

Alejandro A. Muñoz · Constanza Celedon-Neghme
Lohengrin A. Cavieres · Mary T. K. Arroyo

Bottom-up effects of nutrient availability on flower production, pollinator visitation, and seed output in a high-Andean shrub

Abstract Soil nutrient availability directly enhances vegetative growth, flowering, and fruiting in alpine ecosystems. However, the impacts of nutrient addition on pollinator visitation, which could affect seed output indirectly, are unknown. In a nutrient addition experiment, we tested the hypothesis that seed output in the insect-pollinated, self-incompatible shrub, *Chuquiraga oppositifolia* (Asteraceae) of the Andes of central Chile, is enhanced by soil nitrogen (N) availability. We aimed to monitor total shrub floral display, size of flower heads (capitula), pollinator visitation patterns, and seed output during three growing seasons on control and N addition shrubs. N addition did not augment floral display, size of capitula, pollinator visitation, or seed output during the first growing season. Seed mass and viability were 25–40% lower in fertilised shrubs. During the second growing season only 33% of the N addition shrubs flowered compared to 71% of controls, and a significant (50%) enhancement in vegetative growth occurred in fertilised shrubs. During the third growing season, floral display in N addition shrubs was more than double that of controls, received more than twice the number of insect pollinator visits, and seed output was three- to four-fold higher compared to controls. A significant (50%) enhancement in vegetative growth again occurred in N addition shrubs. Results of this study strongly suggest that soil N availability produces strong positive

bottom-up effects on the reproductive output of the alpine shrub *C. oppositifolia*. Despite taking considerably longer to be manifest in comparison to the previously reported top-down indirect negative effects of lizard predators in the same study system, our results suggest that both bottom-up and top-down forces are important in controlling the reproductive output of an alpine shrub.

Keywords Bottom-up limitation · Central Chilean Andes · Nutrient addition · Plant–pollinator interactions · Plant reproductive output

Introduction

Plants inhabiting alpine and arctic ecosystems face short growing seasons, low temperatures, and low nutrient soils (Billings and Mooney 1968; Körner 1999). Soil nutrient availability is known to limit productivity in alpine (e.g., Atkin and Collier 1992; Bowman et al. 1993, 1995; Bowman and Conant 1994; Wyka and Galen 2000; Seastedt and Vaccaro 2001; Gerdol et al. 2002) and arctic (e.g., Jonasson 1992; Parsons et al. 1994; Havström et al. 1993; Press et al. 1998) ecosystems. Changes in plant community structure, such as shifts in plant composition, species richness, and relative abundance (Theodose and Bowman 1997; Press et al. 1998; Bowman and Bilbrough 2001; Nilsson et al. 2002) have also been reported following nutrient addition.

Enhanced nutrient availability in the alpine ecosystem can also affect reproductive processes. Increased soil nutrient availability can result in the allocation of additional resources to greater fruit and seed production (Campbell and Halama 1993; but see Gerdol et al. 2000) or to the maturation of existing fruits, thereby reducing levels of seed abortion (Stephenson 1981). However, seed set could also be augmented indirectly. An increase in the number and size of flowers resulting from greater nutrient supply (as shown by Campbell and Halama

A. A. Muñoz (✉) · C. Celedon-Neghme · L. A. Cavieres
ECOBIOSIS, Departamento de Botánica,
Facultad de Ciencias Naturales y Oceanográficas,
Universidad de Concepción, Casilla 160-C,
Concepción, Chile
E-mail: aamunoz@rdc.cl
Tel.: +56-41-204418
Fax: +56-41-246005

M. T. K. Arroyo
CMEB, Laboratorio de Sistemática y Ecología Vegetal,
Departamento de Ciencias Ecológicas,
Facultad de Ciencias, Universidad de Chile,
Casilla 653, Santiago, Chile

1993; Parsons et al. 1995; Nagy and Proctor 1997; Wyka and Galen 2000; Heer and Körner 2002) will increase total floral display, which in turn would contribute to greater floral attractiveness to pollinators at the individual plant level, resulting in greater pollinator visitation. Much research has shown that floral display and morphology can greatly influence pollinator visitation levels (e.g., Galen 1985; Galen et al. 1987; Schemske and Horvitz 1988; Johnston 1991; L€ehtila and Strauss 1997). However, for this enhanced pollinator visitation to ultimately result in greater seed output, the increase in number of pollinator visits (or visit time) at the plant level should outweigh the enhancement in floral display.

Notwithstanding, the impact of nutrient augmentation on pollinator visitation patterns in alpine species is unknown. Increased pollinator visitation would be especially critical in the alpine ecosystem where entomophily is common (Arroyo et al. 1982; K€orner 1999), pollinator abundance and activity levels are often low (e.g., Arroyo et al. 1985; Bingham and Orthner 1998; Mu˜oz and Arroyo 2004), seed set is often pollen-limited (e.g., Totland and Sottocornola 2001; Mu˜oz and Arroyo, unpublished data), and outbreeding is the dominant breeding system among long-lived species (Arroyo and Squeo 1990; Gugerli 1998).

In the alpine ecosystem, most of the few nutrient addition studies concerning reproduction have considered herbaceous species (see however, Wookey et al. 1993; Parsons et al. 1994 in the arctic). Alpine ecosystems are characterised by a wide range of life-forms, among which dwarf and low-rounded shrubs are usually important and dominant in lower alpine belts (K€orner 1999; Cavieres et al. 2000). Woody alpine species tend to grow slower than herbaceous species and often have preformed vegetative buds (K€orner 1999). Thus, responses to added nutrients are likely to be different than in herbaceous species both at the vegetative and reproductive levels. A comprehensive picture for the nutrient effects on reproduction, and on vegetative growth in high-altitude ecosystems, thus, requires increasing the knowledge base for the shrub life-form as well as incorporating studies on pollinator visitation rates.

The primary objective of this study was to test the hypothesis that reproductive output in the insect-pollinated, self-incompatible, alpine shrub *Chuquiraga oppositifolia* (Asteraceae), a dominant species of the lower alpine belt throughout central Chile, is enhanced by soil nutrient addition. We specifically manipulated soil nitrogen (N) as it is considered the most limiting nutrient in cold environments (Holzmann and Haselwandter 1988; K€orner 1999). In particular, we were interested in determining whether any such potential effects are produced indirectly as a result of increased pollinator visitation. We aimed to monitor total floral display and size of capitula, insect pollinator visitation patterns, and seed output following N addition for treatment and control shrubs over three growing seasons: 2001–2002, 2002–2003, and 2003–2004. Since we found no significant effects on reproductive parameters in the first growing

season (2001–2002), vegetative growth was monitored in treatment and control shrubs in the second (2002–2003) and third (2003–2004) growing seasons following nutrient addition to better understand the processes investigated.

Specifically we asked the following questions. Does N addition to the soil prior to the commencement of vegetative growth in the 2001–2002 growing season (1) result in the production of a greater number (floral display) and size of capitula, (2) enhance pollinator visitation patterns, and (3) result in the production of a greater number of fruits (achenes) and/or higher quality achenes, in terms of size and viability, during that season as well as during subsequent seasons. Secondly, we sought to determine whether prior nutrient application in the first growing season affected vegetative growth in a second and third growing season.

Materials and methods

Study system

Research was conducted on an 18-ha site at 2,600 m altitude in the lower alpine belt, Valle Nevado area (33°21' S, 70°16' W), Andes of central Chile, ca. 50 km east of Santiago. Climate is alpine with Mediterranean influence, with a mean annual precipitation at 2,400 m of 431 mm, falling predominantly as snow during the winter months from June through August, although snow may fall as early as April and as late as October. The growing season (corresponding to the snow-free period) at this altitude commonly extends from mid-October to mid-May (Arroyo et al. 1981). The study site is south-facing, with gentle (<15°) slopes. Vegetation is dominated by low <45 cm spiny shrubs of *C. oppositifolia*, *Anarthrophyllum cumingii*, and *Berberis empetrifolia*. Herbaceous species, such as *Acaena pinnatifida*, *Phacelia secunda*, *Stachys philippiana*, and various species of *Adesmia* and *Senecio*, are also abundant. We chose to work with the small <45 cm high asteraceous shrub *C. oppositifolia* (16% cover), an insect-pollinated (Arroyo et al. 1982; Rozzi 1990), fully self-incompatible (Rozzi 1990), and the dominant shrub species in the lower alpine belt (Cavieres et al. 2000). *C. oppositifolia* flowers late in the season (January–April) (Arroyo et al. 1981). The conspicuous shrubs become covered with yellow capitula containing 12 florets on average, which have been reported to be pollinated by bumblebees and solitary bees (Apidae, Anthophoridae, Megachilidae), flies (Syrphidae, Bombyllidae), and butterflies (Satyridae) (Arroyo et al. 1982; Rozzi 1990; Mu˜oz 2003). The main pollinators have been shown to transport pollen (Rozzi 1990).

Field experimental design

To assess the effects of N addition on *C. oppositifolia*, we conducted a manipulative field experiment over three

growing seasons: October–April of 2001–2002, 2002–2003, and 2003–2004. Forty-eight shrubs of comparable size, each separated by at least 15 m from one another, were selected. Shrubs were assigned to one of two treatments, each with 24 replicates: (1) Control (no N added), and (2) + Nitrogen (N added). Shrubs under the two treatments did not differ in size (area), with means of $8,561 \pm 1,471 \text{ cm}^2$ (1 SE) and $11,645 \pm 2,866 \text{ cm}^2$ in Control and + Nitrogen shrubs, respectively (Mann–Whitney U -test, $U=198.5$, $Z=-0.790$, $P=0.430$). In late October 2001 (prior to the commencement of that year’s growth), on an overcast and cold day, we applied 30 g of N in the form of urea-N pellets (NPK 40-0-0) dissolved in 2 l of water (15 mg ml^{-1}) to the soil around each of the 24 shrubs. The amount of urea-N applied was similar to that added in various N addition experiments conducted in other alpine ecosystems (e.g., Bowman et al. 1993; Bowman and Conant 1994; Theodose and Bowman 1997). The fertiliser was added (once during the entire season) in eight 5-cm holes dug around the periphery of each shrub. The holes were subsequently filled with soil to minimise N volatilisation. The 24 control shrubs received 2 l of pure water each.

To assess the effect of Urea-N fertiliser application on soil inorganic N levels, we collected ca. 20 g soil samples from 20 non-experimental shrubs. Urea-N was applied to 50% (10) of these. One and 3 months after nutrient addition, soil samples were taken and analysed for NH_4^+ -N (ammonium) and NO_3^- -N (nitrate). NH_4^+ and NO_3^- were extracted with a 1% KAl (SO_4)₂ solution, and determined by means of the steam MgO-Devarda fractionated micro-distillation method (Page 1983). Urea-N fertiliser addition produced a ca. 200-fold increase in NH_4^+ concentration 1 month following N addition, from a mean of 0.59 ppm of NH_4^+ prior to N addition to a mean of 104.66 ppm. NH_4^+ was >125 times higher than control levels 2 months later, with a mean of 73.62 ppm (Kruskal–Wallis, $H=19.878$, $P<0.001$). In contrast, soil NH_4^+ concentration did not change significantly under Control (untreated) shrubs during the same period (Kruskal–Wallis, $H=0.086$, $P=0.958$). Fertiliser addition enhanced NO_3^- concentration six-fold after 1 month following Urea-N application from 1.94 to 11.09 ppm of NO_3^- , increasing to a high of 19.71 ppm 2 months later (Kruskal–Wallis, $H=21.375$, $P<0.001$). In contrast, soil NO_3^- concentration did not change significantly under untreated shrubs during the same period (Kruskal–Wallis, $H=5.160$, $P=0.080$).

Floral display and size of capitula

We initially evaluated predicted effects of N addition on the number and size of capitula produced by *C. oppositifolia* shrubs during the 2001–2002 growing season, i.e., when N was initially applied; this way being able to assess potential short-term responses. In the second growing season after N application (2002–2003),

floral display and size of capitula were not monitored because only ca. 50% of the shrubs under study flowered that season. Flowering of *C. oppositifolia* shrubs was low during that season in the general study area. Number and size of capitula produced by *C. oppositifolia* shrubs were again monitored during the third growing season (2003–2004) after N application. Thus, we were able to determine both short-term (2001–2002) as well as long-term (2003–2004) effects of N addition on the reproductive performance of *C. oppositifolia*.

We quantified total floral display by counting all fully opened capitula on each shrub during the flowering period of the 2001–2002 growing season (late January to mid-March 2002). We also quantified the size of capitula and the number of open florets/capitulum. For this, during early January 2002, we tagged 50 randomly selected twiglets per + Nitrogen and Control shrub, each with its apical floral bud. Each twiglet can produce only one capitulum. Maximum diameter of capitula was measured using a hand calliper (precision 0.05 mm) during the days when the capitula exhibited abundant pollen presentation and receptive stigmas. Much temporal variation was observed regarding the timing of capitulum opening both within and among shrubs. Thus, measurements on each individual shrub necessarily spanned a number of days. During the 2003–2004 growing season again we quantified total floral display on each monitored shrub. To measure maximum diameter of capitula and number of open florets/capitulum, 30 randomly selected capitula per + Nitrogen and Control shrub were tagged in late January 2004. Maximum diameter was determined as described above between late January and mid-February 2004.

We analysed differences in total floral display, size of capitula, and mean number of open florets/capitulum in Control and + Nitrogen shrubs using Student’s t -tests (Zar 1996; StatSoft 1998), after testing for normality and homogeneity of variances using the Shapiro–Wilks and Bartlett tests, respectively. Data were transformed appropriately when the normality condition was not met. When this assumption was not met with the transformed data, Mann–Whitney U -tests were applied.

Pollinator visitation patterns

We determined insect pollinator visitation rates, and duration of pollinator visits to shrubs for + Nitrogen and Control shrubs following Arroyo et al. (1982, 1985) and Rozzi (1990). “Pollinators” were defined as insects that made contact with the anthers and/or stigmas of florets of capitula and carried pollen. We conducted 10-min observations on each shrub between 9:00 a.m. and 5:00 p.m. over the period late January to early March 2002 and late January to mid-February 2004. We defined a pollinator visit to a shrub as one in which an insect landed on at least one open capitulum of the shrub and became covered with pollen. Pollinators that only flew above or around the shrub and then left were

not included in the analyses. Observations were made at least 6–8 m away from the target shrubs using binoculars, so as to avoid potentially disturbing pollinator behaviour. Overall, we performed 397 and 270 10-min observations in the 2001–2002 and 2003–2004 growing seasons, respectively. Response variables were expressed as mean values for each shrub for 10-min observation periods. We analysed differences in mean pollinator visitation rates and duration of visits to Control and + Nitrogen shrubs as described above.

Seed output

To evaluate the effects of N addition on seed production in *C. oppositifolia*, we monitored seed output in open pollinated capitula in + Nitrogen and Control shrubs. In February to early March of 2002 and 2004 we covered all monitored capitula following withering with 8×6 cm yellow mesh bags so as to prevent the potential loss of the developing wind-dispersed achenes (one-seeded fruit). We retrieved all bags in April–May 2002 and late March 2004, and analysed each capitulum for seed output (number of achenes). Seed output was expressed as (1) Percentage of capitula per shrub that set one or more achenes (%CA), (2) Mean number of achenes per capitulum per shrub (NAC), and (3) Percentage seed set (%SS), which was measured as the percentage of ovaries of open florets per shrub that set seeds (i.e., that could potentially have been fertilised). When calculating NAC, we included all monitored capitula regardless of whether the capitulum produced any achene or not (i.e., including the zero-achene capitula). We compared seed output in Control versus + Nitrogen shrubs as described above.

Seed quality (size and viability)

We compared the quality of achenes produced by Control and + Nitrogen shrubs during the 2001–2002 and 2003–2004 growing seasons in terms of their biomass and viability. We individually weighed achenes (including the pappus) to the nearest 1 mg. Because the number of mature achenes per shrub collected in the mesh bags was low, we pooled all achenes per treatment for the viability test. Percentage viability of 50 achenes from + Nitrogen and Control shrubs was assessed with the tetrazolium test (Baskin and Baskin 1998) and compared using a proportions test (Zar 1996).

Vegetative growth

We compared vegetative growth of shrubs using a random sample of ten non-flowering twiglets, obtained from each of the shrubs under study during late February 2003 and 2004. While under winter snow, *C. oppositifolia* loses its leaves completely, with the

appearance of new shoots and leaves the following spring from pre-formed buds (Muñoz and Arroyo, personal observations). This allowed us to estimate annual vegetative growth. We dried twiglets to constant weight in an oven at 70°C for 72 h and weighed them to the nearest 1 mg. Mean dry weight of twiglets was compared for Control and + Nitrogen shrubs through Student's *t*-tests or Mann–Whitney *U*-tests.

Results

Effects on floral display and size of capitula

The flowering response variables assessed in the first growing season (2001–2002) did not differ in the Control and N addition (+ Nitrogen) shrubs of *C. oppositifolia* (Fig. 1). This was true for mean floral display (Mann–Whitney *U*-test, $U=218.0$, $Z=0.063$, $P=0.950$), and size of capitula, expressed as mean maximum diameter

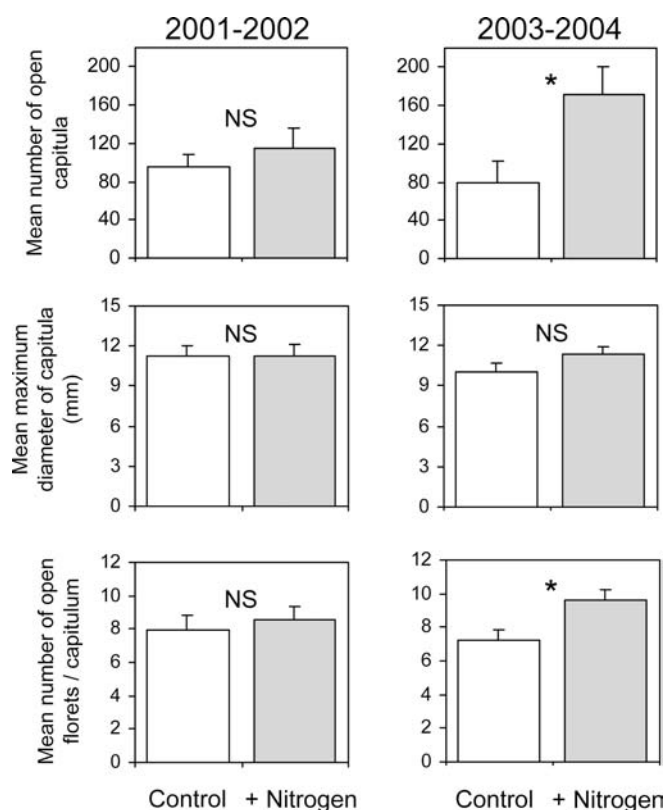


Fig. 1 Mean floral display (mean number of open capitula/shrub), size of capitula expressed as mean maximum diameter of capitula, and number of open florets/capitulum in Control and N addition (+ Nitrogen) shrubs of *Chuquiraga oppositifolia* during the 2001–2002 and 2003–2004 growing seasons in the lower alpine belt, at 2,600 m altitude, Andes of central Chile. Nitrogen was added to the soils around shrubs at the start of the 2001–2002 growing season (October 2001). Twenty-four replicate shrubs per treatment were monitored during 2001–2002 while 14 (Control) and 16 (+ Nitrogen) replicate shrubs were monitored during 2003–2004. Bars are means + 1 SE. * significant differences ($P < 0.05$), NS no significant differences between treatments

of capitula (Student's t -test, $t = -0.006$, $df = 45$, $P = 0.995$, Fig. 1). Likewise, mean number of total open florets/capitulum did not differ either ($t = -0.801$, $df = 45$, $P = 0.428$, Fig. 1).

During the second growing season (i.e., October 2002 to April 2003) flowering of shrubs was low; only 25 of the 48 test shrubs (52%) flowered. Of these, 17 shrubs corresponded to Control replicates and 8 to + Nitrogen shrubs. Given the low number of replicate shrubs in flower, we decided not to monitor flowering variables during the 2002–2003 growing season. Nevertheless, a proportions test ($Z = 2.31$, $P < 0.05$) revealed that flowering was significantly lower in + Nitrogen shrubs (33%) versus Control shrubs (71%).

By the third growing season (2003–2004), two of three flowering response variables assessed differed significantly between the Control and N addition shrubs (Fig. 1). Mean floral display in N addition shrubs was more than double that shown by Control shrubs ($t = -2.780$, $df = 28$, $P = 0.010$). However, mean maximum diameter of capitula did not differ in Control and + Nitrogen shrubs ($t = -1.711$, $df = 28$, $P = 0.098$, Fig. 1). Mean number of total open florets/capitulum was ca. 30% greater in + Nitrogen shrubs than in Controls ($t = -2.731$, $df = 28$, $P = 0.011$, Fig. 1).

Effects on pollinator visitation patterns

C. oppositifolia was visited by 18 and 16 different pollinator taxa during the first and third growing seasons,

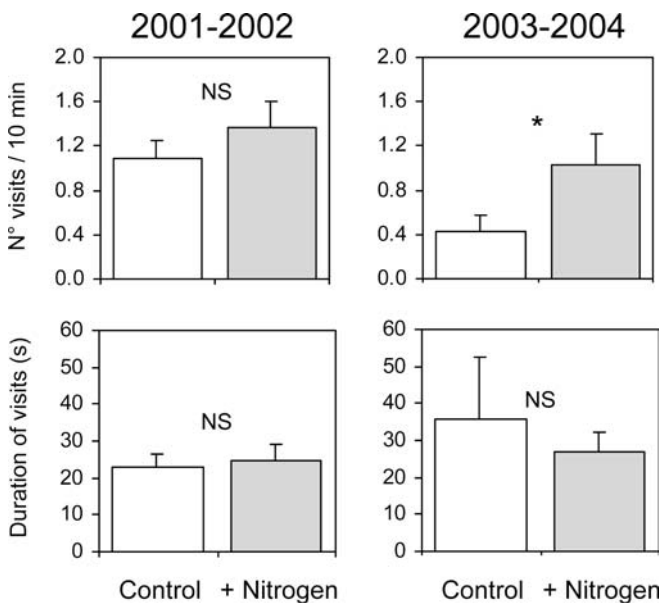


Fig. 2 Effects of nitrogen addition to soils around shrubs of *C. oppositifolia* on insect pollinator visitation rates (number of pollinator individuals visiting shrubs/10 min), and duration of visits to shrubs (s) during the 2001–2002 and 2003–2004 growing seasons in the lower alpine belt, at 2,600 m altitude, Andes of central Chile. Bars are means + 1 SE. Symbols as in Fig. 1

respectively. During the 2001–2002 season, the most frequent pollinators were the satyrid butterfly *Cosmosatyrus chilensis* (28.0% of the total number of visits), the syrphid fly *Scaeva melanostoma* (22.7%), and the andrenid bee *Heterosarus* sp. (22.2%). Less frequent visitors were the andrenid bee *Liphanthus* sp. (5.3%), bombyliid flies *Villa* spp. (4.8%), other bombyliid flies (4.6%), and the bumblebee *Bombus dahlbomii* (2.4%). During the 2003–2004 season, the most important pollinators were again the fly *S. melanostoma* (37.0% of the total number of visits) and the butterfly *Cosmosatyrus chilensis* (21.0%), while the bee *Heterosarus* sp. proved to be an infrequent visitor that season (4.0%). Other fairly important visitors were the flies *Villa* spp. (10.0%), other bombyliid flies (8.0%), and the bumblebee *B. dahlbomii* (5.0%).

During the first growing season (2001–2002) pollinator visitation rates did not differ between Control and + Nitrogen shrubs ($t = -0.807$, $df = 45$, $P = 0.424$, Fig. 2). Likewise, the duration of pollinator visits was not significantly different (Mann–Whitney $U = 263.5$, $Z = 0.266$, $P = 0.790$, Fig. 2). In contrast, on average,

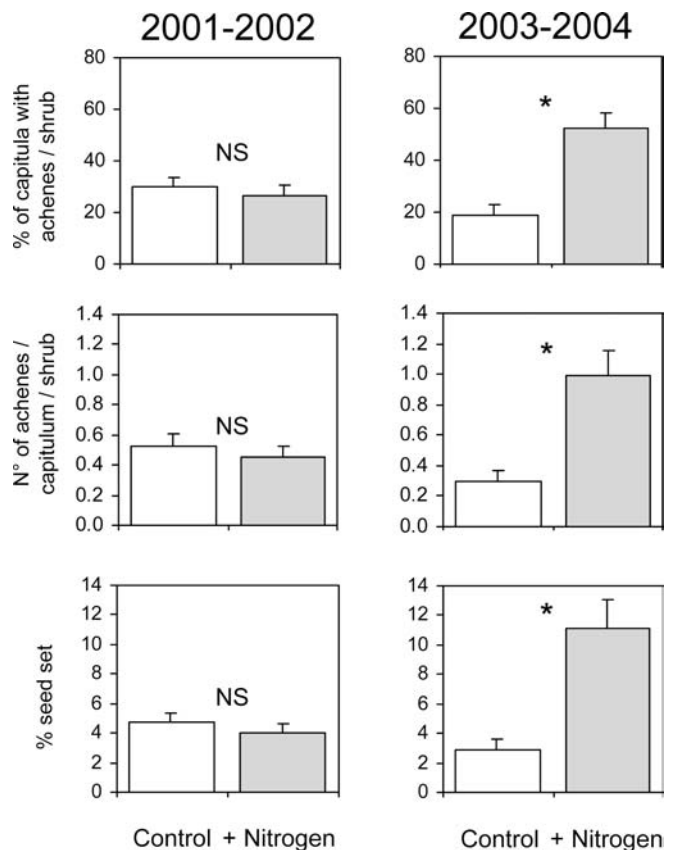


Fig. 3 Seed output expressed as the percentage of capitula per shrub that set one or more achenes (%CA), mean NAC per shrub, and percentage seed set (%SS) in Control and N addition (+ Nitrogen) shrubs of *C. oppositifolia* during the 2001–2002 and 2003–2004 growing seasons in the lower alpine belt, at 2,600 m altitude, Andes of central Chile. Bars are means + 1 SE. Symbols as in Fig. 1

+ Nitrogen shrubs received more than twice the number of pollinator visits compared to Control shrubs ($U=43.5$, $Z=-2.661$, $P=0.008$, Fig. 2) during the third growing season (2003–2004). However, the duration of pollinator visits did not differ ($t=-0.125$, $df=25$, $P=0.902$, Fig. 2).

Effects on seed output, size, and viability

Seed output between fertilised and control shrubs did not differ significantly during the 2001–2002 growing season (Fig. 3). Mean %CA was similar between Control and + Nitrogen shrubs ($t=0.822$, $df=45$, $P=0.415$, Fig. 3). Similarly, N addition did not affect the mean NAC ($U=255.0$, $Z=0.681$, $P=0.496$, Fig. 3). Percentage seed set (%SS) did not differ either ($t=1.003$, $df=46$, $P=0.321$, Fig. 3).

However, by the third season following N addition, seed output, expressed as %CA and NAC, was ca. three-fold higher on fertilised shrubs compared to Control shrubs (t for %CA = -4.750 , $df=26$, $P<0.001$, and t for NAC = -3.970 , $df=26$, $P<0.001$, Fig. 3). Further, when expressed as %SS, differences proved even more striking with an almost four-fold increment between Control and + Nitrogen shrubs ($U=26.5$, $Z=-3.285$, $P<0.001$, Fig. 3).

Despite the lack of differences in seed number in the 2001–2002 growing season, counter intuitively, achenes produced by shrubs that received fertiliser were significantly smaller than achenes produced by Control shrubs

($U=153.0$, $Z=2.784$, $P<0.001$, Fig. 4). Further, percentage viability of seeds produced by + Nitrogen shrubs was significantly lower (Proportions test, $Z=2.36$, $P<0.05$, Fig. 4) than that of seeds produced by Control shrubs.

On the other hand, the large differences between Control and + Nitrogen shrubs in the number of seeds produced during the third (2003–2004) growing season (Fig. 3) were not coupled with differences in seed quality. Mean achene mass of shrubs that had received fertiliser was similar to that of control shrubs ($U=78.5$, $Z=-0.897$, $P=0.369$, Fig. 4). Likewise, percentage viability of seeds did not differ between Control and + Nitrogen shrubs (Proportions test, $Z=0.63$, $P>0.05$, Fig. 4).

Effects on vegetative growth

In February 2003, mean dry weight of twiglets of shrubs that had received N the previous season (2001–2002) was ca. 50% greater than for Control shrubs ($U=110.0$, $Z=-3.67$, $P<0.001$, Fig. 5), suggesting a significant enhancement in vegetative growth of + Nitrogen shrubs. A positive effect of initial N addition on vegetative growth was also suggested in the third season (February 2004) following N addition, with mean dry weight of twiglets of + Nitrogen shrubs again being ca. 50% greater than for Control shrubs ($U=29.0$, $Z=-3.451$, $P<0.001$, Fig. 5).

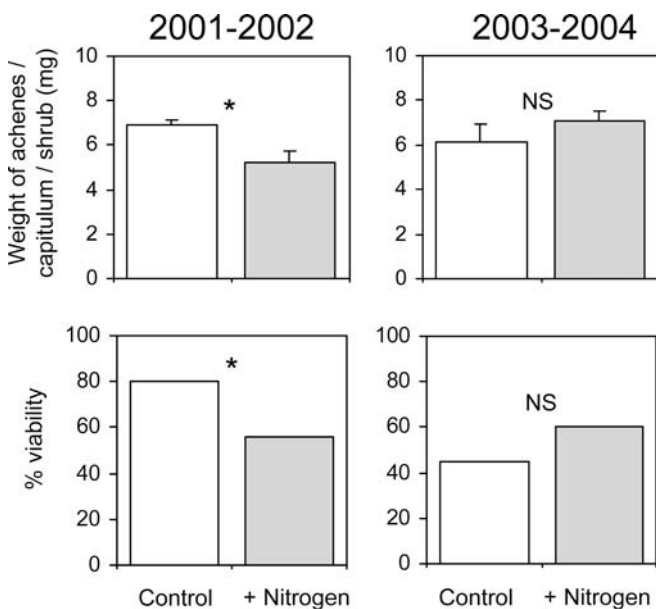


Fig. 4 Effects of nitrogen addition to soils around shrubs of *C. oppositifolia* on the quality of seeds produced expressed as mean weight of achenes per capitulum per shrub and percentage viability (%alive) during the 2001–2002 and 2003–2004 growing season in the lower alpine belt, at 2,600 m altitude, Andes of central Chile. Bars are means + 1 SE. Symbols as in Fig. 1

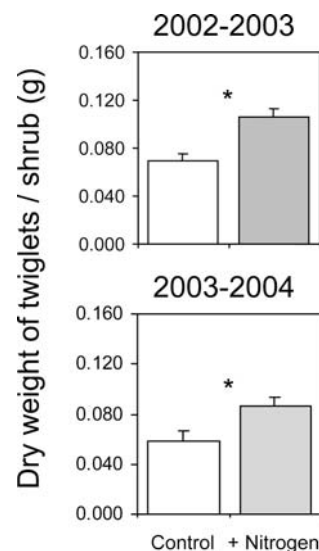


Fig. 5 Effects of nitrogen addition to soils around shrubs of *C. oppositifolia* on vegetative growth expressed as the mean dry weight of twiglets produced during the 2002–2003 and 2003–2004 growing seasons in the lower alpine belt, at 2,600 m altitude, Andes of central Chile. Bars are means + 1 SE. Symbols as in Fig. 1

Discussion

Fertiliser application produced strong positive bottom-up effects on the reproductive output of the alpine shrub *C. oppositifolia*, including greatly enhanced floral display, pollinator visitation, and seed production, suggesting that seed output in this alpine species is resource limited. However, positive effects of N addition were only evident after three growing seasons (2003–2004) following its application whereby mean floral display in N addition shrubs was more than double that of control shrubs. Further, N addition shrubs received more than twice the number of insect pollinator visits and seed output was ca. three- to four-fold higher compared to controls. These strong relatively long-term positive effects can be contrasted with the non-significant and even a few negative effects detected over the first and second growing seasons. Floral display, size of capitula, pollinator visitation patterns, and seed output were not affected by N addition in the same season of its application (2001–2002).

The reduction in seed mass and viability in N addition shrubs during the first growing season was an unexpected result. The potentially toxic effects of an initial 200- and six-fold increase in soil NH_4^+ and NO_3^- levels, respectively, may have caused a reduction in the quality of seeds. It has been reported that many plant species are adversely affected by high NH_4^+ concentrations and/or ammonium: nitrate ratios (e.g., Marschner 1995; Dorland et al. 2003). High levels of inorganic N may have been caused by high rates of mineralisation by microorganisms in the soils of the lower alpine belt at 2,600 m altitude, which are not particularly cold, especially during the summer months (Cavieres et al. 1998).

To our knowledge, there has been no previous attempt to simultaneously evaluate the potential effects of soil nutrient availability on floral attractiveness, visitation rates and seed output in an alpine plant. In fact, relatively few studies on the effects of nutrient augmentation on reproductive parameters have been conducted in alpine plants in general (e.g., Wyka and Galen 2000; Bechtold et al. 2002; Heer and Körner 2002), and alpine shrubs in particular (but see Gerdol et al. 2000). Indeed, most studies designed to detect the effect of nutrient availability on reproduction in alpine plants, even in herbaceous species (where studies are more abundant), have only used fruit and seed set as response variables. An exception is Campbell and Halama's (1993) study in the perennial herb *Oxytropis sericea*. These authors evaluated whether nutrient availability influenced seed production indirectly by increasing flowering; however they did not actually measure pollinator visitation patterns to flowers of plants that had received nutrients, as was done here. In that study no effect of nutrient application on corolla size was detected. However, increased flower production per se had a strong effect on total seed production.

Here, we were particularly interested in determining whether nutrient availability could influence seed output indirectly via increased floral attractiveness to insect pollinators (i.e., increased floral display and size of capitula), resulting in higher pollination levels, and ultimately in greater seed output. Much research has shown floral display to influence pollinator visitation levels (e.g., Galen 1985; Galen et al. 1987; Schemske and Horvitz 1988; Johnston 1991; L  htila and Strauss 1997). Further, numerous studies have reported increased pollinator visitation to lead to greater seed output (e.g., Johnson et al. 1995; Bosch and Waser 2001; Brown et al. 2002; Mu  oz and Arroyo 2004), which can be explained by the fact that seed set is often limited by a low abundance and activity of pollinators (see reviews by Burd 1994; Larson and Barrett 2000). Particularly, in alpine environments pollinator abundance and activity levels are characteristically low (e.g., Arroyo et al. 1985; Rozzi 1990; Bingham and Orthner 1998; Mu  oz 2003) with many studies showing pollen limitation of seed output among alpine plants (e.g., Galen 1985; Campbell and Halama 1993; Totland and Sottocornola 2001). A supplemental hand-pollination experiment conducted concurrently at the study site resulted in a two- to threefold increase in seed output in hand-pollinated shrubs, indicating that seed output in *C. oppositifolia* is pollen-limited (Mu  oz and Arroyo, unpublished data).

Given this strong pollen-limitation in this shrub species, in our study, the three- to four-fold enhancement in seed output of fertilised shrubs cannot be considered an unexpected result, and therefore may be, at least partially, explained by the more than two-fold increase in insect pollinator visitation to this self-incompatible alpine shrub. In addition, the enhanced pollinator visitation most probably was caused by the more than two-fold increase in floral display (total number of capitula per shrub). However, it could be asked as to what extent an increase in the number of pollinators visiting shrubs, which roughly matches that of the floral display, could explain an increase in seed output at the individual shrub level? This would require pollinators to be more efficient in ovary fertilisation during each visit. Although this possibility cannot be discarded, other non-mutually exclusive mechanisms could also explain the increased seed output: (a) enhanced resource provisioning of ovaries via the allocation of the greater quantity of nutrients available following fertilisation, resulting in decreased abortion and maturation of developing seeds (see Stephenson 1981) and (b) a direct consequence of the enhanced number of capitula and florets per capitulum (Campbell and Halama 1993). We cannot dismiss any of these possibilities, with all three probably having influenced seed output in fertilised shrubs.

Our results, together with those of the above-mentioned supplementary hand-pollination experiment, contribute to the ongoing debate and research on the relative importance of pollen versus resource limitation

of plant reproduction (e.g., Willson and Price 1980; Bierzychudek 1981; Galen 1985; Zimmerman and Pyke 1988; Campbell and Halama 1993; Mattila and Kuitunen 2000; Montgomery et al. 2003; see review in Larson and Barrett 2000). Logically, plant reproduction cannot be co-limited by both resources and pollinators (Campbell and Halama 1993). The observed enhancement in seed output in fertilised shrubs suggests that an increase in nutrient availability, not only provided more resources for seed maturation, but also resulted in increased floral display (a two-step release from resource limitation), and this, in turn might have relieved these shrubs from pollinator limitation. Therefore, the reproductive output of *C. oppositifolia* could be augmented either through direct liberation from pollinator limitation via supplemental hand-pollination or indirectly via first releasing these shrubs from resource limitation (this study).

Positive reproductive responses to nutrient supply in the dwarf shrub *C. oppositifolia* were slow to become manifest, appearing only by the third growing season following nutrient addition to the soil. This could perhaps be explained by the fact that nutrient availability may be less important in controlling plant growth and reproduction in cold environments than inherent developmental constraints, which may limit the ability of plants to respond quickly to nutrient supply (Chapin et al. 1986; Körner 1989). Plants native to ecosystems with low nutrient supply, such as arctic and alpine tundra, often show a limited response to increases in soil nutrients as a consequence of adaptations that promote conservative use, loss, and uptake of nutrients (Chapin 1980; Theodose and Bowman 1997). Indeed, in a worldwide comparison of plant tissue N concentrations, Körner (1989) reported that plants from high elevations tend to have higher foliar N concentrations than their lowland congeners, which may be related to the fact that in cold infertile habitats, plants tend to grow slowly and build up higher concentrations of N by “luxury consumption” (Chapin et al. 1986; Bowman et al. 1993; Körner 1989, 1999; Theodose and Bowman 1997). The fact that *C. oppositifolia* only showed positive vegetative responses to N fertilisation in the second growing season (even though applied N had quickly broken down in the soil) is consistent with these general observations for high elevation plants.

Nutrient enrichment of the soil produced a 50% enhancement in our estimation of vegetative growth that measured the level of current-year individual shoot growth in the two seasons following its application. This result concurs with those of many other studies conducted in alpine (e.g., Bowman et al. 1993; Bowman and Conant 1994) and arctic (e.g., Parsons et al. 1994; Press et al. 1998) ecosystems. However, we cannot discard the possibility that although twiglets of + Nitrogen shrubs grew more than those of Control shrubs, the former produced fewer twiglets such that total biomass or primary production did not differ between treatment shrubs. Thus, the use of shoot biomass gives us only a

partial picture of the effects of N fertilisation on overall vegetative growth at the individual level.

Finally, our results also contribute to the debate on the relative importance of top-down (predation) versus bottom-up (resource availability) forces in plant communities (e.g., Hunter and Price 1992; Polis and Strong 1996; Dyer and Letourneau 1999; Schmitz et al. 2000; Dawes-Gromadzki 2002; Muñoz 2003). A predator (lizard and insectivorous bird) exclusion experiment conducted in conjunction with the present research showed that pollinator visitation and seed output can be several times lower on shrubs of *C. oppositifolia* growing adjacent to lizard-inhabited rocks compared to those of shrubs growing in plots from where lizard predators were excluded (Muñoz and Arroyo 2004). Thus, the results of the present study, together with those of the predator exclusion experiment, suggest that both bottom-up (i.e., soil nutrient availability) and top-down forces (i.e., lizard predator presence), acting on seed output in the shrub *C. oppositifolia* in the central Chilean Andes, are important. The temporal scales at which these two forces operate are strikingly different. Top-down effects of lizard predators were observed quickly and within single growing seasons (Muñoz 2003). Lizards, by their mere presence on rocks next to shrubs and resultant risk of predation, were capable of altering pollinator behaviour, ultimately producing strong negative indirect effects on the plant fitness of *C. oppositifolia* (Muñoz and Arroyo 2004). In sharp contrast, the positive bottom-up effects of soil N availability on seed output took much longer to appear, with significant effects on floral display, size of capitula, pollinator visitation, and seed output, only being manifest three growing seasons after N application. Despite differences in the time-scale of operation, our results strongly suggest that both forces (i.e., lizard predator presence and soil nutrient availability) are important in regulating the reproductive output of the shrub *C. oppositifolia* in the central Chilean Andes.

Acknowledgements We thank N. Binfa, P. Chacón, M. Correa, L. Díaz, S. Henríquez, K. Maldonado, B. Muñoz, A. Rivera, and L. Sotomayor for help in the field. Research benefited from discussions with J.J. Armesto, R.O. Bustamante, F.M. Jaksic, C.G. Jones, and M.F. Willson. R.O. Bustamante and two anonymous reviewers made highly valuable comments on earlier versions of the manuscript. R. Leatherbee is acknowledged for permission to conduct work on El Colorado Ski Complex (ANDACOR S.A) property. Research was supported by FONDECYT 2010032 doctoral research grant to A.A.M, Chilean Millennium Scientific Initiative grants P99-103-F-ICM and P02-051-F ICM to M.T.K.A., and FONDECYT 1030821 research grant to L.A.C. A.A.M is currently a Postdoctoral fellow at Universidad de Concepción, Chile, supported through Mecesus grant UCO9906.

References

- Arroyo MTK, Squeo FA (1990) Relationship between plant breeding systems and pollination. In: Kawano S (ed) Biological approaches and evolutionary trends in plants. Academic, New York, pp 205–227

- Arroyo MTK, Armesto JJ, Villagrán C (1981) Plant phenological patterns in the high Andean Cordillera of central Chile. *J Ecol* 69:205–223
- Arroyo MTK, Primack R, Armesto J (1982) Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. *Am J Bot* 69:82–97
- Arroyo MTK, Armesto JJ, Primack RB (1985) Community studies in pollination ecology in the high temperate Andes of central Chile. II. Effect of temperature on visitation rates and pollination possibilities. *Plant Syst Evol* 149:187–203
- Atkin OK, Collier DE (1992) Relationship between soil-nitrogen and floristic variation in late snow areas of the Kosciusko alpine region. *Aust J Bot* 40:139–149
- Baskin CC, Baskin JM (1998) Seeds. Ecology, biogeography, and evolution of dormancy and germination. Academic, New York
- Bechtold HA, Forbis TA, Bowman WD, Diggle PK (2002) Lack of reproductive plasticity in alpine *Saxifraga rhomboidea* (Saxifragaceae). *Nordic J Bot* 22:361–368
- Bierzzychudek P (1981) Pollinator limitation of plant reproductive effort. *Am Nat* 117:838–840
- Billings WD, Mooney HA (1968) The ecology of arctic and alpine plants. *Biol Rev* 43:481–529
- Bingham RA, Orthner AR (1998) Efficient pollination of alpine plants. *Nature* 391:238–239
- Bosch M, Waser NM (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia* 126:76–83
- Bowman WD, Bilibrough CJ (2001) Influence of a pulsed nitrogen supply on growth and nitrogen uptake in alpine graminoids. *Plant Soil* 233:283–290
- Bowman WD, Conant RT (1994) Shoot growth dynamics and photosynthetic response to increased nitrogen availability in the alpine willow *Salix glauca*. *Oecologia* 97:93–99
- Bowman WD, Theodose TA, Schardt JC, Conant RT (1993) Constraints of nutrient availability on primary production in two alpine tundra communities. *Ecology* 74:2085–2097
- Bowman WD, Theodose TA, Fisk MC (1995) Physiological and production responses of plant growth forms to increases in limiting resources in alpine tundra: implications for differential community response to environmental change. *Oecologia* 101:217–227
- Brown BJ, Mitchell RJ, Graham SA (2002) Competition for pollination between an invasive species (purple loosestrife) and its native congener. *Ecology* 83:2328–2336
- Burd M (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot Rev* 60:83–139
- Campbell DR, Halama KJ (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* 74:1043–1051
- Cavieres LA, Peñaloza A, Papić C, Tambutti M (1998) Efecto nodriza de *Laretia acaulis* en plantas de la zona andina de Chile central. *Rev Chil Hist Nat* 71:337–347
- Cavieres LA, Peñaloza A, Arroyo MK (2000) Altitudinal vegetation belts in the high-Andes of central Chile (33°). *Rev Chil Hist Nat* 73:331–344
- Chapin FS III (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst* 11:233–260
- Chapin FS III, Vitousek PM, Van Cleve K (1986) The nature of nutrient limitation in plant communities. *Am Nat* 127:48–58
- Dawes-Gromadzki TZ (2002) Trophic trickles rather than cascades: conditional top-down and bottom-up dynamics in an Australian chenopod shrubland. *Aust Ecol* 27:490–508
- Dorland E, Robbink R, Messelink JH, Verhoeven JTA (2003) Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *J Appl Ecol* 40:804–814
- Dyer LA, Letourneau DK (1999) Relative strengths of top-down and bottom-up forces in a tropical forest community. *Oecologia* 119:265–274
- Galen C (1985) Regulation of seed set in *Polemonium viscosum*: floral scents, pollination, and resources. *Ecology* 66:792–797
- Galen C, Zimmer KA, Newport ME (1987) Pollination in floral scent morphs of *Polemonium viscosum*: a mechanism for disruptive selection on flower size. *Evolution* 41:599–606
- Gerdol R, Brancaloni L, Menghini M, Marchesini R (2000) Response of dwarf shrubs to neighbour removal and nutrient addition and their influence on community structure in a subalpine heath. *J Ecol* 88:256–266
- Gerdol R, Brancaloni L, Marchesini R, Bragazzi L (2002) Nutrient and carbon relations in subalpine dwarf shrubs after neighbour removal or fertilization in northern Italy. *Oecologia* 130:476–483
- Gugerli F (1998) Effect of elevation on sexual reproduction in alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). *Oecologia* 114:60–66
- Havström M, Callaghan TV, Jonasson S (1993) Differential growth responses of *Cassiope tetragona*, an arctic dwarf-shrub, to environmental perturbations among three contrasting high- and subarctic sites. *Oikos* 66:389–402
- Heer C, Körner C (2002) High elevation pioneer plants are sensitive to mineral nutrient addition. *Basic Appl Ecol* 3:39–47
- Holzmann HP and Haselwandter K (1988) Contribution of nitrogen fixation to nitrogen nutrition in an alpine sedge community (*Caricetum curvulae*). *Oecologia* 76:298–302
- Hunter MD, Price PW (1992) Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73:724–732
- Johnson SG, Delph LF, Elderkin CL (1995) The effect of petal size manipulations on pollen removal, seed set, and insect-visitor behavior in *Campanula americana*. *Oecologia* 102:174–179
- Johnston MO (1991) Natural selection of floral traits in two species of *Lobelia* with different pollinators. *Evolution* 45:1468–1479
- Jonasson S (1992) Plant responses to fertilization and species removal in tundra related to community structure and clonality. *Oikos* 63:420–429
- Körner C (1989) The nutritional status of plants from high altitudes. *Oecologia* 81:379–391
- Körner C (1999) Alpine plant life. Functional plant ecology of high mountain ecosystems. Springer, Berlin Heidelberg New York
- Larson BMH, Barrett SCH (2000) A comparative analysis of pollen limitation in flowering plants. *Biol J Linn Soc* 69:503–520
- Lähtilä K, Strauss SY (1997) Leaf damage by herbivores affect attractiveness to pollinators in wild radish *Raphanus raphanistrum*. *Oecologia* 111:396–403
- Marschner H (1995) Mineral nutrition of higher plants. Academic, London
- Mattila E, Kuitunen MT (2000) Nutrient versus pollination limitation in *Platanthera bifolia* and *Dactylorhiza incarnata* (Orchidaceae). *Oikos* 89:360–366
- Montgomery BR, Kelly D, Robertson AW, Ladley JJ (2003) Pollinator behaviour, not increased resources, boosts seed set on forest edges in a New Zealand Loranthaceous mistletoe. *N Z J Bot* 41:277–286
- Muñoz AA (2003) Evaluación experimental de la importancia de efectos indirectos descendentes y ascendentes sobre el éxito reproductivo de *Chuquiraga oppositifolia* (Asteraceae) en la Cordillera de Los Andes en Chile central. Ph.D. dissertation, Facultad de Ciencias, Universidad de Chile, Santiago
- Muñoz AA, Arroyo MTK (2004) Negative impacts of a vertebrate predator on insect pollinator visitation and seed output in *Chuquiraga oppositifolia*, a high Andean shrub. *Oecologia* 138:66–73
- Nagy L, Proctor J (1997) Plant growth and reproduction on a toxic alpine ultramafic soil: adaptation to nutrient limitation. *New Phytol* 137:267–274
- Nilsson MC, Wardle DA, Zackrisson O, Jäderlund A (2002) Effects of alleviation of ecological stresses on an alpine tundra community over an eight-year period. *Oikos* 97:3–17
- Page AL (1983) Methods of soil analysis. Part 2: Chemical and microbiological properties. American Society of Agronomy, Madison

- Parsons AN, Welker JM, Wookey PA, Press MC, Callaghan TV, Lee JA (1994) Growth responses of four sub-Arctic dwarf shrubs to simulated environmental change. *J Ecol* 82:307–318
- Parsons AN, Press MC, Wookey PA, Robinson CH, Callaghan TV, Lee JA (1995) Growth responses of *Calamagrostis lapponica* to simulated environmental change in the Sub-arctic. *Oikos* 72:61–66
- Polis GA, Strong DR (1996) Food web complexity and community dynamics. *Am Nat* 147:813–846
- Press MC, Potter JA, Burke MJW, Callaghan TV, Lee JA (1998) Responses of a subarctic dwarf shrub heath community to simulated environmental change. *J Ecol* 86:315–327
- Rozzi R (1990) Períodos de floración y especies de polinizadores en poblaciones de *Anarthrophyllum cumingii* y *Chuquiraga oppositifolia* que crecen sobre laderas de exposición norte y sur. Masters thesis, Facultad de Ciencias, Universidad de Chile, Santiago
- Schemske DW, Horvitz CC (1988) Plant–animal interactions and fruit production in a neotropical herb: a path analysis. *Ecology* 69:1128–1137
- Schmitz OJ, Hambäck PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *Am Nat* 155:141–153
- Seastedt TR, Vaccaro L (2001) Plant species richness, productivity, and nitrogen and phosphorus limitations across a snowpack gradient in alpine tundra, Colorado, USA. *Arct Antarct Alp Res* 33:100–106
- Statsoft (1998) Statistica. Version 5.1. Statsoft, USA
- Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. *Annu Rev Ecol Syst* 12:253–279
- Theodose TA, Bowman WD (1997) Nutrient availability, plant abundance, and species diversity in two alpine tundra communities. *Ecology* 78:1861–1872
- Totland Ø, Sottocornola M (2001) Pollen limitation of the reproductive success in two sympatric alpine willows (Salicaceae) with contrasting pollination strategies. *Am J Bot* 88:1011–1015
- Willson MF, Price PW (1980) Resource limitation of fruit and seed production in some *Asclepias* species. *Can J Bot* 58:2229–2233
- Wookey PA, Parsons AN, Welker JM, Potter JA, Callaghan TV, Lee JA, Press MC (1993) Comparative responses of phenology and reproductive development to simulated environmental change in sub-arctic and high arctic plants. *Oikos* 67:490–502
- Wyka T, Galen C (2000) Current and future costs of reproduction in *Oxytropis sericea*, a perennial plant from the Colorado Rocky Mountains, USA. *Arct Antarct Alp Res* 32:438–448
- Zar JH (1996) Biostatistical analysis, 3rd edn. Prentice Hall, New Jersey
- Zimmerman M, Pyke GH (1988) Reproduction in *Polemonium*: assessing the factors limiting seed set. *Am Nat* 131:723–738