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Direct and indirect interactions co-determine species composition in nurse plant systems

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Facilitation by nurse plants plays an important role in determining community composition in severe environments. Although the unidirectional effect of nurses on beneficiary species has received considerable research interest, nurse-mediated interactions among beneficiary species (so-called indirect interactions) are less known. Consequently, community composition in nurse plant systems is generally considered as a simple consequence of the facilitative effect of the nurse even though beneficiary species may significantly contribute to community assembly and modulate the direct nurse effects on the community. In an observational study we assessed nurse effects and nurse-mediated beneficiary interactions in two contrasting nurse plant systems in dry environments using a newly developed framework. We quantified plant–plant interaction intensity using the relative interaction index (RII) at the community and species level for three *Retama sphaerocarpa* shrub size-classes in a semiarid shrubland and four *Arenaria tetraquetra* cushion plant communities differing in aspect and elevation in dry alpine gravel habitats. The observed RII was split into nurse and beneficiary effects, and related to individual mass, species frequency and abundance using generalized linear mixed models. Results showed predominantly positive nurse effects and negative beneficiary interactions. The effect size of nurse plants, however, was significantly higher than the effect size of beneficiary species in both systems. Individual plant mass and abundance of species was dependent on the combined effects of nurse and beneficiary species whereas species occurrence was related to nurse effects only. Despite evident differences, the semiarid and alpine nurse plant systems showed strong functional parallelisms. We found interdependence between the effects of nurse and beneficiary species on beneficiary plant assemblages emphasizing their combined role on community assembly in both systems. Our results highlight the need to consider indirect interactions to understand fully plant community dynamics.

Since all plants use the same resources, competition for light, nutrients, and water as well as for physical space is common in plant communities (Whittaker 1965). However, an increasing number of studies have shown that at least some species do not only consume resources but can also increase their availability or at least dampen their loss to other species (Callaway 2007) thereby benefiting them. For example water in shallow soil layers can be increased by plants due to redistribution of soil water (Prieto et al. 2012) or reduced evaporation caused by shading (Moro et al. 1997), while nutrient availability can be increased by nitrogen-fixing species (Temperton et al. 2007), the effect of mycorrhiza (Nara and Hogetsu 2004, Casanova-Katny et al. 2011) or accretion of soil organic matter (Pugnaire et al. 2004). These and other positive effects of plants on resource availability may generally be to their own benefit (Kylafis and Loreau 2011) but, as a side effect, they can also benefit other species. A notable example of this phenomenon is facilitation in nurse plant systems where a dominant species creates environmental conditions that often benefit a large number of subordinate species (Pugnaire et al. 2011). Nurse plants are so termed because of their positive 'nursing' effects on their own seedlings (Niering et al. 1963), although current definitions of nurse plants generally include both intra- and inter-specific effects.

The positive effect of nurses on subordinate, beneficiary species has been widely addressed (reviewed by Callaway 2007). However, community assembly in nurse plant systems and its consequences for species composition and diversity is not only determined by the unidirectional, positive effect of nurses. All individuals sharing resources potentially interact with each other implying also effects of beneficiaries on the nurse as well as interactions among beneficiaries. The few studies available on the effect of beneficiaries on the nurse suggest a full range of interactions: positive (Pugnaire et al. 1996b), neutral (Lortie and Turkington 2008), or negative (Holzapfel and Mahall 1999, Michalet et al. 2011). Furthermore, these interactions may also change in sign and intensity with ontogeny and do likely become more competitive with increasing age of the beneficiary (Barnes and Archer 1999). Most empirical evidence, however, shows predominantly negative consequences for the nurse because of resource competition (Callaway 2007).

Studies that focus on interactions among beneficiary species (also called indirect interactions since they are mediated by the nurse, Miller 1994) are even fewer (Brooker et al. 2008). Aguiar and Sala (1994) showed competition between adult tussock grasses and grass seedlings in the understory of *Mulinum spinosum* shrubs in the Patagonian steppe. In a similar ecosystem Armas et al. (2008) showed that grass species growing underneath the nurse shrub *Adesmia volckmanni* competed for resources, but that facilitation by the nurse surpassed the competitive effects among beneficiaries, allowing their coexistence in the nurse plant system. Negative effects of adult beneficiary species on seedling emergence and establishment were also shown by Maestre et al. (2004) in a *Pinus halepensis* plantation where understory vegetation inhibited the establishment of *Pistacia lentiscus* seedlings. Such evidence suggests that increased productivity, often along with higher plant density and an altered species composition, may result in intense competition between beneficiary species. However, Saccone et al. (2010) found direct and indirect positive effects among *Acer negundo* individuals in floodplains in France, indicating that indirect interactions in facilitative systems do not necessarily need to be competitive. Positive indirect interactions can for example be the result of a reduced plant density under the influence of the nurse resulting in a competitive release for the beneficiary species (Saccone et al. 2010). Nevertheless, such indirect interactions have been seldom quantified or considered when addressing community structure and composition in nurse plant systems even though they could have profound consequences for community assembly.

A likely reason for the lack of studies on indirect interactions and their consequences for community structure is the difficulty of disentangling these effects in empirical studies. For example, the experimental removal of either the nurse or the beneficiary species in order to separate the effects is insufficient as the removal of the nurse does not eliminate the entire nurse effect (Michalet et al. 2006). Furthermore, in many systems the removal of nurse or beneficiary species is not possible without significant disturbance of the whole nurse plant system. Therefore, alternative approaches are needed to address indirect interactions, in particular non-destructive methods that allow their application in all types of nurse plant systems.

Here we developed and applied a new framework based on observational data for disentangling indirect (beneficiary) interactions from direct (nurse) effects. We used this framework to quantify the impact of both, nurse and beneficiary effects on the frequency, biomass, and abundance of beneficiary species in two well-known facilitative systems in dry environments, a nurse shrub system in a semiarid lowland valley and an alpine cushion plant system in dry, high elevation mountains. These systems provide distinct but stressful and/or disturbed environmental conditions where nurse plants are important drivers of plant community structure and composition (Flores and Jurado 2003, Cavieres and Badano 2009). In both systems the positive net effect of nurse plants has been extensively studied (Reid et al. 2010, Pugnaire et al. 2011), but the relative contributions of direct and indirect effects on beneficiary species and final community structure have been hardly ever quantified irrespective of the ecosystem. There is little and inconsistent evidence on the impact of indirect interactions in nurse shrub systems in dry environments (Tielbörger and Kadmon 2000, Soliveres et al. 2011, Armas et al. 2008), and we are not aware of any study investigating indirect effects in alpine cushion plant systems (likely due to the difficulties for experimental manipulations in this system). In addition, the two systems facilitate the study of the interrelationship between direct and indirect effects by means of gradients of environmental severity and ontogeny. These factors are known to affect net interaction intensity significantly (Callaway et al. 2002, Schiffers and Tielbörger 2006, Armas and Pugnaire 2009) and may, therefore, also be associated with variation in both the direct nurse effect and indirect beneficiary effects on beneficiary species.

Within a nurse plant system we expected that the net effect of the nurse on subordinate species would be facilitative both at the whole community and at the species level. However, we expected that an average of species-level interactions would underestimate community-level facilitation as species-level interactions do usually not consider cases of obligate facilitation, even though they may be common in harsh environments (Butterfield 2009). We further hypothesized that the (direct) nurse effects would generally be positive whereas competition would prevail among beneficiary species. We expected a rather weak relationship between direct and indirect interaction intensity, as interactions among beneficiary species are assumed to be driven by productivity (Michalet et al. 2006), and productivity of a nurse plant system is not only determined by the nurse effect but also by local environmental conditions. As a consequence of the expected direct and indirect interactions, we hypothesized that frequency, biomass, and abundance of beneficiary species would be highest when nurse effects are most positive and beneficiary effects least negative.

Methods

Study sites

Field work was conducted in two different nurse plant systems: 1) a *Retama sphaerocarpa* shrubland in a semiarid lowland valley, and 2) an *Arenaria tetraquetra* cushion plant community in the alpine belt. The *R. sphaerocarpa* shrubland was located in Rambla del Saltador, Almería province, SE Spain (37°08′N, 2°22′W) at 630 m elevation. Climate is semiarid, with a dry summer season between June and September. Mean annual rainfall is 256 mm and annual mean temperature is 15.8°C (Pugnaire and Lázaro 2000). The soil at the valley bottom is a sandy loam of alluvial origin with poor water holding capacity and low soil organic matter and nutrient concentrations (Puigdefábregas et al. 1996). The sparse vegetation at the valley bottom is dominated by *R. sphaerocarpa*, a leguminous shrub with very deep roots known to be involved in the redistribution of water in the soil (Prieto et al. 2010). Shrubs are haphazardly distributed (but see Haase et al. 1996), and surrounded by large open areas. Both shrub understory and the surrounding open area are mainly colonized by annual grasses and herbs, the vegetation however being denser and more species-rich below shrubs than in open areas (Pugnaire et al. 1996a). This facilitation effect of the shrub could be due to higher water availability (Prieto et al. 2011) as well as higher soil organic matter and nutrient concentration (Pugnaire et al. 1996a, 2004) along with ameliorated environmental conditions (Moro et al. 1997, Pugnaire et al. 2004). Facilitation intensity of *R. sphaerocarpa* is significantly related to shrub size, indicating an accumulation of positive effects on growth conditions (e.g. increasing water and nutrient availability with shrub age) in the understory over time (Pugnaire et al. 1996a, Pugnaire and Lázaro 2000). We studied three different size classes according to mean canopy diameter and height (mean ± 1SE), i.e. small shrubs (126 ± 17 cm diameter, 94 ± 12 cm height); shrubs of medium size (238 ± 7 cm diameter, 188 ± 6 cm height); and large shrubs (440 ± 23 cm diameter, 286 ± 7 cm height).

Some 90 km from the *R. sphaerocarpa* field site, communities of *Arenaria tetraquetra*, a cushion plant species, were selected in the Sierra Nevada Mountains, Granada province, southeastern Spain. Two study sites were established at the northern hillside of Pico Veleta at elevations of 2720 m (37°05′N, 03°23′W) and 3240 m a.s.l. (37°03′N, 03°22′W), and another two sites at the southern hillside of Mulhacen peak at elevations of 2575 m (37°00′N, 03°19′W) and 3110 m a.s.l. (37°02′N, 03°18′W). Climatic conditions are alpine with summer drought in July and August. Mean annual precipitation in Pradollano (2500 m a.s.l.) close to our low elevation site in the northern slope is 690 mm and annual mean temperature is 3.9°C. High elevation sites experience higher amounts of precipitation ($+33.3$ mm year^{-1} per 100 m) and lower mean temperatures (-0.61°C per 100 m) compared to low elevation sites (Delgado Calvo-Flores et al. 1988) which result in a better water balance for plants. *Arenaria tetraquetra* communities are found in low-productivity, siliceous gravel habitats with poorly developed soils and generally low soil organic matter and water content (Schöb et al. 2012). The plant community in this habitat is dominated by the cushion-forming species *A. tetraquetra* ssp. *amabilis* which is randomly distributed in large open areas. Both cushions and open areas are sparsely colonized, mostly by perennial herbs and grasses of which more than 40% are endemic to the Sierra Nevada range (Schöb unpubl.). Species richness and plant density in *A. tetraquetra* cushions are generally higher than in the surrounding open areas – a facilitative effect of cushions that could be attributed to higher levels of soil organic matter and water content in cushions compared to open microsites (Schöb et al. 2012).

Data collection

In the *R. sphaerocarpa* shrubland and within an area of approximately 10 ha we selected eight shrubs for each of the three different size classes. We determined aboveground standing mass and abundance (i.e. number of individuals) of all vascular plant species in the understory of each shrub in two spatially separated 400 cm^2 quadrats. Quadrats were placed in the northern side of the shrub at an intermediate distance between the center of the shrub and the projected canopy edge. Similarly, we randomly placed two quadrats of same size in open areas nearby shrubs and determined aboveground standing mass and abundance of vascular plants growing inside. Measurements were conducted between 22 and 24 April 2010 at peak standing biomass and samples were dried at 70°C for 48 h. Mean values of each pair of quadrats were used in statistical analyses.

In the *A. tetraquetra* community we selected 10 large cushions in every site and within an area of approximately 0.5 ha per site. Mean cushion size (± 1SE) across all sites was 240 ± 13 cm^2 and did not differ significantly among sites ($F_{3,36} = 1.7$, $p = 0.19$). We determined aboveground standing mass of all vascular plant species growing inside each cushion and its paired 400 cm^2 quadrat in the neighboring open area. The 10 sampling quadrats per site in the open area were placed at approx. 50 cm from the paired cushions. For comparison of standing biomass between paired cushion and open plots we extrapolated mass data in cushion plots to an area of 400 cm^2. For extrapolation we used a linear relationship between area of cushions and biomass of beneficiary species because there was good reason to prefer a linear model (AIC = 247) over a more complex model including a quadratic term (AIC = 299). Frequency and abundance of each species in cushions and open areas in each site was determined in 100 (85 at the north-low site) paired cushion and open plots. Each open area was of equal shape and size to that of its paired cushion (Schöb et al. 2012). Biomass sampling was conducted at the peak of aboveground standing biomass, on 29–30 July 2010 at the two sites on the southern slope and on 9–10 August 2010 at the two sites on the northern slope.

Individual plant mass was determined for the most abundant species occurring both under the nurse and in paired open areas. We selected seven species in the *R. sphaerocarpa* shrubland and eight in the *A. tetraquetra* community (Supplementary material Appendix 1 Table A1). For each target species we determined aboveground mass of individuals growing outside the sampling quadrats: we randomly selected 10 individuals per shrub size class and open area in the *R. sphaerocarpa* shrubland, and five individuals per cushion and open area per site in the *A. tetraquetra* community. The reduced number of replicates in the *A. tetraquetra* community was for conservation reasons, as five out of the eight target species are endemic to the Sierra Nevada Mountains (Blanca et al. 2009), and five are near threatened or vulnerable according to IUCN criteria (Cabezudo et al. 2005).

Uncoupling indirect beneficiary effects from direct nurse effects

We developed an analytical framework to disentangle direct nurse effects from nurse-mediated interactions between beneficiary species in observational data (see Supplementary material Appendix 2 for a detailed layout). The framework uses four plant performance variables based on standing biomass. Two variables were sampled at the community level: Bn (aboveground plant mass in 20 × 20 cm quadrats in the nurse understory), Bo (aboveground plant mass in 20 × 20 cm quadrats in a nearby open area). Another two variables were sampled at the individual plant level: iBn (mean individual plant mass of the selected target species in the nurse understory), and iBo (mean individual plant mass of the same target species in the open area). In order to calculate effect sizes these four variables need to be greater than 0 meaning that the species and communities sampled do actually need to exist with and without the nurse. Furthermore, we estimated two other variables at the individual plant level: iBwn (individual mass of the target species in the understory without nurse), and iBwb (individual mass of the target species in the understory without beneficiaries). In order to estimate iBwn and iBwb, and to quantify the nurse-mediated effect among beneficiary species we made two assumptions: 1) individual plant performance under the influence of the nurse is the product of direct nurse and nurse-mediated beneficiary effects whereas individual plant performance in areas without nurse is nearly unaffected by plant–plant interactions because of low plant density in open areas in our severe environments; 2) the direct effect of nurses on beneficiaries is equal for all the selected beneficiary species and can be quantified at community level by comparing communities under and outside the influence of the nurse. However, we are aware that this is likely a simplification of reality. The outcome of plant–plant interactions is known to be species-specific in particular due to the tradeoff between stress-tolerance versus competitive-response ability (Liancourt et al. 2005). However, species-specificity can either be due to the direct nurse or the indirect beneficiary effects on beneficiary species. Stress-tolerant species may profit less from ameliorated growth conditions than stress-intolerant species resulting, indeed, in species-specific direct nurse effects (Liancourt et al. 2005). However, as most (if not all) species are probably not in their fundamental niche optima in such extreme environments (Lortie et al. 2004), all species will likely benefit from the ameliorative direct nurse effects. Therefore, even though the direct nurse effect may not be exactly the same for all the target species, it is most likely positive and within a rather narrow range of magnitude in our study systems. In contrast, more intense competition under ameliorated conditions provided by the nurse may lead to species-specific facilitation favoring species with higher competitive-response ability (Liancourt et al. 2005). This latter effect, however, is not related to the direct nurse effect but to the interactions among beneficiary species.

For calculating separated nurse and beneficiary effects, we estimated first the direct nurse effect (NE) by dividing mean community-level standing biomass of the nurse understory by mean community-level standing biomass of the corresponding open area for each size class or site (y) as

$$NE_y = \frac{\overline{Bn}_y}{\overline{Bo}_y} \tag{1}$$

At the individual plant level, individual mass in the open (iBo) is assumed to be unaffected by co-occurring individuals. On the contrary, individual plant mass in the nurse understory is influenced by both the nurse effect and the nurse-mediated indirect effect among beneficiary species (BE). Therefore,

$$\frac{iBn}{iBo} = NE \times BE \tag{2}$$

and BE for each size class or site (y) can be calculated as

$$BE_y = \frac{\overline{iBn}_y}{\overline{iBo}_y \times NE_y} = \frac{\overline{iBn}_y \times \overline{Bo}_y}{\overline{iBo}_y \times \overline{Bn}_y} \tag{3}$$

It is important to note here that even if beneficiary effects could be considered direct effects between beneficiary species, they are mediated by the nurse and therefore they are indirect sensu Miller (1994).

Finally, we estimated the theoretical mass of each target individual without nurse but with co-occurring beneficiary plants (iBwn) and the theoretical mass of each target individual growing under the influence of a nurse but without neighboring beneficiary plants (iBwb) by dividing the observed individual mass under a nurse (iBn) either by the nurse effect (NE) or the beneficiary effect (BE) of the corresponding size class or site (y):

$$iBwn = \frac{iBn}{NE_y} \tag{4}$$

$$iBwb = \frac{iBn}{BE_y} \tag{5}$$

Intensity of direct and indirect plant–plant interactions

Intensity of plant–plant interactions was assessed on the community level for each paired nurse and open plot and on the species level for each pair of individuals of each target species sampled in the understory of the nurse and in the open area by means of the relative interaction index (RII; Armas et al. 2004) as

$$\text{Community RII} = \frac{Bn - Bo}{Bn + Bo} \tag{6}$$

$$\text{Species RII} = \frac{iBn - iBo}{iBn + iBo} \tag{7}$$

Based on individual plant mass values estimated with Eq. 4 and 5 and individual plant mass values from open areas, we assessed interaction intensities for each pair of individuals of each target species by adapting Eq. 7:

$$RII_{nurse} = \frac{iBwb - iBo}{iBwb + iBo} \tag{8}$$

$$RII_{beneficiaries} = \frac{iBwn - iBo}{iBwn + iBo} \tag{9}$$

where RII_{nurse} quantifies the intensity of the direct nurse effect and $RII_{beneficiaries}$ quantifies the intensity of the indirect beneficiary effect.

Statistical analyses

To test whether community-level interaction intensity corresponds to mean species-level interaction intensity we performed linear mixed models relating mean Species RII to Community RII, including study system as random effect to control for system-specific differences. Mean Species RII was calculated for each size class or site either by simple or abundance-weighted averaging of the RIIs of all the target species. For mean abundance-weighted Species RII, the RII of each species was weighted by its abundance in the particular size class/site of each system. Significance of the random effect was tested with a log-likelihood test comparing models with and without the random effect (Bolker et al. 2009).

To test for interdependence between the nurse effect ($\text{RII}_{\text{nurse}}$) and the beneficiary effect ($\text{RII}_{\text{beneficiaries}}$) on target species we used a linear mixed model based on mean values per species and size class/site in each system and included 'species' nested within 'system' as random effects. Significance of the random effect 'system' was tested again with a log-likelihood test comparing two models with and without 'system' included as the random effect.

To reveal the effects of nurse and beneficiaries on individual plant mass we used linear mixed models with the mean biomass of a target species in a specific size class/site as response variable and the corresponding nurse ($\text{RII}_{\text{nurse}}$) and beneficiary effect ($\text{RII}_{\text{beneficiaries}}$) as explanatory variables. Species identity was included as a random effect to control for the significant biomass differences among species.

With a similar model structure we tested for nurse and beneficiary effects on species frequency and abundance, in which case we applied generalized linear mixed models with a Poisson error structure and a log-link function. Models included either the number of occurrences or the number of individuals in a specific size class or site as response variable and the logarithm of the number of samples included as an offset in order to get either the frequency of occurrence or the number of individuals per sample as response.

Statistical analyses were performed with R software ver. 2.14.0 (R Development Core Team) using the packages 'car' (Fox and Weisberg 2011) for type-II ANOVA, 'effects' (Fox 2003) for constructing effect plots, 'lme4' (Bates et al. 2011) for generalized linear mixed models, and 'nlme' (Pinheiro et al. 2011) for linear mixed models.

Results

Community RII could not be assessed accurately from a subset of species-level interactions, either by simple- or abundance-weighted averaging of species, because Species RII underestimated facilitation at the community level (Fig. 1). Nevertheless, Species RII followed the trend of Community RII (Fig. 1). Our two nurse plant systems showed no significant differences in the relationship between community- and species-level interactions (simple mean Species RII: L = 0.51, p = 0.48; abundance-weighted mean Species RII: L = 0.36, p = 0.55).

Whereas nurse effects were positive for most target species in most size classes and sites, the effects among beneficiary species were overwhelmingly negative, and only in a few cases did a selected target species experience positive effects from both the nurse and co-occurring beneficiary species (Fig. 2). Competition among beneficiary

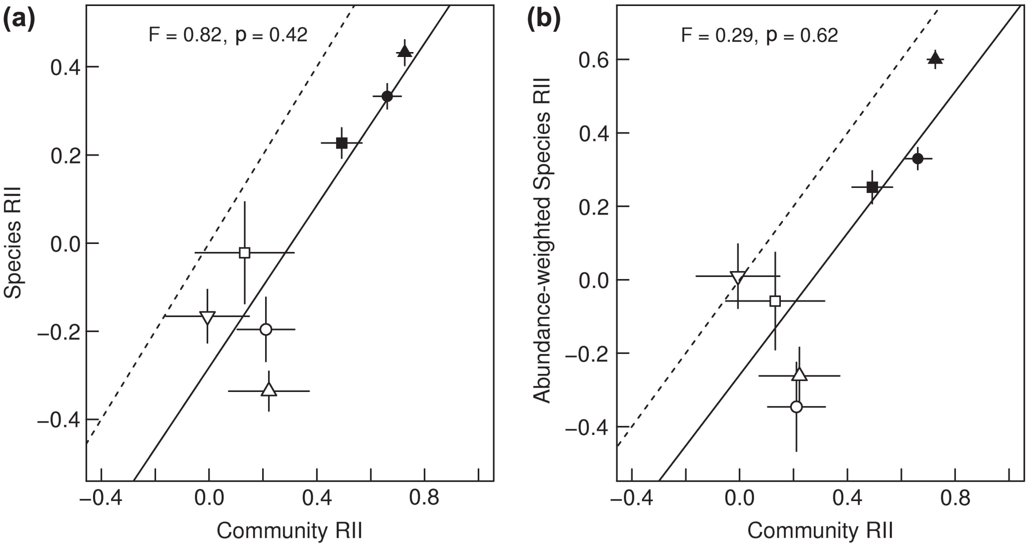

Figure 1. Community-level interaction intensity in relation to either a simple mean species-level RII (a) or an abundance-weighted mean species-level RII (b). Statistical tests of linear mixed models with the two study systems as random effect are shown (n = 7). The solid lines show the linear relationship between Community and Species RII. The dashed lines indicate the 1:1 line where Species RII would be equal to the Community RII. Values above the dashed line indicate that Species RII overestimates facilitation intensity whereas values below the dashed line indicate underestimation of facilitation intensity by Species RII compared to Community RII. Symbols indicate size class or site means ± 1 SE. Solid symbols: *Retama sphaerocarpa* shrubland; clear symbols: *Arenaria tetraquetra* ssp. *amabilis* community. ■ (small shrub), ● (medium shrub), ▲ (large shrub), ○ (North-Low), □ (North-High), ∇ (South-Low), Δ (South-High).

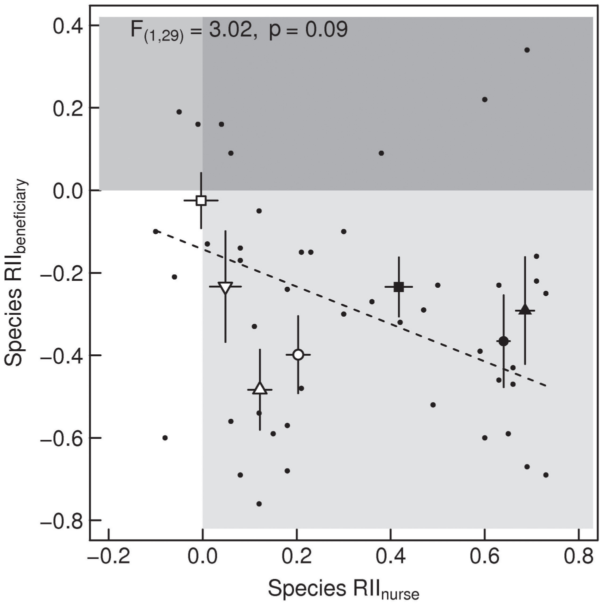

Figure 2. Beneficiary effect vs nurse effect quantified with RII. Statistical test of a linear mixed model is shown (n = 45), with the 15 target species nested within the two study systems included as random effects. White background indicates competitive effects by nurse and beneficiary plants; light grey background indicates facilitative nurse effects and competitive beneficiary effects; medium grey indicates competitive nurse effects and facilitative beneficiary effects; dark grey indicates facilitation by nurse and beneficiary plants. Each dot represents a particular species in a particular size class or site, whereas symbols represent size class or site means ± 1 SE. Symbols as in Fig. 1.

species was marginally related to the intensity of facilitation by nurses ($F_{1,29} = 3.02$, $p = 0.09$), showing a tendency for increased competition intensity among beneficiaries if facilitation intensity by the nurse increased. Log-likelihood tests revealed no significant differences between the two nurse plant systems ($L = 0.50$, $p = 0.48$).

The individual mass of a given species was dependent on the combined effects of the nurse and co-occurring beneficiary species (Fig. 3a) with a significant interaction term (Table 1). Positive effects of both nurse and beneficiary species increased individual plant mass, but the effect size of nurses was more than five times higher than the effect size of beneficiaries (1.28 for RII_{nurse} vs 0.23 for $RII_{beneficiaries}$). The significant interaction term indicates a reduced nurse effect on biomass under strong competition from beneficiaries, compared to the nurse effect under conditions with moderate or no beneficiary competition (Fig. 3a).

The presence of a given species was significantly related to the nurse effect (Fig. 3b) but not to beneficiary effects (Table 1). The stronger the facilitation by the nurse, the more frequent was a beneficiary species. Species abundance was affected by the nurse and co-occurring beneficiaries, with significant interaction effects (Fig. 3c and Table 1). Nurses had, again, approximately a five times stronger effect on species abundance than beneficiaries (regression coefficients of $RII_{nurse} = 1.87$ and of $RII_{beneficiaries} = 0.37$). The significant interaction term indicates an increased intensity of the positive nurse effect on species abundance under strong competition from beneficiaries, compared to the intensity of the nurse effect under conditions with moderate or no competition among beneficiaries (Fig. 3c).

Discussion

Our data support the idea that plant–plant interactions are strong drivers of plant community structure and composition in nurse plant systems with similar effects irrespective of the environment or type of nurse plant community, such as a semiarid shrubland and an alpine cushion plant community. Our results revealed mostly positive nurse effects on beneficiary species but also predominantly competition among beneficiary species under influence of the nurse, in line with previous reports (Aguiar and Sala 1994, Rice and Nagy 2000, Maestre et al. 2004, Armas et al. 2008, Soliveres et al. 2011, Luzuriaga et al. 2012; but see Tielbörger and Kadmon 2000). The results further revealed combined and interdependent effects of the nurse on beneficiaries and among beneficiary species for species frequency, biomass and abundance. Consequently, our results indicate that the combination of nurse effects and nurse-mediated beneficiary interactions affects final community composition in a nurse plant system.

Interdependence of direct and indirect effects

Our results indicate a weak negative relationship between the intensity of nurse and beneficiary effects, which may be related to the effect of nurse plants on productivity. Both the nurse plants studied are known to influence understory productivity (Pugnaire et al. 1996a, Badano and Marquet 2009), and for the leguminous shrub *R. sphaerocarpa* it has even been shown that productivity of the nurse understory community increases with shrub age (Pugnaire et al. 1996a). The fact that *R. sphaerocarpa* is able to perform nitrogen fixation by rhizobial nodules may also be related to the increased nurse effect observed for *R. sphaerocarpa* compared to *A. tetraquetra*. These changes in the intensity of nurse effects within or between species affect productivity in the nurse understory which in turn may influence the interaction outcome among beneficiary species. However, the weak relationship indicates that the intensity of indirect interactions is not only driven by the nurse effect but is also likely dependent on the local environmental conditions at the site affecting productivity of the nurse plant system. Furthermore, the neighboring beneficiary community with which a beneficiary target species is interacting may have a major influence on the interaction outcome. For example, a higher density of neighboring species may result in increased competition on the target species or different combinations of neighboring species may vary in their effects on the target species.

Effects of direct and indirect interactions on beneficiary species

Biomass of beneficiary species was highest with the highest levels of facilitation from both nurse and neighboring beneficiary species, whereas the lowest values were observed under most intense competition from both nurse and beneficiaries. Under such conditions, individual mass of

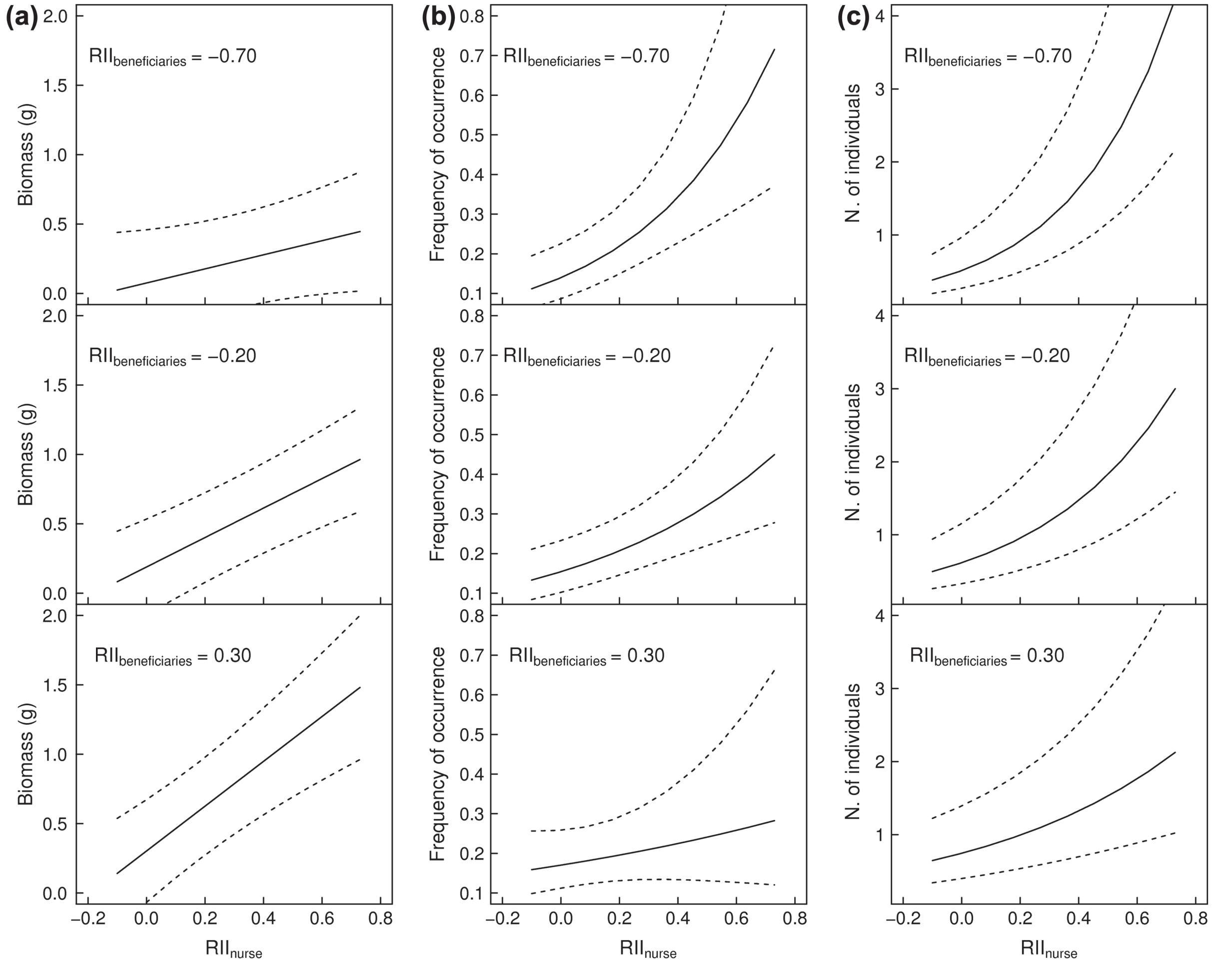

Figure 3. Individual biomass (a), frequency (b), and abundance (c) of beneficiary species in response to the intensity of nurse and beneficiary effects. Model predictions and 95% CIs between the nurse effect (RII_{nurse}) and the beneficiary response for three arbitrary levels of beneficiary effects ($RII_{beneficiaries}$) are shown. The underlying model is a linear mixed model with species as the random effect (n = 45; see Table 1 for statistical results).

beneficiary species reached values close to zero, showing hardly viable conditions for additional plant growth. This is in line with lowest levels of species frequency and abundance observed under these conditions that point towards competitive exclusion of the corresponding target species.

A strong negative effect of beneficiary species along with a strong positive effect of nurses led to a high frequency and abundance of beneficiary species, whereas positive effects of beneficiary species together with strong facilitation from the nurse resulted in an overall reduced species frequency or abundance. We suppose that this may be due to intraspecific facilitation among beneficiary species. Such species-specific facilitation could result in local dominance of single beneficiary species and potentially lead to monospecific stands. In support of this argument, our results revealed highly positive nurse effects together with positive beneficiary effects for *Avena sterilis* in the *R. sphaerocarpa* system in medium-sized and large shrubs, where *A. sterilis* was dominant in terms of individual mass and community-level standing biomass (Supplementary material Appendix 1 Table A1). In contrast, most co-occurring beneficiary species experienced high levels of competition within the size classes where *A. sterilis* was dominant. Under such conditions, it is likely that the dominant beneficiary species may competitively exclude other beneficiary species (Grime 2001), which would also explain the low frequency

Table 1. Results of mixed models relating the intensity of the effects of the nurse (RII_{nurse}) and beneficiary plants ($RII_{beneficiaries}$) to individual biomass, frequency, and abundance of beneficiary species. Species identity was treated as random variable (n = 45).

		Individual biomass			Frequency			Abundance		
	DF	χ^2	p	coef	χ^2	p	coef	χ^2	p	coef
RII_{nurse}	1	21.2	< 0.001	1.28	14.88	< 0.001	1.16	102.1	< 0.001	1.87
$RII_{beneficiary}$	1	10.8	< 0.001	0.23	0.05	0.820	0.20	9.6	0.002	0.37
$RII_{nurse} \times RII_{beneficiary}$	1	5.6	0.018	1.11	2.77	0.096	1.55	10.4	0.001	1.46

coef = regression coefficient, i.e. slope of the regression line, indicating the effect size of the corresponding factor.

and abundance of other species observed under these particular situations. In fact, this suggests that indirect interactions among beneficiaries, such as the strong competition between *A. sterilis* and other beneficiary species, are an important source of the species-specific effects observed in nurse plant systems (Temperton and Zirr 2004, Liancourt et al. 2005).

Limitations of the framework

We think that our methodological approach to separate nurse from beneficiary effects can provide useful insights into the processes driving plant community dynamics. Nevertheless, the observational method to assess effects among beneficiary species could have a potential constraint. Even if direct positive interactions among beneficiaries exist (Saccone et al. 2010) positive $RII_{beneficiaries}$ values could have originated from disproportionately high nurse effects. Based on our second assumption we attributed the same nurse effect to all beneficiary species independent of their identity, assuming that habitat amelioration by the nurse would affect all beneficiary species in similar ways. This is certainly a simplification of reality as previous studies have shown that nurses may have different effects on species (Liancourt et al. 2005). However, under very severe environmental conditions such as those prevailing at our study sites, it is very likely that any improvement of environmental conditions by nurse plants would benefit all beneficiary species. Furthermore, by selecting target species with different distributional optima we may have been able to balance potential species-specific differences in response to nurse effects. Accordingly, our model output does likely resemble the relative effects of nurse and beneficiaries on mean beneficiary species, whereas particular species may show slightly different results. However, since we found very few cases with positive beneficiary interactions, our results also indicate that the method may have worked well and that it can provide simplistic but sound results. Nevertheless, coupling this kind of analysis with experimental manipulations, including removal of nurses or in particular beneficiary species may provide a stronger test for our questions. However, as removal of nurse plants without significant disturbance of the beneficiary community is often unlikely (e.g. in tree-understory systems) or unfeasible (e.g. in cushion plant systems, where beneficiary species often root within the cushion), we consider our observational approach a valuable, non-destructive alternative to address the interplay between direct and indirect interactions.

Species- versus community-level measures of species interactions

Our data can further help to solve apparent discrepancies between species-level analyses of plant interactions and community-level interactions, as recently argued by Maestre et al. (2009) and Armas et al. (2011) in terms of the stress-gradient hypothesis. Community-level analyses comparing the nurse plant system with neighboring open systems reveal the effect of the nurse whereas species-level studies measuring performance of single species with and without nurse are highly influenced by nurse and beneficiary species affecting plant performance. Even a representative set of species cannot fully reflect community-level effects as extreme cases of facilitation (or competition), i.e. species that only grow with nurse (or without nurse) are a priori overlooked in species-level studies. In our case, underestimating facilitation with our species-level approach clearly shows the consequences of neglecting obligate facilitation which was found in both systems. In consequence, as the stress-gradient hypothesis is a community-level concept (Callaway 2007), species-level studies that do not support the stress-gradient hypothesis may be seriously confounded by indirect interactions.

Conclusion

Our results highlight the importance of indirect interactions for plant community structure and composition, exemplified by the interdependence between nurse and beneficiary interactions in two very contrasting nurse plant systems. The functional parallelism found between our two disparate ecosystems emphasizes the generality of our conclusions. Furthermore, the interdependence between interactions among all individuals in a nurse plant system, including positive and negative interactions, indicates the suitability of nurse plant systems to address interaction networks consisting of mutualistic (+/+), antagonistic (+/−) and competitive (−/−) interactions.

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Supplementary material (Appendix oik-00390 at <www.oikosoffice.lu.se/appendix>). Appendix 1 and 2.