

Original Research Article

Impacts of seven years of experimental warming and nutrient addition on neighbourhood species interactions and community structure in two contrasting alpine plant communities



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ABSTRACT

Global change is predicted to have major impacts on alpine and arctic ecosystems. Plant fitness and growth will be determined by how plants interact with each other at smaller scales. Local-scale neighbourhood interactions may be altered by environmental perturbations, which could fundamentally affect community structure. This study examined the effects of seven years of experimental warming and nutrient addition on overall changes in the community structure and patterns of interspecific interaction between neighbouring plant species in two contrasting alpine plant communities, mesic meadow and poor heath, in subarctic Sweden. We used a network approach to quantify the dissimilarity of plant interaction networks and the average number of interspecific neighbourhood interactions over time in response to different environmental perturbations. The results revealed that combined warming and nutrient addition had significant negative effects on how dissimilar plant interaction networks were over time compared with the control. Moreover, plant–plant neighbourhood interaction networks were more dissimilar over time in nutrient-poor heath than in nutrient-rich mesic meadow. In addition, nutrient addition alone and combined nutrient addition and warming significantly affected neighbourhood species interactions in both plant communities. Surprisingly, changes in interspecific neighbourhood interactions over time in both communities were very similar, suggesting that the nutrient-poor heath is as robust to experimental environmental perturbation as the mesic meadow. Comparisons of changes in neighbouring species interactions with changes in evenness and richness at the same scale, in order to determine whether diversity drove such changes in local-scale interaction patterns, provided moderate evidence that diversity was behind the changes in local-scale interspecific neighbourhood interactions. This implied that species might interact at smaller scales than those at which community measures were made. Overall, these results demonstrated that global change involving increased nutrient deposition and warming is likely to affect species interactions and alter community structure in plant communities, whether rich or poor in nutrients and species.

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1. Introduction

Global change is predicted to significantly affect plant community structure, mediated through plant traits, their interactions and growth (Baruah et al., 2017; Niu and Wan 2008). A better understanding is needed of how interactions among plants can shift due to global change. Plant interactions

shape large-scale evolutionary processes, which are important in maintaining species diversity (Isbell et al., 2011). The local-scale processes and biotic interactions among plants are very complex and changes are therefore difficult to predict (Ricklefs, 2004), but such interactions are key to regional diversity (Tilman et al., 1997; Allesina and Levine 2011). It is thus very important to study changes in these local-scale interactions, which can consist of positive and negative interactions and complex combinations of both (Callaway, 1995). For example, global changes or even local changes can cause demographic change due to increased negative

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plant interactions and changes in litter accumulation (Olsen and Klanderud, 2014).

Plant–plant interactions are an important component in understanding the response of plant communities to predicted global change, which will affect plant communities either directly or indirectly (Adler et al., 2012; Brooker 2006; Gilman et al., 2010; Lortie et al., 2004). Plant–plant interactions play an important role in regulating plant community structure. Many studies have demonstrated the importance of network analysis in understanding community dynamics (Albert and Barabási, 2002), in particular in response to environmental change (Tylianakis et al., 2008; Bascompte and Jordano 2013). Due to the nested structure of plant networks, plant–plant interactions can be facilitative and thereby prevent extinction (Verdú and Valiente-Banuet, 2008).

It is unclear, however, to what extent community responses are sensitive to variations in local-scale neighbourhood interactions. It has been demonstrated that species competition increases as the number of neighbour species increases and that the degree of competition can vary depending on the type of communities present (Wiegand et al., 2007). Thus the importance of neighbourhood interactions cannot be ignored in light of climate change. Although measurements of diversity using indices such as evenness can roughly sum up the community structure and link it to the productivity of the community, such measures may miss interactions at smaller, local scales (McKenna and Yurkonis, 2016). Changing neighbourhood species interactions could substantially affect community productivity and plant diversity–productivity relationships if the scale of interactions between plant species in a community is localised within a few metres (Lamošová et al., 2010; Zhang et al., 2014). Such local-scale interactions in a particular habitat could be identified with the help of neighbourhood interaction analyses. Moreover, in a habitat it might be possible for evenness to remain the same over the years, but neighbourhood associations between species in that habitat can potentially decrease. This might occur for example, in a hypothetical case where there are different plant species in a small habitat, but not all are neighbours to all others. This is possible when there are non-random aggregations of a particular species and its con-specifics, such that all plant species are far apart and hence there are no direct neighbours (at a scale of 0.1 m). Such non-random aggregations of conspecifics might allow weaker competitors to persist through delayed competitive exclusion (Stoll and Prati, 2001). The resulting communities would presumably be more diverse, yet less productive, than non-aggregated communities (McKenna and Yurkonis, 2016). Diverse communities are generally more productive and stable, with a linear relationship between plant productivity and diversity (Mulder et al., 2004). However, recent studies suggest that the diversity–productivity relationship may be affected if neighbour species compete at a very small spatial scale (Zhang et al., 2014). The spatial pattern of plants in a particular habitat can have a significant effect on the outcome of competition and, consequently, alter the biodiversity yield (Lamošová et al., 2010). Hence the importance of such local-scale neighbourhood species associations should not be ignored.

Many experiments and studies have been conducted on the potential effects of global warming on biodiversity in arctic and alpine environments (Alatalo 1998; Alatalo et al., 2014b, 2015; Elmendorf et al., 2012; Jägerbrand et al., 2009). The network approach of calculating interactions in a plant community can reveal changes in the overall community structure and would also allow the robustness of plant communities to environmental change to be analysed (Proulx et al., 2005). Robustness can be measured by perturbing the current network structure, for example by removing focal species in the network or by changing the environment of the plant communities by increasing/decreasing stress levels over time. Whether a plant community

is robust to changes in the environment would be manifested in the number of plant–plant interactions in the plant community.

In this study, a factorial experimental design was used, with warming and nutrient addition as treatments, to study and compare changes in neighbourhood species interactions over time in two contrasting alpine tundra plant communities: a species-rich meadow and a species-poor heath. Specifically, with the experimental set-up, we tested the following five hypotheses: 1) The overall topological structure of networks differs over time in response to warming and nutrient addition and such changes in topological structure vary between the two contrasting plant communities. 2) Interspecific neighbourhood interactions change significantly in mesic meadow and poor heath communities due to warming and nutrient addition; 3) Changes in interspecific neighbourhood interactions at a scale of 0.1 m differ between nutrient-limited poor heath and mesic meadow; 4) Changes in neighbourhood interactions are independent of changes in diversity; 5) Interspecific interactions among dominant species respond to changes in the experimental environmental change in both mesic meadow and poor heath communities.

2. Methods

2.1. Study area

Fieldwork took place at Latnjajure field station, which is located in the Latnjavagge valley (68°21'N, 18°29'E; 1000 m above sea level) in northern Sweden. A total of 40 plots (1 m × 1 m) with homogeneous vegetation cover were chosen in July 1995 at sites representing contrasting resource availability: 20 plots for a mesic meadow community with higher nutrient availability and 20 plots for a poor heath with lower nutrient availability. We have previously reported on the vascular plant abundance and diversity in these communities (Alatalo et al., 2014a, 2015). Treatments were randomly assigned in a factorial design experiment to eight control (CTR) plots and four plots for each of three experimental treatments: warming (T), nutrient addition (N) and combined warming and nutrient addition (TN). Warming was induced by open top chambers (OTCs), which increased the air temperature by 1.5–3.0 °C during the snow-free summer period. Nutrients were added in similar amounts as used in previous studies in Alaskan Tundra (Chapin et al., 1995) and consisted of application (per m²) of 5 g nitrogen (as NH₄NO₃) and 5 g phosphorus (P₂O₅) dissolved in 10 L snowmelt water. As part of a BACI (Before-After-Control-Impact) approach (Underwood, 1996), in 1995 all plots were analysed with a point frame method (described below) to determine species occurrence under natural conditions, prior to application of experimental treatments. The OTCs were then left on plots with warming treatments year-round, while the nutrient solution was applied directly after the initial vegetation analyses in 1995 and a few days after snowmelt in subsequent years (1996–2001). The nutrient treatments were terminated after the 2001 season.

2.2. Measurements

All vascular plants in the plots were identified to species level and cover of each species was assessed, using a 1 m × 1 m frame with 100 grid points, in the middle of the 1995, 1999 and 2001 growing seasons (Walker, 1996). To ensure accuracy and reproducibility, the same grid frame was used for each measurement, and fixed points at the corner of each plot allowed the frame to be placed in the same position within the plot on each measuring occasion, allowing us to 'hit' (measure) the same points in the grid in each plot in different years. Only the first hit of each species was recorded. This method has been shown to be accurate in detecting

changes in tundra vegetation (May and Hollister, 2012). However, it should be noted that it is a crude method for comparing relative changes over time.

2.3. Methods and statistical analysis

To quantify the number of interspecific neighbouring species around a focal plant in a particular plot, we used the help of networks. Network analysis is one of the best ways to construct interactions on a spatial scale and involves analysis of collections of units which interact in a system (Proulx et al., 2005). The systems in our study were the plots and the units were the plant species. However, network analysis does not test explicitly whether the presence of other plant species in a plant's local neighbourhood at a scale of 0.1 m can affect its growth either positively or negatively. Our analysis and calculation of neighbourhood interactions were based on the assumption that a plant species at a scale of 0.1 m from another focal plant might significantly affect its fitness (Wiegand et al., 2007). We call this effect (whether neutral, positive or negative) a 'neighbourhood interaction'. With this particular assumption, due to experimental perturbation such local-scale neighbourhood interactions will increase (decrease) over time. For example, a particular plant species may have at most eight different neighbouring interactions, but under environmental perturbation this can change over time to, for example, six new neighbouring species interactions or remain at eight interactions depending on competition or facilitation (or neutral). However, there can be many more reasons for a change in neighbouring species interactions that were not considered in the present study. We calculated the average number of neighbour species per species or, in other words, the average number of interspecific neighbourhood interactions, in each treatment plot. We considered how interspecific neighbourhood interactions in the two different plant communities changed over time in response to the experimental environmental changes. We included only hetero-specifics in our interaction network, such that each interaction was

an interspecific interaction at 0.1 m. Decreases in the number of species interactions would suggest that, over time, the experimental treatments had caused some species to outcompete others and thus fewer interactions remained. Thus in principle, such an analysis can also indicate whether a plant community is robust to changes in the environment (Proulx et al., 2005). In addition to these analyses, we identified species that are most abundant in mesic meadow and in poor heath and called them 'dominant species'. We then evaluated how interactions of these dominant species were affected over time due to the treatments.

To calculate the average neighbourhood interspecific interactions for each plant species present in the 1 m x 1 m plot, we checked for interspecific neighbours around each plant species at 0.1 m above, below and around the focal species. We then created an interaction matrix for each treatment plot where the columns and rows represented the species present in the particular plot. For example, if there were four species (S1–S4) present in the plot, the interaction matrix was a 4×4 square matrix (Fig. 1). Each element in the matrix had a value of either 1 or 0, where 1 represented the presence of a neighbour species, and hence presence of an interaction, and 0 represented the absence of the neighbour species, and hence no interaction. We then calculated the number of neighbouring plant species for each focal plant in each treatment plot and from that calculated the average number of neighbouring species interactions in each treatment plot. All statistical analyses were performed in R 3.2.1 (R Core Team, 2015).

The response variable data, which were average number of neighbourhood species interactions and network dissimilarity, were normally distributed. We assessed the normality of these response variables statistically by the Shapiro-Wilks normality test and visually by quantile plots. To assess whether the overall structure of the networks differed over time due to warming (T), nutrient addition (TN) and the combined treatments (TN), compared with the control in each of the communities, we used a hypothesis-testing approach. We quantified differences in the topological structure of networks using an approach where

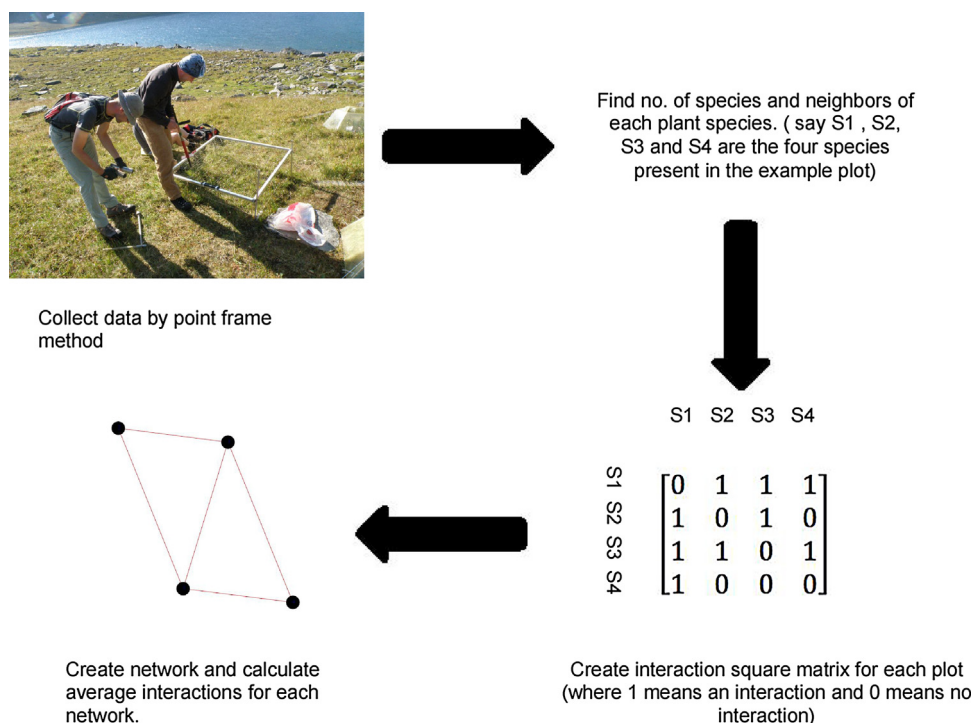


Fig 1. Graphical illustration of the methodology used to calculate plant neighbourhood species interactions in each plot from data collected by the point-frame method in the mesic meadow and poor heath communities.

topological dissimilarity of a network with another network was evaluated by a function called $D(G, G')$. This function quantified structural topological differences between two networks G and G' (Schieber et al., 2017). Network dissimilarity in this case was calculated from the reference year 1995 (or time 0) when the treatments started. For instance, *dissimilarity* (D) of a network evaluated in a treatment plot in 1999 compared with 1995 was given by the function $D_{1999} = D(G_{1995}, G_{1999})$, where G_{1995} was the network for a treatment plot in 1995 and G_{1999} was the network in the same treatment plot in 1999. Similarly, the network dissimilarity for 2001 was $D_{2001} = D(G_{1995}, G_{2001})$. With this, we checked whether trends in network dissimilarity changed differently in the treatment plots (T, N, TN) compared with control plots (CTR) over time. Next, we created a mixed-effects model with *network dissimilarity* (D) as the response variable, a categorical variable called *treatment* (CTR, N, T, TN), a continuous variable called *time* (0, 4, 6) (since we were interested in overall trends and not year-by-year estimates) and another categorical variable called *community* (mesic, poor heath) as fixed effects and individual *plots* as random effects. With this mixed-effects model, we examined whether trends in structural topological differences differed in the treatment plots compared with control plots and whether such topological differences varied differently in the two communities. The responses in terms of network dissimilarity were analysed using the restricted maximum likelihood (REML) approach from the lme4 package (Bates et al., 2012). Model details are shown with estimates of fixed effects and their p-values in Supplementary Table S4.

After looking at the overall changes in the structure of a network in response to external environmental change, we then compared whether the differences in interspecific neighbourhood interactions due to the treatments (T, N, TN) were significantly different from those in control treatments (CTR) over time within each plant community and between the two plant communities. We first converted the categorical variable *Year* (1995, 1999, 2001) to a continuous variable *time* (0, 4, 6), as we were interested in the trend in interspecific neighbourhood interactions in response to the treatments. We then created a mixed-effects model with the response variable *average neighbourhood species interactions*, a categorical *treatment* variable (with levels CTR, N, T, TN), a continuous variable called *time* and another categorical variable called *community* (with levels mesic and poor) as fixed effects and individual *plots* as random effects. With this mixed-effects model we tested the second and third hypotheses: whether there was a treatment effect on the neighbourhood species interactions and whether the response of the neighbourhood interactions differed in the two communities. The responses in terms of neighbourhood interactions were analysed using the REML approach from the lme4 package (Bates et al., 2012). Model details are shown in Table 1. Multiple comparisons were performed within the linear model framework using the lsmeans package (Lenth, 2016) whenever the interaction terms were significant. Significance of the fixed effects was assessed based on the coefficients and p-values of the model (Table 1).

To compare whether changes in neighbourhood species interactions might actually be driven by changes in diversity, we analysed evenness and richness data from both plant communities. First, we calculated normalised changes in evenness and normalised changes in neighbourhood species interactions so that they could be compared at the same scale. We then calculated changes in neighbourhood species interactions, evenness and richness data relative to 1995, which we set as time point 0 and normalised the change for 1999 (time point 4) and 2001 (time point 6). For example, if n denotes the metric of measurement (evenness, richness or neighbourhood interactions), then normalised change

Table 1

Model coefficients and model variables of the full community-wide model. The response variable was *average neighbourhood species interactions* and fixed effects were *Treatment* (control (CTR), nutrient addition (N), warming (T) and combined warming and nutrient addition (TN)) of four levels, *community* of two levels (*mesic meadow* and *poor heath*), *time* as a continuous variable *contTime* and random effect of individual *plots*.

Mesic Meadow Model Variables	Full model		
	Coefficient	Std. Error	p-value
<i>Intercept</i>	4.60	0.59	0.000 ***
<i>Treatment (N)</i>	0.41	1.03	0.69
<i>Treatment (T)</i>	-0.80	1.03	0.44
<i>Treatment (TN)</i>	0.04	1.03	0.96
<i>contTime</i>	0.03	0.81	0.0001
<i>mesic meadow</i>	3.75	0.04	0.36
<i>Treatment (N): mesic meadow</i>	-0.10	1.41	0.94
<i>Treatment (T): mesic meadow</i>	0.51	1.41	0.71
<i>Treatment (TN): mesic meadow</i>	-0.79	1.41	0.57
<i>Treatment (N): contTime</i>	-0.26	0.080	0.001 ***
<i>Treatment (T): contTime</i>	-0.12	0.080	0.14
<i>Treatment (TN): contTime</i>	-0.38	0.080	0.0000***

in n (evenness, richness or neighbourhood interactions) over a period of four years, i.e. 1995 to 1999, is given by:

$$\left(\frac{n_{1999} - n_{1995}}{n_{1995}} \right) \Delta t$$

where n_{1999} denotes data for 1999, n_{1995} denotes data for 1995 and Δt is the time period, in this case four years. Similarly, normalised change for a period of six years is simply $\left(\frac{n_{2001} - n_{1995}}{n_{1995}} \right) \Delta t$, with $\Delta t = 6$.

For the neighbourhood species interactions and richness, normalised change over periods of four and six years was calculated similarly. This normalisation allowed neighbourhood species interactions, evenness and richness to be compared directly. To analyse whether changes in neighbourhood species interactions differed from the changes in evenness and richness, we again used a hypothesis-testing approach and created another mixed model with *normalised change* as the response variable, *treatment* (CTR, N, T, TN) as the categorical variable, *time* as a continuous variable, another categorical variable *metric* of levels (evenness/richness, neighbourhood) as fixed effects and *plots* as the random effect. If the interaction term of *metric*, *treatment* and *time* or *metric* and *treatment* in the mixed model was significant, we concluded that the normalised change in neighbourhood species interactions differed from the changes in evenness or richness in the treatment plots and over time, respectively. This analysis was performed separately for the mesic meadow and poor heath, but we did not compare these communities, as the main aim was to assess whether there were differences in changes in neighbourhood interactions with changes in evenness or with changes in richness. This particular analysis was performed to determine whether changes in neighbourhood species interactions were driven by changes in diversity. Details of the model can be found in Supplementary Tables S1, S2, S3A, and S3B.

Finally, we complemented our analyses by identifying the dominant species in each of the plant communities. We then assessed how their interactions changed over time and whether their interactions changed differently in response to the treatments compared with the control. For this final analysis, we created another mixed-effects model with the response variable *dominant species interaction*, fixed effects of *time* as the continuous variable, *treatment* (CTR, N, T, TN) as the categorical variable and *plots* as the random effect. Details of all models for each *dominant species* are given in Supplementary Table S5A-E.

3. Results

3.1. Impacts on trends in network dissimilarity in the mesic meadow and poor heath

The trends in network dissimilarity were significantly negatively affected by the combined nutrient addition and warming treatment (TN) in both communities (estimate=0.0178, $p=0.01$) compared with the controls. However, warming alone (T) and nutrient addition (N) alone did not have significant impacts on network dissimilarity in either of the communities (Fig. 2, Table S4).

Moreover, network dissimilarity over time increased significantly in poor heath compared with mesic meadow (estimate=0.018, $p=0.0007$). Multiple comparisons between treatments of the two communities and within the linear mixed model framework showed that dissimilarity in networks in poor heath increased more than those in mesic meadow over time in response to warming (T) (estimate=-0.018, $p=0.004$) and combined warming and nutrient addition (TN) (estimate=-0.12, $p=0.0007$). However, network dissimilarity differed only moderately in response to nutrient addition (N) (estimate=-0.06, $p=0.054$) between the two communities and the network dissimilarity of the control plots (CTR) over time between the two communities did not differ (estimate=-0.029, $p=0.3$).

3.2. Impact on plant neighbourhood species interactions in the mesic meadow and poor heath

Nutrient addition (N) (estimate=-0.26, $p=0.0019$) and combined nutrient addition and warming (TN) (estimate=-0.38, $p<0.00001$) had significant negative effects on neighbourhood interactions in both the mesic meadow and poor heath. However, warming alone (T) did not have a significant effect on neighbourhood interactions in either of the plant communities compared with the control plots (estimate=-0.11, $p=0.14$) (Fig. 3, Table 1).

3.3. Community differences in response to experimental environmental change

Mesic meadow had, on average, a higher number of neighbourhood interactions than poor heath (intercept estimate=3.75, $p=0.36$). However, nutrient addition (N) (estimate=-0.1, $p=0.9$), warming (T) (estimate=0.51, $p=0.76$) and combined warming and nutrient addition (TN) (estimate=-0.79, $p=0.57$) did not significantly alter trends of neighbourhood interactions over time in mesic meadow when compared with poor heath (Fig. 3, Table 1).

3.4. Comparisons between neighbourhood interactions and richness

Trends in neighbourhood species interactions over time differed significantly from changes in richness for the nutrient addition (N) addition treatment (estimate=-0.29, $p=0.03$).

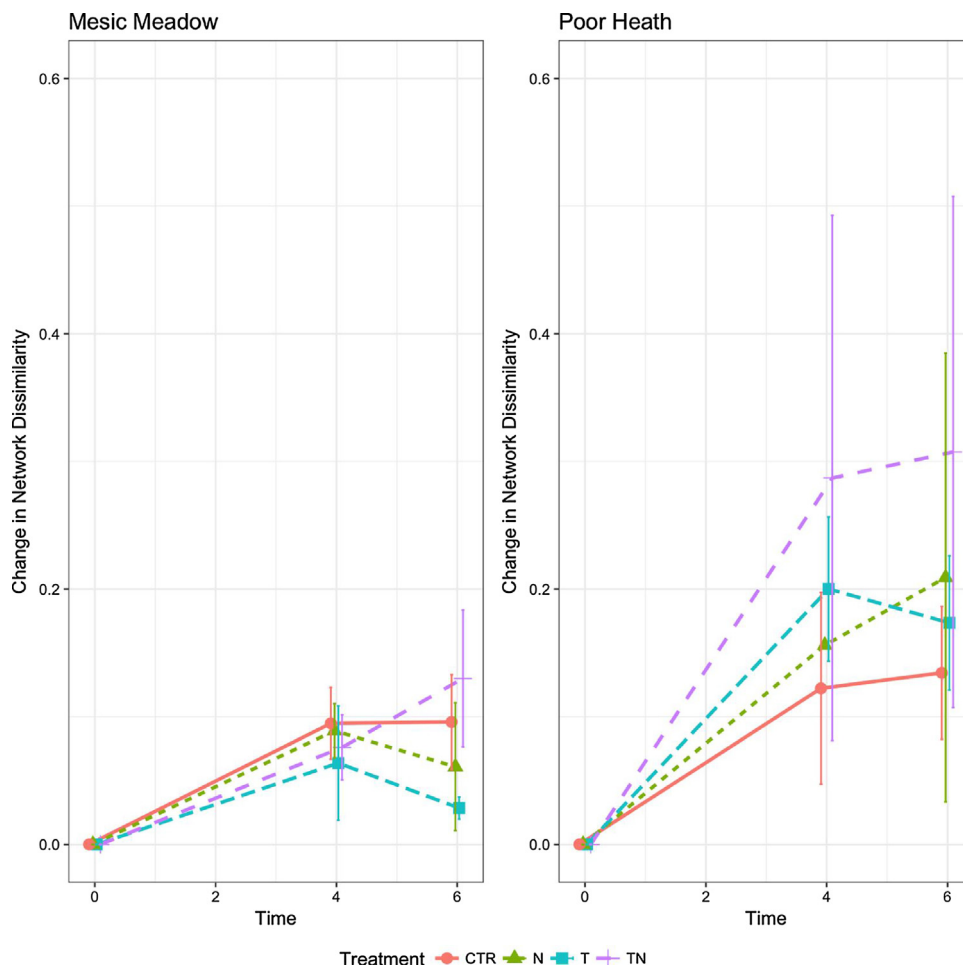


Fig. 2. Interaction plot for the control (CTR), nutrient addition (N), warming (T) and combined warming and nutrient addition (TN) treatments in the mesic meadow and poor heath plant communities. Y-axis shows network dissimilarity for all treatment plots over a period of six years relative to the reference year 1995 (i.e., time 0).

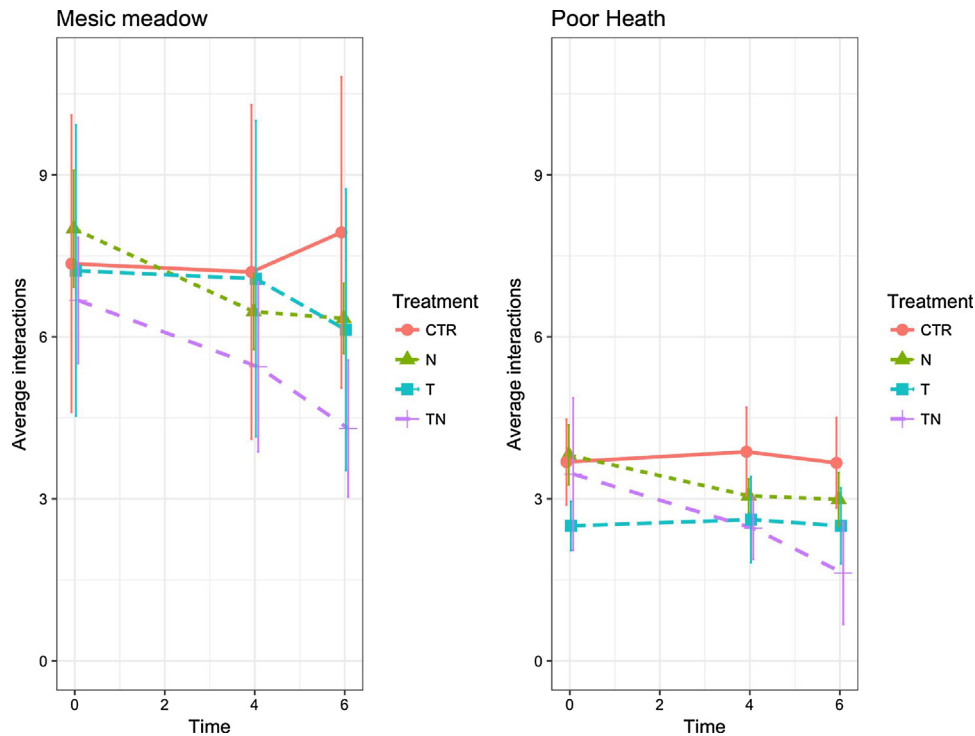


Fig. 3. Interaction plot for the control (CTR), nutrient addition (N), warming (T) and combined warming and nutrient addition (TN) treatments in the mesic meadow and poor heath plant communities. Y-axis shows average number of estimated plant species interactions for all treatment plots over a period of six years relative to the reference year 1995 (i.e., time 0).

However, for warming (T) (estimate = -0.13 , $p=0.3$) and combined nutrient addition and warming (TN) (estimate = -0.15 , $p=0.2$), neighbourhood interactions were not significantly different from changes in richness in the mesic meadow (Fig. S1, Table S3B). For the poor heath, trends in changes in richness and changes in neighbourhood interactions over time were different (Fig. S1), but these differences were not significant for any of the treatments (Table S3A).

3.5. Comparisons between neighbourhood interactions and evenness

For the mesic meadow, changes in neighbourhood interactions differed significantly negatively with warming (T) (estimate = -0.223 , $p=0.05$), and nutrient addition (N) (estimate = -0.249 , $p=0.045$) compared with changes in evenness over time. However for the combined treatment of nutrient addition and warming (TN), changes in neighbourhood interactions were not significantly different from changes in evenness (estimate = -0.21 , $p=0.08$) (Table S1, Fig. 4).

For the poor heath, changes in neighbourhood interactions in response to combined nutrient addition and warming (TN) were significantly different from changes in evenness (estimate = -0.676 , $p < 0.0001$) over time (Fig. 4, Table S2). However, changes in neighbourhood interactions in response to warming (T) and nutrient addition (N) were not significantly different from changes in evenness.

3.6. Dominant species and their interactions in response to experimental environmental change

The dominant species in the mesic meadow were *Cassiope tetragona*, *Carex bigelowii* and *Carex vaginata*. In poor heath, *Calamogrostis lapponica* and *Betula nana* were the two species that were mostly dominant.

Neighbourhood interactions of *C. tetragona* were significantly negatively affected by combined warming and nutrient addition (TN) (estimate = -0.79 , $p=0.005$) and by nutrient addition (N) (estimate = -0.54 , $p=0.01$), but not by warming (T) alone (estimate = -0.05 , $p=0.79$). Neighbourhood interactions of *C. bigelowii* over time were significantly negatively affected by nutrient addition (estimate = -1.07 , $p=0.006$) and combined nutrient addition and warming (TN) (estimate = -0.83 , $p=0.03$), but not by warming alone (T) (estimate = -0.65 , $p=0.08$).

There was no significant impact on neighbourhood interactions of *C. vaginata* (p -values > 0.2) (Fig. 5, Table S5A-C) or *C. lapponica* over time in any of the experimental treatments (Fig. 5). Neighbourhood interactions of *B. nana* over time were significantly negatively affected by warming (T) (estimate = -0.30 , $p=0.025$) and combined warming and nutrient addition (TN) (estimate = -0.43 , $p=0.002$), but not by nutrient addition (N) alone (estimate = -0.19 , $p=0.15$) (Fig. 5, Table S5D-E).

4. Discussion

In the two plant communities examined in this study, plant-plant neighbourhood interaction networks became more dissimilar over time in response to combined nutrient addition and warming compared with the control. Specifically, structural changes in the plant-plant interaction networks over time in the poor heath in response to the treatments were greater, leading to more dissimilarity in the interaction networks compared with the mesic meadow. When underlying neighbourhood species interactions of each network in the treatment and control plots were examined, it was found that decreasing abiotic stress levels led to fewer neighbourhood species interactions on average over time in both plant communities. The changes in neighbouring species interactions over time were not significantly different between the two communities, suggesting that the species-poor

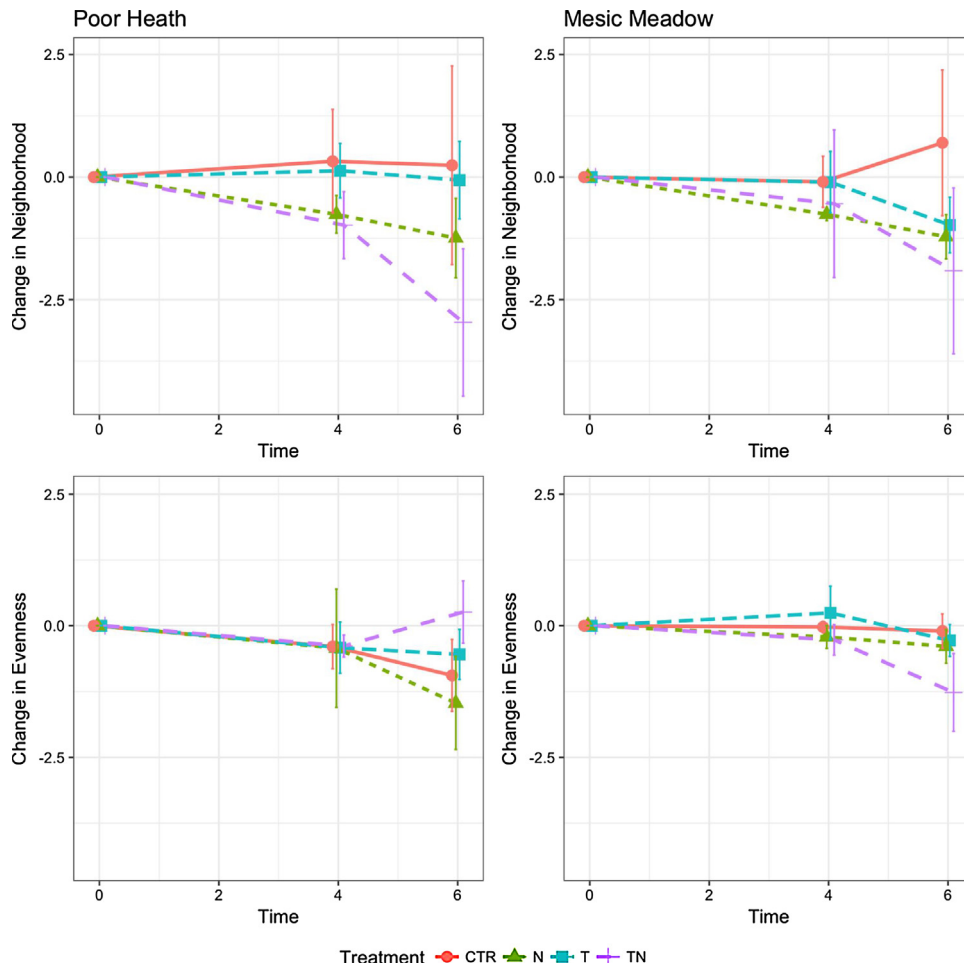


Fig. 4. Comparison between changes in evenness and changes in plant neighbourhood species interactions over time in the mesic meadow and poor heath communities, relative to the reference year 1995 (time 0).

and nutrient-poor heath community was as robust to changes brought about by experimental perturbation as the mesic meadow.

The analytical approach used in our study took into account the number of interspecific neighbour species around a single plant species in both plant communities (mesic meadow and poor heath) and in all three treatments (T, N, TN). The results showed that in the control plots, the plant species had more connections over time than those in the treatment plots. In the treatment plots, neighbouring species interactions changed negatively over time due to experimental perturbation (warming), but such changes were not very different from those in the control plots. However, in some treatment plots, particularly with nutrient addition and combined nutrient addition and warming, the changes over time differed significantly negatively from those in the control plots. Over the seven-year study period, neighbouring species interactions decreased significantly in the nutrient addition and combined nutrient addition and warming plots. Such changes in neighbourhood species interactions were most likely driven by changes in dominance structure in the functional groups (Alatalo et al., 2015; Little et al., 2015).

The poor heath community was originally very nutrient-limited and in a more stressed condition. The number of neighbourhood species interactions declined over the years in the combined nutrient addition and warming treatment to levels significantly lower than those in control plots. A probable reason is a shift in the community from a deciduous shrub-dominated system to a grass- and sedge-dominated community (Alatalo et al., 2015), as also

reported in other studies of comparable length (five and seven years) (Capioli et al., 2013; Jägerbrand et al., 2009), and in short-term studies in Tibet (Ganjurjav et al., 2016; Peng et al., 2017).

On average, the mesic meadow had a higher number of average neighbouring interactions than the poor heath (Table 1, coefficient of the intercept; Fig. 3). This was because the nutrient-rich mesic meadow had a higher number of species than the poor heath (see Alatalo et al., 2015; Little et al., 2015). However, although the mesic meadow had higher nutrient levels on average and a higher number of average neighbourhood interactions, the changes in average neighbourhood interactions over time in response to the treatments were similar, indicating that in both communities the decreases in stress levels decreased the interactions by a similar amount over time. Thus there was no community specific response to experimental environmental change in terms of neighbourhood species interactions in either the poor heath or mesic meadow.

A decrease in the number of neighbourhood species interactions could signal a decrease in productivity (Zhang et al., 2014; McKenna and Yurkonis, 2016), although follow-up studies are needed to confirm this. Local-scale neighbourhood species interactions are important, as some specific species can be reducers or accumulators of diversity (Wiegand et al., 2007). Moreover, non-random aggregations of plant species have been shown to decrease biomass production (Lamošová et al., 2010; Zhang et al., 2014) and hence fine-scale local species interactions might drive community change and decrease productivity. However, a recent study did not find strong evidence of local-

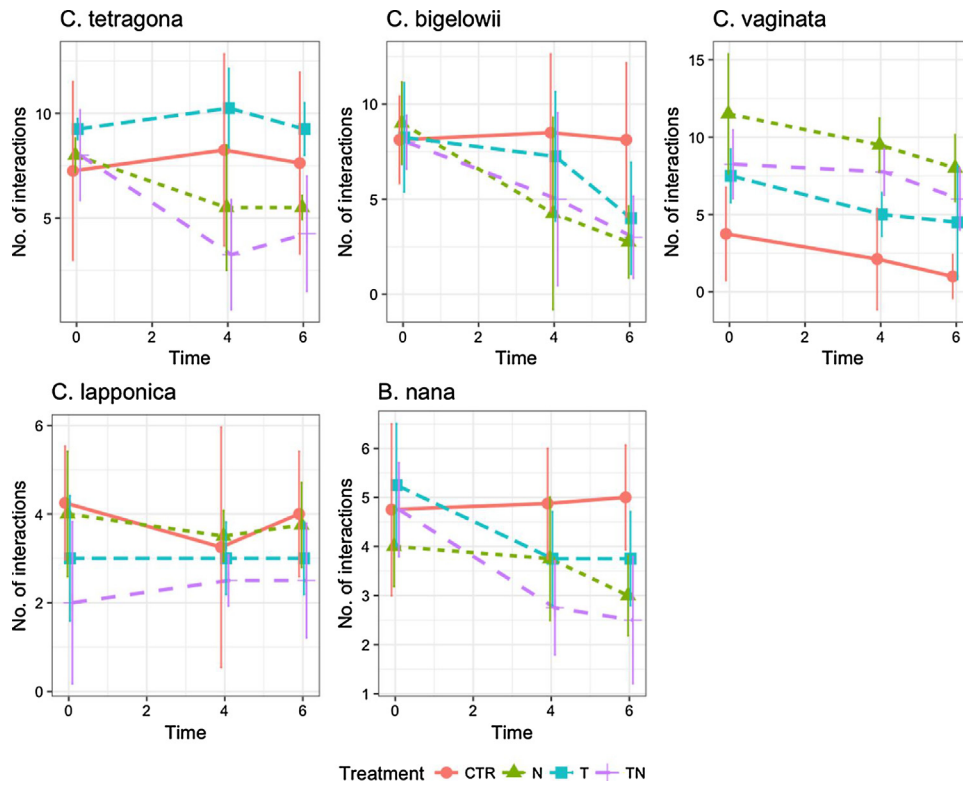


Fig. 5. Interaction plot for all dominant species in both the mesic and poor heath plant community. *Cassiope tetragona*, *Carex bigelowii* and *Carex vaginata* were the dominant species present in the mesic meadow and *Calamogristis lapponica* and *Betula nana* were the dominant species in the poor heath. Y-axis denotes interspecific interactions of each dominant species at 0.1 m scale and changes over a period of six years relative to the reference year 1995 in response to the environmental treatments (control (CTR), nutrient addition (N), warming (T) and combined warming and nutrient addition (TN)).

scale neighbourhood species interactions altering community-scale measures like diversity and productivity, although it suggested that such species interactions might still affect these measures when taking into account different non-random aggregations (McKenna and Yurkonis, 2016). Moreover, such non-random local-scale species interactions have been shown to alter resource use and hence significantly affect other ecosystem functions like microbial diversity (Massaccesi et al., 2015), insect interactions (Parachnowitsch et al., 2014) and root biomass production (Orwin et al., 2014).

In the present study, there was moderate evidence that changes in neighbourhood species interactions are independent of changes in the diversity index of evenness and richness. Since we explicitly considered interspecific interactions, changes in neighbourhood interactions theoretically should not be significantly different to changes in richness, because index of richness and our interspecific neighbour interactions metric consider changes at the species level. Thus in the poor heath we found that changes in richness were comparable to changes in neighbourhood interactions even at 0.1 m scale in all treatments. This means that richness in nutrient-limited and species-poor plant communities might explain changes in species interactions at 0.1 m scale. However, in the more nutrient-rich and species-rich mesic meadow, we found that in the nutrient addition plots (and very weakly in the combined warming and nutrient addition plots) neighbourhood changes at 0.1 m scale changed independently of changes in richness in response to the treatments.

Changes in neighbourhood interactions were significantly different from changes in evenness in the nutrient addition and warming plots in the poor heath, suggesting that the pattern of changes in interactions is independent of evenness. The corresponding pattern of changes in neighbourhood interactions over

time in the mesic meadow was found to be significantly different (negative) from changes in evenness in the nutrient addition plots and warming plots (and weakly for combined TN plots). This suggested that diversity (both richness and evenness) in species-rich plots might not be the driver of small-scale interactions when environmental stress declined over time. This pattern of changes in such local-scale neighbourhood interactions was probably because external environmental change perturbed the whole community and restructured it in a way that changed interactions (example, causing non-random aggregations), while maintaining diversity. This could happen due to fine-scale local species interactions in treatment plots, which community measures like evenness or richness did not take into account.

The decline in neighbourhood species interactions in this study could be linked to increases in certain types of functional groups like grasses (and decreases in sedges, see example, Alatalo et al., 2014a, 2015), which might imply non-random aggregations of a certain type of functional group at the local scale. Alatalo et al. (2014a) reported significant increases in dominance of grass cover (and decreases in sedges) due to nutrient addition over time in mesic meadow. Thus increases in both the abundance and richness of a particular functional type (grasses in this case) led to decreases in the richness and abundance of other functional types (sedges and deciduous shrubs in this case), keeping the overall richness and evenness more or less the same (Alatalo et al., 2014a; Little et al., 2015). However, neighbouring species interactions at a scale of 0.1 m decreased significantly over time for the nutrient addition treatments in the mesic meadow, probably due to non-random aggregations of a particular species of a particular functional type. Within the same functional type (grasses for example), aggregations of a grass species might have occurred at 0.1 m scale due to decreases in stress levels and increased competition. This might

have led to the species outcompeting another species of a different functional type at a scale of 0.1 m in response to declining stress levels. Hence aggregation of that particular species might have occurred at 0.1 m scale that drove species from other functional types to the edges of the plot. This would lead to richness or evenness to be more or less constant but would directly lead to lower interspecific neighbourhood interactions. Such local-scale aggregations might ultimately affect community productivity, although further studies are needed to link changes in neighbourhood species interactions directly to changes in community productivity under the umbrella of external environmental change.

Our results suggest that decreasing levels of environmental stress might affect plant–plant species interactions at local scale. The changes we observed in neighbourhood species interactions might have been due to interspecific competition. Following changes in stress levels, decreases in neighbourhood species interactions could arise because of competition, as the best competitors that can adapt to the changes come to dominate the community (Alatalo et al., 2015; Little et al., 2015). We did not measure nutrient content in this study, but measurements in a previous study showed that the poor heath is a more nutrient-limited ecosystem, with lower soil moisture content, a shallower organic soil horizon and three-fold lower nitrogen mineralisation rate than the meadow ecosystem (Björk et al., 2007).

In alpine meadows and arctic plant communities, diversity frequently declines with nutrient addition (Theodose and Bowman 1997; Wardle et al., 2012). Combined nutrient addition and warming had also been reported in other studies to have the largest significant negative effects on plant neighbourhood species interactions (Press et al., 1998; Klanderud and Totland 2005). Decreases in neighbourhood species interactions could possibly also lead to decreases in diversity over time, which may lead to ecosystem functioning being hampered over time (Hooper et al., 2005; Balvanera et al., 2006; Lamošová et al., 2010; Zhang et al., 2014; McKenna and Yurkonis 2016). The differences in response to the treatments in the two communities were not significantly different from those reported previously, but most previous studies have been performed on alpine heath (Alatalo et al., 2015; Press et al., 1998; Klanderud and Totland 2005).

Finally, in our study the dominant species in the alpine meadow were *Cassiope tetragona*, *Carex bigelowii* and *Carex vaginata*. *Carex bigelowii* and *C. vaginata* are both sedges. In fact, *C. bigelowii*'s neighbourhood interactions over time were significantly affected by nutrient addition, indicating that aggregations of *C. bigelowii* might have occurred at 0.1 m scale and hence decreases in interspecific interactions might have resulted from a decline in the stress levels. *Carex vaginata*'s interactions also decreased over time in response to nutrient addition, but this decrease was not strongly significant. In the poor heath, *Calamagrostis lapponica* and *Betula nana* were the most dominant species. *C. lapponica* is a grass species, while *B. nana* is a deciduous shrub. The neighbourhood interactions of both species decreased in response to the treatments, but the decrease in interactions was more significant for *B. nana* in the warming and nutrient addition treatments.

Overall, our results and analysis suggest that over time, environmental change will have effects on neighbourhood species interactions in sub-arctic and alpine environments, with combined effects of warming and nutrient addition most likely leading to increases in competition. Initial abiotic stress levels will most likely influence the impact on plant interactions. Furthermore, climate change will most likely increase the variability and frequency of extreme events (IPCC 2007; Abeli et al., 2014), and thus a constant level of warming is not a very realistic scenario for the future. However, at present there are no data from long-term experiments applying different warming scenarios and only a few

results from multi-year experiments in alpine and arctic areas (Alatalo et al., 2014a, 2016; Jonasson et al., 1999; Orsenigo et al., 2014).

5. Conclusions

Future global warming and increased nutrient deposition are predicted to cause major changes in plant species interactions and community structure. This study confirmed that, over seven years of experimental warming and, in particular, nutrient addition, there were definite changes in local-scale species interactions in the two communities studied. Moreover, simply assessing diversity and community-scale measures may not be enough, as local-scale species interactions changed differently in response to environmental change in the two contrasting plant communities in this study. However, the changes in local-scale interactions in both communities were fairly similar, indicating that both were robust to environmental perturbation. Decreases were observed in plant neighbourhood species interactions, which might indicate increased competition in the sub-arctic and alpine plant communities studied. This might have been driven by increases in plant species of a particular functional type relative to increases in another species of a different functional type that expanded when environmental conditions shifted to a more favourable state for that species.

Authors' contributions

JMA and UM designed the experiment. JMA, UM and AKJ carried out the fieldwork. GKB carried out data analyses. GKB and JMA drafted the manuscript. All authors read, commented upon and approved the final manuscript.

Competing financial interests

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ecocom.2017.11.003>.

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