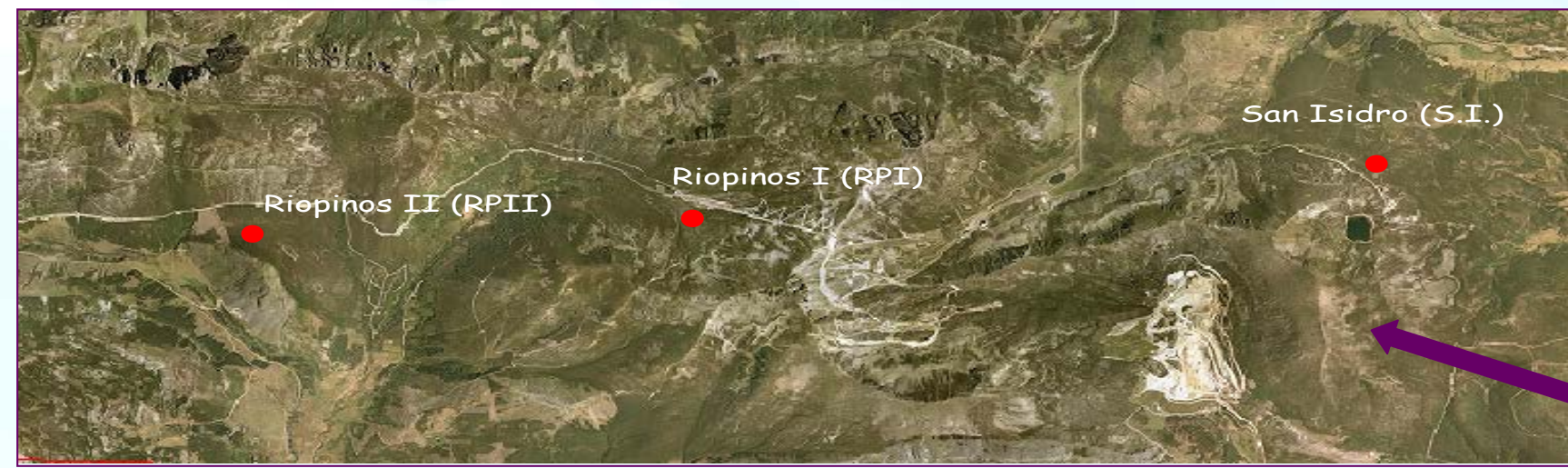


INTRODUCTION

Elevated nitrogen (N) inputs into terrestrial ecosystems generally cause harmful effects to the ecosystems' health. Particularly, those adapted to low levels of N availability, such as montane heathlands, are more vulnerable to increased N inputs. Furthermore, the life-cycle stage of the heathland vegetation might influence its susceptibility to N loading. The N critical load is a valuable tool to assess the heathland resistance to changes in N availability.

In this study we established, based on empirical evidences, the N critical load for montane heathlands located at their southern-most distribution area, in relation to *Calluna vulgaris* heathland life-cycle stages: young- and mature-phase.

STUDY AREA



Distribution of *Calluna vulgaris* in Europe

Soils are Umbrisols developed over shales and sandstones (San Isidro) and quartzite rocks (Riopinos I and Riopinos II). Vegetation is dominated by *Calluna vulgaris*. N background deposition has been calculated in 4.6 kg N ha⁻¹ yr⁻¹.

METHODOLOGY



In each plot (2x 2 m) we measured plant and soil response variables during 2015



Experimental plots



Vegetation cover sampling

Vegetation response variables: species cover (*Calluna vulgaris*, *Erica tetralix*, *Vaccinium myrtillus*, *Cetraria* sp., *Cladonia* sp.); life form cover (annual and perennial forbs, annual and perennial graminoids, woody, bryophytes, and lichens); plant species richness; *Calluna* flowering; current year's *Calluna* shoot length; *Calluna* shoot N and P contents, and N:P ratios.



Calluna shoot

Soil response variables: litter N and P contents, N:P ratios; extractable NH₄⁺ and NO₃⁻; total N; soil organic C; available P; microbial biomass N and C, C:N ratio; enzymatic activities (acid phosphatase, urease, and β-glucosidase).



5 cm

Estimation of N critical load

* One way ANOVA including treatment as fixed factor

* **N critical load:** the lowest N treatment (N₁₀, N₂₀ and N₅₀) at which the response variable showed a significant change with respect to the control treatment (N₀).

RESULTS

A significant increase in shoot N content was found at N₁₀ treatment in **young heathlands**. It was necessary a higher N load (N₂₀) to detect changes in *Calluna* vital rates (flowering, growth), and soil variables (litter N content and soil ammonium). However, in **mature stands** a significant increase was found at N₁₀ treatment in *Calluna* vital rates (flowering and growth) and *Calluna* chemistry (shoot N content). Changes in soil variables (ammonium and microbial C:N ratio) were only found at N₅₀ treatment. No changes were found in species composition and richness



View of flowering of mature stands in Riopinos I

Conclusions

The current N critical loads in montane heathlands are established within the range 10-20 kg N ha⁻¹ yr⁻¹ for young and mature stands. However, the effects of higher N availability were different depending of growth phase. Mature heathlands showed more sensitivity to low N input than young ones. The main indicators of the impact of higher N inputs were: *Calluna* flowering, growth and shoot N content.

Young heathlands

N treatment				
	N10	N20	N50	p-value
No. flowers	ns	↑ ***	↑ ***	0.000
Calluna shoot length	ns	ns	↑ ***	0.000
Calluna shoot N content	↑ ***	↑ ***	↑ ***	0.000
Litter N	ns	ns	↑ **	0.005
Calluna N:P ratio	ns	↑ **	↑ ***	0.000
NH ₄ ⁺	ns	ns	↑ *	0.028

Mature heathlands

N treatment				
	N10	N20	N50	p-value
No. flowers	↑ ***	↑ ***	↑ ***	0.000
Calluna shoot length	↑ ***	↑ ***	↑ ***	0.000
Calluna shoot N content	↑ **	↑ **	↑ ***	0.000
Calluna N:P ratio	ns	↑ *	↑ *	0.011
Microbial C:N ratio	ns	ns	↓ **	0.034

Direction of response (↑ increased or ↓ decreased) and significance level [*** (p < 0.001), ** (0.01 > p > 0.001), * (0.05 > p > 0.01), and ns (p > 0.05)] of different N treatments (N₁₀, N₂₀ and N₅₀) respect to the control N treatment (N₀). Significance level of 'N treatment' effect for each response variable is presented as p-value.