Elevated CO_2 and Extreme Climatic Events Modify Nitrogen Content and Ruminal Protein Digestion of Temperate Grassland

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Elevated CO_2 and extreme climatic events modify nitrogen content and ruminal protein digestion of temperate grassland

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Key words: atmospheric CO_2, drought, grassland, nitrogen, rumen fermentation, protein digestion, ruminant

Abstract
This study was aimed at analyzing changes in nitrogen (N) content and *in vitro* protein rumen digestion of an upland grassland exposed to climate changes in controlled conditions. Monoliths of grassland were inserted in 12 macrocosms in which climatic conditions for the 2050s were simulated (i.e., +2.3°C and 33 mm less precipitation compared to the current climatic conditions). Six of them were subjected to ambient CO_2 (390 ppm) while the other six were subjected to elevated CO_2 (520 ppm). After four months, an extreme climatic event (ECE) consisting of four weeks of reducted precipitation (-50%) followed by two weeks without irrigation combined with a heat wave (+6°C) were applied in three macrocosms at ambient CO_2 and three macrocosms at elevated CO_2. Then, all the macrocosms were irrigated to allow the vegetation to recover. The N content and *in vitro* parameters of rumen protein digestion were measured on plant samples collected before the extreme event (two cuts) and after recovery. Our results indicate that, irrespective of the sampling date, elevated CO_2 results in a decrease in plant N content ($P < 0.01$). Inversely, the application of the extreme event resulted in a large increase in N content ($P < 0.001$) without a significant interaction with the CO_2 effect. These changes significantly impacted ruminal protein digestion as evidenced by changes in the production of the fermentation end-products indicators of the proteolysis, namely ammonia and iso-volatile fatty acids. We conclude that several components of climate change can impact the nitrogenous quality of the forage and its use by ruminants.

Introduction
Climate change is predicted to increase average temperature, atmospheric CO_2 concentration, and the intensity and frequency of extreme climatic events (ECE) such as droughts and heat waves (Planton et al., 2008). All these changes are capable of altering grassland production and forage quality including plant nitrogen (N) content and the subsequent protein digestion by ruminants. Interactions of ECE with elevated CO_2 in terms of forage quality has been under-researched, especially for permanent grasslands (Dumont et al., 2015). Drivers of forage quality under such climatic conditions need to be better understood in order to adapt grass-based ruminant systems to the context of global climate change. The aim of this study was to analyze changes in the N content of an upland grassland when exposed under controlled conditions to an elevated atmospheric CO_2 level, with and without a simulated ECE event. The impact of these changes on ruminant protein digestion was investigated using an *in vitro* rumen fermentation assay.

Methods and Study Site
The experiment was previously described by Roy et al. (2016) and Volaire et al. (2020). In brief, 48 monoliths (1 m^2 each) of soil and their resident plant communities were excavated at 0.6 m depth, from an upland semi-natural grassland site (Redon, 45°43′N, 03°01′E, 800 m a.s.l.) near Saint-Genès-Champanelle (France). Monoliths were transported to the Ecotron near Montpellier (43°40′N, 03°52′E), and four of them were inserted in each of the 12 Ecotron macrocosms, where they acclimated to the climatic conditions fixed according to future scenarios projected for the 2050s (+2.3 °C and -33 mm, compared to the 1990–2009 annual air temperature and precipitation means, respectively) (Ciais et al., 2005). From mid-March to the end of the experiment, six macrocosms were exposed to elevated CO_2 (520 ppm) while the other six were exposed to current atmospheric CO_2 (390 ppm). An ECE was applied to three randomly selected macrocosms out of the six at each CO_2 concentration. This ECE corresponded to a reduction in precipitation by 50% during four weeks in midsummer (from 25 June to 21 July) followed by two weeks (from 22 July to 4 August) with no irrigation and a concomitant air temperature increase (+3.4 °C compared with the 2050s average). After the ECE, from 5 to 31 August, irrigation was progressively increased in the treatment with ECE to obtain the same

cumulative precipitation as in the control treatment without ECE. Until the end of the experiment on 3 November, all macrocosms were exposed to the same climatic conditions, replicating the model projections for the 2050s.

On three dates (26 April, 9 June, 3 November), above-ground biomass was cut on a fixed center square (0.5 × 0.5 m^2) in each monolith at 6 cm cutting height using a precision mower. The April cut was an expression of the winter and spring growth when no treatments were applied except for one month of CO_2 treatment, while the June cut was the expression of spring and early summer growth and of CO_2 treatment. The November cut was the expression of summer and fall growth, and included the ECE, the CO_2 treatment and the recovery phase. The cut material was separated into two subsamples: the first sample was oven-dried at 60°C for 72 h and used for the determination of the N content; the second was freeze-dried and used for the *in vitro* rumen fermentation assay.

The N content was determined in the isotopic platform of INRAE Nancy using a stable isotope ratio mass-spectrometer (Isoprime 100, IsoPrime, Manchester, UK). The *in vitro* rumen fermentation assay was performed as described by Niderkorn et al. (2011). Briefly, ground freeze-dried samples (0.6 g) were incubated in anaerobic conditions at 39°C in culture bottles containing 40 ml of buffered rumen juice from sheep, and the concentrations of total volatile fatty acids (VFA), *iso*-VFA and ammonia (NH_3) in the fermentation medium were measured after 24 h of fermentation. The VFA and *iso*-VFA (isobutyrate, isovalerate) were measured by gas chromatography and NH_3 was measured by the Berthelot reaction (Park et al., 2009). All variables were analyzed using a mixed model (MIXED procedure, SAS Enterprise Guide 5.1, SAS Institute Inc., Cary, NC, USA). Each macrocosm was considered as an experimental unit. The CO_2 concentration, the effect of the ECE and their interaction were used as fixed effects, and the cuts nested within the macrocosm were used as random factors. Significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$.

Results

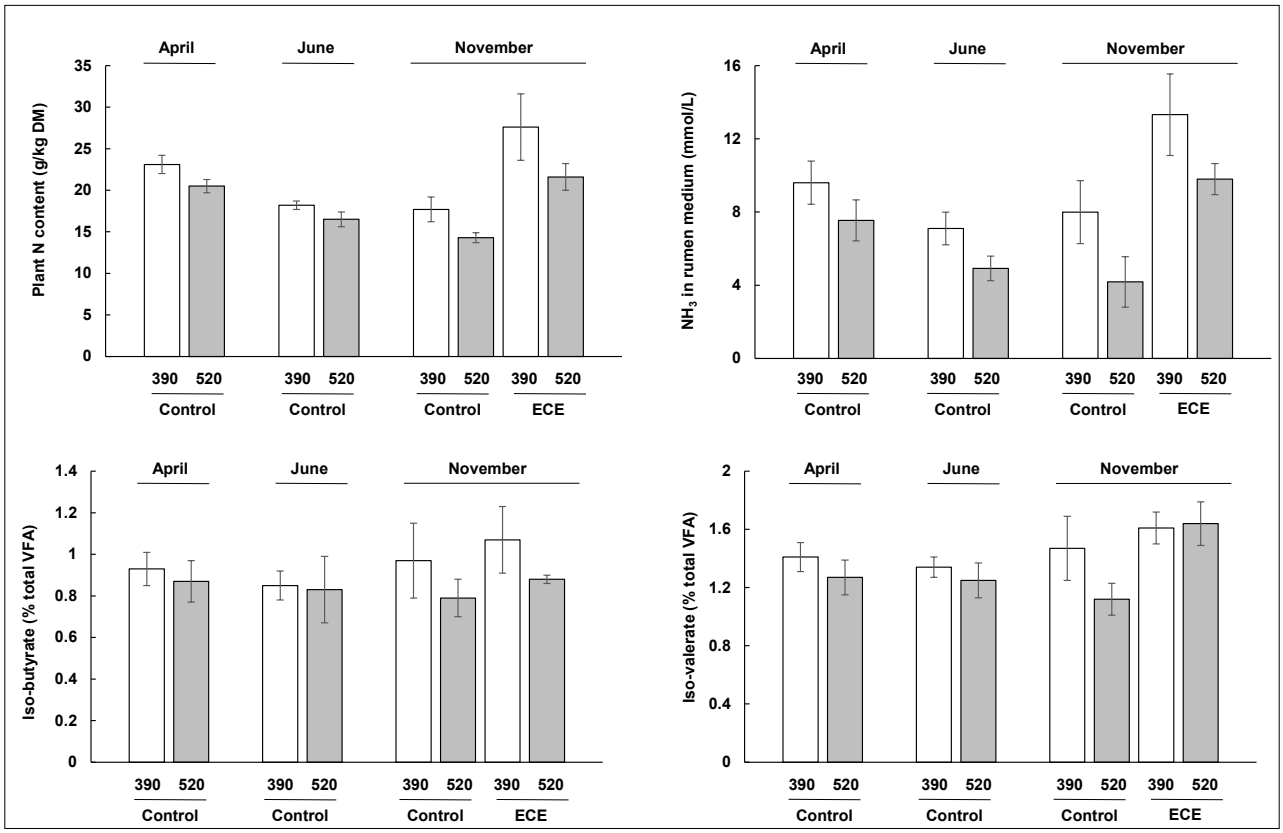

Figure 1: Plant nitrogen (N) content and fermentation end-products (ammonia (NH_3), *iso*-butyrate and *iso*-valerate) produced during *in vitro* rumen incubation of plant communities subject to two levels of CO_2 concentration (390 and 520 ppm) with or without an extreme climatic event (ECE) at three different cuts.

Table 1. Effects of factors (*P*-values) on plant nitrogen (N) content and fermentation end-products (total volatile fatty acids (VFA), *iso*-butyrate, *iso*-valerate and ammonia (NH_3)) produced during *in vitro* rumen incubation of plant communities subject to two levels of CO_2 concentration (390 and 520 ppm) with or without an extreme climatic event (ECE) at three different cuts

	Cut April	Cut June	Cut November		
	CO_2 effect	CO_2 effect	CO_2 effect	ECE effect	CO_2 × ECE effect
N	<0.001	0.003	0.007	<0.001	0.346
NH_3	0.014	0.001	0.005	<0.001	0.882
Total VFA	0.056	0.577	0.256	0.186	0.871
Iso-butyrate	0.308	0.755	0.063	0.289	0.999
Iso-valerate	0.062	0.134	0.111	0.006	0.067

The N content in the above-ground biomass was significantly lower at 520 ppm CO_2 concentration compared to the 390 ppm level (Figure 1 and Table 1). The reductions were evident in the cuts of April (-11%), June (-9%) and November (-21%). After the recovery of the ECE in November, the N content strongly increased (+54%) without significant interaction of CO_2 × ECE. Increasing the level of CO_2 strongly decreased the NH_3 concentration in the incubation medium for the cuts of April, June and November (-21%, -31% and -34%, respectively). For the cut of April, increasing the level of CO_2 also tended to increase the total VFA concentration and to decrease the proportion of *iso*-valerate, while the proportion of *iso*-butyrate tended to decrease for the cut of November. The ECE increased the NH_3 concentration in the incubation medium (+90%) and the proportions of *iso*-valerate (+25%) and there was a trend for the effect of interaction CO_2 × ECE on *iso*-valerate concentration.

Discussion

Contrasted effects of elevated CO_2 and ECE were observed on the N content of above-ground biomass. The lower N concentration under elevated CO_2 compared to the control was shown for the different cuts and is consistent with results reported in the meta-analyses of Dumont et al. (2015) and Dellar et al. (2018). The reductions were not a consequence of significant changes in legume proportions (data not shown) but they can be attributed to increased herbage growth and changes in photosynthetic N use efficiency (Leakey et al., 2009). In addition, we observed a clear increase in N concentration in above-ground biomass for the cut of November when the ECE was applied. The dehydration of plant material during ECE may have led to the asynchrony between plant growth and soil microbe functioning. Plant litter and microbial detritus are sources of energy available for microbial recovery during rehydration, which appears to be faster than in plants (Hofer et al., 2017). Microbial mineralization of the organic matter produces inorganic N that can be taken up by plants. It has also been shown that the maintenance of root exudates during drought can explain above-ground recovery, since root functionality can ensure increased N availability (Karlowsky et al., 2018).

The changes in plant N content strongly affected parameters of protein digestion in the rumen. In particular, the ruminal NH_3 concentration followed the N content, as it is a main end-product of protein degradability through amino acid deamination. The increase of *iso*-valerate with the ECE indicates the increased degradation of protein since this branched-chain volatile fatty acid results from deamination and oxidative decarboxylation of the branched-chain amino acids leucine (Andries et al., 1987). To clarify the impact of elevated CO_2 and ECE on the N value of plant biomass for the ruminant use, it has to be noted that a part of the NH_3 produced is incorporated into the rumen microbial biomass, but the surplus is converted into urea. Urea is largely excreted into the environment, representing N loss and pollutant emission. Indeed, the fraction of urinary N not used by soil microbes and plants is transformed into N_2O, a potent GHG, through microbial nitrification and denitrification (Firestone et al., 1980).

In conclusion, our study shows that the impact of different drivers of climate change, namely elevated CO_2 concentration and ECE (drought and heat wave), have contrasted impacts on N content in the plant biomass of grasslands, and affect differently the subsequent digestive use of N by the ruminants.

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