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Disruption of trophic interactions involving the heather beetle by atmospheric nitrogen deposition☆

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ABSTRACT

Elevated nitrogen (N) deposition impacts the structure and functioning of heathland ecosystems across Europe. *Calluna* plants under high N-inputs are very sensitive to secondary stress factors, including defoliation attacks by the heather beetle. These attacks result in serious damage or death of *Calluna*, its rapid replacement by grasses, and the subsequent loss of heathland. We know very little about the mechanisms that control the populations and trigger outbreaks of the heather beetle, impeding proper management measures to mitigate the damage. We investigated the effects of N deposition on the relationships between the heather beetle, its host plant, and two arthropod predators at building (rejuvenated through fire) and mature heathlands. The study combines field manipulation experiments simulating a range of N deposition rates (0, 1, 2, 5 g N m^{-2} $year^{-1}$ for 2 years, and 5.6 g N m^{-2} $year^{-1}$ for 10 years), and food-choice laboratory experiments testing the preferences of adults and larvae of the heather beetle for N-treated *Calluna* plants, and the preferences of predators for larvae grown on plants with different N-content. The larvae of the heather beetle achieved the highest abundances after the long-term (10-year) addition of N at mature *Calluna* plots in the field. Contrary to the adults, the larvae foraged preferentially on the most N-rich *Calluna* shoots under laboratory conditions. Predators showed no aggregative numerical responses to the accumulation of heather beetle larvae at high N-input experimental plots. During the feeding trials, predators consumed a small number of larvae, both in total and per individual, and systematically avoided eating the larvae reared on high-N *Calluna* shoots. Our study showed that the most severe defoliation damage by the heather beetle is inflicted at the larval stage under prolonged availability of high-N inputs, and that arthropod predators might not act as effective regulators of the beetle's populations.

1. Introduction

Global rates of atmospheric nitrogen (N) deposition from anthropogenic emissions of total N (NO_y and NH_x, primarily associated with fossil fuel combustion and food production) are projected to double by 2050 (Galloway et al., 2004), exceeding the critical load thresholds that have detrimental effects on human health and the environment (Dentener et al., 2006; Galloway et al., 2008; Sala et al., 2010). Elevated N inputs impact both ecosystem composition and functioning, by (1) altering multiple fundamental processes such as decomposition, microbial activity, plant growth and productivity, and species interactions, and by (2) increasing plant susceptibility to secondary stress and disturbance factors (i.e., frost, drought, pathogens, and pests) (Bobbink et al., 2010; Krupa, 2003; Ochoa-Hueso et al., 2011; Throop and Lerdau, 2004). Especially vulnerable to excess N accumulation are N-limited ecosystems adapted to low levels of N availability (Sala et al., 2010), like semi-natural heathlands dominated by heather, *Calluna vulgaris* (L.) Hull (hereafter *Calluna*) (Fagúndez, 2013; Phoenix et al., 2012; Stevens et al., 2016). Both N-manipulation experiments (e.g., Calvo et al., 2005, 2007; Härdtle et al., 2009; Power et al., 2004) and field-scale surveys (e.g., Jones and Power, 2012; Southon et al., 2013) evidenced N-driven changes in the composition, diversity and functioning of heathlands, highlighting atmospheric N deposition as a major driver of heathland decline across Europe.

Many studies have revealed increases in the annual shoot growth, leaf nutrient content, and flowering of *Calluna* plants in

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response to the experimental addition of N (e.g., Friedrich et al., 2011; Power et al., 1998a; Uren et al., 1997; von Oheimb et al., 2010). And yet, few studies have explicitly linked the enriched *Calluna* nutritional quality induced by N to its greater sensitivity to biotic stress, particularly to insect attack (Hartley et al., 2003; Kerslake et al., 1998; Power et al., 1998b). Similarly, growing number of researches investigate the response of heathland vegetation to N deposition in combination with multiple drivers of global change (e.g., land-use change: Härdtle et al., 2009; climate change: Meyer-Grünefeldt et al., 2015), overlooking possible interactions with insect herbivory (see Gladbach, 2010; Peñuelas et al., 2004). This is despite defoliation attacks by the main insect herbivore of *Calluna*, the heather beetle, *Lochmaea suturalis* (Thomson, 1866) (Coleoptera: Chrysomelidae), being expected to intensify in the next decades in response to higher N loading and warming (Peñuelas et al., 2004; Phoenix et al., 2012; but see Scherber et al., 2013). Enhanced availability of N very likely improves performance of the heather beetle (e.g., accelerates larval growth, increases body size and survival rate, and augments population numbers) (Berdowski, 1993; Brunsting and Heil, 1985; Power et al., 1998b), and might strengthen the effects of defoliation on the vegetation.

Populations of the heather beetle periodically reach outbreak densities as high as 2000 individuals per square meter (Berdowski, 1993) that result in severe damage or death of *Calluna* plants, their rapid replacement by grasses, and the subsequent loss of heathland (Berdowski and Zeilinga, 1987; Bobbink and Heil, 1993; Brunsting and Heil, 1985; but see Scandrett and Gimingham, 1991). Based on past defoliation episodes, numerous variables have been suggested to influence the magnitude (i.e., incidence, extent, and intensity) of a heather beetle outbreak (e.g., nitrogen and sulphur deposition: Berdowski, 1993; temperature: Staley, 2000; precipitation: Berdowski, 1993; von Melber and Heimbach, 1984), but proper experimental work is needed to support these claims. Also, the extent to what *Calluna* plants of different ages (i.e., growth phases; Gimingham, 1972) are more or less susceptible and resilient to heather beetle attacks remains untested. So far, our knowledge on the ecology of the heather beetle and the factors that trigger and end an outbreak is scarce (Pinder et al., 2015a), and, as a consequence, current management advice to mitigate the damage is conflicting and insufficient (Pinder et al., 2015b; Rosenburgh and Marrs, 2010). Moreover, the latest systematic review by Pinder et al. (2015a) established that we know very little about the relationships between the heather beetle and its natural enemies (predators, parasites, and pathogens), and whether these might act as effective population regulators (Scandrett and Gimingham, 1991). Up to now, no specialist predators of the heather beetle have been documented in the field, and only a few generalist arthropod predators (e.g., ladybird beetles, ground beetles, and true bugs) have been mentioned in the literature as potential control agents of the beetle's populations (Cameron et al., 1944).

This study offers new insights into the relationships between the heather beetle, its host plant and two generalist arthropod predators (ground beetles and harvestmen) that are highly abundant in *Calluna* heathlands (Cuesta, 2015), mediated by N deposition and the age of heathland vegetation (see Jones and Power, 2015). Specifically, we aim to investigate: (i) whether the heather beetle displays behavioural adaptations to locate and forage preferentially on the most N-rich plant tissues, as a response to low natural availability of N (Chen et al., 2010; Fernandez and Hilker, 2007; Ikonen, 2002); (ii) whether predators can feed preferentially on preys of high nutritional quality raised on N-enriched plants to regulate their intake of nutrients and compensate the limited availability of N in the ecosystem (Mayntz and Toft, 2001; Mayntz et al., 2005); (iii) whether predators show aggregative numerical responses to high prey accumulation triggered by plant N-enrichment, maximizing their efficiency as natural enemies (Monsrud and Toft, 1999); and (iv) whether the abundances of the heather beetle and its predators are likewise impacted by different rates of N loading (Haddad et al., 2000) and by N-driven changes in heathland vegetation at different growth phases of *Calluna* development.

2. Material and methods

2.1. Study area and experimental design

The study was performed in three *Calluna* heathland sites located in the Cantabrian Mountains (NW Spain; 43°02-03′N, 5°21-26′W; 1560–1660 m a.s.l.; 18–35 ha), and subjected to minimal regular free-range grazing by cattle and horses (1–2 LU ha^{-1} $year^{-1}$) in June–September. Estimations of current total atmospheric N deposition in the study area (7.5–15 kg N ha^{-1} $year^{-1}$; García-Gómez et al., 2014) are close to exceed the critical load that threatens the persistence of European dry *Calluna* heathlands (i.e., 10–20 kg N ha^{-1} $year^{-1}$; Bobbink et al., 2010). In each site, we selected two differently-aged heathland areas: (1) rejuvenated through prescribed fire in 2005, and (2) mature showing first signs of degeneration after 30–40 years of land use abandonment (i.e., building- and mature-phase; Gimingham, 1972). We randomly established a total of 90 2 × 2 m plots and performed a manipulative experiment to modify plant N availability, consisting of five different N-addition treatments (i.e., 3 plots per treatment, age class, and site): 0 g m^{-2} $year^{-1}$ (control, hereafter ‘N0’), 1 g m^{-2} $year^{-1}$ (‘N1’), 2 g m^{-2} $year^{-1}$ (‘N2’), and 5 g m^{-2} $year^{-1}$ (‘N5’) of granules of ammonium nitrate monthly added in June–November from 2013 to 2014; and 5.6 g m^{-2} $year^{-1}$ (‘N5.6’) monthly added in May–October from 2005 to 2014 (see Cuesta et al., 2008), equivalent to the predicted N input by 2050 (Galloway et al., 2004).

2.2. Food-choice laboratory experiments

To test whether the heather beetle might preferentially forage on *Calluna* plants grown under certain N-input conditions, we conducted two series of 96 h food-choice (“cafeteria”) experiments (July–August 2014): (1) newly molted third-instar larvae and (2) adults were allowed to choose between pairs of current-year apical shoots obtained from the field experimental plots: 10 pair combinations (N0 vs. N1, N2, N5, N5.6; N1 vs. N2, N5, N5.6; N2 vs. N5, N5.6; and N5 vs. N5.6) x 3 replicates x 3 larvae (i.e., 90 larvae in total), and 4 pair combinations (N0 vs. N1, N2, N5, N5.6) x 3 replicates x 3 adults (i.e., 36 adults in total). First- and second-instar larvae were collected outside the experimental plots in the three study sites by vegetation beating while holding a 23 × 35 cm tray underneath, reared in pint-sized BugDorm plastic containers (720 ml; BugDorm Store, Megaview Inc., Taiwan) at 20–25 °C and 15:9 h L:D regime in the laboratory, and fed on control *Calluna* shoots until they reached early third-instar larval or adult stages for the cafeteria experiments. As the number of third-instar larvae grown under laboratory conditions tripled the adults, higher number of feeding pair combinations were feasible for the larvae. The experiments were set up in BugDorm containers with moist filter paper to prevent desiccation. All the apical shoots used in the experiments were as similar as possible in weight (0.52 ± 0.20 g) and morphological characteristics (e.g., number of twigs and amount of leaves). For each apical shoot we assessed defoliation by calculating shoot weight loss as the difference between the initial and final shoot weight during the cafeteria experiments, after correcting for natural weight loss (36.2%) due to shoot dehydration

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