{1,40} = 2\cdot3$, $P = 0\cdot14$, respectively; Fig. 6). Leaf N concentration and N/P ratios of *Lolium* were significantly reduced under competition with *Oxalis* ($F_{1,38} = 5\cdot2$, $P = 0\cdot03$ and $F_{1,38} = 5\cdot1$, $P = 0\cdot03$, respectively; Fig. 3A, C), while leaf P concentration was not affected by competition ($F_{1,38} = 0\cdot05$, $P = 0\cdot55$; Fig. 3B).

Overall, biomass responses to nutrients and to competition were similar for both species ($F_{\mathrm{N\times SP}\ 1,75} = 0\cdot61$, $P = 0\cdot08$; $F_{\mathrm{C\times SP}\ 1,75} = 0\cdot3$, $P = 0\cdot21$; three-way ANOVA

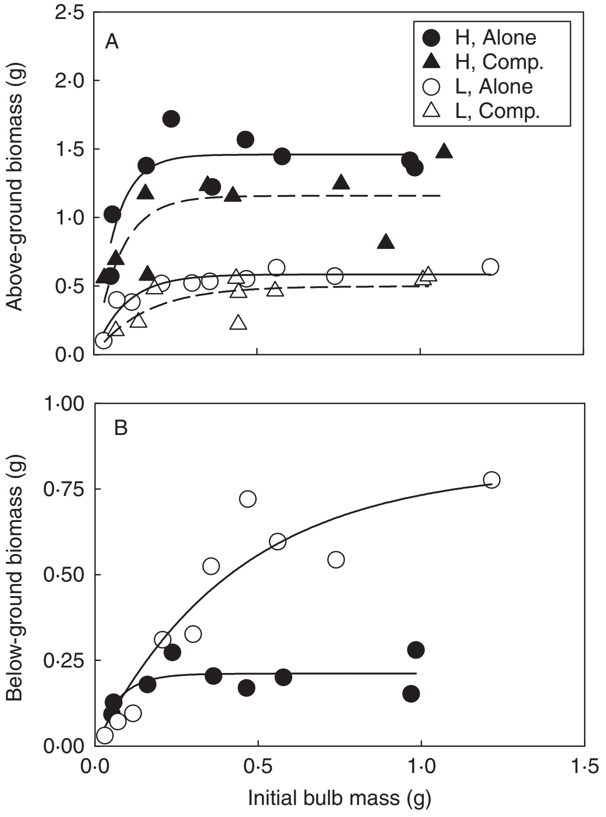

FIG. 5. Relationship between (A) above-ground biomass and (B) below-ground biomass and the mass of the mother bulb of *Oxalis* plants grown under high or low nutrientsand either alone or in competition with *Lolium*. For each treatment data were fitted to an exponential rise function, $y = a(1 - e^{-bx})$.

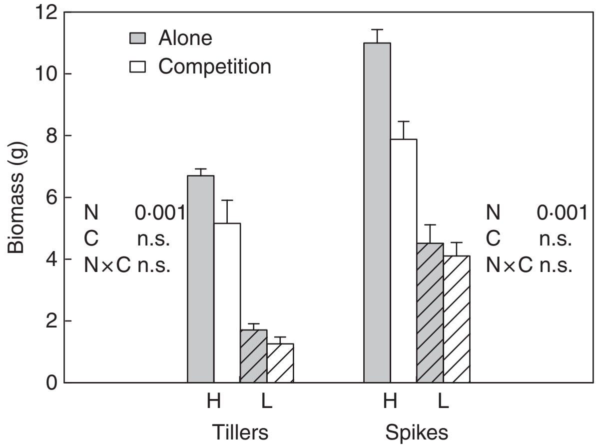

FIG. 6. Total biomass of tillers and spikes of *Lolium* at its peak biomass when grown under high (H, no hatching) and low (L, hatched) nutrients and either alone or in competition with *Oxalis*. Error bars are s.e. of the mean. *P*-values for the effects of competition (C), nutrients (N) and their interaction are also indicated (n.s., not significant).

with species, nutrients and competition; Figs 1 and 2). Decreases in leaf N concentration under low nutrients were greater for *Oxalis* than for *Lolium* ($F_{\mathrm{N}\times\mathrm{SP}\ 1,75} = 5{\cdot}2$, $P = 0{\cdot}03$; Fig. 3A). In contrast, under low nutrients leaf P concentration decreased in *Oxalis* while it increased in *Lolium* ($F_{\mathrm{N}\times\mathrm{SP}\ 1,75} = 22{\cdot}8$, $P = 0{\cdot}0001$; Fig. 3B). Consequently, nutrient effects on the N/P ratio were stronger for *Lolium* than for *Oxalis* ($F_{\mathrm{N}\times\mathrm{SP}\ 1,75} = 4{\cdot}6$, $P = 0{\cdot}04$; Fig. 3C).

Oxalis-invaded soils sampled in Menorca during 2003 had significantly higher P concentrations than paired non-invaded soils (one-tail paired *t*-test; $t_{22} = 1{\cdot}7$, $P = 0{\cdot}02$; Fig. 7).

DISCUSSION

The invasive success of alien species has been associated, among other factors, to disturbance-related increases in resource availability and reductions of competition with native species (Hobbs and Huenneke, 1992). Our results show that *Oxalis pes-caprae* growth and reproductive output is enhanced by increased nutrient availability and absence of competition with the common annual grass *Lolium rigidum*.

Decreases in nutrient availability caused stronger negative effects on *Oxalis* vegetative biomass than did increases in competition with *Lolium. Oxalis* responded to increased nutrient availability with increases in above- and below-ground biomass, decreases in root mass ratio, and increases in leaf N and P concentrations. However, competition with *Lolium* had a much stronger negative effect on the total number of daughter bulbs and their total biomass than was caused by reduced nutrients: when *Oxalis* was grown in competition with *Lolium* the total number and biomass of daughter bulbs was dramatically reduced regardless of nutrient availability. In a glasshouse experiment using the same pot size, Vilà *et al.* (2006*a*) reported that *Oxalis* daughter-bulb production per plant decreased from an average of 50 bulbs when one mother plant was grown per pot (which is consistent with our results) to approx. 30 when there were two mother plants per pot, and to approx. ten when there were fifteen plants per pot. In our experiment, the number of daughter bulbs decreased to below ten bulbs per plant when *Oxalis* was grown with a single *Lolium* plant. These results suggest that the effect of intraspecific competition is much smaller than the effect of interspecific competition. The relative responses to competition and nutrient availability in plants reproducing exclusively by bulbs are poorly documented and, to our knowledge, no study has addressed these interactions in an invasive bulb-reproducing plant. Our results are in accordance with studies on asexual-reproducing plants; increases in asexual propagules and vegetative spread in response to nutrient addition have been documented by Wookey *et al.* (1994), Ronsheim and Bever (2000) and Hansjörg *et al.* (2002). Ronsheim (1996) also reported decreases in aerial bulbil production under intraspecific competition in *Allium vineale*, although nutrient effects were not studied. The strong negative effect of competition by *Lolium* (relative to nutrient availability) on *Oxalis* reproductive output increased even more under high nutrients: not only was the number of daughter bulbs reduced to that produced under low nutrients and competition, but the bulbs were significantly smaller. Consistent with these results, Hansjörg *et al.* (2002) also reported stronger negative effects of competition by natives relative to nutrient reductions on vegetative growth of the invasive clonal forb *Rorippa austriaca*.

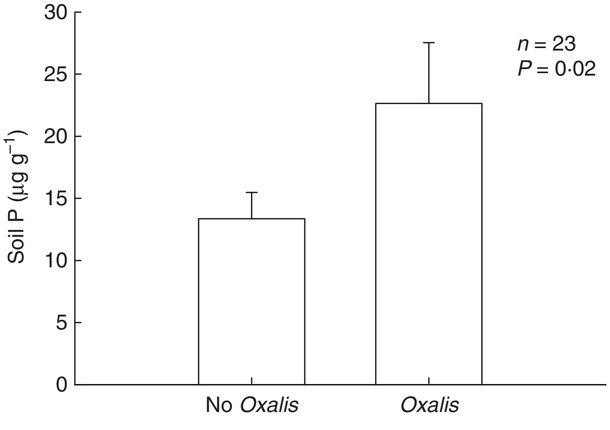

FIG. 7. Average soil available P from field sites either invaded or not invaded by *Oxalis*. Error bars are s.e. of the mean. Although overall averages are shown, field sampling and statistics were conducted with a paired design (*Oxalis*-invaded, non-invaded) for a total of 23 pairs.

The larger size of daughter bulbs produced under low nutrients regardless of competition may be ecologically relevant for the maintenance of subsequent populations in nutrient-deficient soils: under stressful conditions a few, large bulbs may increase population emergence relative to many, small bulbs. Indeed, emergence and growth of *Oxalis* have been shown to depend on bulb size and the depth at which the bulb resides in the soil (Lane, 1984). Smaller bulbs take longer to emerge at the soil surface as initial below-ground growth depends on stored reserves in the bulb. Consistent with this, the few bulbs in the current experiment that failed to emerge were in the smallest category. As reported by Lane (1984), we also found that for bulbs smaller than 0·2 g, increases in mother-bulb mass resulted in higher vegetative biomass. Smaller plants are more likely to exhibit lower daughter-bulb production as bulblet development depends on tuber size, which in turn depends on mother-bulb stored resources and, upon depletion of these, on photosynthates from above-ground structures (Chawdhry and Sagar, 1973). Consistent with this, under low nutrients and no competition, reduced mother-bulb size significantly reduced the biomass of daughter bulbs. Together these results suggest that increased bulb size under nutrient deficient conditions may improve emergence, growth and reproductive output of future plants in the field. Increased bulb size under low nutrients could be an evolutionary response of *Oxalis*, as soils from its native South African range are notoriously nutrient-poor (Read and Mitchell, 1983).

It is unlikely that the decrease of bulb size under high nutrients limits bulb emergence in the more fertile field soils that *Oxalis* tends to prefer in its invasive range. This is because average bulb size under the high nutrient treatment was about 0·2 g, which is the threshold above which bulb size does not affect above-ground growth and daughter-bulb production. Further, disturbances involving soil mixing (e.g. ploughing) bring bulbs close to the soil surface, potentially offsetting the small size effect.

Some invasive species have been shown to be more responsive to nutrient addition than co-occurring native species (e.g. Leishman and Thomson, 2005). This was not the case in our experiment as biomass responses were generally similar for both species. Further, while in *Oxalis* leaf P concentration under low nutrients at the first harvest decreased, there was an increase in *Lolium*. We suspect that this increase was due to a concentration effect, as *Lolium* plants had not yet reached their maximum growth at the first harvested and their above-ground biomass was significantly reduced.

Invasive species are often superior competitors than natives. If this is the case, invaders should affect native plant performance more, and be more resistant to their presence than vice versa (Vilà and Weiner, 2004). This was not the case in our study system: *Lolium* competition reduced both *Oxalis* vegetative biomass and asexual reproductive output while *Oxalis* competition reduced the biomass of *Lolium* at the first harvest but, as predicted, not its final biomass and reproductive output. Although the advantages of initial plant size have been highly emphasized (Gerry and Wilson, 1995), it is probable that the rapid growth of *Oxalis* (with peak growth in the glasshouse by mid-January) relative to *Lolium* (peak growth in April) resulted in only a transient competitive effect of *Oxalis* on *Lolium*. Thereafter, upon *Oxalis* senescence and during bulb maturation, *Lolium* continued to grow to its peak regardless of prior competition with *Oxalis*. While the winter phenology of *Oxalis* minimizes competition with native species, overlap between *Oxalis* and native species occurs during the later stages of the life cycle of *Oxalis* in the spring and early summer, particularly when native species germinate early after warm winters. If climate change advances the phenology of Mediterranean native species (Peñuelas *et al.*, 2002; Gordo and Sanz, 2005), these results point to a potential increase of the competitive effect of native species on *Oxalis*.

Many alien species are known to alter nutrient cycling in invaded areas (Ehrenfeld, 2003). In the field it was found that *Oxalis*-invaded soils had significantly greater available P than non invaded soils. *Oxalis* is known to accumulate oxalic acid in its leaves (Marshall, 1987), which is a chelating agent known for its ability to improve P availability (Cannon *et al.*, 1995). The annual return of *Oxalis* leaf litter and its oxalate to the soil may, therefore, be the mechanism responsible for the observed P increase. Interestingly, in the greenhouse, while soil P amelioration was expected to be minimal, *Lolium* leaf P did not decrease when grown in competition, which is in contrast to the significant decrease in leaf N. These results are consistent with our field data and suggest that *Oxalis* may improve P availability in the field. Such an effect may explain the low observed differences in plant species' diversity in *Oxalis*-invaded and non-invaded communities (Vilà *et al.*, 2006*b*). Field experiments are needed to test if P availability counterbalances the competitive effect of *Oxalis*. Such an effect has been suggested by Bellingham *et al.* (2005), who observed soil P accumulation in communities invaded by *Buddleja davidii* without any changes in native species' diversity relative to non-invaded communities.

In conclusion, our results show that the reproductive output of *Oxalis* was negatively influenced by reduced nutrient availability and, particularly, by competition with the native *Lolium*. These results are consistent with the distribution of *Oxalis* in the field (preferentially in abandoned fields and orchards; Gimeno *et al.*, 2006) and could have important implications for management. For instance, because of the winter phenology of *Oxalis*, ploughing of agricultural fields in the autumn may enhance *Oxalis* invasion due to the relatively high fertility in these fields and the removal of native competitors. The results also suggest that planting native grass species may improve restoration of abandoned orchards, particularly if climate change advances the phenology of native species.

ACKNOWLEDGEMENTS

We are thankful to Josep Matas from the University of Barcelona and Ignasi Bartomeus for their assistance in the greenhouse and in the laboratory; two anonymous referees provided valuable comments to earlier drafts of the paper. Support to AS was provided by the Government of Catalonia via a Visiting Research Professorship awarded to MV. Partial research support was provided by the Integrated European Project ALARM — Assessing Large Scale Risks to Biodiversity with tested Methods (http://www.alarmproject.net), contract 506675 to MV and by a grant from DGICYT to DV (AGL2002-02136 AGR-FOR). Thanks to the Institute of Environmental Science and Technology (ICTA) of the Autonomous University of Barcelona for their hospitality and stimulation during AS's sabbatical.

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