Nurse-based restoration of degraded tropical forests with tussock grasses: experimental support from the Andean cloud forest

Fabien Anthelme[1,2*], Lorena Gómez-Aparicio[3] & Rommel Montúfar[2]

[1]Institut de Recherche pour le Développement (IRD), UMR AMAP/DIADE, Boulevard de la Lironde, TA A-51/PS2, F-34398 Montpellier Cedex 5, France

[2]Pontificia Universidad Católica del Ecuador, Facultad de Ciencias Exactas y Naturales, Av. 12 de Octubre y Roca, Quito, Ecuador

[3]Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, PO Box 1052, Sevilla E-41080, Spain

*Corresponding author: fabien.anthelme@ird.fr

Running head: Facilitation in the wet tropics

Word count:

Summary: 334

Main text: 4882

Acknowledgements: 66

References: 1168

Tables and figure legends: 373

Total: 6995

Number of tables: 1

Number of figures: 5

Summary

1. The degradation of the Andean cloud forest raises strong biological conservation issues and threatens the sustainability of a crucial water resource. The idea that nurse-based restoration can accelerate the recovery of these forests is underexplored, despite its promise as a restoration technique. Recent conceptual models predict that facilitation among plants may be an important mechanism, but there is a lack of strong empirical support. We gathered experimental data to test this prediction and explore the relevance of using nurse-based forest restoration in these environments.

2. A 20-month factorial experimental design in the Andean tropical cloud forest was established. We measured the survival and estimated the biomass production of transplanted seedlings of a keystone canopy forest species, *Ceroxylon echinulatum* (Arecaceae), in a deforested area in the presence/absence of herbivory, a potential nurse plant (the tussock grass *Setaria sphacelata*, Poaceae), and artificial shade.

3. The joint effects of deforestation and herbivory led to the death of all seedlings, whereas most seedlings survived in the adjacent forest, which was used as the control. The presence of nurse plants led to significantly higher survival and growth of *Ceroxylon* seedlings throughout the experiment, regardless of herbivore presence.

4. The nurse effects were explained by a reduction of the relative abiotic stress experienced by the seedlings outside the forest, i.e. the consistently decreasing maximum vapour pressure deficit. Furthermore, nurse tussocks delayed and reduced the effects of herbivory by offering physical protection and a refuge for seedlings against detection by herbivores. However, the effects of herbivory and abiotic stress on facilitation were not additive.

5. *Synthesis and applications*. Facilitation in degraded cloud forest can be intense as soon as the beneficiary plants are driven away from their physiological optimum (relative abiotic stress) and/or are confronted by herbivory. Using pre-established exotic tussock grasses as a

nurse-based restoration technique in degraded cloud forest is a low-cost, non-detrimental (to biodiversity) option, especially in the absence of nurse trees and shrubs. The success of this method requires transplanting seedlings at the base of tussocks.

Key-words: *Ceroxylon echinulatum*, competition, herbivory, deforestation, facilitation, plant–plant interactions, rehabilitation, *Setaria sphacelata*, stress-gradient hypothesis, tussock grass

Introduction

At a time when the pace of deforestation is accelerating in tropical forests (Hansen *et al.* 2013), identifying feasible techniques to restore degraded forests is more topical than ever. In comparison with lowland tropical forest, restoration techniques in tropical montane cloud forest (TMCF) have been largely overlooked thus far, although secondary successions after deforestation are expected to be very distinctive (Sarmiento 1997; Aide, Ruiz-Jaen & Grau 2010). This raises strong conservation issues because TMCFs, which form 14.2% of tropical forests worldwide in their broader sense (Mulligan 2010), (1) provide a crucial water resource by capturing water via condensation from clouds and fog, and (2) are a remarkable biodiversity hotspot (Bubb *et al.* 2004).

TMCFs are becoming increasingly degraded, and at one of the highest rates of deforestation worldwide (Mosandl *et al.* 2008). In the majority of cases, this is taking place for the conversion of TMCF to agricultural and pastoral land (Bubb *et al.* 2004). As a flagship example, each of the five tropical Andean countries (from Venezuela to Bolivia) have lost at least 50 000 km² of their initial TMCF cover, largely because of human activities (Mulligan 2010). In the Andes, the pastures generated from the clearance of TMCF are dominated by three planted African tussock grasses (Poaceae) – *Setaria sphacelata*, *Pennisetum clandestinum* and *Melinis minutiflora* – which hinder secondary succession (Sarmiento 1997; Bubb et al. 2004; Aide, Ruiz-Jaen & Grau 2010). Faced with this global threat on water resources and biodiversity, and despite the fact that some researchers consider their removal a prerequisite ahead of a possible succession toward forest (Sarmiento 1997), we suggest that using these tussock grasses as nurse plants to restore degraded Andean TMCF could be a promising method. Indeed, tussocks have already been reported as nurse plants in other stressed and grazed regions (e.g. Callaway, Kikvidze & Kikodze 2000; Milchunas & Noy-Meir 2002). Also, nurse-based restoration has recently been shown to be successful in a number of degraded ecosystems, thus enriching forest restoration options rather than focusing solely on removal techniques (Padilla & Pugnaire 2006; Gómez-Aparicio 2009; Prévosto *et al.* 2012).

However, applying nurse-based restoration in such a productive environment is challenging, since facilitation among plants is primarily expected to be high in environments limited by resources for plants (stress-gradient hypothesis: Bertness & Callaway 1994), while the direction of interactions in productive environments is more obscure, with fewer data available (Smit, Rietkerk & Wassen 2009; Holmgren *et al.* 2012; Shoo & Catterall 2013). Accordingly, connecting theory on plant–plant interactions with nurse-based restoration is a necessary step before proposing generalized techniques in tropical, productive environments.

In the last 20 years, a number of conceptual attempts have been made to evaluate the importance of facilitation in productive, grazed environments. Among them, Bertness & Callaway (1994) proposed that facilitation may increase with herbivory, at least up to a certain threshold of herbivory intensity (Smit, den Ouden, & Müller-Schärer 2007). A more recent conceptual model hypothesized that facilitation in productive environments may be high as soon as beneficiary species deviate from their physiological optimum (Holmgren & Scheffer 2010). From this viewpoint, facilitation would be modulated by the relative abiotic stress exerted on beneficiary plants (Liancourt, Callaway & Michalet 2005; Smit, Rietkerk & Wassen, 2009; Gross *et al.* 2010). These conceptual advances suggest that both herbivory and relative abiotic stress may generate patterns of intense facilitation in productive environments affected by abrupt environmental changes. They require robust empirical data, which might open up interesting management perspectives for the ecological restoration of degraded TMCF (Shoo & Catterall 2013).

By examining patterns and mechanisms of interactions between a dominant, exotic tussock grass, *Setaria sphacelata* Stapf & C.E. Hubb (Poaceae), and seedlings of a keystone palm tree in an Andean TMCF (*Ceroxylon echinulatum* Galeano, Arecaceae), our objective was to test the potential use of facilitative interactions as an efficient technique for the restoration of degraded forests in tropical, productive environments. To achieve this, we first aimed to quantify the impacts of deforestation and herbivory on the performance of palm seedlings, within which our first underlying hypothesis was that both of these stressors may take seedlings away from their physiological limits. We then aimed to examine patterns and related mechanisms of plant–plant interactions after deforestation. Thus, our second hypothesis was that tussock grasses may act both as grazing refuges and microclimatic refuges for palm seedlings.

Material and methods

STUDY AREA

The study was located in the private Inti-Llacta Nature Reserve, on the western flanks of the Ecuadorian Andes (00°02'N, 78°43'W). The area lies at 1860 m a.s.l. and regular precipitation averages 3200 mm annually (Anthelme *et al.* 2011). The Reserve is representative of fragmented Andean cloud forest, with as much as 41% of its forests having been clear-cut between 1966 and 1990. Forested patches are dominated by secondary forest elements such as the trees *Cecropia* spp. (Cecropiaceae) and *Alnus acuminata* (Betulaceae), the understorey palm *Chamadorea pinnatifrons*, and the mid-storey palm *Prestoea acuminata* (Arecaceae). A small number of deforested areas in the Reserve remain grazed by large, domestic herbivores (cows and horses). This is because the objective to protect the natural environment must be counterbalanced by the needs of the local people to maintain agricultural activities for subsistence. In terms of their vegetation, they are dominated by spotted patterns of the exotic tussock grass *Setaria sphacelata* (Poaceae), surrounded by short herbaceous vegetation; shrubs and juvenile trees are almost absent, probably because they are inhibited by the presence of the tussock grasses [pers. obs.; see Sarmiento (1997) for patterns at the regional scale].

INTERACTING SPECIES

The genus *Ceroxylon* comprises 12 species, all generally restricted to the Andean TMCFs (Mulligan 2010; Sanín & Galeano 2011). *Ceroxylon echinulatum* is endemic to the cloud forests of Ecuador and northwest Peru, and its distribution is patchy and highly fragmented. It is a slow-growing, dioecious palm tree that frequently reaches or grows through the canopy, can form dense populations, and shows little resilience to deforestation (Montúfar *et al.* 2011; Sanín *et al.* 2013). Similar to other species that have been selected for restoration purposes (e.g. *Psidium guajaba*; Sarmiento 1997), *C. echinulatum* is a keystone species of the cloud forest, in particular because of its large production of fleshy fruits consumed by efficient seed dispersers (Henderson 2002; Sanín & Galeano 2011). Thus, the species is an interesting phytometer to determine to what extent nurse plants can facilitate the recovery of TMCF (Anthelme *et al.* 2011). Recent observations in deforested areas of the Andean cloud forest showed that, despite the presence of some spared adult palms with fruits and young seedlings (only beneath female adult palms), populations of *Ceroxylon* spp. are unable to regenerate in the long term (Anthelme *et al.* 2011; Sanín *et al.* 2013).

Setaria sphacelata is a tall C_4 tussock grass that was introduced in Ecuador around 1980 and has become the dominant grass in pastures created following the deforestation of TMCF (Brenner *et al.* 2012). The species has a relatively low nutrition value for large herbivores because of the low nutrient content of its leaves, particularly the older ones located at the tussock base (Hacker 1987). Spatial patterns observed in the field show common positive spatial associations between seedlings of *C. echinulatum* and mature tussocks of *S. sphacelata*, but only at the foot of female trees of *C. echinulatum* (pers. obs.). Therefore, *S.*

sphacelata was hypothesized to be a biotic refuge for other plants, especially the lower, older segments of tussocks [shared defense mechanism *sensu* Callaway (2007)].

IN SITU MANIPULATION EXPERIMENT

An *in situ* experiment was implemented in a deforested area of approximately 1 ha, dominated by an assemblage of short native/exotic grasses and *S. sphacelata* (approximately 25% cover at the soil surface), and in the adjacent forest. The deforested area was regularly grazed by five or six cows and two horses. In March 2009, 600 natural seedlings of *C. echinulatum* were collected under 20 mother trees in the forest and grown together in a greenhouse at the site under study. After three months, 210 of these seedlings were transplanted to the deforested area and in the adjacent forest for the experiment. We selected "seedling" (individuals with two or three undivided leaves; Anthelme, Montúfar Galárraga & Pintaud 2010) as our target life stage because of the expectation of greater sensitivity to interactions with neighbouring plants than older life stages (e.g. Venn, Morgan & Green 2009).

We used a randomized block design (30 blocks) that manipulated the biotic stress (herbivory) and abiotic stress applied to transplanted seedlings in the deforested area (Fig. 1). Seedlings were transplanted in each block within a 3×2 factorial design, with three levels of neighbours (OPEN: microsites occupied by short, herbaceous vegetation; NURSE: presence of *S. sphacelata*; SHADE: presence of artificial shade retaining approximately 50% of light intensity; Fig. 1) and two levels of herbivory (presence/absence, achieved using metal exclusion fences). To this design we added an additional treatment in the adjacent secondary forest, where domestic herbivores were absent (FOREST; 30 transplanted seedlings; Fig. 1). One seedling was transplanted for each replication of each treatment (total: 210). Comparing FOREST and OPEN treatments allowed the effects on seedlings of abiotic stress as a result deforestation to be measured. In the deforested area, possible amelioration of the abiotic microenvironment by artificial shade or the presence of nurse plants was assessed by comparing the performance of seedlings in the OPEN vs. the NURSE and SHADE treatments, respectively. The level of light interception by artificial shade was equivalent to the shading effects provided by *S. sphacelata* on palm seedlings. To test for the effects of herbivory in the three neighbouring treatments in the deforested area, we replicated them outside metal fences in each of the 30 blocks (treatments $\mathrm{OPEN}_{\mathrm{H}}$", $\mathrm{NURSE}_{\mathrm{H}}$ and $\mathrm{SHADE}_{\mathrm{H}}$, respectively; Fig. 1).

The performance of transplanted seedlings was monitored every two months between March 2009 and March 2010, as well as in November 2010. For each seedling of *C. echinulatum*, we measured two performance variables to obtain a clear picture of the effects of *S. sphacelata*: survival, and the green area of leaves (cm^2). The green area (GA) of each surviving individual reflected the photosynthetic potential of each individual. Leaves were extrapolated to be diamond-shaped and showing little morphological variation (Sanín & Galeano 2011; pers. obs.). Accordingly, their GA was calculated as

$$\mathrm{GA\ (one\ leaf)} = L \times 0.5l \times X \times Y,$$

where L is the length of the leaf, l is its width, X is the portion of leaf material removed by herbivores (ratio of leaves grazed, estimated visually in each leaf and taken separately as the index of leaf predation), and Y is the portion of the leaf that was not green. Variables X and Y were estimated visually and systematically for every single leaf of each individual. The GAs of all leaves of each individual were summed to provide the GA at the individual level.

In order to explore the mechanisms underlying the patterns of interactions found, we measured atmospheric water stress, light intensity and soil temperature for each treatment without herbivores, every 30 minutes over 12 days in a relatively dry period (March 2009). Water stress was estimated at 15 cm above the soil surface (approximately the height of seedling leaves) via the vapour pressure deficit (VPD) (kPa), which was calculated with atmospheric temperature and relative humidity (Anthelme, Michalet & Saadou 2007). Atmospheric temperature and relative humidity were measured with HOBO-Pro RH/Temp data loggers (Onset, Bourne, MA, USA), with at least four simultaneous measurements assigned randomly in each treatment. Light intensity (lux) was measured using UA-002 data loggers (Onset, Bourne, MA, USA), with eight simultaneous measurements for each treatment. Data loggers in the NURSE treatment were placed directly under the peripheral cover of *S. sphacelata* stems. Temperature in the soil at a depth of 5 cm was measured with U23-01 data loggers (Onset, Bourne, MA, USA), as the average value of three simultaneous measurements in each treatment.

DATA ANALYSES

In the deforested area, the effects of herbivory (yes/no) and neighbourhood (OPEN/SHADE/NURSE) on seedling survival were analysed using the Mixed Effects Cox model (Therneau & Grambsch 2000), which is a modification (by allowing the inclusion of random covariates, i.e., the "blocks" in this study) of the commonly used Cox's Proportional Hazards model. To test the significance of each fixed variable, we performed a likelihood ratio test (LRT) by comparing the deviances of a pair of nested models: a null model including only the random variable, and an alternative model including the random variable plus each fixed factor. The interaction between the fixed factors was included only if the LRT of the model indicated significant difference with both the simpler models. The effects of herbivory and neighbourhood on GA were analysed using a randomized-block linear model (fixed effects) with comparisons between pairs of treatments within blocks provided by simultaneous post-hoc Tukey tests. This latter analysis was conducted using data from July 2009, after which the mortality of seedlings did not permit the performance of further statistical tests.

The block structure of our design in the deforested area allowed us to make paired comparisons among treatments (see Fig. 1). We calculated the intensity of the facilitative effects on *Ceroxylon* seedlings generated by (1) *S. sphacelata*, and (2) artificial shade, by comparing seedling performance in these treatments with that in the OPEN$_H$ and OPEN treatments. Additionally, we compared each above treatment with FOREST in order to determine to what extent the performance of seedlings under conditions of shade or the presence of nurse plants can challenge the performance of seedlings in their natural habitat (with random assignment of forest replicates to each of the 30 blocks). These paired comparisons were made with the Relative Interaction Index (RII; Armas, Ordiales & Pugnaire 2004). RII was calculated with the survival and GA performance variables as

$$\mathrm{RII} = (Bw - Bo) / (Bw + Bo),$$

where Bw and Bo represent the performance of potential beneficiary species within and outside the nurse/artificial shade areas, respectively. RII values less than 0 reflect competitive interactions, while RII values greater than 0 reflect facilitative interactions. As seedlings in the forest were not allocated to a specific block, we assigned them randomly to each block and calculated additional RII values with the FOREST treatment taken as a control (absence of deforestation and herbivory).

Variation in the ratio of leaves grazed throughout the experiment was analysed with two-sample *t*-tests (and one-sample *t*-tests when all values of one treatment reached the maximum value of 100%). Variation of each of the abiotic parameters among the OPEN, NURSE and FOREST treatments were analysed with ANOVA and two-sample *t*-tests for average and maximal values. Correlations between abiotic parameters were tested with linear regressions. All analyses were made using MINITAB 15 and R 2.14.1 software (R Development Core Team 2011).

Results

THE EFFECTS OF DEFORESTATION AND HERBIVORES ON SEEDLINGS

Throughout the experiment, the seedlings in FOREST displayed higher survival than the seedlings in OPEN$_{\mathrm{H}}$ (two-sample *t*-tests: $P < 0.001$ at each date; Fig. 2a). They were almost unaffected by mortality (survival ratio after 20 months: 0.86 ± 0.06 SE), whereas in OPEN$_{\mathrm{H}}$ most of the seedlings died at the very beginning of the experiment (survival after two months: 0.07 ± 0.05) and all individuals died after 10 months (Fig. 2a; for other treatments see Fig. S1 in the Supporting Information). Cumulative variation in the area of leaves was also significantly different between the two treatments (two-sample *t*-tests: $P < 0.001$ at each date; Fig. 2b). Whereas GA regularly increased in FOREST, (+70.90 cm^2 ± 0.13 after 20 months), it reached its minimum value after four months in OPEN$_{\mathrm{H}}$ and maintained it up until the end of the experiment (−51.92 cm^2).

EFFECTS OF SHADE AND NURSE PLANTS WITH AND WITHOUT HERBIVORY

The presence of a neighbourhood had a significant positive effect on the survival, as well as the GA, of seedlings; whereas, the presence of herbivores impacted negatively upon the performance of seedlings ($P_{\mathrm{survival}} < 0.001$ and $P_{\mathrm{GA}} < 0.01$ in both cases; Table 1). The interaction between the neighbourhood and herbivory variables was significant only for GA ($P_{\mathrm{survival}} = 0.099$ and $P_{\mathrm{GA}} = 0.032$, respectively). The net effects of NURSE vs. OPEN (and NURSE$_{\mathrm{H}}$ vs. OPEN$_{\mathrm{H}}$) on the survival and the GA of seedlings were significantly positive throughout the experiment (e.g. survival after 20 months of experiment: $\mathrm{RII}_{\mathrm{NURSE}} = 0.60$; $\mathrm{RII}_{\mathrm{NURSEH}} = 0.20$; GA after four months: $\mathrm{RII}_{\mathrm{NURSE}} = 0.40$; one sample *t*-tests “not superior than zero”: $P < 0.01$; Figs. 3a, c and e). Meanwhile, the effect of SHADE vs. OPEN was significantly positive throughout the experiment when considering survival, reaching a plateau from 10 months ($\mathrm{RII}_{\mathrm{SHADE}}$: 0.37 ± 0.11; Fig. 3b), but was significantly lower than the effect of NURSE vs. OPEN, except after 12 months (simultaneous post-hoc Tukey tests: $P < 0.05$). The SHADE$_{\mathrm{H}}$ treatment did not influence survival (Fig. 3d). The effect of SHADE on the GA of seedlings was increasingly positive up until between 2 and 10 months of the experiment, after which the low number of surviving seedlings yielded insufficient repetitions for interpretation (RII after 10 months: 0.46 ± 0.14; Fig. 3f).

The NURSE treatment took the survival of seedlings to a similar level as FOREST (after 20 months: $RII_{NURSE\ vs.\ FOREST} = 0.20$, one sample T-test: $P > 0.05$; Fig. 3a); however, the GA was increasingly lower in the former throughout the experiment (after 20 months: $RII_{NURSE\ vs.\ FOREST} = -0.47$; Fig. 3e). In contrast, in the presence of herbivory, the effect of *S. sphacelata* ($NURSE_H$) was highly negative when compared to FOREST, from the very beginning of the experiment ($RII_{2\ months}$: −0.93 ± 0.05; Fig 3c). RII_{GA} was not calculable for treatments with herbivory because of too few data being available.

ABIOTIC CHARACTERIZATION OF EXPERIMENTAL TREATMENTS

Light intensity reached an average value of 1331 ± 90 lux in the FOREST treatment, which was 9 times lower than in OPEN and 3.7 times lower than in NURSE (Fig. 4). The VPD in OPEN was significantly higher than that in FOREST during all periods of the day (Figs. 4a–c; two-sample *t*-tests at each time: $P < 0.05$). These differences peaked at 12:00 when average VPD in the OPEN treatment reached 0.59 ± 0.11 kPa, compared to 0.01 ± 0.00 kPa in FOREST. The NURSE treatment reduced the average VPD by more than half (two-sample *t*-test: $P < 0.001$), and buffered the maximum VPD to 0.29 ± 0.07 kPa at 12:00. In OPEN, the light intensity was a major driver of VPD variation, with or without the presence of *S. sphacelata* ($R^2 = 0.22$ and 0.30, respectively; see Fig. S2). In FOREST, variation in VPD was barely influenced by light intensity ($R^2 = 0.01$). The average soil temperature was higher in OPEN than in FOREST, while NURSE provided intermediate values (ANOVA: $P < 0.001$).

HERBIVORE DAMAGE TO PALM SEEDLINGS

In the deforested area with the presence of herbivores, the leaf area removed by herbivores reached 100% within only six months after transplantation, regardless of the presence/absence of artificial shade (Fig. 5; OPEN$_H$ vs. SHADE$_H$; $P > 0.05$ on all dates). In contrast, the impact of herbivores was significantly attenuated and delayed by the presence of *S. sphacelata* (NURSE$_H$: 78% ± 8 of leaf area grazed after 20 months; NURSE$_H$ vs. OPEN$_H$: $P < 0.01$ on all dates), but it remained significantly higher than in FOREST on all dates (FOREST: 17% ± 6 of leaf area grazed after 20 months; $P < 0.001$).

Discussion

PALM SEEDLINGS BEYOND THEIR PHYSIOLOGICAL LIMITS UNDER DEFORESTATION AND HERBIVORY

As is the case for most plants in TMCF, *C. echinulatum* is a shade-tolerant species during its earlier life stages, with seedlings particularly intolerant to drought and full light intensity (Holmgren *et al.* 2012). These traits explain the high mortality of palm seedlings observed in deforested, non-grazed areas after 20 months of the experiment (93%; see Fig. S1). Their mortality is likely the result of higher maximum levels of atmospheric water stress, light intensity, and soil temperature (our data), as well as lower minimum levels of soil humidity (Anthelme *et al.* 2011). The additional presence of herbivores in deforested areas eliminated all remaining palm seedlings, thus corroborating our first hypothesis that the combination of deforestation and herbivory is responsible for the absence of *C. echinulatum* regeneration in deforested areas. This interpretation is supported by a significant loss in the GA of leaves observed in open, grazed areas with time following the transplantation of seedlings. In this situation, the loss of leaf area and exacerbated atmospheric water stress took palm seedlings away from their physiological limits, making their survival and growth dependent on habitat amelioration, especially by the presence of nurse plants (Gross *et al.* 2010; Holmgren *et al.* 2012). Similar to the case for seedlings of *C. echinulatum*, many other tree seedlings present in these cloud forests may also not be able to withstand the stresses generated by deforestation and grazing. Therefore, facilitation among plants must be considered a consistent option for the restoration of TMCF.

FACILITATION THROUGH REDUCTION OF ABIOTIC STRESS AND HERBIVORY

The tussock grass *S. sphacelata* was shown to be an efficient nurse plant for palm seedlings in deforested TMCF without herbivory. The mechanisms involved are a reduction of atmospheric water stress (by half; our data), and the provision for soils of a similar humidity as that observed in the adjacent forest during the driest period of the day (Anthelme *et al.* 2011). This direct habitat amelioration effect explains the increasing positive effect of *S. sphacelata* observed on *Ceroxylon* seedlings with time, which is expected to persist in the long term. Owing to the fact that the nurse-plant effects were significantly more positive than those of artificial shade, they were likely not only caused by the shading effects of *S. sphacelata*, but also (1) by the dense aggregation of tussock aerial stems, which maintains high atmospheric humidity in its core (Anthelme, Michalet & Saadou 2007) and (2) by the more humid and cooler soil provided by tussock grasses, via specific mechanisms such as hydraulic lift (Prieto, Armas & Pugnaire 2012). Therefore, we provide for the first time experimental evidence for strong facilitative interactions among plants through (abiotic) habitat amelioration in a tropical, productive environment. Our results support the conceptual model of Holmgren & Scheffer (2010). They are also in line with the stress-gradient hypothesis (more facilitation with more abiotic stress) provided that the relative abiotic stress endured by plants – not only the absolute harshness defining an ecosystem – is taken into account in the stress-interaction relationship (see also Smit, Rietkerk & Wassen 2009; Bagousse-Pinguet *et al.* 2014).

In the presence of herbivores, the effects of *S. sphacelata* on *Ceroxylon* seedlings remained positive throughout the 20-month experiment. The facilitative mechanisms involved are therefore partly indirect, i.e. encompassing a third biotic component (herbivores). First, they are likely related to "shared defense" because the oldest leaves at the base of *Setaria* tussocks are barely eaten by large herbivores (Hacker 1987). A second indirect mechanism of facilitation likely to occur is "external avoidance" (*sensu* Milchunas & Noy-Meir 2002) because seedlings are partially hidden from herbivores by tussocks. These interpretations are supported by the fact that artificial shade, which generated no hiding or protective effects on seedlings, did not increase the performance of seedlings in the presence of herbivores (see Fig. 1; on the contrary, we observed that horses were rather attracted to our "shade" design, in such a way that reduced facilitation might in part be indebted to the methodology used). Consequently, facilitation by nurse tussocks can be explained by both a reduction in relative abiotic stress [model of Holmgren & Scheffer (2010)] and a reduction in the effects of herbivory, up to a certain threshold [model of Smit, den Ouden, & Müller-Schärer (2007)]. This corroborated our second hypothesis.

However, combining the effects of abiotic stress and herbivory resulted in a reduction in the facilitative effects exerted by the nurse tussocks, at least when considering the GA of leaves (see the significant 'Herbivory*Neighbourhood' effects in Table 1). This result is in line with various studies showing that intense herbivory in an already (abiotically) stressed habitat reduces facilitation among plants (Graff & Aguiar 2011; Soliveres *et al.* 2011). Furthermore, it lends support to the conceptual and mathematical models that predict the combined influence of stress and disturbance on facilitation among plants to not simply be additive (Smit, den Ouden, & Müller-Schärer 2007; Malkinson & Tielbörger 2010; Bagousse-Pinguet *et al.* 2014). More experimental data are required to fully understand this complex relationship. We suggest that the level of protection against herbivory provided by the nurse tussocks may decline at higher intensities of herbivory because *S. sphacelata*, which is usually a low palatable species, would switch to become a viable option as a source of nutrition under such conditions.

The facilitative effects generated by *S. sphacelata* on *C. echinulatum* under herbivory gradually faded with time during the experiment, in such a way that we can reasonably expect there to be no nurse-plant effects in the long term. This trend might reduce the relevance of using facilitative interactions for long-term restoration projects (e.g. Prévosto *et al.* 2012). However, while this uncertainty surrounding facilitative effects may generally represent a strong limitation when considering their use for restoration, recent studies on the sister species *Ceroxylon quindiuense* demonstrated that juvenile individuals of more than 2 m in height allocate the majority of their resources to developing subterranean meristems. This strategy permits them to be highly resilient to damage of their aerial meristems (Sanín *et al.* 2013). Furthermore, leaves of tropical forest plants, when aging, develop more chemical and physical defences to protect themselves from herbivory (Coley & Barone 1996), and older palm leaves may be more resilient to predation (Mendoza, Piñero & Sarukhan 1987). Therefore, we assume that the facilitative effects provided by *S. sphacelata* on *C. echinulatum* should ensure the long-term survival of these individuals by protecting them until they reach a life stage at which they are more resistant to herbivory (at least at a height of 1 m, which is the height almost all *Setaria* tussocks reach).

FROM THEORY TO APPLICATION: IS RESTORATION OF DEGRADED CLOUD FOREST ACHIEVABLE WITH TUSSOCK GRASSES?

Recent reviews have evidenced the important role that facilitative interactions can play in the restoration of degraded ecosystems, including in tropical (dry) regions (Padilla & Pugnaire 2006; Gómez-Aparicio 2009). A meta-analysis that took into account various degraded ecosystems worldwide demonstrated that trees may be predominantly facilitated by nurse shrubs or nurse trees whereas herbs – in particular perennial grasses – may have mostly a negative effect on the survival and growth of trees (Gómez-Aparicio 2009). However, the combined effects of deforestation and herbivory in TMCF generate patterns of impoverished plant communities without trees and shrubs, and dominated by invasive tussocks at the regional scale (Aide, Ruiz-Jaen & Grau 2010; Brenner *et al.* 2012). Therefore, two options for forest nurse-based restoration are possible: planting nurse trees/shrubs, or using existing tussock grasses as potential nurse plants.

The first option should yield the most efficient results in terms of restoration given the higher nurse potential of these life forms. One experiment using the native nitrogen-fixing tree *Alnus acuminata* (Betulaceae) for this purpose in the Colombian cloud forest yielded positive results, but did not consider the effects of herbivores, which were absent from the study site (Murcia 1997). In comparison, empirical data taking into account the effects of herbivores on the regeneration of forest tree species show that shrubs are better nurse plants than trees, especially because of their multi-stemmed architecture and their crown being located closer to the soil surface (Gómez-Aparicio, Zamora & Gómez 2005). From this viewpoint, and at similar cost, planting shrubs rather than trees may yield better results in grazed deforested areas. However, the expense of planting trees or shrubs reduces the feasibility of each of these restoration techniques (Aide, Ruiz-Jaen & Grau 2010).

An alternative nurse-based restoration option highlighted by our data is to consider existing tussock grasses as potential nurse beds for forest restoration. Even though tussock grasses have in general been found to be competitive with other plants, by developing high densities of roots and aerial stems (Maestre, Bautista & Cortina 2003; Hooper, Legendre & Condit 2005), observed patterns in the deforested cloud forest show positive spatial associations between *S. sphacelata* and young individuals of *C. echinulatum*, but only at the base of mother palms where palm seeds and seedlings were found aggregated, probably because of a deficit in the number of animal dispersers (Anthelme *et al.* 2011; pers. obs.). This demonstrates that the success of restoration using nurse tussocks requires transplanting palm seedlings under the cover of tussocks, thus compensating the limited dispersal generally observed in degraded cloud forest (e.g., Sarmiento 1997). The level of investment needed is minor, with the possibility of collecting seedlings directly in adjacent forests, or at the foot of mother trees in deforested areas. The fact that exotic tussock grasses may be detrimental to the biodiversity of open areas in the tropics (e.g., Hooper, Legendre & Condit 2005) is not relevant here as the tussocks are already established in the deforested areas at high levels of abundance. On the contrary, using pre-established *S. sphacelata* as nurse plants for *C. echinulatum* may lead to a reduction of its abundance along with succession.

Acknowledgments

We thank the people of the Inti-Llacta Reserve for their kind reception and J. Lincango, S. Cauvy-Fraunié, and C. Gully for their help with data collection. We are also grateful to P. Liancourt for extensive comments on the manuscript, and to the LucidPapers team for English revision. This research was funded by the Ecuadorian government (ECOFONDO grant no. 019-ECO7-Inv1), and the project PALMS FP7-ENV-2007-I (www.fp7-palms.org).

Data accessibility

Dataset on palm seedling and their microenvironment: DRYAD entry doi:

References

Aide, T.M., Ruiz-Jaen, M.C. & Grau, H.R. (2010) What is the state of tropical montane cloud forest restoration. *Tropical Montane Cloud Forests: Science for Conservation and Management* (eds L.A. Bruijnzeel, F.N. Scatena & L.S. Hamilton), pp. 101-110. Cambridge University Press, Cambridge.

Anthelme, F., Lincango, G., Gully, C., Duarte, N. & Montúfar, R. (2011) How anthropogenic disturbances affect the resilience of a keystone palm tree in the threatened Andean cloud forest? *Biological Conservation*, **144**, 1059-1067.

Anthelme, F., Michalet, R. & Saadou, M. (2007) Positive associations involving the tussock grass *Panicum turgidum* Forssk. in the Aïr-Ténéré Reserve, Niger. *Journal of Arid Environments*, **68**, 348–362.

Anthelme, F., Montúfar Galárraga, R., & Pintaud, J.C. (2010) Caracterización de la resiliencia ecológica de poblaciones de palmeras. *Ecología en Bolivia*, **45**, 23-29.

Armas, C., Ordiales, R. & Pugnaire, F.I. (2004) Measuring plant interactions: a new comparative index. *Ecology*, **85**, 2682–2686.

Bagousse-Pinguet, Y., Xiao, S., Brooker, R.W., Gross, N., Liancourt, P., Straile, D. & Michalet, R. (2014) Facilitation displaces hotspots of diversity and allows communities to persist in heavily stressed and disturbed environments. Journal of Vegetation Science, 25, 66-76.

Bertness, M. & Callaway, R.M. (1994) Positive interactions in communities. *Trends in Ecology and Evolution*, **9**, 191-193.

Brenner, S. Roos, K., Vos, I., König, M., Rollenbeck, R., Scheibe, R., Beck, E. & Bendix, J. (2012) Simulating canopy photosynthesis for two competing species of an anthropogenic grassland community in the Andes of southern Ecuador. *Ecological Modelling*, **239**, 14-26.

Bubb, P., May I., Miles, L. & Sayer, J. (2004) *Cloud forest agenda*. UNEP World Conservation Monitoring Centre, Cambridge.

Callaway, R.M. (2007) *Positive interactions and interdependence in plant communities*. Springer, Dordrecht.

Callaway, R.M., Kikvidze, Z. & Kikodze, D. (2000) Facilitation by unpalatable weeds may conserve plant diversity in overgrazed meadows in the Caucasus Mountains. *Oikos*, **89**, 275-282.

Coley, P.D. & Barone, J.A. (1996) Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics*, **27**, 305-335.

Gómez-Aparicio, L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology*, **97**, 1202-1214.

Gómez-Aparicio, L., Zamora, R. & Gómez, J.M. (2005) The regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. *Biological Conservation*, **121**, 195-206.

Graff, P. & Aguiar, M.R. (2011) Testing the role of biotic stress in the Stress-gradient hypothesis. Processes and patterns in arid rangelands. *Oikos*, **120**, 1023-1030.

Gross, N., Liancourt, P., Choler, P., Suding, K.N. & Lavorel, S. (2010) Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions. *Perspectives in Plant Ecology, Evolution, and Systematics*, **12**, 9-19.

Hacker, J.B. (1987) Genetic shift in *Setaria sphacelata* in response to grazing. *Australian Journal of Agricultural Research*, **38**, 907-916.

Hansen, M.C., Potapov, P.V., Moore, R., Hancher, M., Turubanova, S.A., Tyukavina,A., Thau, D., Stehman, V., Goetz, S.J., Loveland, T.R., Kommareddy, A., Egorov, A., Chini, L., Justice, C.O. & Townshend, J.R.G. (2013) High-resolution global maps of 21st-century forest cover change. *Science*, **342**, 850-853.

Holmgren, M, Gómez-Aparicio, L., Quero, J.L. & Valladares, F. (2012) Non-linear effects of drought under shade: reconciling physiological and ecological models in plant communities. *Oecologia*, **169**, 293-305.

Holmgren, M. & Scheffer, M. (2010) Strong facilitation in mild environments: the stress gradient hypothesis revisited. *Journal of Ecology*, **98**, 1269-1275.

Hooper, E., Legendre, P. & Condit, R. (2005) Barriers to forest regeneration of deforested and abandoned land in Panama. *Journal of Applied Ecology*, **42**, 1165-1174.

Liancourt, P., Callaway, R.M., Michalet, R. (2005) Stress tolerance and competitive response ability determine the outcome of biotic interactions. *Ecology*, **86**, 1611–1618.

Maestre, F.T., Bautista, S. & Cortina, J. (2003) Positive, negative, and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. *Ecology*, **84**, 3186-3197.

Malkinson, D. & Tielbörger, K. (2010) What does the stress-gradient hypothesis predict? Resolving the discrepancies. *Oikos*, **119**, 1546-1552.

Mendoza, A., Piñero, D. & Sarukhan, J. (1987) Effects of experimental defoliation on growth, reproduction and survival of *Astrocaryum mexicanum*. *Journal of Ecology*, **75**, 545-554.

Milchunas, D.G. & Noy-Meir, I. (2002) Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos*, **99**, 113–130.

Montúfar, R., Anthelme, F., Pintaud, J.C. & Balslev, H. (2011) Effects of disturbance on resilience of tropical American palm populations and communities. *The Botanical Review*, **77**, 426–461.

Mosandl, R., Günter, S., Stimm, B. & Weber, M. (2008) Ecuador suffers the highest deforestation rate in South America. *Gradients in a Tropical Mountain Ecosystem of Ecuador* (eds Beck, E., Bendix, J., Kottke, I., Makeschin, F. & Mosandl, R.), pp. 37–40. Ecological Studies, vol. 198. Springer, Berlin,.

Mulligan, M. (2010) Modelling the tropics-wide extent and distribution of cloud forest and cloud forest loss, with implications for conservation priority. *Tropical Montane Cloud Forests: Science for Conservation and Management* (eds L.A. Bruijnzeel, F.N. Scatena & L.S. Hamilton), pp. 14-38. Cambridge University Press, Cambridge.

Murcia, C. (1997) Evaluation of Andean alder as a catalyst for the recovery of tropical cloud forests in Colombia. *Forest Ecology and Management*, **99**, 163-170.

Padilla, F.M. & Pugnaire, F.I. (2006) The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment*, **4**, 196-202.

Prévosto, B., Monnier, Y., Ripert, C. & Fernandez, C. (2012) To what extent do time, species identity and selected plant response variables influence woody plant interactions? *Journal of Applied Ecology*, **49**, 1344-1355.

Prieto, I., Armas, C. & Pugnaire, F I. (2012) Water release through plant roots: new insights into its consequences at the plant and ecosystem level. *New Phytologist*, **193**, 830-841.

R Development Core Team. (2011) R: A Language and Environment for Statistical Computing. Vienna, Austria. R Foundation for Statistical Computing, Vienna, Austria. Available at: www.r-project.org.

Sanín, M.J., Anthelme, F., Pintaud, J.C., Galeano, G., & Bernal, R. (2013) Juvenile Resilience and Adult Longevity Explain Residual Populations of the Andean Wax Palm *Ceroxylon quindiuense* after Deforestation. *Plos One*, **8**, e74139.

Sanín, M.J & Galeano, G. (2011) A revision of the Andean wax palms, *Ceroxylon* (Arecaceae). *Phytotaxa*, **34**, 1-64.

Sarmiento, F.O. (1997) Arrested succession in pastures hinders regeneration of Tropandean forests and shreds mountain landscapes. *Environmental Conservation*, **24**, 14-23.

Shoo, L.P. & Catterall, C.P. (2013) Stimulating Natural Regeneration of Tropical Forest on Degraded Land: Approaches, Outcomes, and Information Gaps. *Restoration Ecology*, **6**, 670-677.

Smit, C., Rietkerk, M. & Wassen, M.J. (2009) Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. *Journal of Ecology*, **97**, 1215-1219.

Smit, C., Vandenberghe, C., den Ouden, J., & Müller-Schärer, H. (2007) Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. *Oecologia*, **152**, 265-273.

Soliveres, S., García-Palacios, P., Castillo-Monroy, A.P., Maestre, F.T., Escudero, A. & Valladares, F. (2011) Temporal dynamics of herbivory and water availability interactively modulate the outcome of a grass shrub interaction in a semi-arid ecosystem. *Oikos*, **120,** 710-719.

Therneau, T.M. & Grambsch, P.M. (2000) *Modeling survival data: extending the Cox model*. Springer-Verlag, New York.

Venn, S.E., Morgan, J.W. & Green, P.T. (2009) Do facilitative interactions with neighboring plants assist the growth of seedlings at high altitudes in alpine Australia? *Arctic, Antarctic, and Alpine Research*, **41**, 381-387.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1. Performance of palm seedlings in each treatment

Fig. S2. Influence of light intensity on VPD.

Table 1. Summary of the models analysing the effects of herbivory (presence/absence), neighbourhood (OPEN/SHADE/NURSE), and their interactions on the performance of *C. echinulatum* seedlings in the deforested area. Survival curves were analysed using the Mixed Effects Cox model, while GA in July 2009 was analysed using a randomized-block linear model (fixed effects) and post-hoc Tukey tests ($P < 0.05$). d.f.: degrees of freedom. Different letters mean significant variations among treatments.

Variables	$\chi^2_{survival}$	$P_{Survival}$	F_{GA}	P_{GA}	d.f.	Comparisons among treatments ($P > 0.05$)
Herbivory (yes/no)	51.15	0.000	8.35	0.006	1	Survival: no > yes GA: no > yes
Neighbourhood (OPEN/SHADE/NURSE)	30.58	0.000	7.84	0.001	2	Survival: NURSE > SHADE > OPEN GA: NURSE > SHADE = OPEN
Herbivory* Neighbourhood	4.61	0.099	3.68	0.032	2	

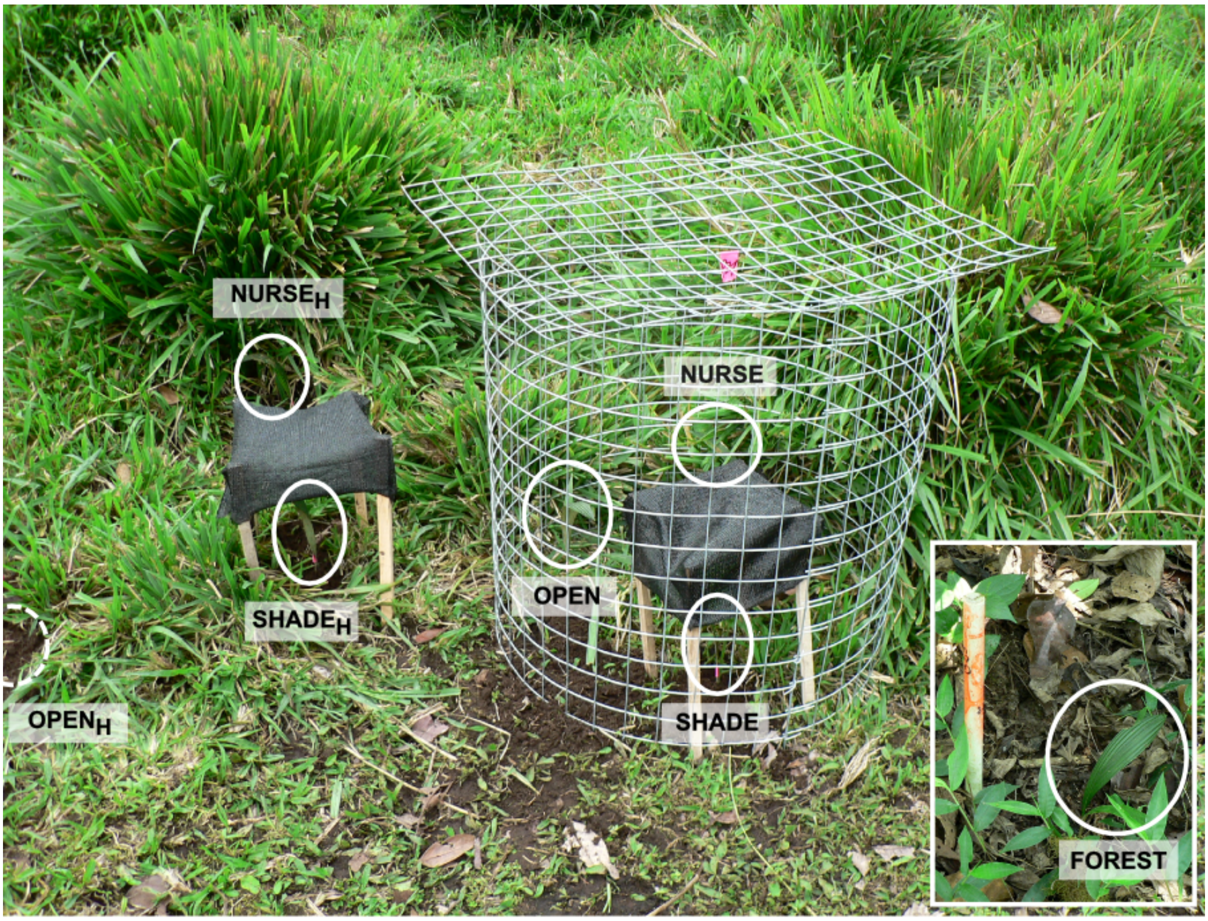

Fig. 1. One of the 30 experimental blocks for transplanted seedlings of *Ceroxylon echinulatum* in the deforested area. Each treatment is annotated in the figure and surrounded with a white circle. Tussocks in the background belong to the species *Setaria sphacelata*. An additional treatment was conducted in the adjacent forest (bottom right-hand corner).

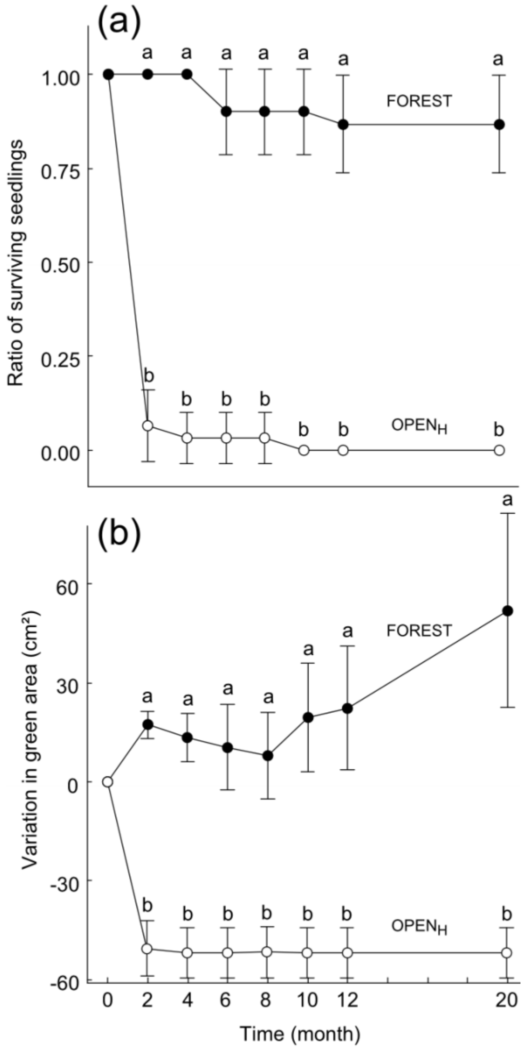

Fig. 2. Performance of transplanted seedlings throughout the 20-month experiment in the FOREST and in OPEN$_H$ treatments: (a) survival; (b) cumulative variation in green area. The bars for each data point show the 95% confidence interval. Different letters indicate significant variation between treatments on each date.

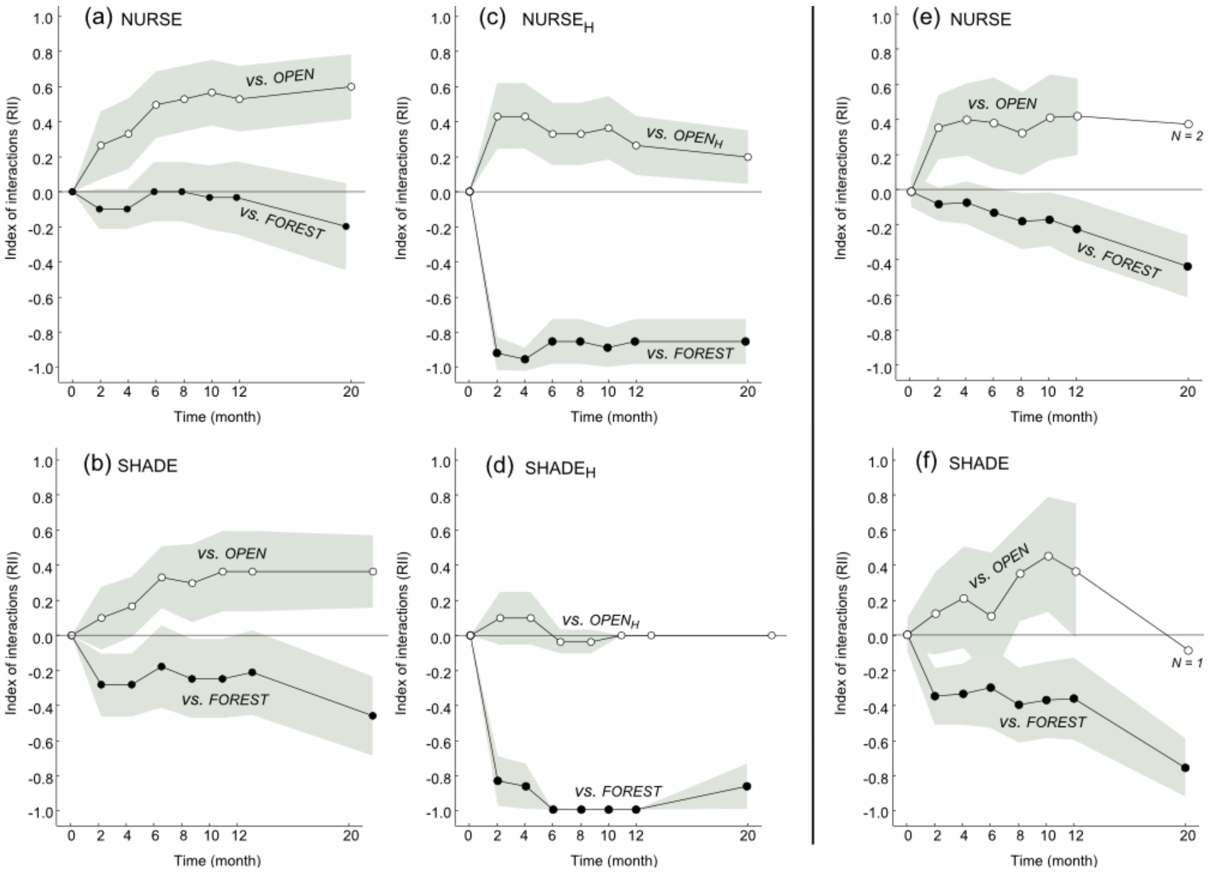

Fig. 3. Effects of NURSE, NURSE$_H$, SHADE and SHADE$_H$ on the performance of transplanted seedlings in the deforested area throughout the 20-month experiment (RII, white dots). For comparison, RII was also calculated between these three treatments and FOREST (black dots). (a, b) Survival in the absence of herbivory; (c, d) survival in the presence of herbivores; (e, f) green area of leaves in the absence of herbivores. The grey shaded areas represent the 95% confidence interval, extrapolated from individual values on each date.

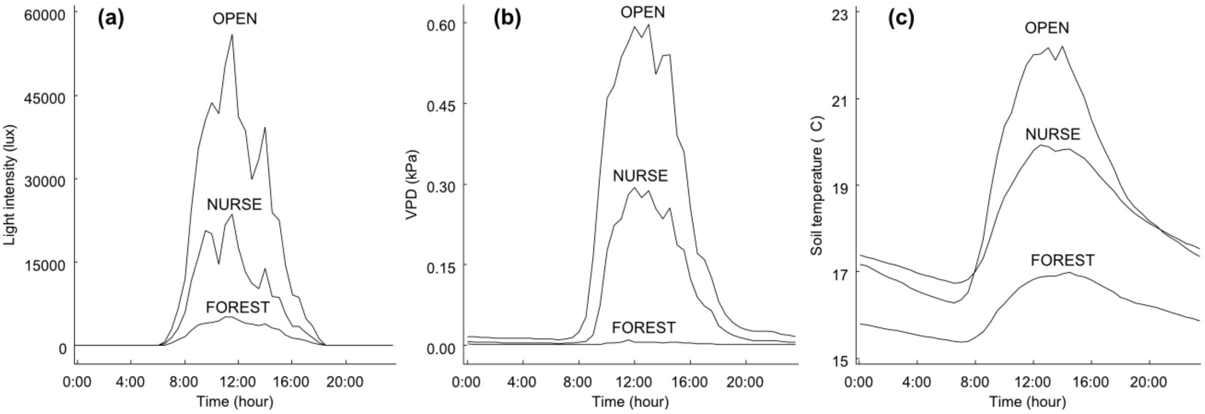

Fig. 4. Mean variation in the abiotic microenvironment with time in the FOREST, NURSE and OPEN treatments: (a) light intensity; (b) VPD; and (c) soil temperature at 5 cm below ground. Time interval: 30 min.

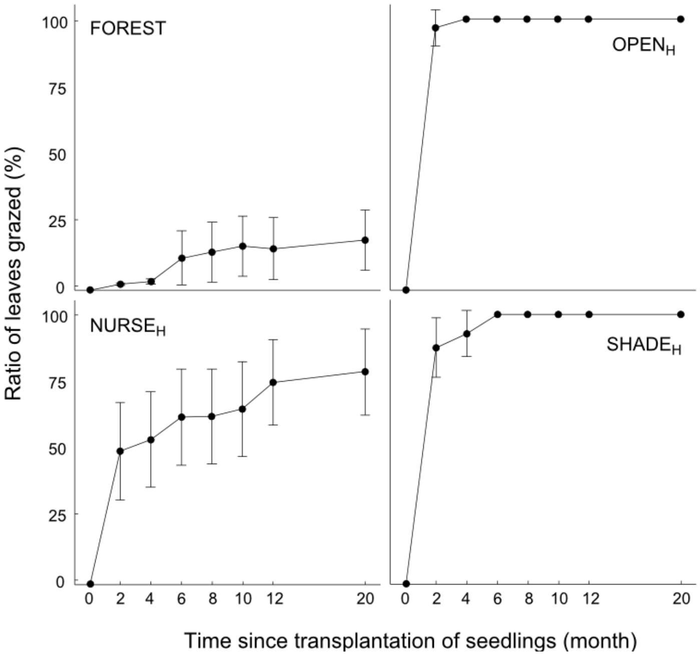

Fig. 5. Dynamics of herbivore damage on transplanted seedlings throughout the 20-month experiment in the FOREST, OPEN$_H$, NURSE$_H$ and SHADE$_H$ treatments. The bars for each data point show the 95% confidence interval.