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# Effects of climate warming and nitrogen deposition on species and community plasticity in grasslands in the French Pyrenees

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**Abstract.** Nitrogen deposition is the third cause of biodiversity loss on natural systems. Many experiments have studied responses of grassland ecosystems to nitrogen deposition, but only a few have addressed the interaction of such process with global warming. This work aims to understand the plastic response of species of a grassland community to the interaction of atmosphere warming and nitrogen deposition through responses in plant functional traits. The experiment was set up in 2012 in a typical subalpine grassland dominated by *Nardus stricta* L. The experimental design includes six randomized blocks with three nitrogen deposition treatments (0, 5 and 30 kg N ha<sup>-1</sup> year<sup>-1</sup> in a dose of 2/3 NH<sub>4</sub><sup>+</sup> and 1/3 NO<sub>3</sub><sup>-</sup>) and two atmosphere warming treatments (ambient and warmed by top open chambers). A survey was performed during the peak biomass period on summer 2015. After completing field measurements, we collected samples from seven out of the 32 recorded species, to measure other plant functional traits. For the remaining species, we acquired the traits from trait databases, in order to calculate the community weighted mean. We want to know if: (1) the most abundant species show high plasticity in response to atmosphere warming and nitrogen deposition; and (2) there is a shift in plant functional traits at the community level in response to environmental changes.

Keywords. Nitrogen deposition - Global warming - Plant functional traits - Community plasticity.

### Effets du réchauffement climatique et des dépôts d'azote sur la plasticité des espèces et des communautés des prairies des Pyrénées françaises

Résumé. Les dépôts d'azote sont la troisième cause de perte de biodiversité dans les systèmes naturels. De nombreuses expériences ont étudié les réponses des écosystèmes des prairies aux dépôts d'azote, mais seulement quelques-unes ont abordé l'interaction de ces processus avec le réchauffement climatique. Ce travail vise à comprendre la réponse plastique d'une communauté de prairie et l'interaction entre le réchauffement atmosphérique et les dépôts d'azote, à travers les traits fonctionnels des plantes. L'expérience a été mise en place en 2012 dans une communauté typique de l'étage subalpin, composée principalement par Nardus stricta L. Le dispositif expérimental comprend six blocs aléatoires avec trois traitements de dépôts d'azote (0, 5 et 30 kg N ha-1 an-1 à une dose de 2/3 NH<sub>4</sub> + et 1/3 NO<sub>3</sub>) et deux traitements de réchauffement atmosphérique (ambiant et réchauffé par chambres ouvertes). Un échantillonnage a été effectué en été 2015 au moment ou la biomasse était à son maximum. Après les mesures sur le terrain, nous avons recueilli des échantillons provenant de sept des 32 espèces recensées, pour mesurer les traits fonctionnels des plantes supplémentaires. Pour les autres espèces, nous avons utilisé les valeurs consignées dans des bases de données de traits, afin de calculer la moyenne pondérée de la communauté. Nous voulons savoir si: (1) les espèces les plus abondantes montrent une grande plasticité en réponse au réchauffement atmosphérique et aux dépôts d'azote; et (2) il y a un changement dans les traits fonctionnels de plantes au niveau de la communauté en réponse aux changements environnementaux.

Mots-clés. Dépôts d'azote – Réchauffement global – Traits fonctionnels de plantes – Plasticité.

#### I - Introduction

Grasslands cover a very large portion of the Earth's surface (about 52 million km2) and play an important role as food source for livestock, habitat for wildlife, environmental protection and conservation of genetic resources (FAO, 2005). The reactive nitrogen (N) deposition has become a serious threat to survival of marine and terrestrial ecosystems. Forecasts indicate that this factor will be the third major cause of biodiversity loss by the year 2100. Moreover, global warming is one of the most important elements of climate change. Nevertheless, there are few studies that analyze the responses of grasslands to the combined effect of warming and N deposition. Measurements of N deposition in the Pyrenees show higher values than expected from model predictions, with amounts of about 8 kg ha<sup>-1</sup> vr<sup>-1</sup> of N currently recorded in the French Pyrenees (Boutin et al., 2015). These values of N may be low when compared to the values of N in some intensive grasslands. Experimental studies show how warming is threatening the vegetation composition (Sebastia et al., 2008), and plant (Sebastia, 2007) and functional (Debouk et al., 2015) diversity of grassland ecosystems in the Catalan Eastern Pyrenees. In order to study the combined effect of N deposition and warming, an experiment was set up in a subalpine grassland in the French Eastern Pyrenees. Here an analysis of the responses of the vegetation to both factors after three years of experimentation is presented. In particular, we report the responses of functional traits of the most abundant plant species in the grassland. In this context, this study aims at analyzing the effects of three nitrogen deposition and two warming scenarios on the plasticity of subalpine grasslands in the Pyrenees.

#### II - Materials and methods

#### 1. Location

The study site is located in the Ariège Region ( $42^{\circ}$  42' 51.1" N - 1° 42' 18.3" W; 1940 m.a.s.l.), in the French Pyrenees. The study area corresponds to a subalpine grassland mostly dominated by *Nardus stricta* L. and a pool of other species with lower abundance. Climate is characterized by mean annual temperature of 5.6 °C and mean annual precipitation of 1100 mm. The study area is moderately grazed by sheep, cattle and horses, but the site has been enclosed since the beginning of the experiment in June 2012 to exclude grazing.

#### 2. Experimental design

We sampled 36 plots in the experimental area three years after the beginning of the experiment. The design includes six randomized blocks with three nitrogen deposition treatments (0, 5 and 30 Kg N ha<sup>-1</sup> year<sup>-1</sup> in a dose of 2/3 NH<sup>4</sup>+ and 1/3 NO<sup>3</sup>-) and two atmosphere warming treatments (ambient and warmed by top open chambers with mean increase of 1.3 °C).

#### 3. Functional trait measurements

We selected ten plant functional traits to answer the objectives of this experiment. In a first stage, we carried out a visual inventory of species cover in the field, and measured plant height, taking the highest and smallest individuals per species and treatment, to evaluate responses related to competitive vigor and stress tolerance (Cornelissen *et al.*, 2003). We then selected 10 individuals of the most abundant species (three grasses, a sedge and a forb) for each treatment within each block, put them in a cooler to keep them fresh, and brought them to the laboratory where we measured additional plant functional traits. The traits measured in the laboratory were: leaf size (LS, one-side projected surface area), specific leaf area (SLA, the ratio between the leaf size and its ovendry mass), and leaf dry matter content (LDMC, oven-dry leaf mass divided by its water-saturated fresh mass). Plant material for each species was then sent to the laboratory to determine leaf nitrogen content (LNC) and carbon content (LCC), corresponding to the total amount of nitrogen and

carbon per unit of dry leaf mass, but this date are not presented here. We used a microbalance to weigh fresh leaves. For leaf projected areas we used a digital scanner (Brother MFC-7860DW Printer) and Adobe Photoshop CS6 software to estimate the leaf surface area. We later dried the samples in an oven at 60°C for 72 hours and we calculated the dry mass. Based on these procedures, we calculated LS, SLA and LDMC. We acquired further traits from literature: growth form (associated with plant strategy, climatic factors and land use), life form (adaptation of plants to climate), vegetation spread and growth habit.

#### 4. Data analysis

We performed regression analysis on each trait and for each species to determine their responses to the treatment factors. In the regression model we included the two treatment factors, warming and N deposition, and their interaction. We also included block as a factor.

#### III - Results and discussion

Our results show that the treatment factors warming and N deposition had a significant effect on the dynamics of traits, except for leaf dry matter content (LDMC), which was the least responsive trait to both factors (Table 1). WSFM was the most responsive trait to both treatment factors for practically all the species (Table 1). SLA was shown to be a highly responsive trait in front of warming by Debouk *et al.* (2015), however in our study only the SLA of *F. eskia*, and to a lesser effect of *C. caryophyllea*, were modified with warming. The interaction between N deposition and warming was significant for many species, particularly *F. nigrescens*, *C. caryophyllea* and *P. erecta*. Generally, these three species were the most affected by warming. Sebastià *et al.* (2008) showed that the first two species became more abundant in a warming experiment. On the other hand, of all the dominant species tested, *F. nigrescens* was the least responsive, particularly to warming. Sebastià *et al.* (2008) already showed how this species went from being dominant in subalpine grasslands in the Pyrenees to becoming scarce under strong warming conditions. However, *F. nigrescens*, together with *N. stricta*, were highly responsive to N deposition.

Table 1. Warming and N deposition effects on plant functional traits of the most abundant grassland species. WSFM: Water Saturated Fresh Mass; LDMC: Leaf Dry Matter Content; ODM: Oven Dry Mass; SLA: Specific Leaf Area (see section 3)

Species	Treatment	WSFM	ODM	Leaf Size	SLA	Height	LDMC
Carex caryophyllea	Warming	***	n.s.	n.s.	**	***	n.s.
	N	***	**	n.s.	n.s.	***	n.s.
	Warming x N	***	*	n.s.	*	n.s.	n.s.
Festuca eskia	Warming	***	***	n.s.	***	***	*
	N	n.s.	n.s.	***	***	***	n.s.
	Warming x N	***	n.s.	***	***	***	n.s.
Festuca nigrescens	Warming	**	n.s.	n.s.	n.s.	**	n.s.
	N	***	**	***	n.s.	n.s.	n.s.
	Warming x N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nardus stricta	Warming	n.s.	n.s.	***	n.s.	n.s.	*
	N	***	***	**	***	***	***
	Warming x N	n.s.	*	n.s.	n.s.	*	n.s.
Potentilla erecta	Warming	***	n.s.	***	n.s.	***	*
	N	***	***	n.s.	n.s.	***	n.s.
	Warming x N	***	n.s.	**	n.s.	***	n.s.

n.s. non significant; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

#### **IV - Conclusions**

Overall, our results show that both N deposition and atmosphere warming have a strong effect on plant functional traits. As different species respond plastically to either one or both of these treatment factors in different ways, we expect that the combination of the two stresses will have in the long term a strong effect on the persistence of the species and the composition of the grassland.

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