

Biol. Lett. doi:10.1098/rsbl.2011.0236 Published online

Do interactions between plant and soil biota change with elevation? A study on *Fagus sylvatica*

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Theoretical models predict weakening of negative biotic interactions and strengthening of positive interactions with increasing abiotic stress. However, most empirical tests have been restricted to plant-plant interactions. No empirical study has examined theoretical predictions of interactions between plants and below-ground micro-organisms, although soil biota strongly regulates plant community composition and dynamics. We examined variability in soil biota effects on tree regeneration across an abiotic gradient. Our candidate tree species was European beech (Fagus sylvatica L.), whose regeneration is extremely responsive to soil biota activity. In a greenhouse experiment, we measured tree survival in sterilized and non-sterilized soils collected across an elevation gradient in the French Alps. Negative effects of soil biota on tree survival decreased with elevation, similar to shifts observed in plant-plant interactions. Hence, soil biota effects must be included in theoretical models of plant biotic interactions to accurately represent and predict the effects of abiotic gradient on plant communities.

Keywords: elevation gradient; forest regeneration; stress gradient hypothesis

1. INTRODUCTION

Biotic interactions have long been recognized as important drivers of plant community dynamics and composition [1]. Many studies have shown that these interactions may be altered by external drivers such as climate and nutrient availability [1]. These studies led to the 'stress gradient hypothesis' (SGH), which postulates that positive interactions predominate under environmentally stressful conditions and that negative interactions prevail under benign conditions [2]. While most studies focused on interactions between plants, other biotic interactions, including insect predation and fungal parasitism, have rarely

Electronic supplementary material is available at http://dx.doi.org/ 10.1098/rsbl.2011.0236 or via http://rsbl.royalsocietypublishing.org. been considered within the SGH framework. This is particularly true for interactions between plant and soil biota.

However, soil biota is an important regulator of plant community composition and dynamics [3]; they can affect plant succession [4], species distribution and invasion processes [5]. The overall soil biota effect on plant performance is determined by the balance between positive and negative effects. For instance, plant recruitment is negatively affected through damping-off disease but positively affected by mycorrhization. Because the soil biota community is influenced by abiotic gradients [6,7], the balance between negative (predation/ parasitism) and positive (mutualism) soil biota effects are likely to vary along abiotic gradients, as it has been reported for plant-plant interactions. Despite these findings, variation in the interactions between plants and below-ground organisms along abiotic gradients has never been investigated.

In this study, we quantified influences of soil biota on tree regeneration across an elevation gradient. We studied regeneration in Fagus sylvatica L., a species known to be highly sensitive to soil biota activity. In particular, we tested (i) whether soil biota affects regeneration of *F. sylvatica*, and (ii) whether the effect of soil biota on regeneration changes across an elevation gradient. To isolate soil biota effects from confounding influences, we compared the effects of sterilized and non-sterilized soils that were collected across an elevation gradient in the French Alps under controlled abiotic conditions (greenhouse). Finally, we explored the effects of abiotic and biotic drivers on the variation in soil biota community as a function of altitude. We focused particularly on the composition of local forest communities (e.g. for host abundances effect).

2. MATERIAL AND METHODS

(a) Description of the gradient

The study was conducted in the French Alps across an altitudinal gradient. Elevation is a complex abiotic gradient including numerous geophysical parameters, such as temperature, precipitation and radiation. The altitudinal gradient can be interpreted as a stress gradient for plant communities because productivity decreases with elevation (most tests of the SGH use productivity as a surrogate for stress) [2]. However, the stress measured at the species level generally differs from the one experienced by the overall community. Our elevation gradient encompassed both the lower and higher limits of the niche of F. sylvatica. Thus, the stress experienced by this species was unlikely to decrease monotonically with elevation. We built a habitat suitability map for *F. sylvatica* for use as an indicator of stress experienced by the species. This model was calibrated using presence/absence data from the French National Forest Inventory with the BIOMOD library in R software [8] and relevant environmental variables (electronic supplementary material, S1). We then predicted the probability of *F* sylvatica presence at the five sites of soil collection and the 95% confidence intervals of each prediction (figure 1c).

(b) Greenhouse experiment

A greenhouse experiment was conducted to test the effects of soil biota (non-sterilized versus sterilized soil) from each elevation. We randomly sampled five soil aliquots (50 ml) at five elevations (500, 980, 1350, 1729 and 2045 m). We used these samples to create two soil biota inocula of 25 ml each. One was sterilized for control. In each pot, we mixed 25 ml inoculum with 750 ml of sterile back-ground soil and incubated the mixture for 7 days in a greenhouse. The mixture was then distributed to five pots for replication. To avoid contamination, all tools, materials and surfaces in contact with the soil samples were sterilized by one of the following four methods: autoclaving for 90 min, flame sterilization, spraying with 90 per cent ETOH and bleach immersion. In each pot, three seeds





Figure 1. (a) Survival of *F* sylvatica seedlings in sterilized versus non-sterilized soil as a function of soil source elevation. The solid line represents predictions of the best-fit model (see §2) and diamonds represent the mean of survival rate at each elevation. The confidence envelope (dashed lines) represents the 95% confidence intervals of the predictive posterior distributions of survival probability. (b) Points represent local relative abundance (\pm s.d.) of *F* sylvatica (diamonds) and HOST CLUSTER (crosses) measured at the five sample sites. (c) Predicted probability of *F* sylvatica presence estimated with BIOMOD; 95% confidence intervals of the prediction are indicated by dashed lines. Black lines, sterilized; grey lines, non-sterilized.

of *F* sylvatica were sown. All seeds were collected on the same site from the centre of its elevation distribution by the National Forest Office in the French Jura. Consequently, effects of local adaptation are eliminated. Experiments were then conducted in a greenhouse maintained at 14°C during the day and 8°C during the night; humidity was maintained between 65 and 95 per cent. Tree survival was monitored over 60 days. We fitted a series of alternative hierarchical Bayesian logistic mixed models to identify the best among three competing hypotheses: (i) soil biota do not affect regeneration of *F* sylvatica (survival), (ii) soil biota affect regeneration of *F* sylvatica, and (iii) the effect of soil biota on regeneration is a function of altitude (electronic supplementary material, S2). For the fixed effects, there was a null model with no effects (sterilized versus non-sterilized soil; *SOIL* for hypothesis (ii)), and a model with effects of non-sterilized soil varying by elevation (*SOIL_ELV* for hypothesis (iii)). The most parsimonious model was selected by deviance information criteria (lowest deviance information criterion (DIC) for the best model).

(c) Potential drivers of soil biota community

Altitudinal variation in the soil biota community interacting with F sylvatica may result from variable abiotic conditions and/or from effects of local forest community composition. Effects of the local forest depend on both F sylvatica tree abundance and on the species composition of the whole local forest community. To assess the effects of local vegetation, we described tree species composition in the surrounding community for each elevation (electronic supplementary material, S3) and calculated the relative density of (i) F sylvatica and (ii) species that occupy a similar position in the tree-pathogen interaction network, viz., Quercus pubescens, Quercus rubra, Quercus robur, Quercus petraea, F sylvatica, and Carpinus betulus (fig. 3 in [9]). This group of potential host species for F sylvatica pathogens is referred to as HOST CLUSTER hereafter.

3. RESULTS

Logistic regression showed a significant negative influence of soil biota on seedling survival structured by elevation (DIC NULL = 223.7 versus DIC SOIL = 219.7 and versus DIC SOIL_ELV = 215.4). The probability of survival was lower in non-sterilized soils than in sterilized soils, and this difference decreased with increasing elevation. The habitat suitability model showed that the negative soil biota effect was limited to lower elevations of *F* sylvatica suitable habitats.

Comparisons with surrounding community composition showed that the strongest negative influences of soil biota were restricted to lower elevations, where the relative abundance of *F. sylvatica* was low, but relative abundances of species from the HOST CLUSTER were high (mainly *Q. petraea*). At high elevations, where the relative abundances of both groups were low, there was no evidence of a negative effect of soil biota on seedling survival.

4. DISCUSSION

Our results showed negative effects of the soil biota community on European beech survival. Model comparisons demonstrated that these negative effects decreased with elevation and disappeared in samples from the highest altitude. This is the first study to show variability in interactions between plants and the soil biota community across an elevation gradient. These findings are consistent with those of numerous plant-plant interaction tests of the SGH. Incorporation of plant-soil biota interactions into the framework of the SGH is of considerable intrinsic interest and calls for further studies into these interactions. In our experiments, many micro-organisms with rapid proliferation rates may have triggered the negative effects observed. For instance, F. sylvatica seedlings are known to host oomycetes from the family Pythiaceae, which are responsible for root and collar rot symptoms in herbaceous and woody plants [10]. Our experiment was probably of insufficient duration to allow positive interactions, such as mycorrhization, to be expressed, and indeed, we observed no mycorrhized roots under microscopic observation. Soil biota inoculum was the only factor varying between pots. Hence, the trend observed was probably the consequence of a decrease in the soil pathogen pressure on *F. sylvatica* with increase in elevation.

Variability in the effects of soil biota on F. sylvatica regeneration across our elevation gradient may have had several root causes. First, a direct effect of climate may explain the trend. Variation in soil temperature or moisture between our sites might lead to differences in local soil biota community composition. For example, parasitic fungal species richness is negatively affected by low winter temperatures [7]. Moreover, several pathogenic species of Pythiaceae that infect members of the HOST CLUSTER are limited by low temperature [11]. Second, climate may also directly influence the plant species composition of the local forest community, which in turn affects the soil biota community; this would be an indirect effect of climate on the soil biota. Pathogen density is generally correlated with host density [12]. However, in our study, the local density of potential conspecific hosts did not appear to be a determinant of soil pathogen pressure. Negative effects were maximal at low elevation where the density of F. sylvatica was low, and although our site at 980 m elevation had a mono-specific F. sylvatica stand, soil pathogen pressure was not elevated at this altitude. The suite of *F. sylvatica* pathogens is influenced not only by F. sylvatica abundance, but also by the composition of the whole local forest stand. At our study sites, abundance of a group of plant species with positions similar to that of F. sylvatica in the treepathogen interaction network (i.e. HOST CLUSTER) decreased with elevation in parallel with decreasing pathogen pressure. It is thus likely that in addition to a direct climate effect, soil pathogens were affected by HOST CLUSTER abundance.

We have shown that negative soil effects are restricted to lower elevations within the realized niche of European beech. This altitude-dependent variation in the effects of micro-organisms may play a significant role in shaping tree species' distributions across elevations and, consequently, the responses of forest communities to climate change. Although our work is the first step in describing the variation in the balance of interactions between soil biota and plants along an abiotic gradient, understanding processes engaged in these phenomena need an accurate description of the protagonists. Studies using diverse microbiological tools are needed to investigate which soil pathogen, parasite and mutualism is involved in the variation of soil biota effects along the abiotic gradient. Eventually, this will allow the incorporation of soil biota effects into the SGH.

We thank P. Tardif for his technical help. This research was funded by the EU BACCARA (N $^\circ$ 226299) and Alcotra

2007-2013 N° 032 projects. W.T. was funded by the ANR DIVERSITALP (ANR-07-BDIV-014), and the EU Ecochange (GOCE-CT-2007-036866) projects.

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