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ScienceDirect
Environmental Pollution 152 (2008) 394–402
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Short- and medium-term effects of experimental nitrogen fertilization on arthropods associated with *Calluna vulgaris* heathlands in north-west Spain

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Received 30 October 2006; received in revised form 7 June 2007; accepted 10 June 2007

We observed consistent species-level and variable trophic-group responses to nitrogen addition in one of the southern-most locations for Calluna vulgaris heathlands within Europe.

Abstract

We studied the short- and medium-term effects of experimental nitrogen fertilization (3 and 15 months after the treatment) on the arthropods of *Calluna vulgaris* heathlands in NW Spain. Three heathland sites were selected with two permanent plots per site: control and fertilized. Ammonium nitrate fertilizer (56 kg N ha^{-1} yr^{-1}) was applied monthly and insects were caught using pitfall traps. We found mainly species-level responses to nitrogen addition. Seven species (e.g. *Lochmaea suturalis*) showed a consistent trend (benefited or harmed) in both periods and were proposed as possible reliable indicators of the effects of nitrogen deposition in these ecosystems. We also found variable arthropod trophic-group responses: (a) herbivores (leaf beetles, true bugs) increased in abundance on a short-term scale; (b) predators (carabid beetles, true bugs) showed opposite and less clear responses in both periods. Further long-term studies are needed to determine the mechanisms underlying the observed arthropod responses.

Keywords: Calluna vulgaris; Heathland; Carabidae; Chrysomelidae; Heteroptera; Nitrogen

1. Introduction

In the last few decades European heathlands have undergone a process of regression and degeneration, mainly associated with the loss of traditional uses that prevented heathland succession to other plant communities (grazing, burning and cutting) (Bartolomé et al., 2005; Webb, 1998), and with an increase in atmospheric nitrogen (N) deposition as a result of pollution (NO_x and NH_x) (Aerts and Heil, 1993).

The consequences of increased N deposition on heathland vegetation have been widely discussed in the literature. N deposition produces a range of effects, including an accumulation of nutrients in the soil (Bobbink and Heil, 1993; Power et al., 1998a), a decrease in mycorrhiza and an increase in the heath aerial/subterranean ratio for heaths (Krupa, 2003). In addition, high N content in plant tissues increases their sensitivity to frost (Bobbink and Heil, 1993; Krupa, 2003) and to phytophage attacks (Adams, 2003; Berdowski, 1993; Krupa, 2003). As a result, the ability of heaths to compete with herbs decreases, favouring plant community substitution in several areas (Adams, 2003) and the disappearance of these heathlands. However, vegetation responses to increased N are only the first step in the chain of functional changes that take place in the ecosystem. Functional changes at the primary producer level affect all other ecological relationships within the ecosystem (Hartley and Jones, 1997).

The effects of increased N deposition on insect communities are poorly known and depend on the trophic level and

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doi:10.1016/j.envpol.2007.06.073

the life form of each group. An increased N content in the vegetation is associated with an increase in the abundance of herbivores (e.g. Berdowski, 1993; Whittaker, 1988), since N constitutes a limiting factor for this trophic group (Hartley and Jones, 1997). Changes in N deposition are also known to have an indirect influence on the abundance of predators (see Bilde and Toft, 1999; Hartley et al., 2003; Sjursen et al., 2005). The aim of this study is to analyse the short- (3 months) and medium-term (15 months) effects of experimental N fertilization on the arthropod fauna of *Calluna vulgaris* heathlands by studying three taxonomic groups of insects (carabid beetles, leaf beetles and true bugs) with different life forms (i.e. linked to different parts of the plant or soil) and food requirements (i.e. herbivores and predators).

Carabid beetles (Coleoptera, Carabidae) are ground-dwelling insects that include mainly predators, but also herbivorous and granivorous species (Campos and Novoa, 2006; Lövei and Sunderland, 1996). Previous studies of this group in heathlands have focused mainly on their responses to different traditional uses and to vegetation development (different phases of *C. vulgaris* growth) (Gardner et al., 1997; Gimingham, 1985; McFerran et al., 1995; Usher and Thompson, 1993).

Leaf beetles (Coleoptera, Chrysomelidae) are mainly located on aerial vegetation and are generally leaf-chewing herbivores. In heathlands dominated by *C. vulgaris*, outbreaks of heather beetle (*Lochmaea suturalis*), a species which feeds monophagously on *C. vulgaris*, are benefited both in frequency and intensity by N deposition (Bobbink and Heil, 1993) and can cause heathland to be replaced by grasslands (Berdowski and Zeilinga, 1987; Diemont and Heil, 1984).

True bugs (Hemiptera, Heteroptera) occur in either soil or vegetation niches and are either herbivores (i.e. sucking and granivorous species) or predators (Schuh and Slater, 1995). Hemiptera assemblages respond positively to changes in plant species composition and particularly to *C. vulgaris* cover in restored moorlands (Littlewood et al., 2006). N addition significantly increases the richness and abundance of this group (Hartley et al., 2003).

The response of these three insect groups to increased N deposition is unknown in the Cantabrian mountain range, although herbivore abundance is expected to increase (see Brunsting and Heil, 1985; Hartley et al., 2003; Whittaker, 1988). Our final goal is to determine the possible value of these groups of insects as indicators of the effects of N deposition on the *C. vulgaris* heathlands of NW Spain.

2. Materials and methods

2.1. Study area

The study was carried out in the Cantabrian mountain range (León, NW Spain). Three heathland sites dominated by *C. vulgaris* were selected, situated at 1560–1660 m.a.s.l. and at least 2.5 km apart: San Isidro (site SI, 30TUN 3082 47694), Riopinos I (site RPI, 30TUN 3035 47687) and Riopinos II (site RPII, 30TUN 3007 47685). The sites were considered mature heathlands as defined by Watt (1955). Two permanent 20 × 20 m plots were established at each site. One served as the control and the other was fertilized with ordinary granules of ammonium nitrate, spread manually every month from July to October 2005 and from June to October 2006. The total concentration of fertilizer ($56\ \mathrm{kg\ N\ ha^{-1}\ yr^{-1}}$) was equivalent to twice the current maximum deposition levels in this area (Rivero Fernández et al., 1996), and application was equally divided over these months.

2.2. Sampling methods

Arthropods were collected using pitfall traps (88 mm depth, 65 mm diameter) partly filled with 25% propylene glycol and covered by 11 × 11 cm roofs. Six traps were placed in each plot (12 traps per site and 36 in total) and the minimum distance between traps was 5 m. Traps were sampled every 20 days from July to October in 2005 and from June to October in 2006. Pitfall trap catches reflect the activity-density of individual species (Thomas et al., 1998) and will be referred to here as abundance or number of individuals. Carabid beetles, leaf beetles and true bugs (Coleoptera: Carabidae, Chrysomelidae; Hemiptera: Heteroptera, respectively) were identified using standard keys for each group: Jeannel (1941–1942), Lindroth (1974) and Trautner and Geigenmüller (1987) for carabid beetles; Doguet (1994) and Warchalowski (2003) for leaf beetles; and Péricart (1972, 1983, 1987, 1999), Stichel (1955–1962) and Wagner and Weber (1964) for true bugs. We followed the nomenclature in Serrano (2003) for carabid beetles, Warchalowski (2003) for leaf beetles and Aukema (2005) for true bugs.

Vegetation sampling was carried out 3 and 15 months after fertilization, in both control and fertilized plots. Ten 1 × 1 m permanent sampling units were established in each plot and the percentage cover of each vascular plant species was visually estimated. These percentages were used to determine the percentage cover of perennial graminoids, perennial forbs and annual forbs, to detect possible changes in habitat structure related to the arthropod fauna (e.g. Brose, 2003; Lassau et al., 2005) due to N addition. The number of flowers and the growth of the shoots of *C. vulgaris* were measured on five randomly selected shoots in each sampling unit.

2.3. Data analyses

Insect abundance data per trap were standardized to 85 trapping days (i.e. minimum number of days traps were active). Two-way ANOVA tests were carried out to assess the short- and medium-term effects of fertilization on (1) the total abundance of carabid beetles, leaf beetles and true bugs, (2) richness of carabid beetles and true bugs and (3) the abundance of each individual species. Before their inclusion in the analyses, insect abundance data per trap were log-transformed [$\ln(x+1)$] to achieve a normal distribution. Due to the spatial configuration of the heathland (i.e. small size and patchy distributed fragments) we considered the individual traps as pseudoreplicates for the analyses. Insects were classified according to their feeding habits in the literature: Campos and Novoa (2006), Ribera et al. (1999) and Thiele (1977) for carabid beetles; Doguet (1994) and Warchalowski (2003) for leaf beetles; and Péricart (1972, 1983, 1987, 1999), Schuh and Slater (1995) and Wagner and Weber (1964) for true bugs. The two main trophic groups obtained were herbivores or mostly plant-eating species (granivorous, leaf-eating and sucking species) and predators or mostly animal material eating species (Tables 1–3).

Similar ANOVA tests were carried out on the vegetation data to assess differences between treatments in terms of (1) the percentage cover of *Erica tetralix*, *C. vulgaris* and *Vaccinium myrtillus*, (2) cover values for perennial graminoids, perennial forbs and annual forbs, (3) the number of flowers and (4) the growth of the shoots of *C. vulgaris*. Vegetation data were arcsine-square root transformed to achieve normality. For all the analyses (i.e. insects and vegetation) the Tukey test was used to detect significant differences ($\alpha = 0.05$). Statistical analyses were carried out using the STATISTICA program, 1998 Edition.

Two canonical correspondence analyses (CCA) were used to detect the main relationships between carabid beetles or true bugs, vegetation characteristics and treatments. Vegetation characteristics included in the CCAs were the same as those used in the ANOVA tests (Table 4). Insect abundance data per plot were standardized to 85 trapping days. The Monte Carlo permutation test was applied to estimate the significance of the CCAs. The analyses were carried out using the R package (R Development Core Team, 2005).