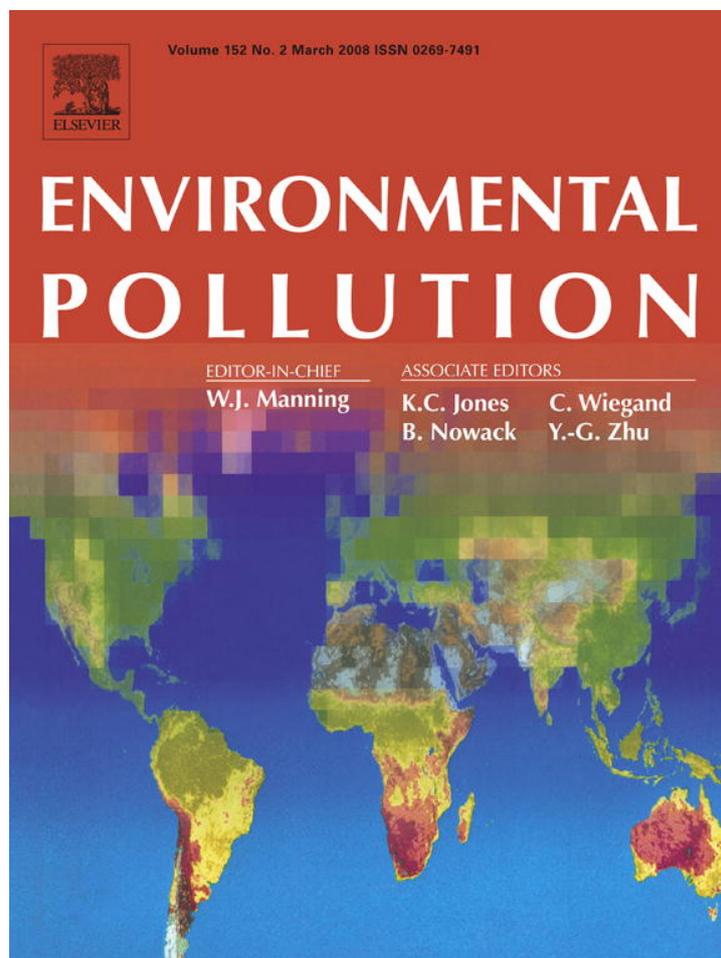


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Short- and medium-term effects of experimental nitrogen fertilization on arthropods associated with *Calluna vulgaris* heathlands in north-west Spain

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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.

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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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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; Sjørusen 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 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).

Table 1
Mean \pm standard deviation number of carabid beetle individuals collected in each treatment 3 and 15 months after fertilization

Family Carabidae	C3	C15	F3	F15	Feeding habit	Abundance behaviour	
						3 months	15 months
<i>Bembidion (Philochtus) guttula</i> (Fabricius, 1792)		0.3 \pm 0.6		0.3 \pm 0.6	P		nc
<i>Bembidion (Metallina) lampros</i> (Herbst, 1784)	0.7 \pm 1.2	0.7 \pm 0.6	0.3 \pm 0.6	0.3 \pm 0.6	P	d	d
<i>Bradycellus (Bradycellus) verbasci</i> (Duftschmid, 1812)	3.0 \pm 1.7	3.0 \pm 2.0	5.0 \pm 2.0	3.0 \pm 1.7	H	i	nc
<i>Calathus (Neocalathus) asturiensis</i> Vuillefroy, 1866	18.3 \pm 25.8	33.0 \pm 43.3	27.0 \pm 43.3	46.0 \pm 53.8	H	i	i
<i>Calathus (Calathus) fuscipes graecus</i> Dejean, 1831				0.3 \pm 0.6	P		i
<i>Calathus (Neocalathus) melanocephalus melanocephalus</i> (Linnaeus, 1758)		1.7 \pm 2.9		1.3 \pm 2.3	P		d
<i>Calathus (Calathus) uniseriatus</i> Vuillefroy, 1866	2.7 \pm 4.6	15.0 \pm 26.0c	8.3 \pm 14.4	32.7 \pm 54.0e**	H	i	i
<i>Carabus (Oreocarabus) amplipennis getschmanni</i> Lapouge, 1924	0.7 \pm 0.6a	4.7 \pm 3.1	4.0 \pm 4.4b*	6.0 \pm 9.5	P	i	i
<i>Carabus (Eucarabus) deyrollei</i> Gory, 1839	0.3 \pm 0.6				P		d
<i>Carabus (Chrysocarabus) lineatus lineatus</i> Dejean, 1826	a		2.0 \pm 3.5b*		P		i
<i>Carabus (Mesocarabus) macrocephalus macrocephalus</i> Dejean, 1826	0.7 \pm 1.2a	3.3 \pm 5.8	3.3 \pm 5.8b*	6.3 \pm 11.0	P	i	i
<i>Cicindela (Cicindela) sylvatica reiseri</i> Mandl, 1970				0.3 \pm 0.6	P		i
<i>Cryobius cantabricus cantabricus</i> (Schaufuss, 1862)	18.0 \pm 8.2	39.3 \pm 37.2c	22.3 \pm 19.4	27.3 \pm 28.6e**	P	i	d
<i>Cymindis (Cymindis) coadunata kricheldorffi</i> Puel, 1935	0.3 \pm 0.6				H	d	
<i>Cymindis (Cymindis) humeralis</i> (Geoffroy, 1785)	0.3 \pm 0.6	1.3 \pm 2.3			H	d	d
<i>Harpalus (Harpalus) rufipalpis rufipalpis</i> Sturm, 1818		1.0 \pm 1.0		0.3 \pm 0.6	H		d
<i>Laemostenus (Pristonychus) terricola terricola</i> (Herbst, 1783)				0.7 \pm 1.2	P		i
<i>Leistus (Leistus) barnevillei</i> Chaudoir, 1867		0.3 \pm 0.6	0.3 \pm 0.6		P	i	d
<i>Nebria (Nebria) asturiensis</i> Bruneau de Miré, 1964		1.0 \pm 1.0		1.0 \pm 1.7	P		nc
<i>Notiophilus biguttatus</i> (Fabricius, 1779)	2.0 \pm 1.0	4.7 \pm 3.2	3.3 \pm 1.5	3.7 \pm 1.5	P	i	d
<i>Olisthopus fuscatus</i> Dejean, 1828	0.7 \pm 1.2	1.0 \pm 1.7	3.0 \pm 3.6	1.3 \pm 2.3	H	i	i
<i>Olisthopus rotundatus</i> (Paykull, 1798)		4.0 \pm 6.1		2.7 \pm 4.6	P		d
<i>Poecilus (Macropoecilus) kugelanni</i> (Panzer, 1797)		1.0 \pm 1.0		1.0 \pm 1.0	P		nc
<i>Poecilus (Poecilus) versicolor</i> (Sturm, 1824)	0.3 \pm 0.6	2.3 \pm 2.5	1.0 \pm 1.0	2.0 \pm 1.0	P	i	d
<i>Pterostichus (Oreophilus) cantaber</i> (Chaudoir, 1868)	0.3 \pm 0.6				P		d
<i>Synuchus vivalis vivalis</i> (Illiger, 1798)	8.3 \pm 12.7	0.3 \pm 0.6	5.0 \pm 7.0		H	d	d
<i>Trechus (Trechus) quadristriatus</i> (Schränk, 1781)	1.0 \pm 1.7a		b*		P	d	
<i>Trechus (Trechus) suturalis</i> Putzeys, 1870		0.3 \pm 0.6			P		d
<i>Zabrus (Iberozaabrus) consanguineus</i> Chevrolat, 1865	0.3 \pm 0.6		0.3 \pm 0.6	0.3 \pm 0.6	H	nc	i
Total number of individuals	174a	355	256b*	411			
Number of species	17	20	14	20			

Treatments: C = control, F = fertilization. Significant differences between treatments at 3 (a,b) and 15 (c,e) months: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Feeding habit of the species: H = herbivore or mostly plant material, P = predator or mostly animal material. Abundance behaviour after N addition: i = increase, d = decrease, nc = no change.

3. Results

3.1. Carabidae

A total of 1196 individuals (29 species) were captured (Table 1). At a short-term scale, overall carabid abundance was significantly higher ($F_{1,30} = 5.8$, $P = 0.02$) in fertilized than in control plots. However, no significant differences were detected in species richness. Six species (*Carabus deyrollei*, *Cymindis coadunata*, *Cymindis humeralis*, *Pterostichus cantaber*, *Trechus quadristriatus* and *Trechus suturalis*; including herbivorous and predator species) were exclusively collected from control plots, while four species (*Calathus fuscipes*, *Carabus lineatus*, *Cicindela sylvatica* and *Laemostenus terricola*; all predators) were exclusively captured in the fertilized plots.

At a short-term scale, most carabid species (11 of 19) increased in abundance in fertilized plots as compared with control plots, significantly so for three predator *Carabus*

species [*C. amplipennis* ($F_{1,30} = 5.7$, $P = 0.02$), *C. lineatus* ($F_{1,30} = 4.3$, $P = 0.04$) and *C. macrocephalus* ($F_{1,30} = 4.9$, $P = 0.04$); Table 1]. Over this period, only *T. quadristriatus* decreased significantly ($F_{1,30} = 5.0$, $P = 0.03$) due to fertilization. At a medium-term scale, one species, *Calathus uniseriatus*, increased significantly ($F_{1,30} = 8.2$, $P < 0.01$) after the treatment, while most species (11 of 24) decreased in abundance in fertilized plots, *Cryobius cantabricus* significantly so ($F_{1,30} = 9.4$, $P < 0.01$).

Regarding the species response to fertilization at both short- and medium-term scales (Table 1), five species increased in abundance [including three species (*C. uniseriatus*, *C. amplipennis* and *C. macrocephalus*) significantly in one of the two periods, and the most abundant species collected, *Calathus asturiensis*, although not significantly so]; and three species decreased, but none statistically significantly so.

From the point of view of the trophic classification of the species we found that an equal number of herbivorous species

Table 2
Mean ± standard deviation number of leaf beetle individuals collected in each treatment 3 and 15 months after fertilization

Family Chrysomelidae	C3	C15	F3	F15	Feeding habit	Abundance behaviour	
						3 months	15 months
<i>Altica longicollis</i> (Allard, 1860)	0.3 ± 0.6		0.3 ± 0.6		H	nc	
<i>Lochmaea suturalis</i> (Thomson, 1866)	1.0 ± 1.0	4.3 ± 4.0	0.3 ± 0.6	3.3 ± 3.2	H	d	d
Larvae of <i>Altica</i> spp.	0.7 ± 1.2	3.3 ± 4.2	1.3 ± 0.6	3.7 ± 3.8	H	i	i
Larvae of <i>Lochmaea suturalis</i>	52.0 ± 46.9a	45.0 ± 7.8	91.3 ± 83.1b**	81.7 ± 35.1	H	i	i
Total number of individuals (adults + larvae)	162a	158	280b**	266			

For letters and symbols see Table 1.

increased or decreased their abundances at short- and medium-term scales due to fertilization (Table 1). Most of the predator species increased in abundance on a short-term scale, but decreased on a medium-term scale.

3.2. Chrysomelidae

A total of 866 individuals belonging to two genera (*Altica* and *Lochmaea*) were caught (Table 2). On a short-term scale, leaf beetle abundance was significantly higher ($F_{1,30} = 11.5$, $P < 0.01$) in fertilized than in control plots. The larvae of *L. suturalis* were clearly dominant (Table 2) and, as expected, were significantly more abundant in fertilized plots ($F_{1,30} = 8.6$, $P < 0.01$) on a short-term scale.

3.3. Heteroptera

A total of 327 individuals (17 species) were collected (Table 3). Neither overall abundance nor species richness responded to fertilization. Two species (*Systellonotus championi*, herbivore, and *Coranus subapterus*, predator) were exclusively captured in control plots, while three species (*Macroplox fasciata*, *Notochilus damryi* and *Deraeocoris ribauti*, all herbivores) were exclusively sampled in fertilized plots.

On a short-term scale, a great number of species (8 of 15) increased in abundance in fertilized plots, *Myrmedobia coleoprata* ($F_{1,30} = 4.5$, $P = 0.04$), *Myrmedobia hispanica* ($F_{1,30} = 6.9$, $P = 0.01$) and *Orthotylus ericetorum* ($F_{1,30} = 10.0$,

Table 3
Mean ± standard deviation number of true bug individuals collected in each treatment 3 and 15 months after fertilization

Hemiptera: Heteroptera	C3	C15	F3	F15	Feeding habit	Abundance behaviour	
						3 months	15 months
Family Lygaeidae							
<i>Kleidocerys ericae</i> (Horváth, 1908)	0.7 ± 0.6	0.3 ± 0.6	1.0 ± 1.7	0.3 ± 0.6	H	i	nc
<i>Macrodemia microptera</i> (Curtis, 1836)	13.7 ± 15.0a	29.7 ± 35.0	6.0 ± 7.8b*	19.7 ± 19.8	H	d	d
<i>Macroplox fasciata</i> (Herrich-Schaeffer, 1835)				0.3 ± 0.6	H		i
<i>Notochilus damryi</i> Puton, 1871			0.3 ± 0.6	0.3 ± 0.6	H	i	i
<i>Scolopostethus decoratus</i> (Hahn, 1833)	0.3 ± 0.6	0.7 ± 1.2	0.7 ± 1.2	0.3 ± 0.6	H	i	d
<i>Stygnocoris fuliginosus</i> (Geoffroy, 1785)	0.7 ± 1.2	1.0 ± 1.0	1.3 ± 2.3	1.7 ± 2.1	H	i	i
<i>Trapezonotus (Trapezonotus) montanus</i> Wagner, 1957		1.7 ± 2.9		3.3 ± 4.2	H		i
Family Microphysidae							
<i>Myrmedobia coleoprata</i> (Fallén, 1807)	0.7 ± 1.2a	0.3 ± 0.6	3.7 ± 4.7b*	2.0 ± 2.0	H	i	i
<i>Myrmedobia exilis</i> (Fallén, 1807)	0.3 ± 0.6		0.3 ± 0.6		H	nc	
<i>Myrmedobia hispanica</i> Péricart, 1972	a	0.3 ± 0.6	2.0 ± 3.5b*	0.3 ± 0.6	H	i	nc
Family Miridae							
<i>Deraeocoris (Deraeocoris) ribauti</i> Wagner, 1943			0.3 ± 0.6		H	i	
<i>Orthotylus (Litocoris) ericetorum</i> (Fallén, 1807)	a	1.0 ± 1.0	1.3 ± 2.3b**	0.3 ± 0.6	H	i	d
<i>Systellonotus championi</i> Reuter, 1903	0.3 ± 0.6	1.0 ± 1.7			H	d	d
Family Nabidae							
<i>Nabis (Nabis) ericetorum</i> Scholtz, 1847	0.3 ± 0.6	1.0 ± 0.0		1.3 ± 2.3	P	d	i
<i>Nabis (Nabicala) flavomarginatus</i> Scholtz, 1847	1.7 ± 1.2	0.3 ± 0.6	1.0 ± 1.0	0.3 ± 0.6	P	d	nc
Family Reduviidae							
<i>Coranus (Coranus) subapterus</i> (De Geer, 1773)	0.3 ± 0.6				P	d	
Family Tingidae							
<i>Acalypta nigrina</i> (Fallén, 1807)	0.7 ± 1.2	2.0 ± 2.0	0.3 ± 0.6	1.3 ± 1.2	H	d	d
Total number of individuals	59	118	55	95			
Number of species	11	12	12	13			

For letters and symbols see Table 1.

Table 4
Mean ± standard deviation percentage cover of the vegetation characteristics in each treatment 3 and 15 months after fertilization

	C3	C15	F3	F15	Monte Carlo results	
					Carabid-CCA	True bug-CCA
Annual forbs (AF)	0.0 ± 0.0a	0.5 ± 0.8	0.3 ± 0.5b*	1.0 ± 1.7	F = 2.9**	F = 0.5
<i>Calluna vulgaris</i> (Ca)	62.8 ± 2.0a	65.3 ± 2.0c	73.8 ± 6.8b**	76.5 ± 4.5e**	F = 1.7	F = 1.7
<i>Erica tetralix</i> (Er)	25.7 ± 22.6a	24.5 ± 22.1c	14.5 ± 12.9b**	15.9 ± 14.3e*	F = 6.9***	F = 3.2***
Number of flowers of <i>C. vulgaris</i> (FI)	7.6 ± 1.9a	7.39 ± 1.9c	9.39 ± 0.6b***	11.9 ± 0.5e***	F = 0.5	F = 0.7
Perennial forbs (PF)	1.2 ± 2.1	1.1 ± 1.9	2.3 ± 3.9	2.3 ± 3.8	F = 3.9*	F = 1.1
Perennial graminoids (PG)	7.2 ± 9.1	5.3 ± 4.5	6.8 ± 7.5	6.7 ± 7.6	F = 6.2***	F = 2.0*
Shoots growth of <i>C. vulgaris</i> (Sh)	2.9 ± 0.2a	2.49 ± 0.5c	3.47 ± 0.6b***	4.0 ± 0.6e***	F = 0.8	F = 0.6
<i>Vaccinium myrtillus</i> (Va)	6.3 ± 7.6	4.5 ± 3.8	7.7 ± 9.8	6.5 ± 7.0	F = 4.2**	F = 1.6

Significant values for Monte Carlo permutation tests for each vegetation variable included in the CCAs are also indicated. For letters and symbols see Table 1.

$P < 0.01$) significantly so. In this period, only *Macrodemia microptera* decreased significantly ($F_{1,30} = 5.5$, $P = 0.03$). On a medium-term scale, a similar number of species benefited or harmed due to N addition, although not significantly so.

Regarding species responses to fertilization on both short- and medium-term scales, three species increased (*M. coleoprata* significantly on a short-term scale) and three species decreased (*M. microptera* significantly on a short-term scale) after the treatment (Table 3).

From the point of view of the trophic classification of the species, we found that on a short-term scale herbivores benefited while predators harmed from N addition (Table 3). On a medium-term scale the trophic groups showed no clear responses to the treatment.

3.4. Vegetation

Vegetation showed a positive response to experimental N addition (Table 4). On a short-term scale, the cover of annual forbs increased significantly ($F_{1,54} = 5.6$, $P = 0.02$) after the treatment; while on both short- and medium-term scales we observed increases in the growth of shoots ($F_{1,294} = 13.5$, $P < 0.001$; $F_{1,294} = 116.5$, $P < 0.001$; short- and medium-term, respectively), the number of flowers ($F_{1,294} = 24.6$, $P < 0.001$; $F_{1,294} = 57.2$, $P < 0.001$) and the cover of *C. vulgaris* ($F_{1,54} = 14.95$, $P < 0.01$; $F_{1,54} = 11.7$, $P < 0.01$). The cover of *E. tetralix* was significantly lower in fertilized than in control plots over both periods ($F_{1,54} = 8.8$, $P < 0.01$; $F_{1,54} = 5.5$, $P = 0.02$). Cover values for *V. myrtillus*, perennial forbs and perennial graminoids increased after the treatment, although not significantly.

3.5. Multivariate analyses

The ordination of carabid beetle or true bug species, vegetation characteristics and treatments was defined by the first two axes of the CCAs (Figs.1 and 2; Table 4). The results of these two analyses showed a clear difference between study sites in first place and partial differences between treatments in second place. For carabid beetles (Fig. 1; Table 4), the fertilized plots at site RPII differed from the control plots due to the presence of *C. lineatus* and low *E. tetralix* cover values. The fertilized plots at RPI differed from the control plots

due to the presence of *C. fuscipes*, *C. uniseriatus*, *C. sylvatica*, *L. terricola* and *Leistus barnevillei*, and were characterized by high cover values for *C. vulgaris* and the absence of *E. tetralix*. For true bugs (Fig. 2; Table 4), the fertilized plots at site RPI differed from the control plots due to the presence of *M. hispanica* and high *C. vulgaris* cover values. The fertilized plots at RPII differed from the control plots due to the presence of *D. ribauti* and *M. fasciata*. On a short-term scale, the fertilized plots at SI were characterized by *Myrmedobia exilis* and high cover values for *E. tetralix* and perennial graminoids.

For carabid beetles (Fig. 1), most of the herbivorous species were located in the positive part of axis I (associated with high values for the number of flowers and the cover of *C. vulgaris*),

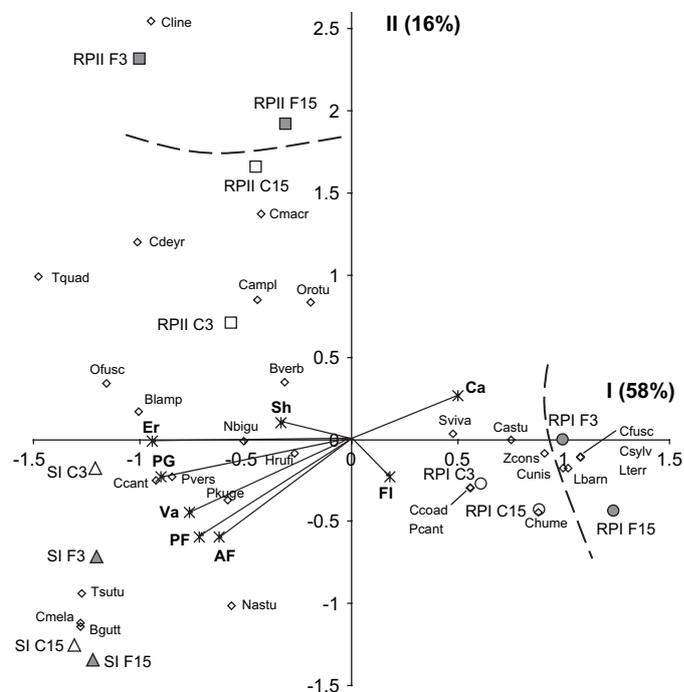


Fig. 1. CCA diagram for carabid species, vegetation characteristics (arrows) and plots (sites: SI, RPI, RPII; and treatments: C = control, F = fertilization, at 3 and 15 months). Variance explained: 58% axis I, 16% axis II; Monte Carlo permutation test: $F = 1.4$, $P = 0.46$. Vegetation characteristics and abbreviations are indicated in Table 4. Carabid species names are a combination of 1 (genus name) and 4 (species name) letters. For example, Blamp = *Bembidion lampros*.

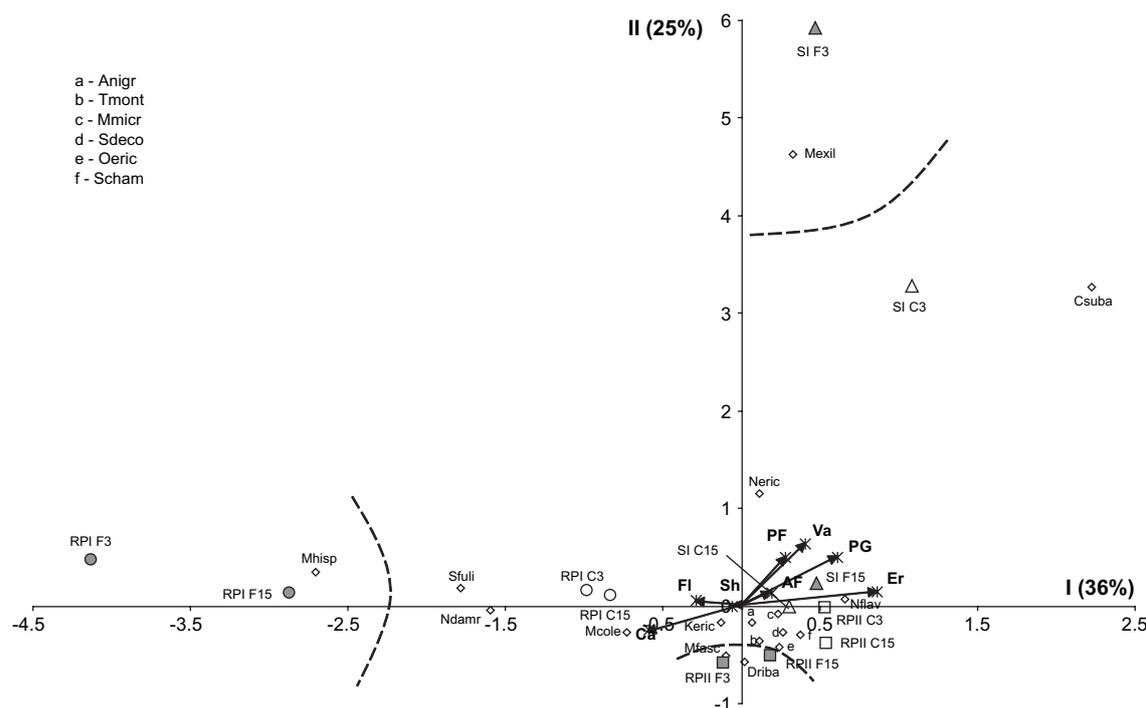


Fig. 2. CCA diagram for true bug species, vegetation characteristics (arrows) and plots. Variance explained: 36% axis I, 25% axis II; Monte Carlo permutation test: $F = 1.7$, $P = 0.03$. For letters and abbreviations see Fig. 1 and Table 4.

while predatory species were located in the negative part of axis I (associated with high cover values for the rest of the vegetation characteristics, except *E. tetralix*). For true bugs (Fig. 2), a similar distribution of trophic groups was obtained related to axis II and vegetation characteristics.

4. Discussion

4.1. Trophic-level responses

Terrestrial ecosystem functioning, including heathlands, is heavily influenced by the availability of limiting nutrients, such as N (Hartley and Jones, 1997). Indeed, greater N availability affects ecosystem productivity and decomposition rates (Adams, 2003), the entire food chain (e.g. Haddad et al., 2000) and “bottom-up” mechanisms for diversity control (Siemann, 1998). Elevated N deposition rates produce functional changes that can be detected at the primary producer level (i.e. via increased vegetation richness, and changes in vegetation production, growth and composition) (Berendse, 1998; Olff et al., 1994; Wilson and Tilman, 1991). This study supports existing evidence for the beneficial effects of N addition on heathland vegetation, as experimental fertilization increased the number of flowers, the growth of shoots and the cover of *C. vulgaris*, comparable with the results of Barker et al. (2004), Calvo et al. (2005) and Carroll et al. (1999). In fact, our preliminary results indicate a slight increase in the total N content in the tissues of *C. vulgaris* on a short-term scale (mean \pm SD: control 0.8 ± 0.04 , fertilized 1.9 ± 0.25 ; Marcos et al. unpublished

results), in accordance with other studies (Hartley et al., 2003; Marcos et al., 2003; Pitcairn et al., 1995).

Changes in primary level production also affect primary level consumers, i.e. herbivores (Hartley and Jones, 1997). Indeed, increasing plant N content results in an improvement in food quality for herbivores, with subsequent effects on herbivore population dynamics, including increased abundance (Haddad et al., 2000; Hartley et al., 2003). On the short-term, this positive relationship applies for leaf beetles and true bugs (in accordance with Siemann, 1998), but not for carabid beetles. Specifically, a clear increase was observed in the number of heather beetle larvae, probably related to increased N content of *C. vulgaris* (Berdowski, 1993; Berdowski and Zeilinga, 1987; Brunsting and Heil, 1985; Power et al., 1998b). However, on a medium-term scale the beneficial effect of N addition was less clear, as similar numbers of carabid beetle and true bug species increased and decreased in abundance. In case of carabid beetles, an increased N availability produces greater flowering of *C. vulgaris* (Calvo et al., 2005), probably resulting in greater seed production and indirectly benefiting granivorous species. However, this expected positive response was not found, possibly because, in general, seeds constitute a low quality food resource in the carabid diet (Fawki et al., 2005). Therefore, it is possible that several species (e.g. *Calathus* spp.) have a broader diet and consumed either seeds or other plant or animal material, depending on food availability (see Fawki et al., 2005; Lövei and Sunderland, 1996) and seasonal variations in seed production (Honek et al., 2006). Consequently, these species are less strongly affected by increased seed production after fertilization. In case

of true bugs, the disappearance of the beneficial effects of N on a medium-term scale could be explained by: (a) the role of N content (i.e. food quality) on nymph survival (see Di Giulio and Edwards, 2003); (b) the need to apply complementary sampling methods for several shrub-canopy active species (see Schmidt and Melber, 2004); or (c) other foraging requirements or diet components not considered here due to the high and incompletely understood diversity of life forms that characterizes the species in this group (see e.g. Schuh and Slater, 1995).

N addition is also expected to have indirect effects on the next trophic level (i.e. predators) (see Siemann, 1998), as a consequence of changes in the abundance (e.g. springtails, Sjursen et al., 2005) and quality of prey (Bilde and Toft, 1999) after fertilization (see also Hartley et al., 2003). In our study, we found contrasting short-term responses to N addition for carabid beetles (increased abundance) and true bugs (decreased abundance). On a medium-term scale, predatory carabid species decreased in abundance while there was no clear response for true bugs. In general, these differences in predator responses between these two groups of insects could be influenced by the following factors: (a) the differential sampling efficiency of pitfall traps (Southwood and Henderson, 2000): more efficient for ground-dwelling predators – e.g. carabid beetles – than for several true bug species that use the vegetation layer to search for prey (Péricart, 1987); (b) the unknown effect of N addition on their immature stages (i.e. larvae or nymphs) whose habitat and food requirements are not completely understood (Di Giulio and Edwards, 2003; Luff, 2005; Saska, 2005). For example, changes in soil properties possibly increase the mortality rate of carabid ground-living larvae (see Luff, 2005), causing a decrease in adult abundance on a medium-term scale. Finally, these differences could also be influenced by (c) microhabitat vegetation differences between heathland sites, as suggested by the results obtained in the CCAs (see e.g. Brose, 2003; Lassau et al., 2005).

4.2. Species-level responses

We reported mainly species-specific responses to fertilization for each arthropod group studied as several species responded positively or negatively depending on their life forms and food requirements (see Berdowski, 1993; Whittaker, 1988). Moreover, seven abundant species showed a consistent trend in their responses on both short- and medium-term scales, and either increased (i.e. benefited) or decreased (i.e. harmed) significantly in one of the periods studied (except *C. asturiensis*). Six of these species were benefited by N addition: *C. asturiensis* and *C. uniseriatus*, both herbivorous carabid species; *C. amplipennis* and *C. macrocephalus*, both predatory carabid species; larvae of the heather beetle, *L. suturalis*; and *M. coleoptrata*, a herbivorous true bug species. One species, *M. microptera*, a herbivorous true bug species, was harmed by fertilization.

Therefore, we propose that these seven arthropod species could be considered as reliable indicators of the effects of N deposition in heathland ecosystems of NW Spain. In recent decades, great attention has been given to defining suitable

indicator species to assess the effects of habitat alteration in terrestrial ecosystems (e.g. McGeoch, 1998). In *C. vulgaris* heathlands, the search for reliable indicators of the effects of N addition has been mainly developed for vegetation species (see Pitcairn et al., 2003), but not specifically for arthropod species, even if they are considered good indicators of environmental change (e.g. for carabid beetles, Rainio and Niemelä, 2003; Taboada, 2007). Finally, our results were obtained after a 15-month period of N addition, and further long-term studies are therefore required (a) to confirm the observed trends in the species or trophic-group responses to fertilization, possibly including complementary sampling methodologies for vegetation-related species, (b) to corroborate the possible indicator value of the species selected, and (c) to assess the ecological mechanisms underlying the response patterns reported here.

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