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Time- and age-related effects of experimentally simulated nitrogen deposition on the functioning of montane heathland ecosystems

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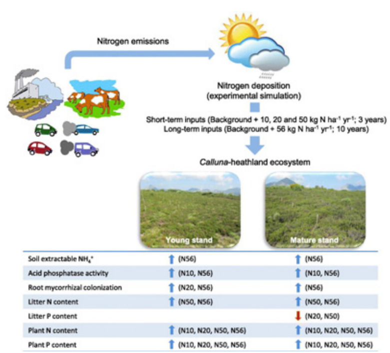
HIGHLIGHTS

- Many biogeochemical properties of marginal heaths did not respond to N fertilization.
- Chronic (10-year) high-N inputs increased soil extractable N-NH_4^+.
- Acid phosphatase enzyme activity responded positively to chronic high-N additions.
- *Calluna* mycorrhizal colonization increased in response to chronic high-N inputs.
- *Calluna* tissue nutrient content increased with N addition especially in young stands.

GRAPHICAL ABSTRACT



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ABSTRACT

Ecosystems adapted to low nitrogen (N) conditions such as *Calluna*-heathlands are especially sensitive to enhanced atmospheric N deposition that affects many aspects of ecosystem functioning like nutrient cycling, soil properties and plant-microbial-enzyme relationships. We investigated the effects of five levels of experimentally-simulated N deposition rates (i.e., N fertilization treatments: 0, 10, 20 and 50 kg N ha^{-1} yr^{-1} for 3 years, and 56 kg N ha^{-1} yr^{-1} for 10 years) on: plant, litter, microbial biomass and soil nutrient contents, soil extracellular enzymatic activities, and plant root ericoid mycorrhizal colonization. The study was conducted in marginal montane *Calluna*-heathlands at different developmental stages resulting from management (young/building-phase and mature-phase). Our findings revealed that many soil properties did not show a statistically significant response to the experimental addition of N, including: total N, organic carbon (C), C:N ratio, extractable N-NO_3^-, available phosphorus (P), urease and β-glucosidase enzyme activities, and microbial biomass C and N. Our results also evidenced a considerable positive impact of chronic (10-year) high-N loading on soil extractable N-NH_4^+, acid phosphatase enzyme activity, *Calluna* root mycorrhizal colonization by ericoid fungi, *Calluna* shoot N and P contents, and litter N content and N:P ratio. The age of heathland vegetation influenced the effects of N addition on ericoid mycorrhizal colonization, resulting in higher colonized roots in young heathlands at the control, low and medium N-input rates; and in mature ones at the high and chronically high N rates. Also, young heathlands exhibited greater soil extractable N-NO_3^-, available P, microbial biomass N, *Calluna* shoot N and P contents, and litter N content, compared to mature ones. Our results highlighted that accounting for the N-input load and duration, as well as the developmental stage

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of the vegetation, is important for assessing the effects of added N, particularly at the heathlands' southern distribution limit.

1. Introduction

The increase in human-induced atmospheric reactive nitrogen (N) in the last century has resulted in a dramatically increase in N deposition rates (Calvo-Fernández et al., 2017), which are expected to rise in future decades at a global scale (Galloway et al., 2004), with slight differences between developing regions and industrialized ones (Vet et al., 2014). Airborne N loading has been identified as one of the most important drivers of biodiversity loss at a global scale (Sala et al., 2000), which in turn is expected to have negative consequences for multiple ecosystem functions. There exists compelling evidence of N-driven damage to ecosystems even at low deposition rates (Bähring et al., 2017; Phoenix et al., 2012). Moreover, chronic N loading has severe impacts on many ecosystem functions when the critical N threshold is exceeded (Gao et al., 2014). These harmful effects are caused by ecosystem eutrophication and soil acidification processes (Bobbink et al., 2010; Stevens et al., 2011; Zhu et al., 2015), altering the biogeochemical cycles of N, carbon (C) and phosphorus (P) (Erisman et al., 2011).

Ecosystems adapted to low levels of nutrient availability, such as heathlands dominated by the dwarf shrub *Calluna vulgaris* (L.) Hull (henceforth referred to as *Calluna*), are particularly sensitive to airborne N deposition (Cuesta et al., 2008; Fagúndez, 2013; Jones and Power, 2012; Meyer-Grünefeldt et al., 2016; Southon et al., 2012). Both field-scale surveys and N-manipulation experiments testing the effects of a variety of N-loading rates over different temporal scales have evidenced substantial N-driven changes in the composition, diversity and functioning of nutrient-poor *Calluna*-heathlands (e.g., Calvo et al., 2005, 2007; Friedrich et al., 2011; Power et al., 2006; Southon et al., 2013), threatening their persistence across Europe (Fagúndez, 2013). Moreover, several studies have evaluated the cumulative effects of N in heathland ecosystems (Johnson et al., 1998; Phoenix et al., 2012; Southon et al., 2012; among others), since chronic N loading is expected to aggravate the impact of N even at low input rates (Phoenix et al., 2012; Power et al., 2006).

Increased N inputs alter a multitude of heathland characteristics such as soil and litter properties (e.g., nutrient availability, enzyme activities or microbial biomass) or plant traits [e.g., growth, flowering, tissue and litter chemistry or plant susceptibility to biotic (e.g., pathogen or pests) and abiotic (e.g., frost or drought) stressors] (Bähring et al., 2017; Jones and Power, 2012; Marcos et al., 2003; Meyer-Grünefeldt et al., 2016; Southon et al., 2013; Taboada et al., 2016). Elevated N inputs stimulate N mineralization rates (Phoenix et al., 2012), resulting in increased soil extractable N-NH_4^+ and N-NO_3^- (Boot et al., 2016; Song et al., 2017; Southon et al., 2013). This enhanced soil N availability may cause either an increase (Du et al., 2014; Haugwitz et al., 2011) or decrease (Ajwa et al., 1999; Boot et al., 2016) in the nutrient contents of the soil microbial biomass, altering the cycles of soil C and N (Contosta et al., 2015; Ramírez et al., 2012; Zhu et al., 2015), and the ericoid mycorrhizal (ERM) fungal community associated with *Calluna* in nutrient-poor environments (Caporn et al., 1995; Yesmin et al., 1996). Since soil microorganisms are considered the primary sources of soil enzymes, and these are involved in nutrient metabolism and decomposition processes (Fatemi et al., 2016; Ramírez et al., 2012; Sinsabaugh and Follstad, 2012; Song et al., 2017; Zhu et al., 2015), an increase in N inputs is expected to alter soil enzymatic activities such as acid phosphatase (P cycle), urease (N cycle) and β-glucosidase (C cycle) (Ajwa et al., 1999; Jian et al., 2016; Ochoa-Hueso et al., 2011, 2014). These variations very likely affect the storage, turnover and uptake of soil nutrients (Cenini et al., 2016; Jones and Power, 2012). As a result, excess N accumulation in heathland ecosystems promotes enhanced rates of nutrient uptake by *Calluna* plants and subsequent increases in foliar tissue N and P contents (Calvo et al., 2007; Jones and Power, 2012; Pilkington et al., 2005b; Rowe et al., 2008; von Oheimb et al., 2010), as well as increases in litter N and P contents (Pilkington et al., 2005b).

Age-related differences in *Calluna* nutrient uptake and growth rate are expected to influence the impacts of N deposition on heathlands (Jones and Power, 2015; Meyer-Grünefeldt et al., 2015), but till now only a limited number of studies have assessed these effects (i.e., Britton et al., 2008; Jones and Power, 2015). European heathlands have traditionally been managed to create pastures for breeding livestock and their nutrient poor status has been preserved through practices as mowing, sod cutting and prescribed burning (Fagúndez, 2013; Härdtle et al., 2006, 2009), resulting in the periodic rejuvenation of heathland vegetation (Gimingham, 1972; Henning et al., 2017). In recent decades, however, land use abandonment has led to heathland management cessation and to *Calluna* plants reaching the mature or degenerate phase of development (sensu Gimingham, 1972; Calvo et al., 2007; Henning et al., 2017). As time progresses since the last management (e.g., prescribed burning, mowing, sod-cutting, and grazing), ageing heathland ecosystems accumulate N in soils and in the vegetation biomass (Härdtle et al., 2009; Jones and Power, 2015). Therefore, specific measures to compensate for atmospheric N deposition are required to remove the excess of N stored in the ecosystem, and, thus to keep a low-N status (Calvo et al., 2005; Härdtle et al., 2006, 2009; Marcos et al., 2009).

In contrast to north-western (e.g., Phoenix et al., 2012; Southon et al., 2012) and central European (e.g., Bähring et al., 2017; de Vries et al., 2009; Friedrich et al., 2011) *Calluna*-heathlands, to date, only one study has been developed on the time-scale and age-related effects of enhanced N deposition in montane *Calluna*-heathlands located at the southern-most limit of their distribution range (Cantabrian Mountains, NW Spain) (i.e., plant-herbivore-predator relationships: Taboada et al., 2016). This is despite these marginal southern *Calluna*-heathlands having been found to respond differently to global change drivers (such as N deposition) as compared to central European ones (Meyer-Grünefeldt et al., 2016). In this study, we evaluated the effects of different levels of experimentally simulated N deposition on the functioning of marginal montane *Calluna*-heathlands, mediated by the age of heathland vegetation resulting from management activities (prescribed burning), with particular attention being paid to the cumulative impact of N loading throughout time. Specifically, we assessed the effects of five levels of N fertilization rates (0, 10, 20 and 50 kg N ha^{-1} yr^{-1} for 3 years, and 56 kg N ha^{-1} yr^{-1} for 10 years) on: (1) soil chemical properties, (2) soil extracellular enzymatic activities, (3) soil microbial biomass C and N, (4) plant mycorrhizal colonization, (5) plant nutrient uptake and (6) litter chemistry. To our knowledge, this is the first study that evaluates the overall impact of cumulative N loading on plant-soil-microbial-enzyme relationships in both young and mature developmental stages of European heathlands.

We hypothesize that an increase in N loading will result in: (1) a subsequent increase in plant-litter-soil N and P contents due to higher nutrient accumulation and immobilization, as well as an increase in plant and litter N:P ratios (Britton et al., 2008; Southon et al., 2013;